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Forum Editorial

Redox Imbalance and Lung Fibrosis

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HUMAN idiopathic pulmonary fibrosis (IPF) is currently an area of intense research interest. Many reasons exist for this fascination: most important, IPF is a disease with an unknown etiology and a poor prognosis. At present, no available treatment is able to improve the prognosis of this disease. IPF belongs in the group of idiopathic interstitial pneumonias; most other diseases of this group, with the exception of acute interstitial pneumonia (AIP), respond to antiinflammatory therapies and have a relatively good prognosis (1, 13). IPF (histopathologic finding: usual interstitial pneumonia, UIP; a previously used term was also cryptogenic fibrosing alveolitis) represents a disorder with characteristic patchy areas of aggressive fibrogenesis (so-called fibroblastic foci) and the presence of a weak inflammatory reaction in the lung. IPF is now regarded as a fibroproliferative disorder triggered by injury to the alveolar epithelium, whereas it was previously considered to be mainly an inflammatory disease. In addition to idiopathic interstitial lung diseases, other interstitial lung diseases include many other disorders that can develop either after exposures to environmental toxins (*e.g*., asbestos fibers), drugs (such as bleomycin and many other cytotoxic drugs), or radiation, or be associated with autoimmune diseases. In addition to emphysema, cigarette smoking causes several interstitial lung diseases (for example, desquamative interstitial pneumonia). Moreover, emphysematous lung contains patchy fibrotic areas in the lung parenchyma, and the airways of these patients (chronic obstructive pulmonary disease, COPD) typically reveal obstruction with airway fibrosis (23). Experimental models of lung fibrosis (such as bleomycin-induced lung fibrosis) have significantly expanded our understanding about the pathogenesis and progression of lung fibrosis. However, many of these models display their own distinct characteristics (such as a diffuse inflammatory reaction) and are not directly comparable to human IPF. This complicates the extrapolation of the results obtained with these experimental models to human lung fibrosis.

Reactive oxygen metabolites are considered among the key mechanisms that modulate the pathogenesis of fibrotic lung diseases, including IPF, asbestosis, and many drug-induced lung reactions (17, 19, 33). Oxidant markers and footprints of oxidative damage and repair can be detected in the lung, airway secretions, and exhaled air of IPF patients (3, 19, 27, 32). Experimentally induced lung fibrosis or the fibrotic areas of IPF patients (or both) also reveal reduced levels of low-molecular-weight antioxidants, such as glutathione (GSH) in the epithelial lining fluid (6), and several antioxidant enzymes and related proteins in the fibrotic areas of the lung (20, 30, 37, 38). Interestingly, only one drug, *N*-acetylcysteine (NAC) (10) has any effect on IPF, although speculation exists that the beneficial effect of NAC may relate to its ability to act as an antidote to the cytotoxic drugs used in the treatment of IPF. However, several studies suggest that an increased oxidant burden occurs in IPF, which subsequently then may have a multitude of consequences [*e.g*., activation of transcription factors and growth factors (such as transforming growth factor-β, TGF-β), which are the major growth promoting mechanisms in lung fibrosis] (19, 21, 28, 31, 36).

Several controversies exist about the pathogenesis and potential therapeutic strategies in human IPF. This exciting Forum Issue gathers together a wide range of studies on the pathogenesis and progression of pulmonary fibrosis and the roles of free radicals in these disorders, as well as proposals for future investigations. Current topics include the role of redox balance in growth-factor activation, apoptosis and coagulation pathways, and the antioxidant defence, including Nrf2 and extracellular superoxide dismutase (ECSOD) in experimental lung fibrosis and human IPF. Several articles deal with potential antifibrotic redox modulators, antioxidants and/or antioxidant mimetics in lung fibrosis, an area of intensive investigation in a number of worldwide laboratories.

The prevailing hypothesis suggests that inflammation plays a minimal, if any, role in the development or progression of IPF. The reasons for these arguments are related first to the findings that very few inflammatory cells can be detected in the IPF/UIP lungs, and second, that antiinflammatory therapies have no impact on the progression of this disease; patients die of this disease within 4–5 years after diagnosis. The role of inflammation in IPF is, however, controversial (15, 34) and studies point to the involvement of mononuclear phagocytes and macrophage colony-stimulating factor (M-CSF) in pulmonary fibrosis (2). The article by Bringardner *et al*. (4) in this issue examines this controversial topic and also proposes that in-

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flammation can be a critical factor in IPF, not only directly but also through several indirect mechanisms.

One of the most important growth-promoting cytokines that have been linked to IPF is TGF-β. This growth factor can also be activated by oxidants (36). TGF-βs are synthesized as inactive precursor proteins, and they are secreted into the extracellular space as large latent complexes containing mature dimeric TGF-β bound to latency-associated protein (LAP) and latent TGF-β–binding protein (LTBP). Multiple factors are involved in the activation of TGF-β (18, 23) (*e.g.*, an alteration in the cellular redox state). The active site of this and many other redox-regulated cytokines and enzymes contains thiol groups that can be attacked by oxidants, which then can lead to conformational changes in the structure of these molecules and enzyme/cytokine activation/inactivation (21, 23). In the review, Koli *et al*. (23) also emphasize that certain common but also diverse features exist in human IPF and fibrotic areas of the emphysematous lung with disease specific changes in TGF-β activation pathways.

