

# Role of Epithelial Cells in Idiopathic Pulmonary Fibrosis From Innocent Targets to Serial Killers

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**Idiopathic pulmonary fibrosis (IPF), a progressive and relentless lung scarring of unknown etiology, has been recognized as the most lethal interstitial lung disease. Despite the growing interest in IPF, the precise molecular mechanisms underlying the development of fibrosis and leading to the irreversible destruction of the lung are still unknown. Recently, it has been proposed that IPF, instead of being a chronic inflammatory disorder, results from multiple cycles of epithelial cell injury and activation. In turn, active alveolar epithelial cells provoke the migration, proliferation, and activation of mesenchymal cells with the formation of fibroblastic/myofibroblastic foci and the exaggerated accumulation of extracellular matrix, mirroring abnormal wound repair. In this article, some characteristics of the alveolar epithelium are briefly outlined, and the fibrogenic mechanisms specifically operated by active abnormal epithelial cells are examined.**

**Keywords:** epithelial cells; idiopathic pulmonary fibrosis; lung fibrosis pathogenesis

Idiopathic pulmonary fibrosis (IPF), with its histopathologic signature of usual interstitial pneumonia (UIP), is a progressive and devastating lung disorder of unknown etiology. Although the natural history and the pathogenic mechanisms remain unknown, the long-prevailing hypothesis sustains the idea that chronic inflammation plays an essential role. According to this hypothesis, the alveolar epithelial alterations—that is, loss of type 1 pneumocytes and proliferation of type 2 pneumocytes characteristic of IPF—are caused by the unresolved inflammatory process, and that alveolar epithelial cells (AECs) are victims of the surrounding injuring/explosive/inflammatory microenvironment.

More recently, however, it has been proposed that IPF probably results from multiple cycles of epithelial cell injury and activation that provoke the migration, proliferation, and activation of mesenchymal cells with the formation of active fibroblastic/myofibroblastic foci, leading to the exaggerated accumulation of extracellular matrix (ECM) and mirroring abnormal wound repair (1).

In this review, we describe AEC development, physiology, and response to injury as well as current data supporting the role of AECs in the pathogenesis of IPF.

## LUNG MORPHOGENESIS AND THE ADULT ALVEOLAR EPITHELIUM

Wound healing involves a coordinated series of tissue movements that bears a striking resemblance to distinct stages in lung embryonic morphogenesis (2). Development of human lung

morphogenesis is initiated around the fifth week of gestation and passes through four distinct structural stages, each of them with a characteristic developmental feature: the pseudoglandular stage (development of bronchial tree); the canalicular stage (development of acini and vascularization); the saccular stage consisting of further differentiation of the acini into saccules, increase in saccules, and vascularization, as well as differentiation of the epithelial cells into type 1 and 2 pneumocytes; and the alveolar stage (increase in number of alveoli and extensive increase in the surface area) (3).

In humans alveoli develop from about 28 wk of gestation. Consequent alveolar multiplication and alveolarization of the lung result in formation of roughly 300 million alveoli by age 2–4 yr. The gas-exchange surfaces develop as thin linings of epithelial–endothelial cells. These two cell types share the basement membrane and together form approximately 70 m<sup>2</sup> of gas-exchange surface.

In adults, type 1 and type 2 alveolar cells constitute the epithelial component of the alveoli. Type 1 cells, which cover more than 90% of the alveolar surface area of the peripheral lung, are highly attenuated cells that interface with pulmonary capillaries, providing an intact surface of minimal thickness readily permeable to gases (Figure 1). The diameter of these cells can reach 100 μm. Type 2 cells, morphologically appearing as large rounded cells, are found in the corners of alveoli and are in close proximity to mesenchymal cells lying beneath them (Figure 2). They are multifunctional cells that synthesize and secrete pulmonary surfactant, serve as progenitor cells for type 1 alveolar cells, directionally transport sodium from apical to basolateral cell surfaces to minimize alveolar fluid, and participate in the effector phase of the immune response by producing molecules involved in innate host defense (4). Importantly, in normal human lungs there are numerous contacts between AECs and lung fibroblasts. These contacts occur at holes in the epithelial basal lamina that seem to be highly organized to maintain communication (5).

## WHAT STEM OR PROGENITOR CELLS ARE RESPONSIBLE FOR ALVEOLAR REPAIR?

Essential to our understanding of the mechanisms involved in the repair of the lung parenchyma is the identification and characterization of the cells that are potentially capable of repopulating the injured lung. However, the turnover of the alveolar epithelium under physiologic conditions and the mechanisms of epithelial regeneration under pathologic situations are poorly understood. It is generally accepted that during homeostasis and in response to lung damage, type 2 pneumocytes serve as progenitor cells. Type 1 AECs are highly vulnerable to injury, whereas the type 2 AECs are more resistant and can therefore function as progenitor cells for regeneration of the alveolar epithelium after injury. These cells proliferate and migrate to recover the denuded basement membrane by forming a layer of cuboidal epithelial cells. Then, they should differentiate to reestablish both type 1 and type 2 pneumocytes into a functional alveolar epithelium.

