Fibrotic process affecting the lung and other tissues is characterized by stimulation of fibroblast proliferation and connective tissue deposition. Conventional therapy consisting of glucocorticoids or cytotoxic agents is usually ineffective in blocking progression of disease. Potential new therapies have emerged from the use of animal models of pulmonary fibrosis and recent advances in the cellular and molecular biology of inflammatory reactions. Such therapies involve the use of substances directed against the action of certain growth factors, cytokines, or oxidants that are elaborated during the fibrotic reaction. In this article, we review possible therapeutic applications of these advances.

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**Key words:** antioxidants; growth factors; pulmonary fibrosis; type I collagen

Diseases characterized by the laying down of excess connective tissue affect many organs and tissues of the body. Although other connective tissue elements increase, the stimulation of fibroblasts to proliferate and deposit excess type I collagen is the most prominent feature of the fibrosing process. This accumulation of collagen disrupts normal organ function.

In the heart, longstanding systemic hypertension results in replacement of myocardial muscle cells by fibrous tissue with resultant diastolic dysfunction and heart failure.1 After abdominal surgery, adhesions may cause intestinal obstruction. In the eye, fibrous opacification of the cornea impairs vision. Cutaneous sclerosis may occur locally, as after burns, or it may be widespread as part of progressive systemic sclerosis. In the liver, the scarring of cirrhosis often results in disruption of structure and function. Although antifibrotic therapy is of potential benefit in any of these conditions, this review will focus on diseases that cause widespread fibrosis in the lungs.

Many conditions of both known and unknown etiology may produce excess connective tissue deposition in the lungs.2 This disorder is variously known as interstitial pulmonary fibrosis or fibrosing alveolitis. These processes may develop over a course measured in days, as in ARDS or over a course measured in years as in the pneumoconioses or idiopathic pulmonary fibrosis (IPF). During the last 3 decades, careful study of human lungs and of animal models of interstitial pulmonary fibrosis by histologic study, ultrastructure, and BAL using the techniques of cell and molecular biology has cast new light on the pathogenesis of these disorders.

The initial injury results in damage to the alveolar epithelium or capillary endothelium. An inflammatory response follows that is initially predominantly neutrophilic but soon goes on to a predominance of lymphocytes and macrophages. Unlike many inflammatory processes in the lungs such as pneumococcal pneumonia, resolution does not follow. Effector substances released during inflammation may injure the lung or perpetuate the inflammation by acting as chemoattractants for inflammatory cells. Products of inflammation such as oxidants and proteases may further damage the lungs. Type II alveolar epithelial cells dedifferentiate, replicate, and spread out to replace the

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sloughed type I alveolar cells. Fibroblasts proliferate in the alveolar walls and in the respiratory airspaces in response to growth factors and deposit new connective tissue that is primarily type I collagen.

The accumulation of collagen disrupts function and there is evidence that inhibition of collagen deposition attenuates the physiologic disturbance.\textsuperscript{3} Fibrosing lung diseases may have serious consequences. The onset of the fibroproliferative phase of ARDS heralds a poor outcome. IPF has a 5-year survival of about 50%\textsuperscript{.4}\n
Current therapy for IPF includes use of corticosteroids and antimetabolites.\textsuperscript{5,8} Other agents used with variable results include colchicine,\textsuperscript{9} cyclosporine,\textsuperscript{10} and penicillamine.\textsuperscript{11} Single lung transplantation holds considerable promise as a treatment for IPF. However, the selection criteria for transplant candidates, the availability of suitable donor lungs, and the costs will likely limit its use.

