What Differentiates Normal Lung Repair and Fibrosis? Inflammation, Resolution of Repair, and Fibrosis

Robert M. Strieter¹

¹Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia

There has been ongoing controversy related to what differentiates normal lung repair and fibrosis. For example, the current prevailing concept has been that idiopathic forms of pulmonary fibrosis are due only to epithelial injury in response to some unknown cause that results in persistent evolving fibrosis without preceding inflammation. This concept would suggest that the lung responds to injury in a different manner than other organs, such as the liver, kidney, and heart. However, that would seem to contradict known established pathological concepts. To address this controversy, concepts were presented as follows: (1) loss of basement membrane integrity is critical in determining the "point of no return," and contributes to the inability to reestablish normal lung architecture with promotion of fibrosis; (2) loss of epithelial cells, endothelial cells, and basement membrane integrity in usual interstitial pneumonia associated with idiopathic pulmonary fibrosis leads to destroyed lung architecture and perpetual fibrosis; (3) transforming growth factor- β is necessary, but not entirely sufficient, to promote permanent fibrosis; (4) persistent injury/antigen/irritant is critical for the propagation of fibrosis; (5) idiopathic pulmonary fibrosis is an example of a process related to the persistence of an "antigen(s)," chronic inflammation, and fibrosis; and (6) unique cells are critical cellular players in the regulation of fibrosis. In keeping with the theme of the Aspen Lung Conference, it is hoped that more questions are raised than answered in this presentation, in support of the continued need for research in this area to address these important concepts.

Keywords: fibrosis; basement membrane; cytokines; inflammation; antigens

I would like to thank the Chair, Dr. David Riches, and the two Co-Chairs, Drs. Gregory P. Cosgrove and Stephen K. Frankel, for inviting me as a State of the Art Speaker to this outstanding 50th Annual Thomas L. Petty Aspen Lung Conference. Dr. Riches gave me the title and the daunting task to present this subject matter. In addition, in keeping with the nature of this conference, Dr. Riches requested me to present concepts that might be perceived as controversial. Concepts that were presented in the presentation were as follows: (1) loss of basement membrane (BM) integrity is critical in determining the "point of no return," and contributing to the inability to reestablish normal lung architecture with promotion of fibrosis; (2) loss of epithelial cell, endothelial cells, and BM integrity in usual interstitial pneumonia (UIP) associated with idiopathic pulmonary fibrosis (IPF) leads to destroyed lung architecture and perpetual fibrosis; (3) transforming growth factor- β (TGF- β) is

necessary, but not entirely sufficient, to promote permanent fibrosis; (4) persistent injury/antigen/irritant is critical for the propagation of fibrosis; (5) IPF is an example of a process related to the persistence of an "antigen(s)," chronic inflammation, and fibrosis; and (6) unique cells are critical cellular players in the regulation of fibrosis. In keeping with the theme of this conference, I hope that I raised more questions than were answered in this presentation to support the continued need for research in this area to address these important concepts.

LOSS OF BM INTEGRITY IS CRITICAL IN DETERMINING THE POINT OF NO RETURN, AND CONTRIBUTES TO THE INABILITY TO REESTABLISH NORMAL LUNG ARCHITECTURE WITH PROMOTION OF FIBROSIS

Normal response to acute lung injury constitutes the host's response to a variety of insults. The lung is not only involved in gas exchange, but has a critical role in mediating host defense (1). The lung is an organ anatomically situated within the body, interposed between the host and its environment. This barrier consists not only of the airway with its mucociliary clearance, but also of the extensive alveolar-capillary membrane (ACM), which is composed of both immune and non-immune cells constantly exposed to both inhaled and hematogenous challenges (1). The pulmonary response to these inflammatory stimuli ultimately impacts on host survival, especially because the lung must maintain its structural integrity for gas exchange.