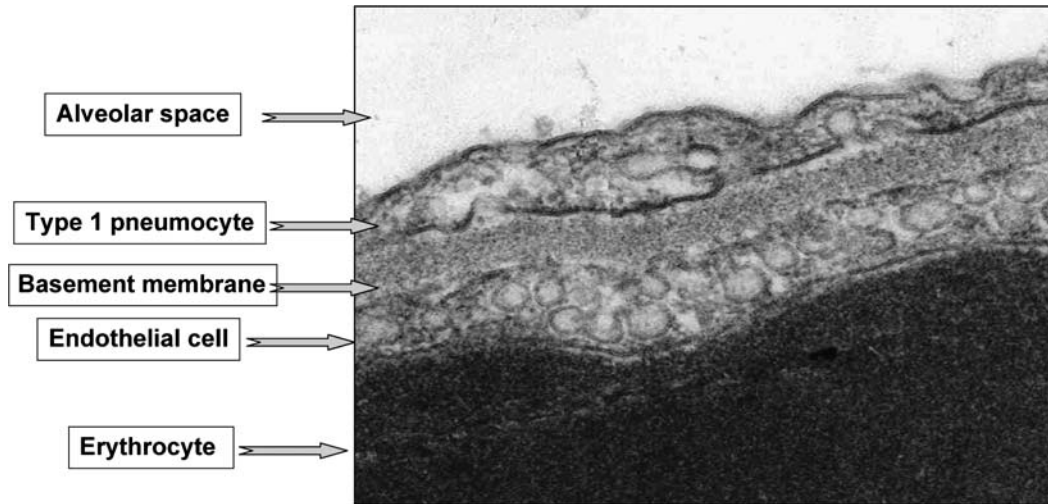
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**Figure 1.** Transmission electron micrograph of an alveolar–capillary unit illustrating the thin lining of the gas-exchange surface. Courtesy of Dr. Teresa Fortoul.

Reddy and colleagues suggested that type 2 AECs isolated from injured lungs could be segregated into at least two subpopulations, based on their E-cadherin expression levels (6). Importantly, E-cadherin–negative AECs are damage resistant, proliferative, and exhibit high levels of telomerase activity, suggesting that they might represent a transiently amplifying progenitor subpopulation of type 2 AECs responsible for repopulation and repair of damaged alveolar epithelium (6).

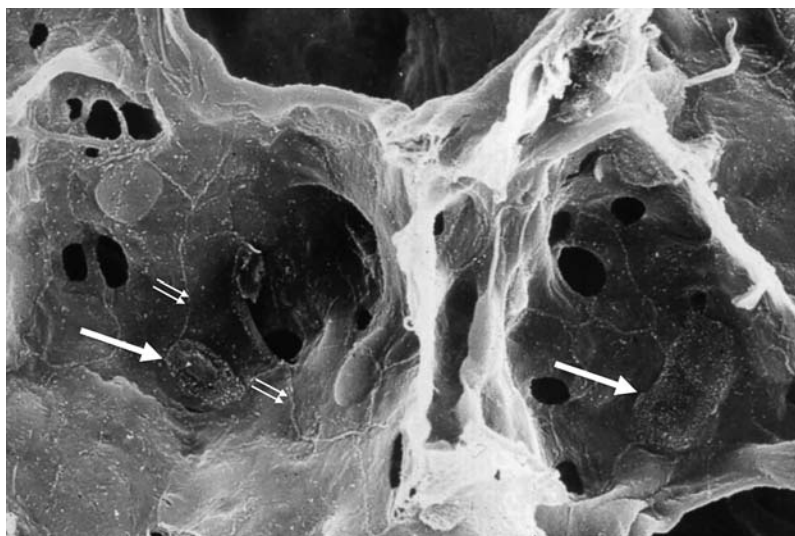
More recently, studies in experimental models and humans after lung transplant suggested that circulating bone marrow–derived stem cells may also participate in alveolar epithelial repopulation. Engraftment by circulating pluripotent cells seems to occur frequently in the lung parenchyma after injury (7, 8), and studies in human recipients of sex-mismatched bone marrow grafts have revealed numerous donor-derived epithelial and endothelial cells in the lungs (9, 10).

However, two almost simultaneous reports in experimental models suggest that the paradigm of epithelial tissue reconstitution by circulating cells derived from bone marrow may not be necessarily true. Using a lineage-specific reporter system based on transgenic mice that express green fluorescent protein (GFP)

only in lung epithelial cells (surfactant protein C [SP-C]–GFP), Kotton and colleagues demonstrated that there was no detectable reconstitution of lung AECs by unfractionated bone marrow cells or purified hematopoietic stem cells (11). This observation included transplant recipients subjected to bleomycin-induced lung injury. Likewise, Chang and colleagues found that all putative marrow-derived pneumocytes resulted from the overlapping fluorescent signals of endogenous pro-SP-C<sup>+</sup> type 2 cells and donor-derived GFP<sup>+</sup> cells, underscoring the technical difficulties associated with evaluating engraftment in lung, and arguing against a contributory role for marrow cells in populating the alveolar epithelium (12).

Although these studies question the contribution of circulating bone marrow–derived progenitor cells to epithelial repopulation, evidence for the existence of side population (SiPo) cells, a rare subset of cells that are highly enriched for stem cell activity, has been found in the lung. SiPo cells are believed to be derived from the bone marrow and can be differentiated from committed-tissue stem cells (13).

Summer and colleagues provided further support that adult lung SiPo cells arise from the bone marrow and that both



**Figure 2.** Scanning electron microscopic image of alveoli. Thin arrows indicate intercellular junctions. Large arrows show type 2 alveolar cells. Courtesy of Dr. Alvaro Osornio.

