Interstitial Fibrosis and Growth Factors

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Interstitial pulmonary fibrosis (IPF) is scarring of the lung caused by a variety of inhaled agents including mineral particles, organic dusts, and oxidant gases. The disease afflicts millions of individuals worldwide, and there are no effective therapeutic approaches. A major reason for this lack of useful treatments is that few of the molecular mechanisms of disease have been defined sufficiently to design appropriate targets for therapy. Our laboratory has focused on the molecular mechanisms through which three selected peptide growth factors could play a role in the development of IPF. Hundreds of growth factors and cytokines could be involved in the complex disease process. We are studying platelet-derived growth factor because it is the most potent mesenchymal cell mitogen yet described, transforming growth factor beta because it is a powerful inducer of extracellular matrix (scar tissue) components by mesenchymal cells, and tumor necrosis factor alpha because it is a pleiotropic cytokine that we and others have shown is essential for the development of IPF in animal models. This review describes some of the evidence from studies in humans, in animal models, and *in vitro*, that supports the growth factor hypothesis. The use of modern molecular and transgenic technologies could elucidate those targets that will allow effective therapeutic approaches. *Key words*: particle inhalation, peptide growth factors, platelet-derived growth factor, pulmonary fibrosis, transforming growth factor beta, tumor necrosis factor. — *Environ Health Perspect* 108(suppl 4):751–762 (2000).
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A great deal of knowledge has been amassed over the past three decades on epidemiologic (*1*), clinical (*2*), pathologic (*3*), and biologic details (*4*) of interstitial pulmonary fibrosis (IPF). IPF by definition, includes idiopathic disease for which the cause is not known, the pneumoconioses (particle-induced disease), and a number of other inflammatory and immunologically mediated interstitial processes. Despite this information, it is obvious that our understanding of disease pathogenesis is insufficient inasmuch as there are no effective therapeutic modalities. It is our goal in this article to present a portion of what is known about IPF but more about what is not known. The key to future success in developing useful treatment strategies for IPF will be to focus on the mediators of inflammation, cell proliferation, and extracellular matrix production, the three major features of the disease process that culminate in the clinical features of IPF. Before any one of these complex processes can be ameliorated or blocked, we must develop a more complete understanding of the fundamental molecular mechanisms that drive development of disease.

The need to investigate the pathogenesis of pulmonary fibrosis is imperative because the clinical outcome for the vast majority of patients with IPF remains poor. Published statistics indicate that our lack of ability to intervene in the fibrotic process leads to premature death. In a retrospective study published 20 years ago, Carrington et al. (*5*) reported on the natural history and treated course of 53 patients with usual interstitial pneumonitis (UIP), and found that only 50% remained alive after 5 years and 18% after 10 years following their diagnosis. More recent 5- and 10-year Kaplan Meier survival analyses figures were 53 and 20%, respectively, in 85 subjects with UIP followed by the United States Armed Forces Institute between 1974 and 1993 according to Travis et al. (*6*). The lack of improvement in IPF mortality over the 20-year interval between the Carrington and Travis reports clearly indicates that currently employed medical regimens are of marginal use. The dismal response to currently available therapies underscores the need to understand the pathobiology of pulmonary fibrosis.

An important cause of interstitial lung disease (ILD) is inhalation of fibrogenic agents encountered in the workplace or in the environment (*7*). Environmental entities known to cause pulmonary fibrosis include organic dusts containing protein antigens and inorganic dusts (e.g., asbestos, silica, metals). Inhalation of each of these substances generates lung damage that with chronic exposure leads to fibrosis. Even though in 30–40% of subjects with fibrotic lung disease, the cause remains unknown (idiopathic) (*8*), there is evidence to suggest that in many of these, environmental exposure may contribute to disease pathogenesis. Metal and wood dust exposures have been found to be an independent risk factor for IPF, with estimates that such exposures may account for 16–23% of the cases (*9*). There are also reports that in some arid regions of the world, including the southwestern United States, nonoccupational exposure to aluminum silicates results in fibrogenic lung disease (*10*). In addition, a few studies suggest that cigarette smoke may contribute to the pathogenesis of IPF (*11*).

Even though the pathologic changes induced by the individual fibrotic agents have recognizable differences, they share a number of common features, including inflammation, mesenchymal cell proliferation, and extracellular matrix deposition. These features are induced by a variety of agents, suggesting that different types of lung injury can converge into common inflammation and repair pathways. Within this context, it is essential to understand the nature of the molecules that mediate the initiation and progression of pulmonary fibrosis induced by environmental agents. Many potential mediators have been identified at increased levels in the lungs of patients with occupational or environmental lung disease and in animal models of disease induced by fibrotic agents. We have proposed that certain growth factors, cytokines, and inflammatory mediators are likely to be involved in fibroproliferative lung disease regardless of the etiology. The challenge is to determine which of these mediators are central to the development of fibrotic lesions after inhalation of environmental toxicants.

Clinical Concepts Associated with IPF

Several texts define the clinical concepts associated with IPF (*4*). However, this review is not the place to dwell on the clinical features, which have a solid base of information that has changed little over the years. Thus, based upon detailed descriptions by Katzenstein et al. (*3*), it is understood that IPF exhibits its earliest clinical manifestations

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as increased accumulations of inflammatory and immune effector cells within the alveolar walls and airspaces (*12*). These changes have been assessed by lung lavage and biopsy procedures that demonstrate dramatic shifts in the percentages of the cells normally inhabiting the lung parenchyma. For example, a normal lavage of the human lung yields a population of about 95% macrophages (generally differentiated), 4% neutrophils, and few lymphocytes. Individuals with IPF can have more than 50% neutrophils, or lymphocytes in the lavage, and this corresponds, respectively, to a higher or lower risk of developing progressive interstitial fibrosis (*13*). Individuals with large numbers of infiltrating lymphocytes appear more likely to respond to treatment with prednisone and/or immune-suppressive drugs (*14*). Regardless of the etiology of the inflammation, if it persists, diffuse alveolar wall thickening and fibrosis is the result. This causes a stiff lung with concomitant abnormalities of gas exchange as the alveolar–capillary membranes widen from increased deposition of extracellular matrix, as the microvascular bed is destroyed by the fibrotic process, and as abnormalities in ventilation–perfusion matching occur (*15*). As the fibrogenesis progresses, scarring becomes more obvious by radiographic studies, and the disease tends to be even more refractory to successful therapeutic intervention (*16*).

The diffuse ILDs that develop according to this common scenario, greatly simplified above, generally are classified into two large groups, those with known and those with unknown causes. Some of the more common causes of IPF are inorganic dusts such as asbestos and silica, organic dusts that cause hypersensitivity pneumonitis, and gases like oxygen, ozone, and sulfur dioxide. Infectious microorganisms, including several viruses, bacteria, and fungi are known to cause IPF. There also are a number of fibrogenic processes for which the etiology remains unknown. Several of the more familiar of these are sarcoidosis, collagen–vascular-associated fibrosis, alveolar proteinosis, and by definition, idiopathic pulmonary fibrosis.

