Nonresolving Fibrotic Disorders: Idiopathic Pulmonary Fibrosis as a Paradigm of Impaired Tissue Regeneration

Louise Hecker, PhD and Victor J. Thannickal, MD

Abstract: The pathogenesis idiopathic pulmonary of fibrosis and related fibrosis lung disorders are complex and poorly understood. This likely involves cellular mechanisms that result in loss of cellular homeostasis leading to aberrant alveolar wall remodeling through the excessive deposition of connective tissue matrices. Impaired tissue regeneration and dysregulation of cell death in lung fibroblasts and epithelial cells appear to be important in the initiation and progression of these disorders. This review summarizes current understanding in this area to stimulate research into the development of novel therapeutic strategies that prevent, halt or reverse the progression of lung fibrosis.

Key Indexing Terms: Pulmonary fibrosis; Lung injury and repair; Apoptosis; Tissue regeneration; Myofibroblasts; Epithelial cells. **[Am J Med Sci 2011;341(6):431–434.]**

Recent progress in basic and translational research has improved our understanding of human fibrotic disorders, including idiopathic pulmonary fibrosis (IPF). Paradigms of IPF pathogenesis have evolved from a focus on chronic inflammation,[1–4] to aberrant wound healing,[5–7] to current concepts of a multifactorial and heterogeneous disease process involving cellular senescence, oxidative stress, cellular plasticity, mechanotransduction and epigenetic mechanisms. A comprehensive and complete discussion of these processes and mechanisms is beyond the scope of this review. In this discussion, we will focus on IPF as a nonresolving fibrotic process in which interactions/relationships between the epithelium and the underlying mesenchyme are altered, resulting in alveolar epithelial cells becoming susceptible to apoptosis, whereas mesenchymal cells acquire an apoptosis-resistant myofibroblast phenotype. First, we will provide an evolutionary perspective on fibrosis to address the question of "why" an adaptive tissue repair process may go awry in IPF and lead to a progressive, irreversible disease process.

AN EVOLUTIONARY PERSPECTIVE ON FIBROSIS

Fibrosis has been defined as "the process by which the body lays down collagen as part of the healing process." It may be a consequence of the normal healing response leading to a scar, or it may be abnormal, as in scleroderma and keloid scar formation (http://dermnetnz.org/pathology/pathology-glossary.html). Thus, although fibrosis is most commonly referred to in its pathological context, the elaboration of extracellular matrix (ECM) proteins by activated (myo)fibroblasts at sites of tissue injury is also part of the normal physiological response to injury. An interrogation of "why" fibrosis has evolved as a biological process in higher multicellular species may provide important insights into the progressive nature of this process in disease states. In mammals, the formation of this scar may serve several useful purposes: (1) it reinforces the initial clot formation at the site of wounding to prevent blood loss; (2) it creates a barrier to prevent the invasion of microbial pathogens and (3) it may provide a "provisional" matrix for subsequent reepithelialization. Fibrosis may also be considered as part of innate host defense against infectious pathogens or the perceived threat of infection in the setting of an open wound. The formation of ECM "scars" around infectious and/or noninfectious (traumatic) injury is a "normal" response that serves to limit the invasion/spread of the pathogen at the site of infection/injury in both animal and plant species.[8] An example of this type of tissue response in humans is in the formation of a fibrous scar around *Mycobacterium tuberculosis* bacilli-containing granulomas.[9] If such a "protective" response did not develop, one might predict that a third of the world's population infected with this pathogen would succumb to disseminated infection. It makes teleological sense that the need for survival of the organism from bleeding and infection would take precedence over a temporary (reversible), and often marginal, loss of organ structure and function. Thus, natural selection may have favored the development of fibrosis at sites of wounding or injury, at the expense of complete (and immediate) restoration of tissue architecture and function.

So, how does this adaptive, protective, physiological process become pathological? On the basis of an understanding of the "why," we can begin to generate some hypotheses of "how" pathological fibrosis might occur. There are several possible hypotheses: (1) the host perceives an ongoing threat, whether real or perceived; (2) the host response becomes uncontrolled or dysregulated and (3) the host response which evolved to protect the organism up until reproductive age could not "anticipate" the changes associated with advancing age.

LOSS OF CELLULAR HOMEOSTASIS AND THE APOPTOSIS PARADOX

A careful consideration of the complexity of cellular organization and structure of the alveolus is essential to an understanding of derangements in alveolar regeneration. Ultrastructural (electron microscopy) studies demonstrate that alveolar lining cells of the air and blood luminal surfaces (the epithelium and endothelium, respectively) are tethered together by an interconnected interstitial mesenchymal cell population that is loosely referred to as "fibroblasts."[10] These fibroblasts make direct contact with both epithelial cells and endothelial cells (and pericytes) on both sides of the alveolar wall, supporting a central role for these

From the Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama.

Supported by National Institutes of Health grants, ROI HL067967 and ROI HL094230.

Correspondence: Louise Hecker, PhD, Department of Pulmonary, Allergy, and Critical Care Medicine, University of Alabama at Birmingham, 1530 Third Avenue South, THT 422, Birmingham, AL 35294-0006 (E-mail: lhecker@uab.edu).

cells in maintaining cellular homeostasis during normal turnover and in response to injury.[11,12]

In IPF, there is loss of cellular homeostasis within the alveolar wall.[13] There is clearly an expansion of mesenchymal cells within the interstitium with more discrete areas of assembled bundles of muscle-like cells within so-called fibroblast foci, a typical feature of usual interstitial pneumonia and a histopathological hallmark of IPF.[14–17] These muscle-like cells have been characterized as myofibroblasts, and their contractile activities contribute to alveolar collapse and the associated restrictive physiology and gas-exchange abnormalities characteristic of IPF. The alveolar epithelial cells appear to be in a state of dysrepair with spatially disorganized areas of rapid proliferation and apoptosis, findings that are perhaps best described as "frustrated regeneration." Whether it is the ineffective epithelial repair that drives myofibroblast activation or, whether it is the unrestrained mesenchymal activation that "frustrates" the epithelium is not entirely clear.