CD45-positive and CD45-negative cell types can be derived from a uniform purified population of hematopoietic stem cells (14). Interestingly, a high number of CD45<sup>+</sup> lung SiPo cells expressed markers of mesodermal or endodermal derivatives ( $\alpha$ -smooth muscle actin [ $\alpha$ -SMA] and cytokeratin), while CD45<sup>-</sup> lung SiPo cells were predominantly cytokeratin negative but  $\alpha$ -SMA positive. Local environmental signals may induce SiPo subtypes into phenotypically distinct cell populations (14). The anatomic niche of lung SiPo cells as well as their putative contribution to alveolar epithelium repopulation *in vivo* is largely unknown.

### INTRAEPIHELIAL $\gamma\delta$ -T CELLS AND ALVEOLAR EPITHELIAL REPAIR

$\gamma\delta$ -T cells constitute a separate lineage of T lymphocytes that differ from conventional  $\alpha\beta$ -T cells with regard to T-cell receptor repertoire and tissue localization. Conventional T cells recognize processed peptides in the context of the major histocompatibility complex (MHC) class I or class II molecules. Instead, most  $\gamma\delta$ -T cells recognize different ligands, and usually in an MHC-nonrestricted fashion.

In epithelial barriers,  $\gamma\delta$ -T cells reside in intimate contact with neighboring epithelial cells and appear to play a distinctive role in maintaining epithelial homeostasis as well as in response to tissue injury. However, much of the information is derived from the skin and the intestine (15). Thus, for example, in mice models of wound healing the absence of intraepithelial  $\gamma\delta$ -T cells causes wounds to heal slower than in normal mice (16). Skin  $\gamma\delta$ -T cells are required for effective keratinocyte proliferation and wound reepithelialization through the production of keratinocyte growth factors-1 and -2 (16).

In the lung,  $\gamma\delta$ -T cells contribute to host defense in a murine model of *Nocardia* lung infection and are needed to prevent dissemination of *Klebsiella* (17). However, most resident pulmonary  $\gamma\delta$ -T cells seem to be situated in nonalveolar locations, and their role in alveolar repair is largely unknown (18). Interestingly, we have found a significant decrease of  $\gamma\delta$ -T cells in the bronchoalveolar lavage of patients with IPF (19) (Figure 3).

### EPITHELIAL-DRIVEN FIBROSIS

An increase in the number of epithelial cells and a remarkable disturbance in the integrity of the alveolar epithelium with presence of several altered phenotypes is a striking feature of IPF lungs (Figures 4 and 5). Gene-expression profiling confirmed an increase of several cytokeratins in IPF lungs (20). Numerous hyperplastic and hypertrophic type 2 pneumocytes, with abundant cytoplasm, large hyperchromatic nuclei, and prominent nucleoli cuboidalize the epithelium mainly in the fibrotic thickened alveolar septa. Also, reactive large and elongated epithelial cells (fibroblast-like?) and flattened and attenuated epithelial

cells, usually overlying the fibroblastic foci, are observed. Some of these cells may represent cells undergoing epithelial-mesenchymal transition, a process recently described in IPF (21). Bronchiolar-type epithelium and hyperplastic epithelial foci with squamous metaplasia lining the enlarged airspaces of honeycomb lesions are also found. In this context, alveolar reepithelialization appears severely disturbed in IPF.

By contrast, reepithelialization of some putatively reversible interstitial lung disorders seems to be extensive and ordered. For example, in cryptogenic organizing pneumonia, regenerating type 2 pneumocytes are layered in an orderly fashion and often adopt a flat shape reminiscent of type 1 pneumocytes (22). It is important to emphasize that cryptogenic organizing pneumonia is characterized by the prominent formation of fibromyxoid lesions, fibroblasts/myofibroblasts embedded in a loose ECM, which, in sharp contrast with fibroblastic foci from IPF, are often reversible.

The reasons and significance of the aberrant reactions of the AECs in IPF are unknown, but some of them may result from the initial insult whereas others may be the consequence of accelerated epithelial cell proliferation/migration, indicating regenerative epithelia after injury. The high rate of proliferating cell nuclear antigen observed in the hyperplastic epithelium of the honeycomb lesions indicates that accelerated cell proliferation occurs in these lesions (23). Also, some AECs may be reprogrammed to an earlier embryonic stage.

### THE INITIAL INSULT: MECHANISMS OF EPITHELIAL DAMAGE/ACTIVATION

The events that provoke the repeated microscopic injuries of AECs, resulting in dysregulated repair, are largely unknown. Taking into account the presumed long preclinical phase of the disease, it is possible that no single mechanism initiates the abnormal response in the lung; rather, a combination of different types of injuries may act on a genetically susceptible individual to trigger the disease.

#### The Viral Connection

Viral replication may be one of the sources of repetitive injury to the alveolar epithelium. However, evidence has been somewhat elusive. Both the protein and the DNA of the Epstein-Barr virus (EBV) have been identified in lung tissue of patients with IPF, with the infection localized to AECs (24). Moreover, in a mouse model resistant to bleomycin, the murine  $\gamma$ -herpesvirus 68, an infecting agent that behaves similarly in mice to EBV in humans, promoted the development of pulmonary fibrosis (25). In a very provocative report, Tang and coworkers tested the presence of eight herpesviruses in lung specimens from 33 patients with IPF (8 with familial IPF and 25 with sporadic IPF) and 25 patients with other diseases as control subjects (26). They found that one