A potential therapeutic agent could act at any of the steps in the pathogenetic pathway that results in fibrosis. Among such agents might be drugs that limit proteolytic or oxidative damage, inhibit the migration of inflammatory cells in the lung, or interfere with the action of inflammatory mediators or with the biosynthesis of collagen. It is not clear whether normal healing can occur after inhibition of collagen deposition; restoring structural integrity may require repair of alveolar epithelium, capillary endothelium, and associated connective tissue matrix. Methods of targeting medications to particular tissues, including the lung, may be important. In the lung, aerosolization offers the opportunity of delivering potent drugs in small amounts with limited systemic side effects, although the delivery of an adequate dose of a drug to the respiratory airspaces may be limiting. Besides the usual aerosol systems, other approaches may be necessary such as delivery of bioactive proteins via viral expression vectors. An adenoviral vector system that contains the transmembrane conductance regulatory protein is in clinical trials for administration to patients with cystic fibrosis.\textsuperscript{12,13}\n
One problem with new therapies for pulmonary fibrosis will be deciding how to measure their efficacy. Pulmonary function tests and standard chest radiographs will be useful but may not be sufficiently sensitive or specific, particularly in early disease with minimal impairment or advanced disease with poor gas exchange. High-resolution CT scans will be more sensitive but not specific. Several biologic markers of inflammation and fibrogenesis are available. When measured in blood, urine, or BAL fluid, they identify specific changes in the inflammatory and repair process.\textsuperscript{14,20} Included are collagen-related peptides,\textsuperscript{17,18} adhesion molecules,\textsuperscript{19} alveolar epithelial cell antigens,\textsuperscript{20} and other relevant cytokines.

**Animal Models and Cell Culture Studies**

Animal models are useful to characterize the mechanisms involved in the development of IPF and to examine the efficacy of specific therapeutic interventions. In each of these models, acute lung injury follows the administration of a noxious agent. After resolution of the resulting intense inflammatory reaction, remodeling of the lung and excess connective tissue accumulation occurs stereotypically after injury. These models may mimic human lung fibrosis that results from a specific injury such as following ARDS or exposure to chemotherapeutic agents. Because human IPF is usually a chronic, insidious disorder, the application of observations derived from animal models of IPF to human disease may have limited relevance. An animal model that behaves similarly to human IPF would aid in the assessment of new therapies. Perhaps a transgenic approach would be useful in this regard. For example, overexpression of human transforming growth factor-\(\alpha\) in respiratory epithelial cells results in pulmonary fibrosis in transgenic mice.\textsuperscript{21}\n
Studies in cell culture employing homogeneous cell populations have greatly contributed to the understanding of the molecular events involved in tissue injury and repair. The results of these studies have suggested possible interventions that could disrupt or modify critical cellular events necessary for connective tissue accumulation in the lung. Because these studies use simple biologic systems, their relevance to the pathogenesis of a disease process occurring in a complex structure like the lung is uncertain. For example, certain effector substances such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) have direct actions on fibroblasts in vitro but cause different responses when functioning as part of a complex inflammatory reaction. This may result from induction of other effector substances or their receptors or from interactions with other cell types. Nevertheless, several novel therapies now being considered for fibrotic illness derive from observations gleaned from experiments in cell culture. We will discuss the potential therapeutic applications derived from such studies to the treatment of IPF.

A working model for activation of collagen deposition in fibrotic processes involves an initial cellular injury associated with a marked inflammatory infiltrate and the elaboration of cytokines, including interleukin 1 (IL-1), TNF-\(\alpha\), and platelet-derived growth factor. These mediators amplify the cellular injury and stimulate fibroblast proliferation. Subsequently, an extracellular matrix is deposited coincident with the appearance of certain fibrogenic cytokines, including transforming growth factor-\(\beta\) (TGF-\(\beta\)) and insulin-related growth factors. Additional work is needed to characterize the specific adhesion molecules that mediate the migration of leukocytes into lung. The same
can be said for the influence of other inflammatory cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF), other interleukins, particularly IL-4, IL-6, and IL-10, and prostaglandins in the process. Other events may also contribute to the disorder, including alterations in the antioxidant barrier, regional blood flow, coagulation, protease activation, and hypoxic conditions. Studies employing animal models suggest that interruption of the initial inflammatory events using inhibitors to specific cytokines prevents the subsequent accumulation of collagen. Whether such an approach is applicable to human IPF is unclear.

**IL-1, TNF-α, and GM-CSF**

IL-1β and TNF-α increase in the lung and the BAL fluid in some patients with fibrosis. These mediators may have a role in initiating and sustaining the fibrogenic injury. Alveolar macrophages derived from patients with IPF and asbestosis release increased quantities of IL-1β and TNF-α as compared with normal controls. The activity of IL-1 likely depends on the concentration of the effector and its inhibitor, the IL-1 receptor antagonist (IL-1ra), in the inflammatory milieu. Macrophages release both IL-1 and the IL-1ra. The IL-1/1ra ratio increased in supernatants obtained from cultured macrophages derived from patients with IPF. Epithelial cells in the lungs of patients with IPF, particularly hyperplastic type II pneumocytes, contain high levels of TNF-α. In contrast, the release of these mediators from macrophages derived from patients with sarcoidosis did not correlate with the clinical status in patients with sarcoidosis.