Acute lung injury and normal repair of the ACM result in rapid restoration of tissue integrity and function following a variety of insults. Although acute inflammation and normal repair represent a complex interplay between humoral, cellular, and extracellular matrix networks, this process usually occurs in a sequential, yet overlapping, manner. After injury to the ACM, the process immediately begins with hemorrhage and extravasation of plasma into lung tissue (1–3). This results in activation of the intrinsic and extrinsic coagulation pathways, leading to fibrin deposition and establishment of a provisional matrix (1–3). Platelet activation and degranulation also occur during coagulation, leading to the release of a number of lipid mediators and cytokines into the provisional matrix (1–3). These lipid mediators and cytokines are either important growth factors or chemotaxins that incite leukocyte, endothelial cell, fibroblast/myofibroblast, and epithelial cell activation (1–3).

The extravasation of leukocytes into the lung is dependent on a dynamic and complex series of events (1, 4, 5). The steps that lead to leukocyte recruitment include the following: endothelial cell activation and expression of endothelial cell-derived adhesion molecules, leukocyte activation, and expression of leukocyte-derived adhesion molecules, leukocyte-endothelial cell adhesion, leukocyte diapedesis, and directional leukocyte migration beyond the vascular compartment via chemotactic gradients

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Correspondence and requests for reprints should be addressed to Robert M. Strieter, M.D., Hospital Drive, Outpatient Clinics Building, 6th Floor, Room 6560, Department of Medicine, University of Virginia School of Medicine, Charlottesville, VA 22908. E-mail: strieter@virginia.edu

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(1). Although adhesion between leukocytes and endothelial cells is a prerequisite interaction for successful leukocyte extravasation into the lung, the subsequent steps leading to diapedesis and migration beyond the vascular compartment are dependent on both the continued expression of specific integrins and the movement along a leukocyte-specific chemokine gradient (1). Neutrophils are usually the first leukocytes to arrive at the site of tissue injury; their primary function is to phagocytose debris and microorganisms (1). However, these leukocytes have the capacity to produce a number of lipid and protein mediators that are instrumental in orchestrating the progression of tissue repair (1). Although neutrophils are important for initial host defense in response to pulmonary injury, the second wave of leukocytes consists of mononuclear cells, with the mononuclear phagocyte representing a pivotal leukocyte in the progression of lung repair (1, 3, 6). This leukocyte has the ability to generate a number of inflammatory and reparative mediators that are important in transforming the provisional matrix into a more mature extracellular matrix (ECM) (3, 6).

The transition of tissue repair of the ACM from acute inflammation to deposition of ECM is an essential event, as this type of ECM resembles granulation tissue that consists of a variety of mediators, appropriate extracellular matrix constituents, fibroblasts/myofibroblasts, endothelial cells, and leukocytes, that form either the connective tissue foundation or act as the stimulus for angiogenesis. The process of vascular remodeling is paramount, as this process sustains a continual supply of oxygen and nutrients to the cellular constituents of tissue (1–3, 5). During the early phases of ECM deposition, the immature connective tissue resembles undifferentiated mesenchyme with the presence of fibrin, fibronectin, a predominance of collagen type III as compared with type I collagen, and a highly vascularized capillary bed (1–3). This phase is followed by transformation to a more mature ECM that is associated with increased deposition of collagen type I, fibronectin, and protease-dependent remodeling of the ECM (1–3). If the BM of the ACM is intact and the stimulus for the original injury has been removed, the deposition of ECM is remodeled/reabsorbed, concurrently with reepithelialization and reendothelialization of the ACM (1–3). The normal repair process is complete when parenchymal cells (epithelium and endothelium) have reestablished their normal spatial orientation on their BM of the ACM (1, 7). An example of this process is pneumococcal pneumonia (1, 7). Although there is a robust inflammatory response to the microorganism, the host response together with appropriate antibiotics leads to rapid clearance of the stimulus (microorganism) with no chronic inflammation (1–3, 7). Epithelial and endothelial injury of the ACM occurs; however, the BM remains intact and rapid reepithelialization and reendothelialization occur on the intact ACM BM with resultant limited fibroblast/myofibroblast activation and deposition of ECM (1, 7). Therefore, resolution in this case is associated with return of the ACM to normal integrity.

