Following the Path of CCL2 from Prostaglandins to Periostin in Lung Fibrosis

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Abstract

Without question, the greatest and most humbling honor of my scientific career was to learn that I was nominated for the *American Thoracic Society Recognition Award for Scientific Accomplishments*. On the occasion of this award, as I look back on the progress made in the last 15 years, I am pleased by the scientific insights; however, I am also saddened that we still have no internationally recognized efficacious therapy. This perspective will highlight the areas my laboratory has addressed regarding the pathogenesis of idiopathic pulmonary fibrosis in hopes of identifying new therapeutic targets.

Clinical Relevance

This review offers a perspective covering fibrosis research in the last 15 years, highlighting in particular the role of chemokines, eicosanoids, viral infections, and matricellular proteins.

CCR2-Binding Chemokines and Lung Fibrosis: More Than Just Migration

In 1997, chemokine biology was a rapidly expanding field (1). Data were accumulating to show that various chemokines were elevated in lungs of patients with idiopathic pulmonary fibrosis (IPF), suggesting that cellular recruitment might contribute to the disease (for review, *see* Reference 2). However, patients with IPF had little evidence of inflammation in the lungs, and anti-inflammatory therapies had little effect (3). Thus, investigators began looking for alternative chemokine functions (for example, CXC chemokines were shown to be angiogenic; for review, *see* Reference 4). Working with the bleomycin and the FITC animal models of lung fibrosis, we observed that CCL2 was elevated in response to fibrogenic injury (5–7). Patients with interstitial lung disease also have elevated CCL2 in bronchoalveolar lavage fluid (BALF) (8–10), and high levels of CCL2 correlate with nonsurvival (11). Fibrotic fibroblasts overexpress CCL2 (12–14) and respond to CCR2 stimulation by increasing sensitivity to transforming growth factor (TGF)-β and by limiting apoptosis (15), observations that help explain aberrant accumulation of activated fibroblasts in IPF.

We were the first to report that CCR2−/− mice were protected from experimental lung fibrosis (7), an observation that was later confirmed (14, 16, 17). However, CCR2−/− mice did not show significant inhibition in early inflammatory cell recruitment. We later realized that CCR2 was important for recruiting a minor population of cells called "fibrocytes" (5, 6, 18–21). Fibrocytes, classified by dual expression of leukocyte CD45 and mesenchymal collagen 1, comprise a small fraction of peripheral blood cells yet are rapidly recruited to injured lungs (19). The first observations regarding fibrocytes in IPF (22) were followed by studies identifying CXCR4 and CCR7 as important receptors mediating their migration (21). Patients with IPF have elevated numbers of fibrocytes in circulation, and increased percentages of fibrocytes predict early mortality (23). Our studies demonstrated that CCL12 recruited fibrocytes in mice via CCR2 (6) and that CCR2 stimulation of fibrocytes increased expression of collagen 1 (5); these findings were confirmed in human studies (24). Taken together, our data show that CCR2-binding chemokines recruit a novel profibrotic cell type and play important roles in cellular activation and differentiation.

Another theory of IPF pathogenesis suggested that fibrosis was regulated by aberrant epithelial cell–fibroblast interactions (3). Our results demonstrated that CCR2−/−, but not CCR2+/+, alveolar epithelial cells (AECs) were capable of limiting fibroproliferation in coculture (25).

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The addition of CCL2 to the CCR2+/+ AECs prevented synthesis of prostaglandin (PG)E_2 (25) and triggered our interest in this antifibrotic mediator.