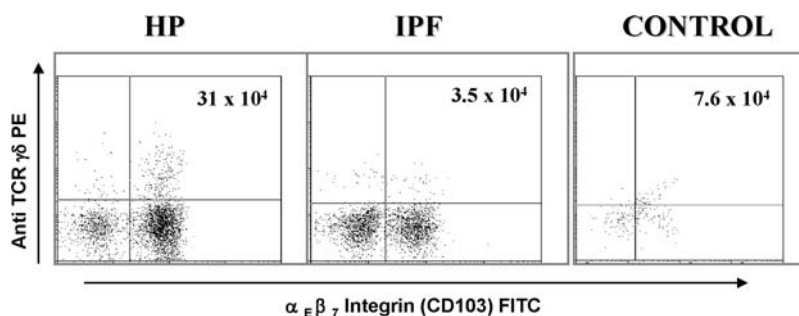
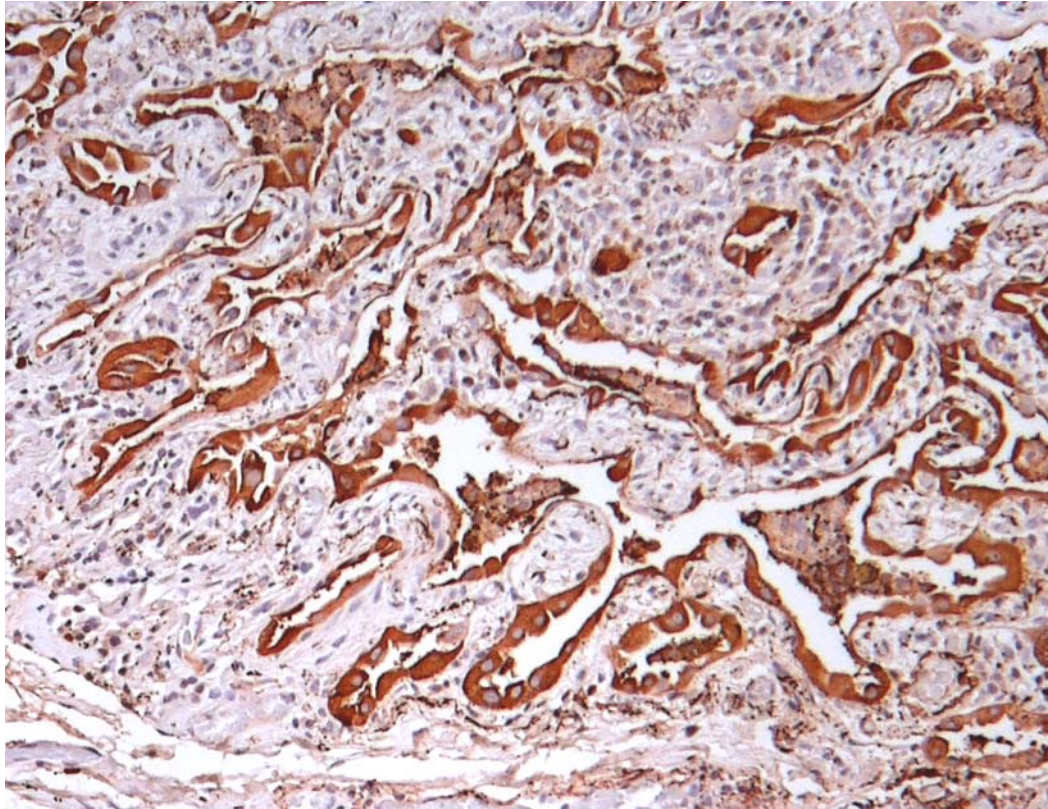


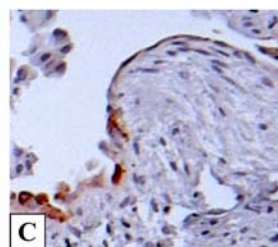
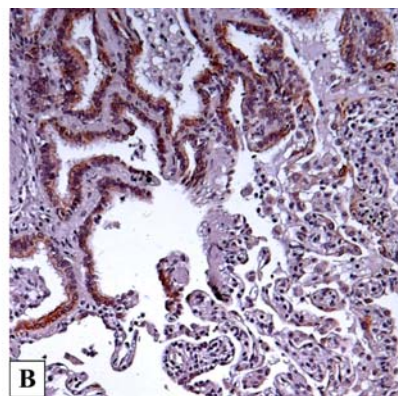
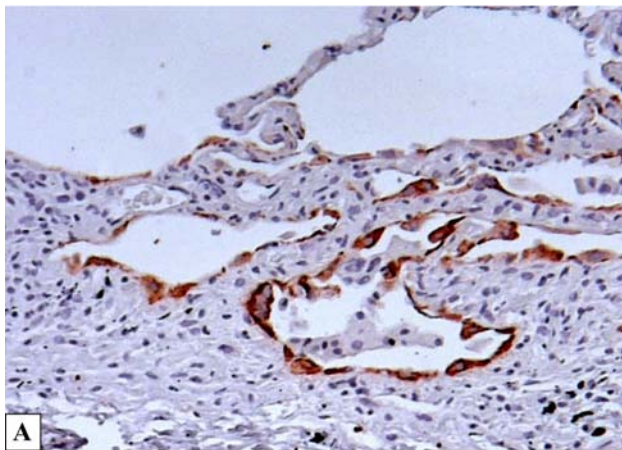
Figure 3. Intraepithelial  $\gamma\delta$  T lymphocytes are decreased in patients with idiopathic pulmonary fibrosis (IPF) compared with normal individuals and patients with hypersensitivity pneumonitis (HP). FITC = fluorescein isothiocyanate; PE = phycoerythrin; TCR = T cell receptor.