The ultimate goal of those studying the diseases noted previously is to develop effective therapeutic strategies for the treatment of IPF. To do so will require a detailed understanding of the molecular mechanisms that control inflammatory cell migration, cell proliferation, and production of extracellular matrix components. These are the three key features controlling fibrogenesis, but each one is highly complex and must be reduced to concepts that can be manipulated effectively. For example, if the alveolar epithelium has been injured (e.g., by asbestos, a virus, or an oxidant gas), inflammatory cells are recruited from the circulation and/or surrounding tissues. Which of the numerous chemotactic cytokines and attachment proteins play essential roles in this central process? The injured epithelium must repair by proliferation and differentiation, while mesenchymal and endothelial cells of the interstitial compartment proceed through multiple rounds of cell division. Dozens of mitogenic factors could control this phase of alveolar repair, which in itself is part of the pathway to fibrosis. Finally, with continuing injury and/or inflammation, the expanded mesenchymal cell population synthesizes and secretes multiple components of the extracellular matrix. Which of these is essential to the production of the scarring that is incompatible with normal lung functions? How is the balance maintained (or lost) among the many types of collagen molecules and the enzymes designed to degrade them (*17*)? Which of the many peptides that induce production of extracellular matrix components are critical in the progression of IPF? These three key features, inflammation, proliferation, and matrix production, are inextricably intertwined. The inflammatory cells are a major source of the mitogens and matrix-inducing peptides (*18*). The proliferating epithelium has been identified as a producer of these molecules as well (*19,20*). In addition, the mesenchymal cells, fibroblasts and myofibroblasts, not only release matrix but also produce significant amounts of the very molecules that control their proliferation and synthesis of the connective tissue components (*21–23*). Thus, there are numerous targets at which therapeutic modalities could be directed. Disruption of any one of the key pathways could provide potential benefits. Some of these approaches currently are in clinical trials. For example, cyclophosphamide appears to decrease lymphocytes in the lung (*24*) and azathioprine suppresses cellular immunity (*25*). Colchicine and methotrexate appear to reduce inflammation by blocking some cell migration and perhaps by reducing elaboration of extracellular matrix (*26*). Penicillamine appears to prevent cross-linking of collagen (*27*) and cyclosporine suppresses T-cell function (*28*). All of these are blunt tools with little evidence of success in most cases. The next generation of therapeutic agents, therefore, must be precisely targeted at the molecular pathways that control the three key features of disease.

It is impossible in this forum to even touch upon all the molecular pathways that are operative during fibrogenesis. At a recent meeting of the American Thoracic Society, numerous abstracts were directed toward a more fundamental understanding of IPF. Thus, the remainder of this article focuses on one small aspect of the fibrogenic process, i.e., the growth factor hypothesis. Even this paradigm is far too extensive for complete consideration here, we focus on approaches to understanding the molecular mechanisms through which three peptide growth factors could be mediating, at least in part, the pathogenesis of the fibroproliferative process that leads to lung fibrosis. The three factors considered here are tumor necrosis factor alpha (TNF-α), platelet-derived growth factors (PDGF), and transforming growth factor beta (TGF-β).

Growth Factors

Tumor Necrosis Factor Alpha

Inflammation is a consistent histopathologic feature of pulmonary fibrosis, though clearly the magnitude of inflammation and the character of inflammatory cells vary depending on the inciting etiology of the fibrosis (*29*). TNF-α, so named because it was first identified as an oncolytic factor for tumors (*30*), is a potent proinflammatory cytokine (*31*). TNF-α is secreted as a 26-kDa membrane-bound glycopeptide that is cleaved by TNF-α converting enzyme to release a 17-kDa monomeric peptide (*32*). Both free and membrane-bound forms are biologically active. Soluble TNF-α forms a homotrimer and induces receptor multimerization at the cell surface (*33*). Two TNF-α receptors have been identified, a 55-kDa receptor (TNFR1) and a 75-kDa receptor (TNFR2), through which TNF-α mediates its biologic responses (*33*). TNFR1 transduces proinflammatory and cytotoxic signals, whereas TNFR2 induces thymocyte proliferation (*34,35*). Most cell types express both TNF-α receptors (*31*).

The mechanism through which TNF-α participates in the development of pulmonary fibrosis appears to be multifactorial, including: induction of inflammatory cell adhesion, enhancement of a procoagulant environment, initiation of a cytokine cascade, immunomodulatory effects on lymphocytes, mitogenic activity for mesenchymal cells, regulation of apoptosis, and regulation of collagen synthesis and resorbtion (*31*). TNF-α is able to recruit into the lung several cell types such as eosinophils and platelets, and these cells can release potent profibrotic mediators (*36*). Platelet aggregation has been observed in the pulmonary vasculature of subjects with pulmonary fibrosis (*37*). Activation of platelets leads to the release of other profibrotic cytokines, including PDGF and TGF-β1, on which we will elaborate in the other two sections of this review. TNF-α has also been shown in a coculture system to induce adhesion of platelets to vascular endothelium (*38*). Eosinophils are a rich source of interleukin (IL)-5, and administration of anti–TNF-α antibodies to mice in a

bleomycin-induced model of lung fibrosis results in diminished IL-5 mRNA expression along with a decrease in lung eosinophils (*39*). Investigators have confirmed a role for eosinophil recruitment in the bleomycin model of pulmonary fibrosis using both anti–IL-5 antibodies and IL-5 knockout mice (*40*). Proinflammatory effects of TNF-α also include induction of other proinflammatory cytokines such as IL-1 and macrophage inflammatory protein 1α (MIP-1α). TNF-α, in combination with IL-6, stimulates MIP-1α expression in leukocytes recovered from lung lavage, and administration of a soluble TNF-α receptor reduces MIP-1α expression in bleomycin-treated mouse lung (*41*). TNF-α also induces IL-6 expression, its cofactor for upregulating MIP-1α (*41*).

In addition to recruitment of inflammatory cells that secrete mesenchymal cell mitogens, TNF-α may act as a fibroblast mitogen through a PDGF-dependent pathway (*42*). Furthermore, TNF-α induces upregulation of TGF-β1 mRNA expression (*43*) and would therefore be expected to upregulate collagen transcription. However, under certain circumstances, TNF-α has been found to decrease collagen transcription (*44,45*), which could also conceivably occur via downregulation of another potent stimulator of collagen expression, namely connective tissue growth factor (CTGF) (*46*). On the other hand, TNF-α in combination with IL-1 has been shown to upregulate collagen transcription in lung fibroblasts under serum-free conditions (*47*). TNF-α also possesses activity that favors dissolution of matrix through induction of the collagenase metalloproteinase-1 (MMP-1) and gelatinase (*48,49*). Blocking TNF-α clearly reduces the early inflammation that ensues following lung injury and thereby reduces fibrosis, yet the net effect of TNF-α on collagen accumulation in the later stages of fibrogenesis is not yet known.

Animal models of pulmonary fibrosis have been instrumental in establishing a role for TNF-α in disease pathogenesis. These models have provided insight into lung fibrogenesis, and include the early time points that are difficult to evaluate using human lung specimens, as most patients seek medical attention late in the course of their disease after considerable lung function has been lost (*50*). For example, bleomycin, a profibrotic chemotherapeutic agent, induces an increase in TNF-α mRNA expression in a bleomycin-sensitive mouse strain but not in a bleomycin-resistant strain (*51,52*). Elevated levels of bioactive TNF-α have been detected in murine lung lavage fluid after bleomycin administration (*41*). Anti–TNF-α antibodies (*53*) and soluble recombinant TNF-α receptors (*37*) abrogate bleomycin-induced fibrosis. Moreover, administration of soluble TNF-α receptors results in a reduction of established bleomycin-induced fibrosis (*37*). More recent data show that double TNFR knockout mice are resistant to bleomycin-induced fibrosis, and thus define an essential role for TNF-α receptors in the pathogenesis of pulmonary fibrosis (*52*). Another mechanism whereby TNF-α may effect lung fibrosis is via induction of apoptosis in inflammatory and other lung cell types. Apoptosis is more prominent in macrophages lavaged from the lungs of a bleomycin-sensitive strain than from the lungs of a bleomycin-resistant strain and double TNF-α receptor knockout mice (*52*).