PGE_2: A Critical Inhibitor of Myofibroblast Differentiation and Function

PGE_2 is synthesized via cyclooxygenase (COX)-mediated metabolism of arachidonic acid. IPF BALF is PGE_2 deficient (26), and IPF fibroblasts are COX-2 deficient, inhibiting their production of PGE_2 (27, 28). This leads to fibroblast activation because PGE_2 normally limits TGF-β–induced myofibroblast differentiation (29, 30). Similarly, COX-2–deficient AECs are impaired in their ability to limit fibroblast proliferation (31). Loss of PGE_2 synthesis worsens fibrosis, as demonstrated by the fact that mice treated with indomethacin have worsened fibrotic outcomes (32); these results are similar to mice genetically deficient in COX-2 (33). Looking forward, therapeutic approaches have shown that early administration of PGE_2 protects from experimental fibrosis (34, 35). New understandings highlight how PGE_2 loss and myofibroblast accumulation occur in IPF. Normally, PGE_2 signaling can induce fibroblast apoptosis (36), but when fibroblasts encounter stiffened extracellular matrix, their ability to generate antifibrotic PGE_2 is suppressed (37, 38). This causes up-regulation of prosurvival molecules (39, 40), leading to proliferation, contraction, and impaired fibroblast apoptosis (36–40).

Our work demonstrated that the antifibrotic effects of plasminogen activation were linked to plasmin activation of hepatocyte growth factor, causing induction of PGE_2 (41). This antifibrotic pathway also explains the beneficial effects of apoptotic cell instillation in fibrotic lungs (42). However, if true, why were wild-type mice, which had the capacity to synthesize PGE_2 and to activate plasminogen, not protected in experimental models of lung fibrosis? This question led to our discovery that E prostanoid (EP)2, an inhibitory PGE_2 receptor, was progressively lost on fibroblasts from fibrotic mice (43); this observation was later confirmed in patients with IPF (44). Now we know that EP2 promoter hypermethylation (45), or loss of protein kinase A expression, explains why IPF fibroblasts are nonresponsive to PGE_2 (44). Fortunately, stimulation of lung fibroblasts in the presence of plasmin can overcome the resistance to PGE_2 signaling by reorganizing protein kinase A signaling (46). Thus, there may yet be therapeutic potential for coordinated delivery of plasmin and PGE_2 or phosphodiesterase inhibitors (47) to combat extracellular matrix deposition in the setting of lung fibrosis.

Leukotrienes: Lipids that Promote Fibrosis

Arachidonic acid can also be metabolized to form leukotrienes (LTs), and patients with IPF have increased LTs in BALF (48, 49), as do animals treated with fibrogenic agents (50, 51). LTs promote fibroblast migration, proliferation, and activation (52–54). We demonstrated that 5-LO−/− mice were protected from bleomycin-induced fibrosis (50) and that cys LTs are important mediators of IL-13–induced fibrosis post-FITC challenge (55), an observation that has been confirmed in other models (56). Accordingly, cys LT1 receptor–deficient mice were protected from lung fibrosis (57). Two other interesting observations were that latent infection with γ herpes virus could stimulate secretion of cys LTs from lung AECs (58) and that cys LTs could mediate the proliferation of fibrocytes (59).

Viral Infections as Cofactors for Fibrogenesis

An ongoing area of our investigation has been to understand how viral infections augment fibrotic responses in the lung. Treatment of mice with a fibrotic insult followed by infection with murine γ herpes virus-68 (γHV-68) can recruit fibrocytes and exacerbate fibrosis (60). Additionally, γHV-68 latent infection before fibrotic insult enhances fibrogenesis (58). Ongoing work suggests that infection with *Pseudomonas aeruginosa* cannot exacerbate experimental lung fibrosis, nor can all viral infections (data not shown); thus, there may be unique properties about herpes viruses that promote fibrogenesis. Growing evidence suggests that herpes viruses are uniquely retained in the lungs of patients with IPF (61–69) and that infection with herpes viruses augments production of TGF-β in human and murine AECs (70, 71). In Th2-biased mice, γHV-68–mediated lung fibrosis is associated with M2 macrophage polarization (72, 73) and chronic viral reactivation (74). More recently, we and others have demonstrated that γHV-68 infection in aged, but not young, mice induces lung fibrosis (75, 76). The mechanisms uncovered in aged mice include induction of endoplasmic reticulum stress in aged AECs (75) and increased expression of TGF-β receptors in lung fibroblasts (76). Our studies also demonstrate that herpes viral infections play a role in fibrotic lung disease that develops after stem cell transplantation (77). Taken together, these results suggest that occult viral infections may play a prominent but underdiagnosed role in fibrotic lung disease.