In contrast, chronic inflammation in interstitial lung disease (ILD) related to persistence or repetitive recurrent injury due to persistent or recurrent exposure to a stimulant, irritant, or antigen is essential to the development of fibrosis representing dysregulated and exaggerated tissue repair culminating in fibrosis (1, 4, 5, 7). The pathogenesis of fibrosis in ILD is associated with the following events: (1) loss of ACM type I epithelial cells and endothelial cells (4, 5, 7–11); (2) loss of the integrity of the BM of the ACM with collapse of the alveolar structures and fusion of their BMs (4, 5, 7–11); (3) proliferation of type II pneumocytes and endothelial cells on an inappropriate ECM without reestablishment of normal alveolar structures (4, 5, 7–11); and (4) recruitment and proliferation of fibroblasts/myofibroblasts

with deposition of mature ECM leading to end-stage alveolar fibrosis (4, 5, 7–11). This process involves the complex and dynamic interplay between diverse immune effector cells and cellular constituents of the ACM and interstitium of the lung, and is sustained in the presence of persistent or recurrent stimuli, irritant, or “antigen” (1, 7). Interaction of these diverse cell populations and the mediators that they produce culminates in lung injury, extracellular matrix deposition, and, ultimately, end-stage fibrosis. However, in many of the idiopathic interstitial pneumonias, the initial stimulus for inflammatory cell recruitment and the mechanism(s) responsible for the perpetuation and evolution of chronic inflammation, granulation tissue formation, and fibrosis have not been fully elucidated. Inhaled antigens, viruses, or toxins may induce an exaggerated immune response leading to the extravasation of activated leukocytes, resulting in lung injury. Aberrant expression of class II MHC molecules on alveolar epithelial cells, endothelial cells, and alveolar macrophages may promote exuberant antigen presentation and immune cell activation, leading to perpetuation of chronic inflammation in ILD. The development of local chemotactic gradients may result in marked expansion of diverse populations of leukocytes within the airspaces and interstitium of the lung. Although the cellular mechanisms by which leukocytes are recruited in ILD have not been fully characterized, cellular constituents of the ACM and interstitium probably are central to recruiting activated leukocytes that further amplify the immune/inflammatory process within the lung (1). In addition to the recruitment of classic inflammatory cells, attention has focused on the role of unique cells that may be critical in the regulation of fibrosis (*see below*).

An example of the above-described process is usual interstitial pneumonia (UIP). UIP is found associated with end-stage asbestosis (e.g., persistent irritant), hypersensitivity pneumonia (e.g., recurrent exposure to antigen), collagen vascular disease, such as rheumatoid arthritis or scleroderma (e.g., recurrent exposure to self-antigen), or idiopathic (e.g., IPF) (4). In each of these circumstances, the stimulus is persistent or repetitive in nature, leading to chronic inflammation, epithelial and endothelial injury of the ACM, loss of the ACM BM, alveolar collapse and fusion, and fibroblast/myofibroblast activation and ECM deposition (4, 5, 7–11). This process results in organization with loss of alveolar structures, and, if anatomically related, there is then entire loss of the anatomic lobular structure (4, 5, 7–11). These events contribute to continued fibroblast/myofibroblast activation, complete loss of the ACM BM, inappropriate reepithelialization of type II pneumocytes resulting in “bronchiolization” without function, inappropriate reendothelialization with aberrant vascular remodeling, and progressive ECM deposition (4, 5, 7–11). The evolution of ECM deposition is associated with a hypocellular state and loss of cells that produce proteinases that could normally be involved in remodeling/reabsorbing the ECM (4, 5, 7–11). The process leads to the end-stage histopathology of UIP.