A murine model of silica-induced lung injury demonstrates an increase in TNF-α mRNA (*54*) and lung lavage protein expression (*55*), which precedes subsequent fibrosis; both are blocked by administration of inhibitors of reactive oxygen intermediates (*56*). *In situ* hybridization has localized the silica-induced TNF-α expression within mononuclear cells located in alveolar spaces and lymphoid nodules (*57*). C57BL/6 silica-sensitive mice exhibit increased TNF-α mRNA expression in total lung RNA following silica exposure as well as increased TNFR2 but not TNFR1 mRNA expression after silica exposure (*55,58*). In contrast, silica-resistant C3H/HeJ mice do not upregulate TNF-α ligand or receptor RNA expression in response to intratracheal instillation of silica. Furthermore, TNF-α receptor double knockout mice bred on a silica-sensitive strain background do not develop fibrosis in response to silica (*58*). These data provide strong evidence for a significant role for TNF-α in silica-induced pulmonary fibrosis. Yet the role of the individual TNF-α receptors in mediating lung fibrosis remains to be clarified.

Human alveolar macrophages exposed to soot or asbestos fibers *in vitro* demonstrate a significant increase in release of TNF-α (*59*), and administration of crocidolite asbestos fibers to rats results in fibrosis, which is preceded by an increase in bronchoalveolar lavage (BAL) fluid TNF-α expression (*60*). These kinds of studies are useful for determining which cells secrete TNF-α in a quantitative way, but it is the experiments using genetically altered mice that offer the most hope for understanding the precise role of TNF-α and other peptide factors in the pathogenesis of IPF. For example, C57Bl/6 mice develop asbestos-induced fibroproliferative lesions at the alveolar duct bifurcations, the predominant site of fiber deposition (*61*). TNF-α expression was localized to macrophages and alveolar epithelial cells in this murine model of chrysotile asbestos-induced fibrosis (*20*). However, J129 mice, which do not develop macrophage infiltration or fibroproliferative lesions in response to asbestos, do not increase their TNF-α expression, as assessed by *in situ* hybridization and immunohistochemistry (*62*). Moreover, double TNF-α receptor knockout mice are protected from the asbestos-induced fibrosis (*20*). Thus, there is convincing evidence of a critical role for TNF-α in the pathogenesis of the inflammatory and subsequent fibroproliferative response in models of pneumoconiosis.

Other animal models of lung fibrosis have also demonstrated an increase in TNF-α expression. These include Sendai virus-induced peribronchiolar fibrosis in rats (*63*), amiodarone-induced pulmonary fibrosis in rats (*64*), and irradiation pneumonitis in which TNF-α mRNA expression is elevated following lung injury before development of fibrosis in a radiation-sensitive mouse strain but not in a resistant murine strain (*65*).

Animal models that involve TNF-α overexpression in the lung have both confirmed that TNF-α overexpression results in pulmonary fibrosis and provided insight into the mechanism of TNF-α–mediated fibrosis. Overexpression of TNF-α specifically in lung epithelial cells using the surfactant protein-C promoter results in a mixed alveolitis with neutrophils, monocytes and lymphocytes, and thickened septal walls along with increased hydroxyproline content as a measure of collagen accumulation (*66*). In agreement with the above findings, TNF-α was overexpressed for 7–10 days, using an adenoviral vector and resulting in murine lung fibrosis in a study by Sime et al. (*43*). An early inflammatory phase with neutrophils, monocytes, and eosinophils is followed by a fibrotic phase following administration of TNF-α using the adenoviral vector. TGF-β is increased in this model, presumably because of TNF-α expression, a concept that will be discussed further.

The "motheaten mouse" is a mutant that develops pulmonary fibrosis with a prominent pathologic feature of macrophage inflammation. Serum levels of TNF-α in motheaten mice are elevated compared to those in wild-type littermates. Furthermore, lung macrophage production of TNF-α in response to lipopolysaccharide is increased in motheaten mice, and TNF-α immunohistochemical staining localizes the increased TNF-α expression within macrophagelike cells in lung tissue sections from motheaten mice (*67*). Moreover, the severity of acute lung injury is diminished in motheaten bone marrow-recipient mice treated with an anti–TNF-α antibody (*67*).

Transgenic mice overexpressing TNF-α on a lung-specific promoter develop mild pulmonary fibrosis in a pattern resembling IPF (*68*). These mice demonstrate enhanced IL-1

expression only during an early time point when lymphocyte proliferation is ongoing. Interestingly, in contrast to the model transiently overexpressing TNF-α in the lung using an adenoviral vector (*43*), TGF-β1 protein expression was decreased in this TNF-α transgenic mouse. Finally, mice deficient in TNF-α demonstrate resistance to bleomycin-induced lymphoid infiltration and increases in lung collagen content as a measure of fibrosis (*69*), which is complementary to the data described above when employing double TNF-α receptor knockout mice. All these transgenic, knockout, and mutant models support the general and fundamental concept that TNF-α is necessary for the development of pulmonary fibrosis.

The essentially universal finding of TNF-α expression in these models of pulmonary fibrosis strongly suggests that TNF-α is required for fibrosis to occur. Yet there is at least one exception, as in a model of murine pulmonary fibrosis induced by an adenoviral vector expressing granulocyte macrophage colony-stimulating factor, where apparently there is no upregulation of TNF-α (*70*). Thus, it is clear that TNF-α overexpression leads to pulmonary fibrosis, including those fibroses caused by inorganic dusts, but whether TNF-α is required for the multiple causes of pulmonary fibrosis remains in question. Other questions concerning TNF-α in the pathogenesis of lung fibrosis remain unanswered. For example: What is the duration of TNF-α overexpression that leads to fibrogenesis? Will continued expression of TNF-α lead to further fibrosis or will the collagenolytic effects of TNF-α in the later stages be protective for the lung? Some investigators have proposed that TNF-α inhibits fibroblast proliferation during a rapid early phase of fibroproliferation following an insult, but TNF-α may stimulate cell proliferation during a later repair phase (*71*). Answers to these challenging questions will require novel approaches using animal models.

The relationships between animal models of TNF-α–induced pulmonary fibrosis and the diverse patterns of human lung disease are not clear, but it is known that specimens of fibrotic human lung from diverse patterns, such as bronchiolitis obliterans with organizing pneumonia (BOOP), the granulomatous lesions of sarcoidosis, and the usual interstitial pneumonitis pattern of IPF, demonstrate upregulation of TNF-α expression (*72*). *In situ* hybridization and immunohistochemical staining have identified mononuclear inflammatory cells and type II epithelial cells as primary sources of TNF-α within the lung (*20,73*). Although some investigators have reported that TNF-α mRNA levels were not elevated in alveolar macrophages obtained from subjects with BOOP or IPF as in normal subjects (*74*) and that TNF-α levels were not elevated in the lung homogenates of subjects with IPF compared with lung homogenates of controls (*75*), others found increased TNF-α mRNA in macrophages derived from the lungs of patients with IPF or asbestosis (*73,76*). Immunohistochemical staining detected increased TNF-α immunoreactivity in hyperplastic alveolar type II cells in humans with IPF compared with those with normal lungs (*77*). In another occupational cause of lung fibrosis, TNF-α mRNA expression is increased in human blood monocytes exposed to beryllium, an inorganic dust that causes a granulomatous pattern of lung fibrosis (*78*).

In agreement with the hypothesis that TNF-α is important in lung fibrogenesis, drugs with antifibrotic properties, such as pentoxyphylline and pirfenidone, which have been employed in animal models of pulmonary fibrosis, demonstrate anti–TNF-α effects (*79,80*). Presently, investigators are studying the effects of inhibiting TNF-α expression in granulomatous lung disease (sarcoidosis) and IPF (*81*) in humans using pentoxyphylline. Pirfenidone appeared to arrest the progression of fibrosis in subjects with IPF who were deteriorating despite treatment with corticosteroids and other immunosupressant drugs (*16*). Inasmuch as animal models have confirmed that blocking TNF-α results in increased susceptibility to respiratory pathogens (*82,83*), we should consider that most animal studies are conducted in pathogen-free environments and investigators must exercise caution if significant and prolonged inhibition of TNF-α is used in the treatment of chronic human fibrotic disorders. Therefore, understanding the roles of individual TNF-α receptors and the TNF-α signal transduction pathways remain important areas of research in lung biology.