Periostin Promotes Lung Fibrosis and Predicts Progression in IPF

As mentioned, aged lungs may be predisposed to fibrosis. We found that periostin, a matricellular protein that cross-links collagen and promotes cellular signaling (78–82), was increased in fibroblasts from old mice (15–18 mo old) compared with young mice (4 mo old; data not shown). Initial studies in patients with IPF demonstrated periostin in areas of fibrotic lung pathology and in serum where it correlated with reductions in lung function (80). We confirmed that levels of periostin were elevated in patients with IPF and could predict disease progression (79). When animal models were initiated, studies in Balb/c mice suggested that periostin influenced chemokine secretion to promote inflammation and/or cellular migration (83). In our studies in C57Bl/6 mice, periostin up-regulated mesenchymal cell proliferation, migration, and extracellular matrix production (79). Interestingly, bone marrow chimeras revealed an important role for periostin production in circulating hematopoietic cells. We found circulating fibrocytes from patients with IPF express abundant periostin mRNA, whereas fibrocytes isolated from control subjects had undetectable levels (79). This suggested that fibrocytes might regulate fibrosis via paracrine secretion of profibrotic mediators rather than via differentiation into

fibroblasts and myofibroblasts (84). Further support for this hypothesis comes from studies of another matricellular protein known as SPARC (secreted protein acidic and rich in cysteine), which also regulates fibrocyte function to promote collagen secretion (85). Taken together, these studies may explain how our adoptive transfer of small numbers of fibrocytes had the ability to increase lung fibrosis (6). Because matricellular proteins may act to increase matrix stiffness, fibroblast activation, and cellular survival (37, 38, 79, 80, 83, 85–88), matricellular proteins may be important therapeutic targets. In fact, our studies demonstrate that treatment of mice with a neutralizing antibody that blocks interaction of periostin with particular cellular integrins can halt progression of established lung fibrosis (79).

Final Thoughts

When reflecting on work from our laboratory and the many other studies I was unable to mention in this perspective, I am encouraged by the rapid accumulation of new knowledge. Molecules like chemokines and matricellular proteins; processes like infection, epithelial cell stress, and matrix stiffening; and cell types like fibrocytes that were essentially unstudied or unknown in this field are now common in our lexicon of fibrotic pathogenesis. Progress in these areas has required critical observation of patients with lung fibrosis and their tissues coupled with careful interpretation of mechanistic studies in animal models. Although some may argue that our animal models do not recapitulate the phenotype of progressive fibrosis seen in patients with IPF, I still believe there is an imperative role for animal models in addressing targeted mechanistic questions (89). I am also reminded that the study of lung fibrosis is a collaborative effort. The ability to test new therapies and to obtain biological samples for further study requires collaborative networks, such as the IPF clinical research network (www.ipfnet.org). Translational research is also benefitted when basic scientists and clinicians partner together to contribute their respective expertise to cross-fertilize and enhance the research and clinical missions. Progress also takes patients, and I am indebted to many for their willingness to participate in research studies. Finally, progress will take resources. The single biggest threat to our ability to understand and treat fibrotic lung diseases will come from a lack of funding. Competition for limited federal funding for scientific research is fierce, and too many deserving studies are going unfunded. Too many talented investigators will be forced to abandon research projects or, even worse, to abandon research altogether. It will be important for researchers to partner with patient advocacy groups to raise awareness and to raise research dollars from multiple sources. If we can do all of this, my hope is that there will be an opportunity in the very near future for another researcher to write a perspective on how we collectively learned to treat pulmonary fibrosis. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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