**Figure 4.** Photomicrograph of an IPF lung stained with surfactant protein A illustrating the chaotic reepithelialization (mostly by hyperplastic type 2 pneumocytes) occurring in this entity.

or more of four herpesviruses were detected in almost all IPF lungs compared with one-third of the control lungs. The positive viruses include cytomegalovirus, EBV, and human herpesvirus (HHV)-7 and HHV-8. Furthermore, two or more herpesviruses were detected in more than half of the patients with IPF. Immunohistochemistry for EBV or HHV-8 antigen showed viral antigen primarily in epithelial cells. However, it is important to

consider that most patients with IPF were under immunosuppressive therapy prior to biopsy, thus making the results somewhat difficult to interpret. Kelly and colleagues found evidence of EBV in IPF lung tissues regardless of previous use of immunosuppressive drugs (27). Recently, Zamo and colleagues failed to find similar results using a combination of techniques for detecting EBV and HHV-8 virus in IPF lungs, suggesting that viral



**Figure 5.** Photomicrographs of IPF lungs showing different epithelial phenotypes. (A) Some reactive large and elongated epithelial cells. (B) Bronchiolization in advanced honeycomb lesions. (C) Flattened and attenuated epithelial cells, overlying a fibroblastic focus.

involvement may not be universal (28). Therefore, the etiologic significance of the viral infection in IPF (if any) remains to be determined.

### **Gastroesophageal Reflux**

Some studies examining the role of gastroesophageal reflux (GER) in individuals with IPF have found a high prevalence of GER compared with normal individuals and patients with other interstitial lung diseases of known cause (29). In a Veterans Administration case-control study, it was found that GER-associated erosive esophagitis was linked with a number of respiratory diseases, including pulmonary fibrosis (30). These studies suggest that acid-aspiration-induced epithelial injury may contribute to the development of IPF. However, abnormal esophageal acid exposure by GER is frequent in the normal population, or in people with other pathologic problems, and its putative role in IPF (if any) still needs to be systematically studied.

### **Tobacco Smoke and Other Exposures**

Case-control studies have suggested that a history of smoking is associated with the development of IPF (31). It also seems that cigarette smoke (which can reach and affect the bronchiolar and alveolar epithelium) can interact with other environmental factors to further modify the development of disease. For example, concomitant exposure to cigarette smoke and livestock significantly increases the risk for IPF (32) as does occupational exposure to metal (33). However, with the exception of cigarette smoking, such exposures seem to explain a minority of cases of IPF.

### **Programmed Epithelial Cell Death**

Epithelial cell apoptosis is a common event and probably an essential feature of IPF. Several mechanisms appear to be implicated in this process including up-regulation of the Fas-signaling pathway in AECs and the release of angiotensin peptides that induce epithelial apoptosis by fibroblasts/myofibroblasts (34). Oxidant stress may also contribute to epithelial cell damage and death. H<sub>2</sub>O<sub>2</sub> secreted from myofibroblasts functions as a diffusible death signal for lung epithelial cells, and oxidative modifications of DNA caused by reactive oxygen species are detectable in the nuclei of AECs in IPF (35).

The main question here is whether these mechanisms of damage are part of the evolution of IPF (a secondary process) or are an early and triggering event. In this context, AEC death has been noticed in otherwise normal areas of the lung parenchyma, suggesting that it may be an initial event in the pathogenesis of IPF (36). However, the precise cellular signals that culminate in the initial AEC dysfunction and death remain completely unknown.

### **Is There an Intrinsic Abnormality in AECs of Patients with IPF?**

Some familial cases of pulmonary fibrosis have been linked to genetic mutations in SP-C. Missense or short-deletion mutations result in the production of abundant misfolded protein, which, by accumulation or complex formation, may cause type 2 epithelial cell injury. In other cases, expression of pro-SP-C and active SP-C has been undetectable, suggesting that abnormalities in SP-C associated with familial pulmonary fibrosis can result from accumulation of an abnormal pro-SP-C as well as from its absence (37). However, these abnormalities have been observed in different types of familial idiopathic interstitial pneumonias and seem to be rare in sporadic IPF (38). Nevertheless, it is tempting to speculate that some subtle genetic abnormalities of the AECs, perhaps made more evident by aging, make them

susceptible to injury and also provoke an uncontrolled state of activation.

### **A SPONTANEOUS MODEL OF IPF-LIKE DISORDER IS ASSOCIATED WITH TYPE 2 PNEUMOCYTE ABNORMALITIES**

Unfortunately, there is not an appropriate experimental model to examine the cellular/molecular mechanisms of IPF and the putative role of epithelial cells. Animal models typically provoked by bleomycin, and less frequently by radiation, silica, or by other means, have been useful for dissecting some of the pathogenic mechanisms of the inflammatory-driven pathway to fibrosis but any of them reproduce the human IPF.

Recently, Williams and colleagues described a spontaneous chronic progressive respiratory disease in domestic cats that shares critical features with human IPF. Interestingly, light microscopic and ultrastructural characteristics of the type 2 pneumocytes were similar to the familial form of IPF in humans associated with a mutation in the SP-C gene. Type 2 cells were hypertrophied and contained many dense cytoplasmic, lamellar-body-like structures. The altered type 2 pneumocyte ultrastructure suggests that spontaneous feline IPF is primarily a defect in these cells (39).