Platelet-Derived Growth Factor

An increase in mesenchymal cell number is a consistent feature of fibrotic lung lesions (*84*). Mesenchymal cells of the lung include fibroblasts, smooth muscle cells, and myofibroblasts. Myofibroblasts are the predominant mesenchymal cell phenotype present in fibrotic lung lesions of various etiologies including bleomycin, oxygen toxicity, asbestos, hypersensitivity pneumonitis, BOOP, systemic scleroderma, sarcoidosis, and IPF (*85,86*). In addition to the mesenchymal cell proliferation in fibrotic lesions, there is also connective tissue deposition. Lung mesenchymal cells play a major role not only in synthesis but also in maintenance of connective tissue through elaboration of appropriate enzymes (*87*).

It has become clear that there are multiple growth factors and cytokines that have the potential to be contributors to the fibrogenic process (*18,36*). In this review, we focus on PDGF. Among a number of mesenchymal cell mitogens, PDGF is the most potent and is a chemoattractant for these cells as well. In part, we have chosen to investigate the role of PDGF rather than that of the many other growth factors potentially active in fibroproliferative disease because it has been shown that other molecules such as IL-1β, TGF-β1, and thrombin exert part of their mitogenic effects on mesenchymal cells *in vitro* indirectly through PDGF and its receptors (*88–90*).

PDGF was first isolated from the alpha granules of blood platelets (*91*). Now it is known that PDGF is secreted by macrophages, endothelial cells, smooth muscle cells, fibroblasts, glial cells, epithelial cells, and some cancer cells (*92*). PDGF is a 29- to 38-kDa glycoprotein dimer composed of A and/or B chains linked by disulfide bonds. The PDGF-A and -B chains share 60% homology and are located on human chromosomes 7 and 22, respectively. The active form may exist as an AA or BB homodimer or as an AB heterodimer. PDGF monomers are inactive (*92*). PDGF is a potent mitogen and chemoattractant for mesenchymal cells and also promotes production of glycosaminoglycans by mesenchymal cells. The introduction of PDGF to mesenchymal cell cultures is followed by the formation of polyribosomes and an increased rate of mRNA translation in the mesenchymal cells, with subsequent cell proliferation (*93*). PDGF acts as a competence factor and stimulates cells to progress from the G1 postmitotic phase to the G1 pre-DNA synthetic phase of the cell cycle (*91*).

PDGF ligands, in dimeric form, bind to receptor dimers composed of alpha and beta subunits (*94,95*). PDGF receptor (PDGFR) activation requires dimerization (*96*). PDGFR-α has a high affinity for A and B chain PDGF, whereas PDGFR-β has a high affinity only for the PDGF-B chain. The PDGF chains, covalently bound in an antiparallel orientation (*97*), bind to the receptor subunits and are subsequently rapidly internalized and degraded with a $t_{1/2}$ of 20 min and 60–90 min, respectively (*98*). Binding of the PDGF dimers to the receptor subunits results in transphosphorylation and subsequent activation of signal transduction pathways, and eventuates in cell proliferation and chemotaxis (*95*).

Lung fibroblasts express receptors for a number of mitogens including IL-1, thrombin, TGF-β, TNF-α, and fibroblast growth factor (bFGF) (*18*). Sources for these cytokines in the lungs are macrophages and other inflammatory cells, epithelial and mesenchymal cells, and matrix deposits in the case of bFGF (*18,92*). A body of data suggests that

several of these molecules (e.g., IL-1β (*88,99*), TGF-β1 (*89,100,101*), thrombin (*90,102*), TNF-α (*42,103*) and bFGF (*104*), which act as mitogens for mesenchymal cells, exert all or part of their growth-promoting activity through induction of PDGF-dependent pathways. The PDGF-dependent proliferative activity of these other mitogens occurs through upregulation of the PDGF-A chain and the PDGFR-α.

It is important to understand that the activity of PDGF also may be modulated by other peptides. For example, α_2-macroglobulin is a large and abundant serum antiprotease that serves as a binding protein for all three PDGF isoforms (*105*). We have shown that the binding of PDGF to α_2-macroglobulin is 80% reversible in 1 M acetic acid and thus is noncovalently bound (*105*). We know that α_2-macroglobulin is produced by macrophages and fibroblasts in the lung, but presently the biologic effects of α_2-macroglobulin on the mitogenic activity of PDGF are not well understood. α_2-Macroglobulin could enhance the activity of PDGF by protecting it from proteolytic degradation or could limit its availability via clearance of PDGF bound to α_2-macroglobulin because α_2-macroglobulin is internalized after binding to its receptor (*22*).

A series of studies in this field has demonstrated that PDGF is produced by these mesenchymal cell populations (*21,106–109*). Accordingly, there are a few reports suggesting that there may be a PDGF-dependent autocrine proliferative mechanism in lung fibroblasts. Chen et al. (*110*) showed that fibroblasts isolated from the lungs of humans (who have died of acute lung injury) proliferate in the absence of exogenous growth factors in serum-free medium, whereas those from the lungs of controls do not. Bishop et al. (*111*) have shown that rat lung fibroblasts and a human fetal lung fibroblast cell line secrete a mesenchymal cell growth factor and proliferate when stretched on a specialized culture membrane. The mitogenic activity in the medium of the stretched cells was partially blocked by an anti-PDGF antibody. Directly relevant to our interest in fibrogenic lung disease caused by environmental agents, we have shown that treatment of fibroblasts with asbestos fibers results in expression of PDGF-A chain and upregulation of PDGFR-α expression, with resultant fibroproliferation (*21,22*). Because PDGF-AA binds only to the PDGFRα, we have proposed that this concomitant upregulation of ligand and receptor is a mechanism through which interstitial asbestos fibers could continuously induce proliferation of mesenchymal cells in an autocrine/paracrine pathway.

We now review the data that support the hypothesis that PDGF is involved in the mesenchymal cell proliferation that is the key to pulmonary fibrosis (Table 1). There is considerable evidence that PDGF plays a role in the development of pulmonary fibrosis of various etiologies in animals and humans, including the recent findings that PDGF and its receptors are upregulated at the sites of fibrotic lung lesions in both human pulmonary fibrosis and in animal models of the disease.

It is obvious that PDGF is not the only growth factor contributing to the pathogenesis of lung fibrosis; however, the *in vitro* and *in vivo* data strongly suggest that PDGF has the potential to be a key factor in mediating mesenchymal cell proliferation in fibroproliferative lung disease. For example,