Studies of fibroblastic foci support the concept of the "apoptosis paradox," whereby alveolar epithelial cells appear to be undergoing increased rates of apoptosis, whereas the adjacent myofibroblasts appear to be resistant to apoptosis.[18–20] Several mechanisms for alveolar epithelial cell apoptosis in IPF have been proposed, including telomere shortening/cellular senescence,[21–24] endoplasmic reticulum stress,[25,26] viral infections[25,27,28] and alterations in the cellular microenvironment perpetuated by activated myofibroblasts that secrete proapoptotic angiotensin peptides,[29] oxidants[30] and Fas ligand.[31] Another potential fate of activated alveolar epithelial cells is epithelial-to-mesenchymal transition,[32,33] thereby contributing to the (myo)fibroblast population in the IPF lung.

Effective apoptotic clearance of myofibroblasts heralds the resolution phase of normal wound healing,[34] although mechanisms that mediate myofibroblast apoptosis in physiologic wound repair remain unclear. It has been postulated that crosslinking of a contracted ECM shields myofibroblasts from biomechanical stress and that loss of mechanical tension may induce myofibroblast apoptosis.[35,36] The myofibroblast in IPF appears to be resistant to apoptosis.[20] As with physiological mechanisms of myofibroblast apoptosis in normal wound healing, the mechanisms of an apparent "apoptosis-resistant" myofibroblast phenotype in progressive fibrotic disorders, including IPF, are not well understood.

Studies by our group have demonstrated that, in addition to myofibroblast differentiation, the profibrotic cytokine, transforming growth factor-β1 (TGF-β1), promotes myofibroblast survival.[37–39] TGF-β1 activates 2 prosurvival signaling pathways, focal adhesion kinase and protein kinase B (PKB/AKT) by mechanisms that involve cell adhesion and release of soluble growth factors, respectively[37,40]; together, both pathways contribute to myofibroblast survival.[38] Importantly, the administration of a protein kinase inhibitor that modulates the activities of these prosurvival pathways attenuates fibrosis in a model of bleomycin-induced lung fibrosis.[41,42] Potential roles of these pathways in apoptosis resistance of myofibroblasts in IPF or in individualized patients require further study.

More recent studies from our laboratory support the role of a member of the nicotinamide adenine dinucleotide phosphate; reduced form oxidase (NOX) family, NOX4, in myofibroblast differentiation/survival. NOX4 was identified as one of the most highly upregulated genes in transcriptomal (Affymetrix, Santa Clara, CA) analyses of human lung fibroblasts treated with TGF-β1.[43] NOX4 activation mediates generation of H_2O_2, myofibroblast differentiation, contractility and ECM production in response to TGF-β1, effects that also seen in human IPF-derived (myo)fibroblasts. In tissues of patients with IPF, the expression of NOX4 is localized to myofibroblasts, both within fibroblastic foci and in remodeled blood vessels, as well as in epithelial cells associated with aberrant bronchiolization.[43–45] Therapeutic targeting of this NOX isoform protects against fibrosis in 2 different animal models of injury-provoked pulmonary fibrosis,[43] providing important proof of concept that this may be an effective antifibrotic strategy.

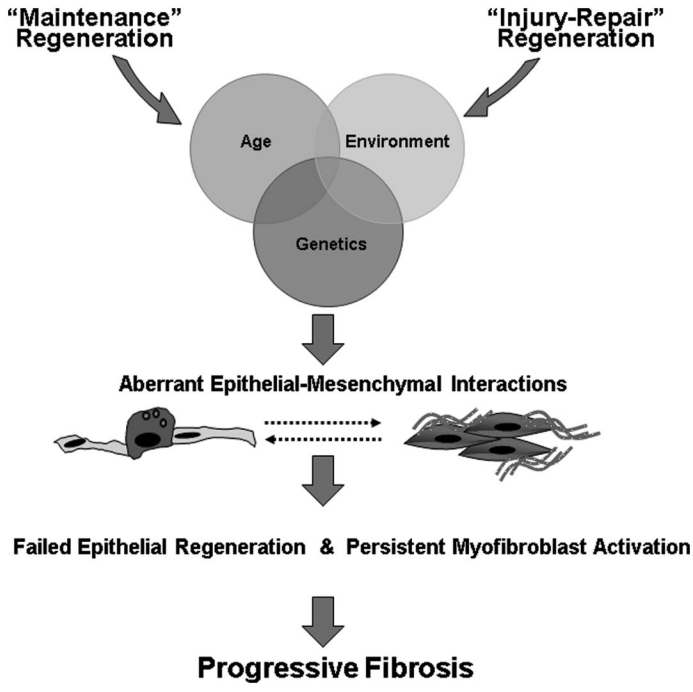

FIGURE 1. Pathogenesis of nonresolving fibrosis: regeneration of the lung may involve both "maintenance" and "injury-repair" responses. These responses can be influenced by age, genetic/epigenetic factors and environmental insults that result in aberrant epithelial-mesenchymal interactions. A failure of epithelial regeneration and persistent mesenchymal activation may reciprocally promote each other, culminating in progressive pulmonary fibrosis.