The BM of the ACM has somehow been underappreciated as the pivotal entity that appears to determine whether the integrity of the alveolar structures returns to normal or dictates the point of no return, with complete loss of the alveolar structures. The BM is made of at least four primary components consisting of type IV collagen, laminin, perlecan, and entactin (12, 13). These components form a complex integrated structure that functions as follows: to provide a scaffold for spatial regulation of cell adhesion, spreading, and motility; formation of a permeability barrier; transmission of extracellular signals to cells via specific receptors; and regulation of extracellular local concentrations of growth factors important to the survival of epithelial and endothelial cells (12, 13). The loss of this struc-

ture is dependent on the magnitude of persistent inflammation and exposure to specific proteinases related to significant loss of the epithelium and endothelium of the ACM (4, 7). Therefore, the integrity of the BM of the ACM will dictate whether normal reepithelialization and endothelialization will occur with preservation of the alveolar structures; or loss of BM integrity of the ACM will contribute to alveolar collapse and fusion of ACM contributing to loss of alveolar structures, including the entire anatomic lobule (1, 4, 7).

LOSS OF EPITHELIAL CELLS, ENDOTHELIAL CELLS, AND BM INTEGRITY IN UIP ASSOCIATED WITH IPF LEADS TO DESTROYED LUNG ARCHITECTURE AND PERPETUAL FIBROSIS

Although there has been a significant amount of literature to address the importance of the epithelium of the ACM in response to injury, the study by Ray and coworkers highlights the importance of protecting both the epithelium and the endothelium in response to injury (14). In this article, the authors generated transgenic mice conditionally expressing the keratinocyte growth factor (KGF) transgene in type II pneumocytes (14). When these mice were exposed to hyperoxia, the type II pneumocytes were markedly protected, as compared with those of mice without transgenic expression of KGF (14). However, despite the protection of the type II pneumocytes with transgenic expression of KGF, all of the mice exposed to hyperoxia died in a similar manner as mice without transgenic expression of KGF (14). The reason for the mortality among the transgenic KGF mice exposed to hyperoxia was related loss of the endothelium in the context of preserved type II pneumocytes (14). These findings support the notion that both cell types of the ACM (epithelium and endothelium) are critical to the integrity of the ACM and alveolar architecture.

Returning to the pathogenesis of UIP associated with IPF, ultrastructural analysis of lung tissue from IPF has demonstrated the following features: (1) type I pneumocyte and endothelial cell injury with destruction of the basement membrane (4, 5, 7–11); (2) intraalveolar exudative organization with fibrosis due to fibroblast/myofibroblast migration through defects in the alveolar wall; and (3) obliteration and fibrosis of the alveoli with fusion of adjacent alveolar BM residual structures, which leads to development of fibroblastic foci with exudative organization composed of parallel arranged fibroblasts/myofibroblasts enmeshed in ECM (4, 5, 7–11). The ultrastructural studies highlight the loss of alveolar structures; however, these studies do not fully provide insight into the anatomic nature of the fibroblastic foci. Cool and colleagues have published the three-dimensional reconstruction of UIP from a patient with IPF (15). This remarkable study demonstrates the following features of UIP in IPF: thickened visceral pleura, extensive vascular remodeling, and fibroblastic foci that appear to be interconnected by fascial planes of connective tissue (15). Moreover, the nature of these interconnected fibroblastic foci highlights that these structures are not anatomically separate entities (15). This allows one to speculate that fibroblastic foci may represent the organizing fibrosis and loss of alveolar architecture of an entire anatomic respiratory lobule of the lung. The anatomic respiratory lobule structure represents the respiratory unit of the lung, consisting of a terminal bronchiole, respiratory bronchioles, alveolar ducts, alveoli with associated pulmonary arteries, alveolar capillaries, and pulmonary venule supported by ACM BM and interstitial ECM (16). Physiologically, these lobular structures are interconnected as demonstrated by the ability of the lung tissue to demonstrate collateral ventilation. Therefore, the respiratory lobule may represent the anatomic target of persistent or recurrent

injury that leads to loss of alveolar structure integrity with organizing fibrosis that ultimately destroys the integrity of the entire respiratory lobule, which leads to the formation of the fibroblastic foci of UIP. In other words, the above-described findings support the notion that UIP is a process that results in targeted loss of respiratory lobules, leading to the formation of fibroblastic foci.

IS TGF- β NECESSARY AND SUFFICIENT TO CONTRIBUTE TO PERPETUAL FIBROSIS AND LOSS OF TISSUE ARCHITECTURE?