Asbestos causes lung fibrosis in humans (asbestosis), and exposure to asbestos fibers has been widely used as a model of lung fibrosis (4, 26). In current issue, two groups have used asbestos exposure or asbestosis as a model for investigating the pathogenetic mechanisms in fibrotic lung (16, 22); the study of Ghio *et al*. (16) also reveals the disruption of the normal iron homeostasis in human asbestosis.

Other mechanisms that have been widely associated with lung fibrosis, also in human lung, include the disturbance of the coagulation pathway (23, 24). The serine protease plasmin is one of the first proteases found to cause TGF-β activation and release the latent TGF-β complex from extracellular matrix (25, 35). Conversely, plasminogen activator inhibitor-1 (PAI-1) is a physiologic inhibitor of plasminogen activators, and its expression is increased in many fibrotic diseases and experimental models of lung fibrosis. Genetic modulation of PAI-1 expression also alters the sensitivity for the development of lung fibrosis. Importantly, these reactions can be modulated by the cellular redox balance and GSH homeostasis (24).

Accumulating evidence suggests that pulmonary fibrosis is partly driven by apoptosis of alveolar cells. However, several aspects of this process have remained unclear, such as the survival of mesenchymal cells and resistance of IPF fibroblasts to apoptosis under these same circumstances (11). Cell survival and tissue repair are also facilitated by mitochondrial biogenesis, although this mechanism is incompletely understood. Mitochondrial biogenesis is a complex phenomenon that is regulated jointly by the nuclear and mitochondrial genomes and coordinated by several transcription factors and activators. These mechanisms can be activated in diverse acute and chronic inhalational lung injuries in association with oxidative stress (7), and this may also occur in the ongoing fibrotic processes in the lung. The exact roles of both of these mechanisms, apoptosis and mitochondrial biogenesis, in the progression of pulmonary fibrosis are, however, largely unknown and worthy of further investigation.

Work carried out with experimental models including bleomycin, asbestos, and/or hyperoxia has expanded our understanding of the mechanisms related to the imbalance of oxidants and antioxidants in the fibrotic lung. These include studies on Nrf2, which is one member of the leucine zipper (bZIP) transcription factor family. Nrf2 regulates the activities of a number of antioxidant enzymes (including superoxide dismutases and several enzymes associated with GSH homeostasis) through binding specific sequences of antioxidant responsive elements (AREs). In the original studies by Kleeberger *et al*., Nrf2 was found to protect against fibrosis, and its deficiency resulted in the overexpression of fibrotic markers such as TGF-β and the enhancement of pulmonary fibrogenesis (8). In the current issue, Walters *et al*. (39) extend these observations on Nrf2 and present exciting proposals for future investigation.

One interesting enzyme that is also associated with Nrf2 regulation is ECSOD, which is the only superoxide scavenging antioxidant enzyme in human lung protecting matrix proteins against degradation (12, 14) The levels of this enzyme are apparently very low in the fibrotic areas of IPF lungs (20). One mechanism through which ECSOD can inhibit fibrosis progression is by protecting heparin/heparan sulfate (a compound that is abundant in ECM and tightly binds to ECSOD) from oxidative fragmentation (22).

It is possible that antioxidant strategies (NAC) may have importance in human IPF, as suggested by the IFIGENIA trial in human IPF (10). However, several other antifibrotic redox modulators (23) and antioxidants/antioxidant mimetics have been tested in experimental models of lung injury/fibrosis (5, 9, 14, 29). Peroxisome proliferator–activated receptor gamma (PPAR) ligands have some potential use as antiinflammatory agents, and activation of PPAR has differential inhibiting effects on cigarette smoke–related inflammation (5). It is possible that PPAR can also have a redox modulatory function in pulmonary fibrosis. Lipoic acid and its reduced product, dihydrolipoic acid, are potent antioxidants, and these agents limit the capability of neutrophil NADPH oxidase to produce superoxide (29). Further studies will likely elucidate whether lipoic acid can diminish excessive superoxide production in the fibrotic lung. Many synthetic antioxidants such as macrocyclics, metalloporphyrins, salens, and nitroxides have already been developed, and these compounds generally can scavenge several kinds of ROS, including superoxide, hydrogen peroxide and peroxynitrite. These catalytic antioxidants have already been explored in many experimental lung injuries (9). They may potentially be of benefit in the prevention of the progression of lung fibrosis but have not been tested in human IPF.

Several questions still remain to be answered, including the genetic and environmental factors contributing to the development of pulmonary fibrosis. The fundamental question also remains: why do exogenous oxidants evoke the disease only in a minority of humans, and why do some individuals develop lung fibrosis when others, especially smokers, develop another type of parenchymal lung disease (emphysema). The reasons for these differences are probably associated with the genetic background and also with variable environmental exposures. Genetic factors are known to contribute to IPF susceptibility. Whether the functional SNPs in *NRF2* and related genes (one example being *ECSOD*) or genes regulating the growth-promoting cytokines in the lung have any role in the pathogenesis of IPF and related disorders is an important area for future investigation. However, because the prevention of many of these diseases is currently impossible, greater efforts

are needed to discover sensitive and specific markers for identifying IPF and better therapies to combat the progression of lung fibrosis. One such group of these therapies in human IPF includes compounds with redox-modulatory effects on the oxidant burden as well as agents that can modify growth-factor activation in human IPF.

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