Nevertheless, the administration of IL-1ra or anti-TNF-α antibodies to a soluble TNF-α receptor inhibited the development of fibrosis in bleomycin- or silica-treated mice. Interestingly, the soluble TNF-α receptor was effective in the treatment of established fibrosis in mice, suggesting that TNF-α is also active during later stages of lung injury. Tetrandrine, a bisbenzisouline quinone alkaloid, inhibits silica-induced fibrosis in rodents, perhaps by decreasing IL-1 production.

The activity of IL-1 and TNF-α may be interdependent because inhibitors directed against either one appear to block the fibrotic response.

GM-CSF induces the differentiation of myeloid stem cells to granulocytes and macrophages. In the lung, GM-CSF generated by T lymphocytes, macrophages, fibroblasts, endothelial cells, and epithelial cells acts on regional inflammatory cells. IL-1 and TNF-α increase GM-CSF production in several cell types and GM-CSF, in turn, induces alveolar macrophages to produce increased amounts of IL-1ra.

This interaction among mediators suggests possible negative feedback control. Such a control system could limit connective tissue accumulation during certain inflammatory reactions. The administration of GM-CSF to mice decreased bleomycin-induced fibrosis, whereas the administration of anti-GM-CSF antibodies increased the deposition of collagen. Although these data suggest that GM-CSF would be useful as an antifibrogenic substance, the subcutaneous administration of GM-CSF to rats produced fibroblast proliferation and induction of α-smooth muscle actin in fibroblasts. This myofibroblast phenotype is typically found in fibrotic lesions in the lung. If GM-CSF proves to have antifibrotic properties, the present cost of GM-CSF would make the clinical use of GM-CSF in chronic IPF prohibitive. It may be useful in a rapidly progressing fibrogenic lesion as in ARDS.

**TGF-β**

The TGF-β cytokines are 25-kDa proteins that, because of their cellular effects, play a central role in the development of IPF and other fibrosing diseases. Accordingly, inhibition of TGF-β action may be an important target for treating IPF. TGF-β is a potent activator of collagen formation by lung fibroblasts in vitro and following subcutaneous implantation. TGF-β activates expression of genes that encode connective tissue proteins and protease inhibitors, and inhibits expression of genes that encode for proteases. These genes contain specific DNA sequences that bind specific nuclear proteins upregulated by TGF-β. For example, we found that TGF-β stimulates transcription of the α1(1) collagen chain by a specific element at 1624 bp upstream to the transcriptional start site.

Many cell types synthesize TGF-β, including platelets, lymphocytes, macrophages, and fibroblasts. Released in a precursor form, it requires proteolytic cleavage for activation. TGF-β stimulates expression of specific genes following binding to specific cell surface TGF-β receptors. The genes encoding these receptors have been isolated and extensively characterized. The complexity of the TGF-β signaling system is notable in view of the identification of two different TGF-β binding proteoglycans (betaglycan and decorin). Betaglycan is associated with the cell surface and released by proteolytic cleavage whereas decorin is associated with the extracellular matrix. When added to cell culture, these proteoglycans inhibit TGF-β action by preventing binding of TGF-β to cell surface receptors. Injection of decorin inhibits scarring associated with an animal model of glomerulonephritis.

Potential antifibrogenic therapy directed at inhibiting the action of TGF-β has attracted considerable recent interest. In human IPF, foci of activated fibroblasts that express high levels of fibronectin and procollagen also contained TGF-β messenger RNA and
protein, suggesting causality. In the bleomycin model of lung fibrosis in rodents, TGF-β is found in regions of active fibrosis. Development of fibrosis in different mouse strains correlated with expression of TGF-β messenger RNA in the lung, although the expression of other effector substances such as TNF-α also correlates with injury. Administration of anti-TGF-β antibodies attenuates connective tissue accumulation following bleomycin treatment.