TGF- β is a pleiotropic cytokine that is the most potent and efficacious cytokine in promoting fibrosis. TGF- β signals through initially binding to TGF- β type II receptor (T β RII), which in turn recruits TGF- β type I receptor (T β RI) (17). In this process, the intrinsic kinase activity of T β RII activates T β RI (17). T β RI cytoplasmic kinase domain activates receptor-associated Smads, Smad2/3, which in turn form heterodimeric complexes with Smad4 (17). The Smad2/3/4 complex is transported to the nucleus and combines with transcriptional complexes of CREB-binding protein (CBP) and p300, which are essential coactivators for Smad-mediated TGF- β -specific gene expression, such as plasminogen activator inhibitor-1, collagen genes, connective tissue growth factor (CTGF), and platelet-derived growth factor B-chain, to name a few (17). The coactivators, CBP and p300, have been determined to be targets of IFN- γ , tumor necrosis factor- α (TNF- α), and prostaglandin E₂ (PGE₂) mediated inhibition of Smad signaling (17). In addition, IFN- γ has been shown to inhibit Smad signaling via induction of the inhibitory Smad, Smad7 (17).

To determine whether TGF- β itself could promote fibrosis in the lung, Sime and associates delivered adenoviral vectors containing either latent TGF- β or the active TGF- β transgenes to the lungs of rats (18). They demonstrated on Day 14 a presence of marked fibrosis in the rats that had received the active TGF- β transgene, as compared with the latent TGF- β transgene (18). Although active TGF- β was found to be important in promoting fibrosis, they also found that inflammation was present, and peaked on Day 7 before maximal fibrosis (18). In another study, Lee and colleagues generated conditional transgenic mice that expressed active TGF- β on the CC10 promoter in airway cells (19). When these mice were induced to express TGF- β , fibrosis was found in a peribronchial distribution within 10 days that extended into the adjacent lung parenchyma (19). However, if they performed the same experiment for 10 days and then turned off the TGF- β transgene, the fibrosis was completely reabsorbed with return of normal peribronchial and adjacent lung parenchymal architecture within 30 days (19). These findings demonstrated that TGF- β was necessary to induce fibrosis, but not entirely sufficient to promote persistent fibrosis. In a third study, Kolb and colleagues demonstrated that adenoviral delivery of human IL-1 β resulted in marked inflammation and destruction of the lung architecture followed by persistent fibrosis that appeared to be mediated by sustained expression of TGF- β (20). Taken together, what contributes to the persistence of fibrosis if TGF- β is necessary but not entirely sufficient to perpetuate fibrosis? The answer is that sustained or recurrent injury to the ACM BM and loss of its integrity lead to inability to effectively reepithelialize or reendothelialize the BM, leading to loss of parenchymal architecture. This scenario would be in the context of recurrent injury/antigen/irritant, which is critical for the propagation of fibrosis. For example, the model by Kolb and colleagues (20) can essentially be recapitulated using a model of acute lung allograft rejection (i.e., the concept of persistent “antigen” = alloantigen) that is not modified by treatment intervention. If a Brown Norway rat left lung donor is trans-

planted into a Lewis rat recipient (21), the donor lung will demonstrate loss of alveolar epithelium and endothelium with destroyed ACM BM and alveolar collapse, which leads to lung architectural destruction and perpetual fibrosis similar to the rats subjected to adenoviral delivery of human IL-1 β (20). This supports the notion that under conditions of persistent stimulus/irritant/antigen this environment results in exuberant inflammation with destruction of ACM BM, leading to destroyed lung architecture: the point of no return. This ultimately results in the default response, fibrosis, as the lung otherwise cannot regenerate alveolar structures.

IS UIP ASSOCIATED WITH IPF A CHRONIC FIBROPROLIFERATIVE PROCESS RELATED TO A PERSISTENT ANTIGEN(S) THAT PROMOTES CHRONIC INFLAMMATION, DESTRUCTION OF LUNG ARCHITECTURE, AND PERSISTENCE OF FIBROSIS?