Table 1. Potential of role of PDGF in IPLF

Type of lung disease	Significant findings
	Human lung disease and isolated cells
Idiopathic pulmonary fibrosis	Alveolar macrophages (AMs) from patients with IPF secrete more PDGF than AM from subjects without IPF (*112*). PDGF-B chain mRNA and protein are localized in the alveolar epithelium, mesothelial cells, and interstitial macrophages (*113*). Greater PDGF ligand positive immunostaining is detected in the macrophages of IPF patients versus controls (*114*). AM from patients with IPF have a 10-fold increase in PDGF-A and -B chain mRNA compared to AMs from subjects with normal lungs, with the B chain 10-fold more abundant than the A chain (*115*). In early-stage IPF, PDGF was located within macrophages, fibroblasts, alveolar type II cells, and vascular endothelial cells, whereas PDGF was only found within macrophages in late-stage IPF (*116*).
Autoimmune-associated pulmonary fibrosis	An increase in PDGF-B chain mRNA was found in lavaged cells from lungs of patients with autoimmune disease and pulmonary fibrosis (*117*). PDGF-AA and -BB, as measured by ELISA and Western blotting, is increased in the lavage fluid of patients with scleroderma compared with that of control subjects (*118*).
Acute lung injury	PDGF activity is present in lung lavage fluid from patients dying with acute lung injury (*119*).
Histiocytosis X	There is an increase in PDGF-B chain mRNA in bronchoalveolar lavage cells from subjects with histiocytosis X (*120*).
Bronchiolitis obliterans	An increase in the PDGF-B chain is detected in lavage fluid post-transplant prior to the development of bronchiolitis (*121*). PDGF-A chain mRNA is detected in inflammatory cells surrounding fibrotic airway lesions (*122*).
IPF/Gray platelet	Abnormal megakaryocytes localized in the pulmonary capillaries secrete PDGF (*123*).
Lung tumor associated	PDGF-B chain and -β receptor mRNA are found in primary lung tumors exhibiting desmoplasia (*124*).
Hermansky-Pudlak syndrome	PDGF-B chain is detected in lung macrophages (*125*).
Asbestos	*In vitro* human lung fibroblast asbestos-induced proliferation is blocked by an antisense oligomer to PDGF-A chain (*126*).
	Rodent models of lung fibrosis
Hyperoxia	PDGF-B chain mRNA is increased in whole lung tissue prior to DNA synthesis and tissue repair (*127*). PDGF-A chain mRNA is increased in whole lung tissue following cell proliferation. Also, PDGF-A chain mRNA is induced by 10% fetal bovine serum in isolated rat lung fibroblasts (*128*).
Residual oil fly ash particles	PDGFR-α is upregulated in the lung *in vivo* following exposure of rats to residual oil fly ash (*129*).
Inflammation	PDGFR-α expression is increased in rat lung through IL-1 (*130*).
Asbestos-exposed	Rat AM secrete more PDGF (all three isoforms) following exposure to asbestos *in vitro* (*134*). Exposure of mineral dust to tracheal explants results in increased expression of PDGF-A and -B chain gene expression (*131*). Increased production of PDGF by rat interstitial macrophages following stimulation with asbestos fibers (*132*). Rat lung myofibroblasts upregulate PDGFR-α mRNA and cell surface receptor expression following exposure to asbestos *in vitro* (*22*). Rat lung myofibroblasts express PDGF-A chain mRNA and secrete PDGF-AA in response to asbestos *in vitro* (*21*). Both PDGF-A and -B chain mRNA and peptide are upregulated at the sites of developing asbestos-induced fibrotic lesions (*19*). PDGFR-α, but not PDGFR-β, mRNA, and peptide are upregulated at the site of asbestos-induced fibrotic lesions (*23*).

significant asbestos exposure results in IPF in humans and rodents, with pathologic and physiologic features that are observed in a wide variety of chronic ILDs (*133*). Morphometric studies have shown that asbestos exposure results in an increase in lung fibroblast numbers and volume at the alveolar duct bifurcations, where the vast majority of asbestos fibers deposit initially (*61*). We and others have reported that lung macrophages release biologically active PDGF when stimulated with asbestos fibers (*134*). Furthermore, asbestos fibers induce proliferation of rat lung fibroblasts in serum-free media through a PDGF-dependent pathway that involves the PDGF-AA isoform and upregulation of the PDGF-α receptor (*21,22*). Our *in vivo* observations using a murine model of aerosolized asbestos exposure demonstrate upregulation of both PDGF-A and -B chains, predominantly in macrophages and epithelial cells, at the alveolar duct bifurcations (*19*) (Figure 1). We have also described upregulation of the PDGF-α, but not the PDGF-β, receptor within interstitial cells at alveolar duct bifurcations after asbestos exposure (*23*). Upregulation of the PDGF ligands and the PDGF-α receptor occurs in concert within hours following asbestos exposure, and this early event precedes the observed asbestos-induced fibrogenesis that can be measured through subsequent days. Thus, PDGF is present at the appropriate place and time during asbestos-induced fibrogenesis and could be mediating fibroproliferation.

In further support of a role for PDGF in lung fibrogenesis, investigators have established that overexpression of PDGF within the lung will result in development of pulmonary fibrosis. Overexpression of PDGF-B chain specifically in murine lung under control of the surfactant protein-C promotor results in the development of enlarged airspaces and thickened septal walls (*135*). In addition, administration of an expression vector encoding PDGF-B chain into the lungs of rats results in the development of pulmonary fibrosis, and administration of a vector with a truncated PDGF-β receptor that will bind PDGF-B chain-containing isoforms, markedly alleviated bleomycin-induced histopathologic changes in murine lung (*136*). Furthermore, transient increases in cellularity and collagen deposition occur along with bromodeoxyurine incorporation within perivascular and peribronchial fibroblasts following intratracheal delivery of recombinant PDGF-BB (*137*). In concert, these data provide convincing evidence that PDGF is capable of mediating lung fibroproliferation. On the other hand, although PDGF is present in fibrotic lesions and is a potent mesenchymal cell mitogen and chemoattractant, it remains to be proven whether this factor is necessary for human lung fibrogenesis to develop.

Finally, certain compounds that inhibit the activity of PDGF block the development of lung fibrosis. The PDGF signal transduction pathway involves tyrosine kinases (*138,139*). Recently, several tyrosine kinase inhibitors have been developed, some of which demonstrate selective inhibition of PDGF receptor tyrosine phosphorylation (*138,139*). In a rat tracheal transplant model of bronchiolitis obliterans, CGP 53716, a PDGF receptor-selective tyrosine kinase inhibitor, blocks the formation of intraluminal fibroproliferation (*140*). A significant step toward demonstrating the critical role played by PDGF in the pathogenesis of lung fibrosis was recently published (*139*), showing that a tyrosine kinase inhibitor of the tyrphostine class (that blocks PDGF signal transduction) blocks mesenchymal cell proliferation and hydroxyproline accumulation, as a measure of collagen accumulation, in a rat model of vanadium pentoxide-induced lung fibrosis. Further supportive evidence for the role of PDGF in the development of lung fibrosis is provided by investigation into the mechanism of the inhibition of bleomycin-induced pulmonary fibrosis in hamsters by the antifibrotic drug pirfenidone (*141*). Pirfenidone did not alter PDGF mRNA expression but did significantly reduce the synthesis of PDGF peptide in the lavage fluid of bleomycin-treated hamsters.

Clearly, it is possible that agents such as pirfenidone or the tyrosine kinase inhibitors discussed above may also diminish fibrogenesis via hitherto unknown effects on other mediators of fibrosis. Therefore, novel approaches such as the generation and evaluation of PDGF transgenic and knockout mice must be developed to define the role of PDGF in lung fibrogenesis. As indicated above, mice that overexpress PDGF-BB using the lung-specific surfactant protein-C promotor demonstrate both thickened alveolar septae and emphysema (*135*). PDGF knockout mice have also been generated for each of the PDGF ligand chains and receptors. Mice that lack the PDGF-A chain are generally not