Major theoretic problems, however, have emerged regarding the potential use of anti-TGF-β substances. Some fibrotic disease states may not involve TGF-β. For example, the data related to the role of TGF-β in scleroderma are conflicting. The amount of TGF-β was reduced in the BAL and in mononuclear cells recovered from patients with scleroderma-related pulmonary fibrosis. In other fibrotic diseases more clearly linked to TGF-β, the long-term use of antibodies to TGF-β is not a feasible approach. Moreover, TGF-β has important immunomodulating properties and mice lacking TGF-β develop a multifocal inflammatory disease and die shortly after birth. A more fruitful strategy may involve the development of specific receptor antagonists or substances that bind TGF-β. But herein also problems have emerged; recent data related to decorin suggest that in some systems it can enhance the action of TGF-β.

Oxygen Radical Injury

Accumulating evidence reveals that oxidant injury is a component of IPF and suggests the possible use of antioxidants as treatment. In IPF, an increase in the oxidant burden may enhance cellular injury and the replacement of differentiated parenchymal cells with fibroblasts and collagen. The mechanism of action of certain injurious agents such as bleomycin and paraquat involves the generation of reactive oxygen species. In addition, exposure to hyperoxia potentiates bleomycin-induced fibrosis, perhaps by further damaging epithelial cells and preventing reepithelialization of the alveolar wall. More directly, repeated treatment of the hamster lung with enzyme-generated oxidants caused pulmonary fibrosis.

Other indirect evidence also implicates reactive oxygen species in the pathogenesis of IPF. Increased serum levels of Cu/Zn superoxide dismutase correlate with disease activity in IPF. This enzyme catalyzes the dismutation of superoxide ion into H₂O₂ and O₂. Curiously, administration of methionyl manganese superoxide dismutase (MnSOD) inhibited bleomycin pulmonary toxicity. In contrast, the administration of oral vitamin E did not increase pulmonary pressure-volume characteristics following bleomycin treatment. The discrepancies between these findings require further study. As compared with controls, increased numbers of alveolar macrophages from patients with IPF express CD11/CD18 adhesion molecules. Expression of this molecule may relate to increased superoxide ion production by these cells. Administration of anti-CD11 antibodies inhibited bleomycin pulmonary fibrosis. The level of glutathione in the lung epithelial lining decreases in patients with IPF, suggesting depletion of the antioxidant barrier.

Taurine, a natural free-amino acid, exhibits antioxidant properties and partially inhibits bleomycin-induced fibrosis in rodents. However, this effect on bleomycin toxicity is not necessarily due to an antioxidant effect because taurine also alters calcium transport. Interestingly, the combination of taurine and niacin further inhibits the development of bleomycin-induced fibrosis. During bleomycin-induced fibrosis, the nicotinamide adenine dinucleotide (NAD) content of the lung decreases. This decrease may relate to the generation of oxidants because the addition of H₂O₂ or hypochorous acid to cells in culture causes a drop in NAD concentration. Treatment with niacin, a precursor for NAD, increases the intracellular levels of both NAD and adenosine triphosphate (ATP) in bleomycin-treated animals.

The antioxidant imbalance in IPF in humans can be reversed at least transiently. One approach involves increasing the glutathione content of the epithelial lining fluid (ELF). Borok et al reported that treatment of patients with IPF with a glutathione aerosol increased total ELF glutathione levels transiently and decreased the spontaneous release of superoxide anion by alveolar macrophages. Meyer and colleagues administered oral N-acetylcysteine to patients and measured glutathione levels in BAL and ELF. They found that administration of N-acetylcysteine resulted in increases in glutathione in the BAL but not in the ELF, although the trend was toward higher values. These studies did not directly assess whether such procedures attenuate the fibrosis in human disease. In mice, oral N-acetylcysteine reduces collagen deposition in bleomycin-induced fibrosis. Although the use of antioxidants is promising, much additional work will be necessary to establish a place for these agents in the treatment of IPF.