UIP is a histopathological entity that has been associated not only with IPF, but with a number of known causes as follows: asbestosis (e.g., persistent irritant); hypersensitivity pneumonia (e.g., persistent or recurrent antigen); and collagen vascular disorders, such as rheumatoid arthritis and scleroderma (e.g., persistent self-antigens) (1, 4, 7). Chronic inflammation and tissue destruction are pathological hallmark features that are associated with the pathogenesis of the above-cited diseases. Does this suggest that UIP associated with IPF is somehow unique, as it has been suggested that UIP of IPF can occur in the absence of preceding inflammation; or are we evaluating the process with a snapshot pathological view of the entire pathogenesis/natural history? Zuo and associates used gene microarrays to analyze eight lung specimens from patients with pulmonary fibrosis (six IPF and two autoimmune related). As anticipated, they found genes that were increased in fibrotic lungs related to smooth muscle markers, proteins involved in ECM formation, degradation, and signaling (22). However, these investigators also found genes normally associated with chronic inflammation and immune responses, such as cytokines, chemokines, antioxidants, complement, amyloid, and immunoglobulins (22). These latter findings of gene expression are reflective of chronic inflammation/immune responsiveness and suggested that there may be evidence of a chronic inflammation/immune response in the lungs of patients with IPF. Supporting this notion is a publication showing significant tertiary lymphoid tissue (i.e., lymphoid neogenesis) in the lungs of patients with IPF (23). In this study, a positive correlation was found for the presence of mature dendritic cells (DCs) and the magnitude of activated lymphocyte infiltration (23). These findings suggested that newly recruited activated DCs and antigen-experienced, preactivated, nonproliferating lymphocytes drive lymphoid neogenesis; and factors present within the lymphoid aggregates appear to be essential to induce DC maturation (23). The accumulation of reactivation of memory T cells in IPF by locally maturing DCs likely plays a central role in sustaining chronic inflammation that could be resistant to antiinflammatory agents (23).

Further support of the concept that a "persistent or recurrent antigen" exists in the lungs of patients with IPF comes from two imaging studies (i.e., high-resolution computed tomography [HRCT]) that demonstrate a direct correlation of the presence of mediastinal lymphadenopathy in patients with IPF (24, 25). Both imaging studies independently demonstrated the baseline prevalence of mediastinal lymphadenopathy of approximately 52.5% in patients with UIP/IPF (24, 25). In the study by Attili and colleagues (24), the follow-up HRCT prevalence of medi-

astinal lymphadenopathy at 1 ± 0.7 years was 57.5% in these patients. Moreover, the mean HRCT ground glass and fibrosis scores were higher in patients with IPF when there were enlarged lymph nodes present; and there was a trend toward increased lymph node enlargement over time that was associated with progression of underlying fibrosis (24). Taken together, the above-noted studies at the molecular, cellular, and imaging levels support the concept of the potential of a persistent or recurrent antigen in the lungs of patients with IPF that correlates with the progression of fibrosis.

M1 AND M2 MACROPHAGES AND FIBROCYTES ARE UNIQUE CELLS THAT ARE CRITICAL CELLULAR PLAYERS IN THE REGULATION OF FIBROSIS

Mantovani and associates were the first to propose a nomenclature system for polarized macrophages that are often found in the context of tumors (26, 27). Although classically activated, M1 macrophages would be expected to have antitumor effects, most of the tumor-associated macrophages appeared to be alternatively activated, and were referred to as M2 macrophages; they appeared to function in promoting tumor growth and metastases (26–28). The M1 macrophage phenotype appears to result from exposure to helper T-cell type 1 or type I cytokines (e.g., IFN- γ), fungal cell wall components, degraded matrix (e.g., hyaluronic acid), LPS, and TNF- α (26–29). M1 macrophages produce large amounts of TNF- α , IL-12, and NO (26–29). Regarding the latter molecule, M1 macrophages express inducible nitric oxide synthase (iNOS), which oxidizes the substrate L-arginine to form NO and L-citrulline (26–34). The large amounts of NO formed are involved in intracellular pathogen killing (26–33). M1 macrophages express low levels of the hemoglobin scavenger receptor CD163 (26–33). M1 macrophages are the prototypical macrophages characterized by features such as phagocytosis, antigen processing and presentation, and T cell activation (26–34). M1 macrophages play an important role in matrix degradation by direct and indirect production of matrix metalloproteinases (MMPs) (MMP-9, MMP-2, MMP-12, and MMP-7) (26–33). M1 macrophages induce myofibroblasts to express MMP-13 and MMP-3 (26–33). The production of MMPs is important for remodeling ECM and is associated with the resolution of fibrosis in many model systems.