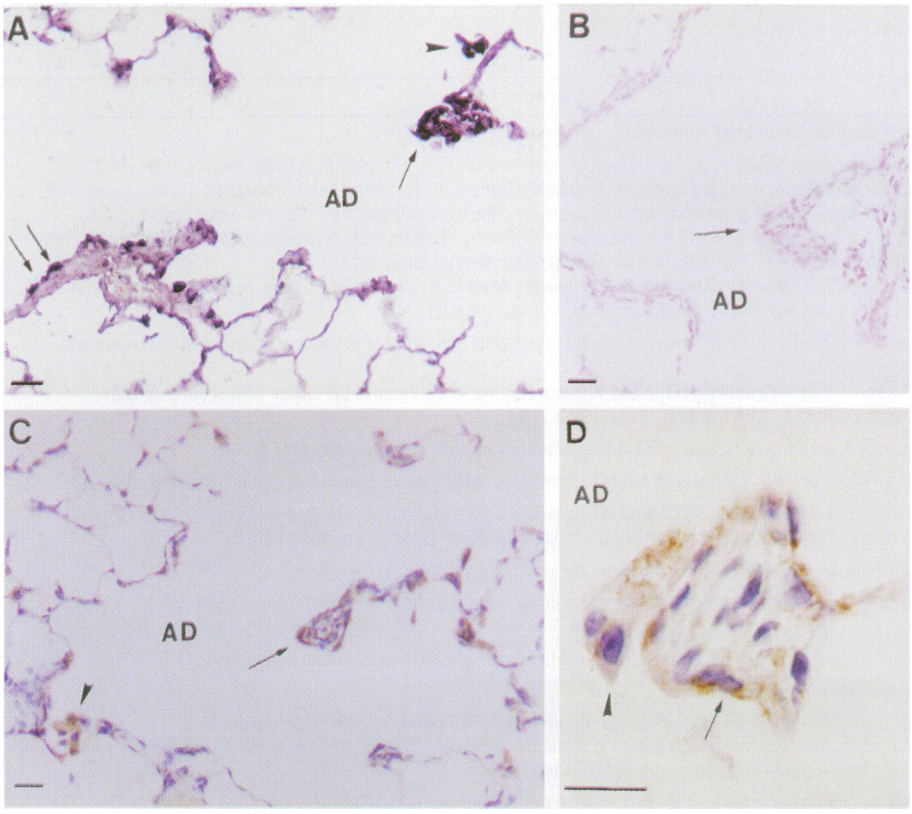

Figure 1. (*A*) The alveolar duct (AD) bifurcations (arrow) in asbestos-exposed rats and mice exhibit strong expression of several growth factors. Here *in situ* hybridization for PDGF-A shows clear upregulation in macrophages (arrowhead), the bifurcations, and bronchiolar epithelium (double arrows) [see Liu et al. (*19*) for details]. (*B*) Sense control for PDGF-A blocks all hybridization. (*C*) Immunohistochemistry for PDGF-A reveals a staining pattern identical to that seen by *in situ* hybridization with macrophages (arrowheads) and the duct bifurcations clearly stained. (*D*) A close-up of a duct bifurcation reveals a fibroproliferative lesion [see Chang et al. (*61*) for details] clearly stained for PDGF-A. The macrophages (arrowhead), alveolar epithelium (arrow) and some interstitial cells exhibit strong staining for PDGF-A. Illustration courtesy of Dr. Jing-Yao Liu (Department of Pathology, Tulane University School of Medicine, New Orleans, Louisana).

viable, and those that are develop pulmonary emphysema apparently because of a failure of alveogenesis along with the lack of lung myofibroblast development (*142,143*). This finding suggests that the PDGF-A chain is involved in the generation of the proliferative mesenchymal cell phenotype observed in pulmonary fibrosis. Mice lacking the PDGF-B chain are also not viable and develop bleeding because of pericyte loss and microaneurism formation (*143*) along with the absence of renal glomeruli (*144*). Investigators recently have overcome the issues of mortality in PDGF knockout mice through generation of chimeras to substantiate a role for the PDGF-β receptor in a murine skin wound model (*145*). The study of PDGF receptor knockout chimeras and other such novel approaches in models of lung fibrogenesis should be equally informative.

Transforming Growth Factor Beta

TGF-β encompasses a family of closely related peptides that bind to the same receptors and have nonidentical but similar biologic activities (*146*). Whereas TGF-β2 and TGF-β3 are expressed constitutively in the lung with little if any alteration in expression in response to fibrogenic stimuli, TGF-β1 expression is often upregulated in the lung at sites of fibrogenesis (*117,147*). Hence, most published literature on TGF-β1 and this review focus on this isoform. TGF-β1 is a 25-kDa peptide dimer secreted by several pulmonary cell types, including macrophages, platelets, epithelial cells, endothelial cells, and fibroblasts (*146*). TGF-β1 is secreted as a latent peptide that is cleaved by proteases, acid hydrolysis, or reactive oxygen species into an active peptide that is recognized by several distinct cell surface receptors that are present on most cell types (*146*). Two recent findings may provide exciting future avenues for regulation of TGF-β1 activity and therefore, lung fibrosis. Thrombospondin was found to be a major activator of TGF-β1 by binding to and inducing a conformational change in the latency-associated peptide of TGF-β (*148*); and the integrin αvβ6, which is upregulated on epithelial cells in response to lung inflammation, induces spatial activation of TGF-β1 (*149*).

TGF-β type I and type II receptors facilitate signaling of each other but favor different biologic activities such as extracellular matrix accumulation for the type I receptor and modulation of cell growth for the type II receptor (*150*). Relatively little was known until recently regarding the expression of the TGF-β receptors during lung fibrogenesis. Northern and Western blot analyses for TGF-β receptor types I and II expression in a rat model of hyperoxia-induced lung injury and repair demonstrate similar distribution for the two receptors but distinct patterns of upregulation during stages of injury and repair (*151*). *In vitro* experiments in mesenchymal and endothelial cells have demonstrated that heteromeric type I–type II receptor complexes are required for the mitogenic activity of TGF-β (*152*). Expression of a truncated type II TGF-β receptor, which acts as a dominant negative mutant in cultured smooth muscle cells, results in suppression of fibronectin and type I collagen production (*153*) and has been helpful in establishing the role of TGF-β1 in regulating the synthesis of these matrix elements. β-Glycan, the TGF-β type III receptor, enhances the binding of TGF-β to its type II receptor and appears to be essential for TGF-β–mediated negative regulation of lung organogenesis (*154*). A TGF-β type V receptor has recently been described and can form heterocomplexes with the type I receptor but may also mediate growth inhibition in the absence of TGF-β type I and type II receptors (*19*). It would be of interest to know whether specific biologic activities of TGF-β, described below, are mediated by distinct TGF-β receptor profiles expressed by cells involved in the pathogenesis of pulmonary fibrosis.

TGF-β can bind to matrix elements such as decorin (*155*) and to the macromolecular anti-protease α_2-macroglobulin (*156*). These binding peptides are likely to modulate the activity of TGF-β. For example, old fibrotic tissue associated with adenocarcinomas stains intensely for decorin but weakly for TGF-β1, suggesting that decorin plays a role as a negative feedback regulator in the expression of TGF-β1 (*155*). Consistent with this hypothesis, repetitive intratracheal administration of decorin attenuates lung fibrosis in a bleomycin model of lung fibrosis in hamsters (*157*).

TGF-β1 has numerous biologic activities including immunomodulatory (*158*), both pro- and antimitogenic (*159,160*), and profibrotic through increases in extracellular matrix synthesis and inhibition of matrix degradation (*161*). TGF-β1 inhibits proliferation of epithelial cells and thus could potentially extend the fibroproliferative phase following lung injury, since reepithelialization is purported to limit the formation of granulation tissue (*162*). TGF-β1 has a bimodal response on fibroblast proliferation, which occurs through a PDGF-dependent pathway. Low doses of TGF-β1 upregulate PDGF-A chain expression, but higher doses of TGF-β1 can decrease the expression of PDGF-α receptor and thus negate its own effects on upregulation of the PDGF ligand (*89*). More recent experiments using embryonic cells lacking the PDGF-α receptor suggest that about half the TGF-β mitogenic activity for these 3T3-like cells is mediated via a PDGF-independent pathway (*163*). Interestingly, TGF-β1 blocks PDGF-dependent proliferation of primary rat lung fibroblasts *in vitro* (*164*).