Modulation of Collagen Biosynthesis

Other potential antifibrogenic substances act directly on collagen biosynthesis. These substances do not affect the inflammatory component of fibrogenic reactions per se. The triple helix domain of the type I collagen molecule contains a repeating amino acid sequence (gly-X-Y) and a high content of proline, alanine, and lysine residues. About 30 to 40% of the proline residues are hydroxylated. Some of the hydroxylated residues are used to form intermolecular cross-links. Incorporation of certain proline analogues into the molecule renders it unstable. Proline analogues inhibit
fibrosis following bleomycin and paraquat but not silica.\textsuperscript{3,8,11} As a potential therapy in humans, proline analogues have limited use because the analogues would presumably incorporate into many proteins causing serious physiologic derangements.

Disruption of cross-link formation between collagen molecules is another treatment strategy. These cross-links are intermolecular bridges catalyzed by lysyl oxidase. Penicillamine functions as a lysyl oxidase inhibitor and is currently used in the treatment of patients with scleroderma.\textsuperscript{11} Its efficacy in other forms of pulmonary fibrosis is uncertain. Penicillamine has limited potential as an antifibrotic agent because of frequent side effects, particularly nephrotoxicity. The development of novel lysyl oxidase inhibitors is another potential therapeutic approach.

Although there is no direct evidence that prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) functions as an antifibrogenic agent \textit{in vitro}, PGE\textsubscript{2} decreases collagen accumulation by lung fibroblasts \textit{in vitro}.\textsuperscript{83,84} Administration of an aerosolized preparation results in increased levels of PGE\textsubscript{2} in the ELF.\textsuperscript{85} However, indomethacin inhibits fibrogenic reactions\textsuperscript{86} and functions, in part, as an inhibitor of cyclooxygenase activity and prostaglandin production. Despite these findings, PGE\textsubscript{2} may be useful as an antifibrogenic agent. Indomethacin may act to inhibit the inflammatory component of the disorder rather than the subsequent fibroproliferative phase. Assessment of potential antifibrotic action for PGE\textsubscript{2} may require human studies because PGE\textsubscript{2} is less effective in inhibiting collagen formation in rodent cells than in human fibroblasts.

\gamma-interferon also decreases collagen production by fibroblasts \textit{in vitro}. \gamma-interferon may influence collagen production \textit{in vivo} and may be a potential therapeutic agent for fibrotic disorders. The production of \gamma-interferon by T lymphocytes is reduced in some patients with IPF.\textsuperscript{87} Recombinant rat \gamma-interferon inhibits collagen accumulation induced by bleomycin in rats.\textsuperscript{88} In addition, the interferon inducers bropirimine\textsuperscript{89} and polyninosinic-polycytidylic acid\textsuperscript{90} decreased collagen accumulation following bleomycin treatment in rodents. Although these results appear encouraging, systemic toxicity will likely limit the use of \gamma-interferon in humans. An aerosolized preparation might be efficacious.

\textbf{ANGIOTENSIN-CONVERTING ENZYME INHIBITORS}

Myocardial fibrosis results from renovascular hypertension or angiotensin infusion in animal models. Administration of captopril, an angiotensin-converting enzyme inhibitor, appears to inhibit this process.\textsuperscript{91-93} The inhibition is independent of blood pressure control.\textsuperscript{94} However, spironolactone but not captopril blocked myocardial fibrosis in primary hyperaldosteronism.\textsuperscript{95} Only limited information is currently available related to the use of captopril in other fibrotic disorders. In the lung, captopril inhibits radiation-induced pulmonary fibrosis in rats.\textsuperscript{96} The administration of captopril but not lisinopril decreases fibroblast proliferation \textit{in vitro} suggesting the mechanism of action is not via angiotensin-converting enzyme inhibition.\textsuperscript{97} Penicillamine, another thiol compound, also decreases thymidine incorporation.

\textbf{CONCLUSIONS}

Several promising new approaches to the treatment of fibrogenic disorders have emerged in recent years. These evolved from a greater understanding of the influence of particular effector substances in the inflammatory milieu. The applicability of insights gained from studies of bleomycin-induced fibrosis to human lung fibrosis requires further examination. For example, we must learn whether antioxidant therapy will reduce the pace of fibrosis in human IPF. The potential to deliver specific inflammatory inhibitors or inhibitors of collagen biosynthesis directly to the lung via aerosolization suggests that lung fibrosis may be more amenable to novel therapies than other internal organs.

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