In contrast, M2 macrophages are more supportive of a fibroproliferative microenvironment (26–33). M2 macrophages secrete the regulatory cytokines platelet-derived growth factor, IL-10, and TGF- β , as well as the soluble IL-1 receptor antagonist, and express the type II IL-1 decoy receptor on their surface (26–33). These two latter molecules attenuate the role of IL-1 in the local microenvironment. In contrast to the expression of MMPs by M1 macrophages, M2 macrophages markedly express antiinflammatory cytokine and tissue inhibitors of metalloproteinase that would impair remodeling of deposited ECM (26–33). In fact, M2 macrophages induce complex ECM deposition when cocultured with myofibroblasts (26–33). M2 macrophages are activated by helper T-cell type 2 cytokines, including IL-4, IL-13, IL-10, and TGF- β , as well as apoptotic cells and corticosteroids (26–33). This latter effect would suggest that therapy with corticosteroids would activate M2 macrophages, which could contribute to reduced efficacy of corticosteroids in the treatment of pulmonary fibrosis. M2 macrophages express the mannose receptor, type I scavenger receptor, and CD163 (26–33). M2 macrophages efficiently inhibit the M1-driven inflammatory process, due, in part, to expression of high levels of arginase-1, which competes with iNOS for L-arginine (26–33). Arginase-1 metabolism of L-arginine can lead to the

formation of L-proline, which in turn can be used by myofibroblasts to produce collagen (26–33). Therefore, the temporal recruitment, spatial presence, and the balance of the M1 vs. M2 macrophages in the local microenvironment of severe lung tissue injury in the context of loss of the ACM BM and perpetual fibrosis in the presence or absence of a persistent stimuli/irritant/antigen may dictate the magnitude of myofibroblast activation and deposition of ECM.

Regarding the importance of the fibroblast and myofibroblast in generating ECM deposition in the lung, a question arises concerning the origin of these cells. There is one classical and two contemporary theories for the origin of fibroblasts/myofibroblasts (5, 35). The classical concept is that tissue injury induces activation of a resident fibroblast to proliferate and express constituents of the ECM. One contemporary theory is that tissue injury with the presence of TGF- β induces epithelial cells to transition to a mesenchymal phenotype, the fibroblast/myofibroblast that subsequently contributes to fibroproliferation (5, 35). Another contemporary theory is that circulating fibrocytes, derived from the bone marrow, are mesenchymal progenitor cells that home to and extravasate into sites of tissue injury, differentiate into fibroblasts/myofibroblasts, and contribute to the generation of ECM during fibroproliferation (5, 35). One study has identified a circulating pool of cells positive for CD45, collagen I, and CXCR4 (CD45⁺Col I⁺CXCR4⁺ cells), termed fibrocytes, that traffic to the lung in response to injury and contribute to fibrosis (36). In this study, they showed that a population of circulating human CD45⁺Col I⁺CXCR4⁺ fibrocytes migrated in response to the CXC chemokine ligand CXCL12, and trafficked to the lungs of SCID mice exposed to bleomycin-induced pulmonary fibrosis (36). They then demonstrated that murine CD45⁺Col I⁺CXCR4⁺ fibrocytes also trafficked to the lungs in response to a bleomycin challenge in immunocompetent mice, with maximal recruitment of CD45⁺Col I⁺CXCR4⁺ fibrocytes in the lungs that directly correlated with increased collagen deposition (36). Treatment of bleomycin-exposed animals with specific neutralizing anti-CXCL12 antibodies inhibited recruitment of circulating CD45⁺Col I⁺CXCR4⁺ fibrocytes into the lungs and attenuated lung fibrosis without perturbing the recruitment of subpopulations of conventional leukocytes (36). To determine whether fibrocytes were significantly present in patients with pulmonary fibrosis, Mehrad and associates assessed the presence of fibrocytes in patients with fibrotic ILD (37). They found enhanced expression of CXCL12 in both the lungs and plasma of patients with pulmonary fibrosis (37). CXCL12 levels were associated with an order of magnitude higher number of circulating fibrocytes in the peripheral blood of these patients (37). Although most of the circulating fibrocytes in patients with ILD were negative for the myofibroblast marker α -smooth muscle actin (α -SMA), there was a trend toward increased numbers of α -SMA⁺ fibrocytes in patients with pulmonary fibrosis (37). Other studies using chimeric bone marrow transplantation and induction of pulmonary fibrosis have shown that bone marrow-derived cells that may be similar to fibrocytes are found in areas of fibrosis (38, 39). However, these studies did not demonstrate whether these bone marrow-derived cells contributed to the pathogenesis of pulmonary fibrosis. Taken together, these data suggest that fibrocytes are involved in the pathogenesis of human pulmonary fibrosis.