### **EPITHELIAL CELL MIGRATION AND PROLIFERATION AS A KEY REPAIR MECHANISM AFTER THE INITIAL INSULT**

The mechanisms by which epithelial cells perceive the necessity for migration and how the appropriate signals result in coordinated movement are unknown. It has been proposed that epithelia may use a highly conserved set of signals and cytoskeletal-based mechanisms to close gaps and form layers. This repertoire is used during embryonic development and may be partially reused again whenever an epithelium needs to be repaired (40). Unfortunately, the molecular events by which bronchiolar cells and AECs proliferate and migrate for lung wound healing are unknown. In adult skin, wounds reepithelialize by lamellipodial crawling of activated keratinocytes where a leading front of these cells drags themselves forward over the wound matrix. By contrast, wounded embryonic epidermis repairs by means of a contractile purse—an assembly of actomyosin cables in the leading-edge cells that are linked by adherens junctions. As the purse-string contracts, the wound epithelium is drawn forward, taking its own basal lamina substratum beneath it as it moves (40). There is probably considerable overlap in these two types of epithelial motility, and it is already apparent that purse-string epithelial repair is not restricted only to embryonic tissues. However, it is unknown whether any of these mechanisms is involved in alveolar epithelial repair. In addition, it must be considered that there is also an epithelial proliferative response, and the released proliferative signals must be coordinated with motility signals to avoid potentially conflicting signals because cells use the same actin-based machinery to move as they use to proliferate.

### **CYTOKINES/GROWTH FACTORS INVOLVED IN AEC MIGRATION AND PROLIFERATION IN IPF LUNGS**

A number of mediators capable of inducing migration and proliferation of AECs have been identified in IPF lungs. The expression of the hepatocyte growth factor, a potent mitogenic/motogenic factor, and its receptor are greatly increased, especially on hyperplastic AECs. Likewise, a new epithelial growth factor, hepatoma-derived growth factor, has been also found highly expressed in the lungs of individuals with IPF, primarily in

the epithelial cells (41). Interestingly, hepatoma-derived growth factor may also induce cell migration (42).

There is increasing evidence that epidermal growth factor (EGF), transforming growth factor (TGF)- $\alpha$  and their common receptor, EGF-R, may regulate epithelial repair *in vivo* and *in vitro*. TGF- $\alpha$  expression is increased in the IPF lungs mainly in the vascular endothelium, type 2 pneumocytes, and fibroblasts. Also, a small but significantly greater expression of EGF-R in the bronchial epithelium and type 2 pneumocytes is observed in patients with IPF (43).

Recent findings suggest that murine AECs express CC chemokine receptor 2 (CCR2) and that this expression mediates chemotaxis/haptotaxis in response to monocyte chemoattractant protein (MCP)-1. Moreover, closure of a mechanical wound in AEC cultures was delayed in the absence of CCR2 and in the presence of blocking antibodies to MCP-1 (44). However, whether the MCP-1/CCR2 axis plays a role in the process of reepithelialization in IPF is unknown.

Some matrix metalloproteinases (MMPs) may participate in the migration of epithelial cells after injury. Particularly, MMP-1 and MMP-7, known to be involved in skin wound healing, are strongly expressed by bronchiolar cells and AECs in IPF lungs (20). We have recently demonstrated that osteopontin, a multifunctional cytokine that mediates diverse biological functions, including cell adhesion, chemotaxis, and signaling, as well as tissue reparative processes, is one of the most up-regulated genes in lungs of patients with IPF, and that it is primarily expressed by AECs (45). Importantly, osteopontin induces both proliferation and migration of AECs. The effect on epithelial cell proliferation is mainly dependent on CD44, whereas migration is dependent on CD44 and integrin signaling. Interestingly, osteopontin colocalized with MMP-7 in AECs of IPF lungs, and application of the weakest link models to microarray data suggested that the genes of both interacted to affect the IPF phenotype.

The balance of urokinase and plasminogen activator inhibitor-1 (PAI-1) is also important in determining the efficiency of AEC migration. Furthermore, recent evidence suggests that efficient alveolar epithelial repair depends on the attainment of a fibrinolytic optimum in the lung microenvironment (46). Excess of local PAI-1 may worsen scarring by delaying or preventing epithelial migration and restoration, an effect that requires the presence of vitronectin (47).

## DIFFERENTIATION OF TYPE 2 INTO TYPE 1 AECs

Differentiation of type 2 AECs into type 1 cells is essential to reestablish a functional alveolar epithelium. However, the events that participate in this process *in vivo* are largely unknown. The ECM is a dynamic structure that not only provides scaffolding for cellular support but also contributes to a variety of cell activities, including cell differentiation. It has been shown that type 2 cells cultured on fibronectin lose typical lamellar bodies, rapidly accumulate cellular protein, and alter expression of genes associated with their differentiated function. With time, the cells assume many type 1 cell-like characteristics (48). In a recent study, it was demonstrated that, independent of individual matrix components, type 2 AECs developed an uncommitted and intermediate type 2/type 1 cell phenotype first. Then, depending on matrix molecules, that is, collagen/fibronectin or laminin-5, cells develop a type 1-like phenotype. By contrast, on other substrates cells regain type 2 cell morphology (49).