The most notable activity of TGF-β1 is its positive effects on extracellular matrix accumulation. TGF-β1 has been shown to influence collagen accumulation at many of the key stages in collagen metabolism and degradation, beginning with induction of procollagen gene transcription (*161,165*). A transgenic mouse containing a rat type 1 alpha-1 collagen promoter fused to the chloramphenicol acetyltransferase (CAT) reporter gene has been studied in dermal wound and bleomycin lung injury models, and revealed that subcutaneous injection of TGF-β1 increases the CAT transgene expression but only in a subpopulation of dermal fibroblasts, even though alpha-1 collagen was expressed throughout the dermis (*166*). Furthermore, CAT expression was increased 6- to 8-fold 2 weeks postbleomycin exposure, and like the observations with dermal injection of TGF-β1, there was a subpopulation of cells around the airways with prominent collagen mRNA expression but little CAT reporter gene expression. These findings indicate that there are distinct groups of mesenchymal cells within the dermis and lung capable of upregulating their collagen expression following stimulation with TGF-β1; however, other mesenchymal cells may require additional elements for collagen gene expression.

Collagen synthesis and degradation is an ongoing process in the lung, and TGF-β1 has been shown to reduce the proportion of procollagen that is degraded within lung fibroblasts prior to secretion (*167*). Furthermore, TGF-β1 is capable of limiting mature extracellular collagen degradation through altering the balance between collagenases and their inhibitors (*161*). TGF-β1 may even induce a negative feedback pathway that limits its own enhancement of procollagen production by inducing prostaglandin E_2 (*161*). Although TGF-β1 is generally viewed as a profibrotic cytokine, investigators have entertained the hypothesis that TGF-β may be important in downregulating the T-helper (Th)1 lymphocyte response in certain fibrotic lung diseases such as sarcoidosis, through inhibition of the production of cytokines (IL-2 and interferon gamma, for example) involved in propagation of granulomatous inflammation (*168*). Thus, TGF-β may limit lung injury by diminishing inflammation and blocking fibroblast proliferation, but it may also promote lung fibrosis though stimulation of matrix accumulation.

Experiments using human lung fibroblast isolates *in vitro* demonstrate that TGF-β1 and -β3 induce similar increases in the percentage of secreted collagens into the culture supernatants along with increases of

collagen deposited into the matrix (*169*). In addition, both of these TGF-β isoforms decreased the secretion of MMP-1 (collagenase), but not MMP-2 (gelatinase A), and increased tissue inhibitor of metaloproteinase (TIMP)-1 but not TIMP-2. Importantly, TGF-β mediates collagen accumulation in human lung fibroblasts through increased collagen synthesis and decreased collagen degradation.

Multiple reports demonstrate increased expression of TGF-β1 mRNA and peptide in fibrotic human lung specimens including those with asbestosis (*170*), silicosis (*171*), titanium dioxide (*131*), IPF, eosinophilic granulomatosis, and sarcoidosis (*172*). TGF-β immunohistochemical staining and mRNA expression are increased in the lungs of humans afflicted with IPF and localize within inflammatory regions and at sites of extracellular matrix expression, thereby implicating TGF-β in the pathobiology occurring at these fibroproliferative sites (*173*). IPF lung specimens reveal TGF-β1 within alveolar type II cells, which coincides with, and may be responsible for, the increase in alpha smooth muscle actin expression within adjacent lung mesenchymal cells (*77*). TGF-β1 peptide levels are elevated in lung lavage fluid from patients with IPF as well as those with sarcoidosis and abnormal lung function studies compared to healthy subjects and those with sarcoidosis and normal lung function (*174*). Patients with eosinophilic granulomatosis had prominent staining for TGF-β1 within macrophages and type II pneumocytes in actively inflamed lesions but not in advanced fibrotic lesions (*175*). Analysis of TGF-β isoform expression was conducted on lung specimens from humans with UIP, nonspecific interstitial pneumonitis, asbestosis, and hypersensitivity pneumonitis and revealed that TGF-β2 and -β3 expression was ubiquitously expressed and was not increased in fibrotic specimens compared with normal lung specimens, whereas TGF-β1 expression was increased within lung macrophages (*176*). Furthermore, TGF-β1 levels were also measured in the lung lavage fluid of 48 patients with IPF by enzyme-linked immunosorbent assay (ELISA), and the authors noted that patients with the highest TGF-β1 levels had significantly shorter survival rates compared with patients who had intermediate and low TGF-β1 levels (*177*). Other investigators reported immunohistochemical localization of TGF-β1 in fibrotic lung within epithelioid histiocytes, type II pneumocytes, bronchiolar epithelial cells, and alveolar macrophages (*174*). Immunohistochemical staining for TGF-β1 in subjects with silicosis demonstrated dense staining within central hyalinized areas of the silicotic nodules and in scar tissue from areas with progressive massive fibrosis, whereas the TGF-β1 staining for subjects with acute silicosis was localized within macrophages and hyperplastic alveolar epithelium (*171*). Thus, TGF-β expression is increased in many cell types during lung fibrogenesis, but specific cell types may vary according to the etiology or stage of the fibrotic disease.

TGF-β1 mRNA expression correlates spatially and temporally with collagen expression in lung development and in animal models of lung fibrosis (*178,179*). Though TGF-β1 mRNA is expressed in the lung constitutively, it is upregulated in several animal models of lung fibrosis including bleomycin (162) and asbestos-induced lung injury (*180*). In normal mouse lung, TGF-β1 and -β3 transcripts are identified within bronchiolar epithelium, but following bleomycin exposure TGF-β1 expression was enhanced, predominantly within macrophages but also within mesenchymal cells, endothelial cells, and mesothelial cells (*147*).

In a murine model of irradiation-induced lung fibrosis, antibodies to the TGF-β1 latent reactive protein and to the active conformation of TGF-β1 demonstrated staining only in a sensitive strain compared with a resistant strain, and localized TGF-β1 peptide expression within fibroblasts at the sites of fibrotic lesions (*181*). TGF-β1 peptide and mRNA expression are increased in rat lung 3–6 weeks following irradiation coinciding with an influx of inflammatory cells in BAL and preceding the fibrosis that appeared 8–10 weeks following irradiation (*137*). TGF-β1 immunohistochemical staining is increased during a fibrotic phase in a murine model of pulmonary tuberculosis and also in a Fas antibody model of apoptosis/fibrosis in mice (*182*). Overexpression of granulocyte macrophage colony-stimulating factor in the lung using an adenoviral vector induced the formation of lung granulation tissue, in part by induction of TGF-β1 expression within lung myofibroblasts; it also caused macrophages lavaged from the lungs of these mice to secrete more TGF-β into their culture media (*70*). The overexpression of TGF-β1 in these animal models of lung fibrosis correlates with the findings in human lung previously discussed.

Data demonstrating that inhibition of TGF-β1 arrests or decreases lung fibrosis have been published but are limited in number. Anti–TGF-β1 antibodies reduce fibrosis in an animal model of bleomycin-induced lung fibrosis (*183,184*). Recently, intratracheal administration of a soluble TGF-β type II receptor significantly reduced bleomycin-induced fibrotic lung histopathogy and hydroxyproline accumulation in hamsters (*185*). Furthermore, taurine and niacine are believed to decrease bleomycin-induced fibrosis in hamsters via inhibition of TGF-β1 mRNA expression (*141*). In addition, measures taken to decrease lung fibrosis appear also to diminish TGF-β expression. In a comparative analysis of TGF-β1 mRNA and protein expression in asbestos-sensitive and -resistant mice, TGF-β1 expression was lower in the resistant strain (*62*). In addition, TGF-β1 expression is essentially lacking in the lungs of the asbestos-exposed TNF-α receptor knockout mice previously described (*20*). This is consistent with the concept that TNF-α upregulates the expression of TGF-β1, an important idea to consider in those systems in which multiple factors are active simultaneously during disease development. In other modes, immunohistochemical localization of TGF-β1 in an ovine model of asbestos-induced lung fibrosis demonstrated increased staining for TGF-β1 within the interstitial matrix (*186*). Investigation into mediators of airway wall fibrosis induced by mineral dusts (asbestos, iron oxide, titanium dioxide) in rat tracheal explants demonstrates increases in TGF-β1 gene expression, which is localized to both the epithelium and subepithelial space (*130*). In a murine model of asbestosis (*187*), TGF-β1 activity was found within myofibroblasts with alpha smooth muscle actin, but in contrast, immunolocalization in a rat silicosis model demonstrated co-localization of TGF-β1 and type 1 collagen mRNA expression within cells not expressing smooth muscle actin. *In vitro*, TGF-β1 induces expression of alpha smooth muscle actin in lung fibroblasts (*188*) and produces a phenotype similar to that of the myofibroblasts encountered in the pathology specimens derived from fibrotic lung (*85*). Furthermore, administration of neutralizing TGF-β1 antibodies to rat lung progenitor fibroblasts in culture resulted in abolition of TGF-β1–induced terminal differentiation to post-mitotic functional fibrocytes. These cells were similar in appearance to the mesenchymal cell phenotype observed in models of irradiation-induced fibrosis (*189*). Thus, lung fibrosis with varying etiologies may lead to expansion of different mesenchymal cell phenotypes (*190*), and TGF-β1 induces alpha smooth muscle actin expression in some lung mesenchymal cells. In concert with this central observation, it is clear that TGF-β1 is overexpressed in smooth muscle actin-expressing and nonsmooth muscle actin-expressing lung mesenchymal cells during lung fibrogenesis.