Because fibrocytes are a distinct population of fibroblast-like progenitor cells in the peripheral blood that have been shown to possess plasticity to differentiate along mesenchymal lineages (e.g., commitment to become myofibroblasts and adipocytes), Hong and colleagues were able to demonstrate the signaling pathways that are necessary to drive fibrocytes to myofibroblast or adipocyte differentiation (40). Myofibroblast differentiation

was mediated in the presence of TGF- β activation through activating Smad2/3 and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) mitogen-activated protein kinase (MAPK) pathways, which in turn induced α -SMA expression (40). They determined that SAPK/JNK signaling acts in a positive feedback loop to modulate Smad2/3 nuclear availability and Smad2/3-dependent transcription (40). Conversely, fibrocyte-to-adipocyte differentiation was found to be driven by the peroxisome proliferator-activated receptor (PPAR)- γ agonist, troglitazone, which was associated with cytoplasmic lipid accumulation and induction of the adipocyte gene, aP2 (40). In contrast, treatment with troglitazone disrupted TGF- β -activated SAPK/JNK signaling, leading to decreased Smad2/3 *trans*-activation activity and α -SMA expression (40). Interestingly, TGF- β was demonstrated to have reciprocal inhibition on fibrocyte differentiation to adipocytes (40). By activating SAPK/JNK signaling, which is normally suppressed during adipogenesis, PPAR- γ -dependent *trans*-activation activity and induction of aP2 expression was disrupted (40). Taken together, within the context of local microenvironmental niche, the delicate balance of PPAR- γ and TGF- β activation drives the selection of an adipocyte or myofibroblast differentiation pathway through SAPK/JNK signaling. These findings have important implications for therapeutic intervention and modulation of these cells from differentiating into myofibroblast-like cells.

In conclusion, loss of BM integrity is critical in determining the point of no return, which reflects the inability to reestablish normal lung architecture with promotion of fibrosis. Loss of epithelial cell, endothelial cells, and BM integrity in UIP associated with IPF leads to destroyed lung architecture and perpetual fibrosis. In fact, the anatomic target that leads to the hallmark features of UIP may be the anatomic respiratory lobule. TGF- β is necessary, but not entirely sufficient, to promote permanent fibrosis. Persistent injury/antigen/irritant is critical for the propagation of fibrosis in the context of the loss of BM integrity and the inability to reepithelialize and reendothelialize this membrane. IPF is an example of a process related to the persistence of an “antigen(s),” chronic inflammation, and fibrosis. Unique cells, such as M1 and M2 macrophages, and fibrocytes, are critical cellular players in the regulation of fibrosis, and their biology has relevance for future consideration of therapeutic targets to attenuate the progression of pulmonary fibrosis.

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