It can be speculated that the transdifferentiation of type 2 into type 1 AECs is profoundly altered in IPF due to the vast ECM abnormalities, interrupted epithelial basement membrane, and the loss of the spatial architecture. In addition, it has been suggested that mechanical distension promotes expression of the

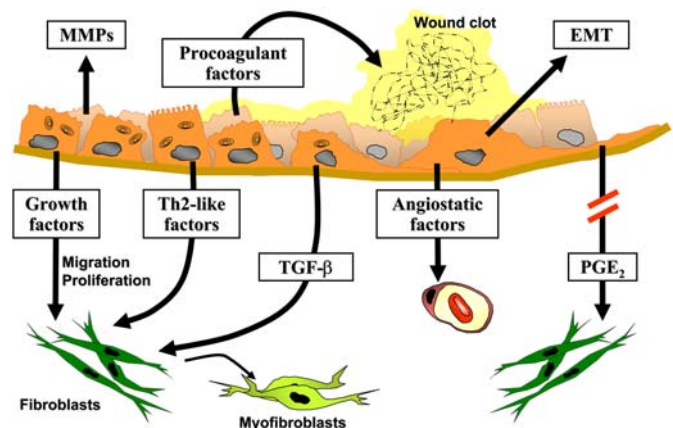
type 1 cell phenotype and inhibits expression of the type 2 cell phenotype, whereas contraction has the opposite effects. However, the mechanisms by which mechanical perturbations of cell surface may cause these changes and whether they occur in fibrosis are unknown (50).

A number of growth factors/cytokines may influence AECs type 2 differentiation. For example, restoration of normal alveolar epithelium after instillation of recombinant human keratinocyte growth factor, a potent epithelial mitogen, is accomplished by terminal differentiation and apoptosis of hyperplastic type 2 AECs *in vivo* (51). In a recent work, it was shown that transdifferentiation of type 2 AECs into type 1-like cells is at least partially induced by the insulin-growth factor (IGF) signaling. The blocking of IGF-I and IGF-1 receptor in type 2 AECs isolated from hyperoxia-exposed lungs resulted in the retention of the cuboidal shape and the typical staining of type 2 cells without the transition to the large and flat shape of type 1 markers (52).

In addition, it has been suggested that leukotriene A4 (LTA4) hydrolase may also be implicated. LTA4 hydrolase, which catalyzes the final step in LTB4 synthesis, accumulates in the nucleus of normal type 2 pneumocytes as well as in type 2 AECs associated with fibrotic regions of lung in patients with IPF (53). In contrast, LTA4 hydrolase localizes predominantly in the cytoplasm in normal type 1 alveolar epithelial as well as in isolated type 2 cells cultured to become type 1-like. These results suggest that, in AECs, the aminopeptidase activity of nuclear LTA4 hydrolase may promote growth, and nuclear export of this activity may be linked to transition from a type 2 to type 1 cell phenotype.

## ACTIVATED ALVEOLAR/BRONCHIOLAR EPITHELIAL CELLS PROVOKE A STRONG PROFIBROTIC MICROENVIRONMENT IN IPF LUNGS

Epithelial cells are the primary source of mediators capable of inducing fibroblast migration, proliferation, and activation as well as ECM accumulation in IPF (Figure 6). These include platelet-derived growth factor, TGF- $\beta$ , tumor necrosis factor  $\alpha$ , endothelin-1, connective tissue growth factor, and osteopontin—all important in the development of pulmonary fibrosis (45, 54).



**Figure 6.** Activated epithelial cells secrete numerous mediators that create a strong profibrotic microenvironment in IPF lungs. Also, they exhibit a defect in the release of some antifibrotic mediators, such as prostaglandin-2. EMT = epithelial-mesenchymal transition; MMPs = matrix metalloproteinases; PGE<sub>2</sub> = prostaglandin-2; TGF- $\beta$  = transforming growth factor  $\beta$ ; Th2 = T-helper cell, type 2.

AECs also produce angiostatic factors, for example, pigment epithelium-derived factor, a TGF- $\beta$  target gene, which may be partially responsible for the absence of capillaries that characterizes the fibroblastic/myofibroblastic foci (55).

There is accumulating evidence suggesting that disordered coagulation and fibrinolysis promoting extravascular fibrin deposition is increased in the alveolar compartment of IPF lungs. Importantly, AECs appear to play a major role in this pathologic process by secreting tissue factor, the primary cellular initiator of the coagulation protease cascade, and PAI-1 (56). Provisional matrix formed by fibrin and fibronectin deposited in the alveolar space can undergo fibrotic repair, which results in the remodeling of alveolar septa. Abnormal fibrin turnover has been also implicated in the surfactant dysfunction that may potentiate the development of microatelectasis in the injured lung.

There is evidence suggesting that polarized T cells participate in the regulation of tissue fibrosis. The development of a Th2 cell response is considered profibrotic because it involves the secretion of at least two putative fibrogenic cytokines, interleukin 4 (IL-4) and IL-13. These interleukins activate fibroblasts and induce the production of ECM components. Importantly, the alveolar epithelium may also generate a Th2-like pattern in IPF lungs. For instance, although in hypersensitivity pneumonitis and sarcoidosis, two potentially reversible interstitial lung disease, type 2 AECs express both IL-4 and IFN- $\gamma$ , only IL-4 is detectable in IPF (57). These results are consistent with a predominantly type 2 pattern of cytokine network in IPF and support a role for epithelial cells in the characteristic imbalance of profibrogenic cytokines in the distal lung of patients with this disease.