Delivery of TGF-β1 into the lungs of rats using the hemaagglutinating virus of Japan and liposome delivery system results in lung fibroblast proliferation and collagen deposition (*191*). In other studies, introduction of TGF-β1 into the trachea using a recombinant adenoviral vector causes histopathologic

changes in rats consistent with pulmonary fibrosis (*192*). Overexpression of TGF-β1 through an adenoviral vector administered to rats by tracheal instillation leads to severe parenchymal and airway fibrosis. Two TGF-β1 constructs were generated for use with the adenoviral vector, one latent and one active by nature of deletion of the fragment encoding for the latency-associated peptide; however, only the active construct induced a fibrotic reaction. The broad spectrum of TGF-β activity in lung biology is apparent from the fate of TGF-β knockout mice. Mice lacking TGF-β1 die secondary to chronic multifocal inflammation and also demonstrate inhibition of lung branching morphogenesis (*158*), whereas mice lacking TGF-β3 die shortly after birth with abnormally developed lungs (*193*).

To date, specific anti–TGF-β–directed therapy has not been forthcoming. Reasons for this are several. TGF-β has numerous biologic effects including profibrogenic, anti-inflammatory, immunoregulatory, and modification of cell adhesion. Ablation of these TGF-β1–mediated activities may be deleterious. This was found to be the case in TGF-β1 null mice, which succumb to severe pulmonary, dermal, and bowel inflammation within weeks after birth (*158*). Anti–TGF-β1 therapy could lead to lung inflammation,

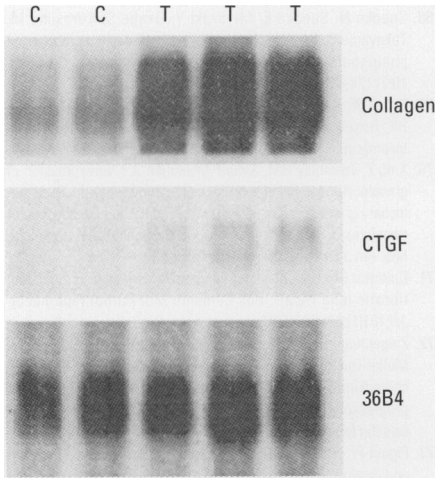

Figure 2. CTGF and alpha 1 type I collagen mRNA expression are increased in mouse lung 7 days after administration of an adenoviral vector containing an active TGF-β1 construct. C57BL/6 mice were given an adenovirus with (T) or without (C) the inclusion of an active TGF-β1 construct via intratracheal injection (109 cfu), which resulted in lung fibrosis only in mice that received the virus with the active TGF-β1 construct. RNA was isolated from the lungs 7 days after virus administration. Administration of the adenovirus with the active TGF-β1 construct resulted in increased expression of CTGF, and alpha-1 type I procollagen mRNA expression, compared to the control adenovirus. 36B4 encodes for a ribosomal protein and is used as a loading control in this experiment. Illustration courtesy of Dr. Saku Warshamana (Department of Pathology, Tulane University School of Medicine, New Orleans, Louisana).

which would be counterproductive in fibrotic lung disease, as inflammation is recognized to be an early and essential component of fibroproliferative lung disease (*29*). Furthermore, since mutant TGF-β type II receptor mice demonstrate increased incidences of breast and lung cancer, it is important to identify the role of the individual TGF-β receptors in lung fibrogenesis in light of this finding (*194*).

Therapy directed toward downstream mediators of TGF-β activity may be more prudent, for example, using CTGF or molecules that may modulate the activity of TGF-β such as decorin (*157*) may be clinically more practical. Decorin is a matrix peptide that binds TGF-β1, and has been shown to reduce but not eliminate histopathologic changes of fibrosis as well as hydroxyproline accumulation and neutrophilic infiltration in hamster lungs treated with bleomycin (*157*). Relaxin has also been studied *in vitro* and *in vivo* and was found to block TGF-β1 effects on collagen metabolism (*195*). Relaxin inhibits induction of collagen types I and III by TGF-β in human lung fibroblasts by 45%, and also stimulates the production of MMP-1. *In vivo*, relaxin blocked bleomycin-induced fibrotic histopathology as well as lung collagen accumulation when assessed by hydroxyproline content (*195*).

Culture of alveolar macrophages derived from the lungs of bleomycin-treated rats demonstrates maximally active TGF-β1 secretion 7 days after bleomycin exposure, coinciding with the expression of plasmin (*176*). These results may point to a key process that regulates TGF-β1 activity and could potentially be targeted therapeutically with protease inhibitors. Since TGF-β requires cleavage of the latent peptide to activate its biologic activity and because plasmin is thought to be a major enzyme involved in this proteolytic cleavage, investigators have studied urokinase-type plasminogen activator knockout mice in a murine model of silica-induced fibrosis (*196*). Although silica induced increases in plasminogen activator activity in wild-type mice, in contrast to other studies, urokinase-type plasminogen activator knockout mice demonstrated increases in total and active TGF-β1 secretion by explanted lung macrophages, and active TGF-β expression was not different from that of silica-treated wild-type mice.

At present several investigators are exploring the role of CTGF in the pathogenesis of lung fibrosis in view of studies demonstrating that CTGF shares many of the profibrogenic activities attributed to TGF-β (*197*). CTGF expression is induced by TGF-β, and CTGF upregulates the synthesis of prominent matrix elements such as collagen and fibronectin (*198*), so it is likely that some of the TGF-β profibrogenic activities are mediated through CTGF. Both TGF-β and CTGF expression are upregulated in the murine bleomycin model of lung fibrosis (*23*), and administration of an active TGF-β1 construct to murine lung using an adenoviral vector results in both increased CTGF mRNA expression and fibrosis (Figure 2).

Attention should now be focused on the contribution of the various TGF-β receptors to lung fibrogenesis and on defining the key factors downstream from TGF-β that modulate the individual activities of TGF-β, including elements in the TGF-β signal transduction pathway. It would be interesting to evaluate the effect of administration of newly described TGF-β1 peptide antagonists at various phases of lung injury and development (*199*) to ascertain whether they block binding of TGF-β to its receptor isoforms equally well. Finally, a study by Awad et al. (*200*) has found that a specific TGF-β1 polymorphism is linked to pretransplant fibrotic pathology and lung transplant allograft fibrosis. Their findings warrant an extended analysis on larger numbers of patients with fibrotic lung disease. The mechanism through which this polymorphism predisposes subjects to develop lung fibrosis remains completely unidentified at this time.

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