However, injured epithelium not only provides activation signals to mesenchymal cells but it also seems to lose its ability to provide its normal and homeostatic fibroblast-suppressive function, primarily mediated by prostaglandin E-2 (PGE2). A growing body of evidence indicates that under physiologic conditions AECs suppress fibroblast migration, proliferation, and activation, including collagen synthesis, through the action of PGE2 (58–60). Two cyclooxygenase (COX) enzymes, COX-1 and COX-2, are responsible for prostanoid production, and both are significantly reduced in bronchiolar epithelial cells of IPF lungs. Moreover, the loss of COX-2 was noticed in epithelial cells but not in alveolar macrophages (61). More recently, the expression of COX-1 and COX-2 was determined in IPF, chronic obstructive pulmonary disease (COPD), and normal lungs by real-time competitive polymerase chain reaction (62). An increased expression of COX-2 was found in COPD without changes in IPF compared with controls. However, after IL-1 $\beta$  stimulation, the expression of COX-2 increased only in COPD and control lungs without significant changes in IPF. In addition, studies in experimental lung fibrosis have indicated that MCP-1/CCR2-mediated signals decrease PGE2 production by AECs. (63).

### AECs AS A SOURCE OF LUNG MYOFIBROBLASTS

Although the role of fibroblasts/myofibroblasts in the pathogenesis of IPF is widely accepted, their origins and activation process during the fibrotic remodeling *in vivo* remain largely undefined and controversial. It has been suggested that epithelial-mesenchymal transition (EMT) may be one of the mechanisms. EMT is a process in which epithelial cells lose their phenotype, acquire fibroblast-like properties, and display reduced cell adhesion and increased motility; it is a key step during embryonic morphogenesis.

Recently, Willis and colleagues demonstrated that TGF- $\beta$ 1 induces EMT in AECs *in vitro*. Importantly, they identified cells coexpressing AEC markers and  $\alpha$ -SMA in regions of hyperplas-

tic epithelium in human IPF lungs, suggesting that EMT may contribute to fibroblast and myofibroblast accumulation in this disease (21). The study however, included only three IPF lungs and will require further corroboration. Additional molecular findings suggest the possibility that EMT may be a source of myofibroblasts in IPF lungs. IPF lungs exhibit an extensive nuclear accumulation of  $\beta$ -catenin in bronchiolar epithelial cells as well as in cuboidal hyperplastic pneumocytes, suggesting involvement of the Wnt/ $\beta$ -catenin signaling pathway (64). Interestingly,  $\beta$ -catenin signaling plays a role in inducing a mesenchymal phenotype in epithelial cells. More recently, up-regulation of N-cadherine, expressed primarily by flattened cells overlying fibroblastic foci, was described (65). A shift of cadherin from the E to the N type is also typical of EMT.

While intriguing, these observations pose many questions. How abundant is EMT in IPF lungs? Are the EMT cells “terminal cells” or do they proliferate to expand the fibroblast/myofibroblast population? Can they revert to AECs if the injury is transient? Does this process occur only when lungs face sustained, chronic injury? Is EMT a process that occurs only in IPF or is it a general biological option in any fibrotic lung disorder? Is EMT a primary event in IPF or is it secondary to the general profibrotic environment and high levels of TGF $\beta$ 1 in IPF lungs?

### EPITHELIAL CELLS IN THE TREATMENT OF IPF

The appropriate repair of the alveolar epithelium at an early stage after injury seems to be a major determinant of lung recovery. In this context, there is some evidence suggesting that endogenous bone marrow-derived cells contribute to repair of the alveolar-capillary units by repopulating type 1 pneumocytes and endothelial cells after endotoxin or elastase inhalation (7, 8). Infusion of exogenous mesenchymal stem cells derived from male BALBc donor mice into female C57BL/6 mice was protective against lung fibrosis. In this study, measurement of male DNA and staining for the Y chromosome suggested that mesenchymal stem cells had engrafted the lung by adopting a type 2 cell phenotype (66). However, as mentioned before, there are some technical uncertainties that preclude the current determination of the real magnitude of the local epithelial chimerism obtained by the use of stem cells (9, 10). Furthermore, the number of local chimeric epithelial cells obtained with adult marrow-derived cells in other respiratory pathologies seems to be small. For example, adult marrow-derived cells containing normal cystic fibrosis transmembrane conductance regulator protein (Cftr) were recently used to repopulate lung epithelium in Cftr-deficient transgenic mice at baseline and in response to naphthalene-induced epithelial injury (67). *In situ* examination of recipient lungs demonstrated rare chimeric airway epithelial cells, and naphthalene-induced airway remodeling did not increase significantly the number of chimeric airway epithelial cells expressing Cftr.

On a different course, tissue engineering has emerged as a therapeutic approach to the regeneration and repair of injured tissues. Currently, coculture of embryonic and other stem/progenitor cells with mature cells or tissues is being increasingly used to drive their differentiation toward required lineages. For example, differentiation of human embryonic stem cells can be directed toward a type 2 AEC phenotype by a combination of soluble factors (68). The same group demonstrated that coculture of murine embryonic stem cells with embryonic mesenchyme from distal lung promotes the differentiation of pneumocytes (69). These cells expressed cytokeratin and thyroid transcription factor 1, an early developmental marker in pulmonary epithelium. Differentiation of type 2 pneumocytes was supported by the presence of SP-C in some of the epithelial cells. Similarly, it has

been demonstrated that fetal lung morphogenetic instructive signals are able to induce mouse embryonic stem cell derivatives to incorporate into the reforming pseudoglandular-like tubular ducts. Cells expressed pan-keratin and SP-C while exhibiting a normal diploid karyotype in coculture support the idea that it may be possible to direct differentiation of mouse embryonic stem cells into respiratory lineages (70).

In general, these results indicate that, under an appropriate and well-controlled microenvironment (i.e., embryonic lung mesenchyme), embryonic stem cells originate lung epithelium *in vitro*. Using this strategy, it is potentially possible to generate large numbers of epithelial cells, providing an alternative for new therapeutic approaches.

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