Modulating the Alveolar Milieu to Enhance Resolution of Fibrotic Lung Injury

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Fibrotic lung injury is often attributed to a myriad of factors, including environmental exposure, age, genetic predisposition, epigenetics, coexisting conditions, acute lung injury, and viral infection. No effective therapies, other than lung transplantation, have proven effective against lung fibrosis. Loss of cellular homeostasis mechanisms in alveolar epithelial type I cells and any inability of type II progenitor cells to resist and repair epithelial injury are indicators that impaired response to injury and regeneration is a critical component of this disorder. The alveolar epithelium has a limited repertoire of responses to injury, which are dictated by the alveolar milieu, a repository of cytokines and growth factors that affect recruitment of other cells to the site of injury, or the proliferation of resident cells at the site of injury. The identification and characterization of the cytokines, growth factors, and other biomarkers that dictate the response to disease is key to understanding, diagnosing, treating, and determining the trajectory of various lung disorders. Corrective therapy of the alveolar milieu may therefore prove to be beneficial in many presently serious and incurable lung diseases that likely begin and progress with injury to the alveolar epithelium.

Keywords: lung fibrosis; alveolar milieu; alveolar epithelium

Pulmonary diseases are one of the leading causes of death worldwide (18.7% of 50.5 million deaths registered in 1990, according to World Health Organization statistics). Terminal lung failure can be a consequence of infectious disease, cancer, and genetic disorders, or interstitial lung disease that starts with inflammation and ends up as pulmonary fibrosis (1). Allogeneic lung transplantation remains the only option for patients with terminal pulmonary failure, despite a scarcity of donated organs. However, lung transplants have relatively low survival rates in comparison to transplantation of other parenchymal organs (2). Therefore, there is an urgent need for novel therapies that will limit progression of epithelial damage to end-stage lung disease.

At the root of many lung fibrotic diseases is the disruption of alveolar homeostasis and the loss of alveolar epithelial type I (AEC1) or alveolar epithelial type II cells (AEC2) coupled with the inability of type II progenitor cells to resist and repair epithelial injury. The alveolar epithelium has a relatively finite repertoire of responses to injury, which are dictated by the alveolar milieu, a repository of cytokines and growth factors that affect recruitment of other cells to the site of injury, or the proliferation of resident cells at the site of injury.

Alveolar lung injury resulting in the loss of homeostasis is often accompanied by inflammation, abnormal repair, endoplasmic reticulum stress, inability of progenitor cells to repopulate injured epithelia, epithelial-to-mesenchymal transition (EMT), activation of resident lung fibroblasts, or recruitment of circulating fibrocytes (3). Alveolar injury can ideally either resolve to heal the gas diffusion surface or, as is the case in disease, chronic wounding of the alveolus can lead to fibrotic scarring and alveolar destruction, which eventually contributes to end-stage lung disease.

The identification and characterization of the cytokines, growth factors, and other biomarkers that dictate the response to disease is key to understanding, diagnosing, treating, and determining the trajectory of various lung disorders. Identification of the soluble factors that promote epithelial versus fibrotic healing offers the translational potential of correcting the alveolar milieu to minimize or ameliorate fibrosis, with the goal of prevention of progression to end-stage disease in currently intractable diseases such as idiopathic pulmonary fibrosis (IPF), bronchopulmonary dysplasia, and chronic obstructive pulmonary disease. In addition, biomarkers of normal versus aberrant epithelial healing could be used to monitor and/or predict disease prognosis.

RESPONSE OF THE ALVEOLAR EPITHELIUM TO INJURY/DISEASE

The alveolar epithelium has a relatively limited repertoire of responses to injury that lead either to resolution or to chronic wounding of the alveolus, which eventually contributes to end-stage lung disease (Figure 1). With the hallmarks of epithelial injury being apoptosis and necrosis, other responses, such as epithelial-to-mesenchymal transition and chronic inflammation, compose the most typical responses of alveolar epithelium to injury or disease (4). In addition to AEC1 and AEC2 participating in maintenance of alveolar homeostasis, fibroblasts and macrophages are garnering much attention as their responses in wound healing and repair have more recently been elucidated. All four of these cell types participate in distinct stages within wound healing. Injury to AEC1 and AEC2 triggers the release of inflammatory mediators, which trigger the activation and migration of inflammatory cells such as macrophages; resident fibroblasts or circulating fibrocytes are then activated to proliferate and/or migrate to wound sites, followed by tissue remodeling, which can result in wound healing or fibrotic scarring (5). Corrective therapy of the alveolar milieu may therefore prove to be beneficial in many presently serious and incurable lung diseases, including bronchopulmonary dysplasia, chronic obstructive pulmonary disease, and IPF, that likely begin and progress with injury to the alveolar epithelium. Our recently published (6) data show that the alveolar milieu can indeed both maintain alveolar epithelial homeostasis and mediate alveolar repair. Importantly, the alveolar milieu, also highly chemoattractive to exogenous progenitor cells, can induce infiltrating stem cells to augment endogenous healing, most probably through specific combinatorial cytokine mediator release, and can also induce expression of AEC2 lineage markers in previously uncommitted stem cells.

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Figure 1. The response of the alveolar epithelium to damage/disease. EMT = epithelial-to-mesenchymal transition.

THE DISTAL ALVEOLAR EPITHELIAL MILIEU IS THE KEY TO ALVEOLAR EPITHELIAL HOMEOSTASIS

The alveolar milieu contains autocrine and exocrine factors that maintain the epithelium in health and dictate the nature of repair in disease. The distal alveolar epithelial milieu is critical for maintaining a functional population of alveolar epithelial cells containing a balance of autocrine and exocrine factors that maintain homeostasis. Some of the cytokines and signaling molecules involved in this response are listed in Table 1. A functional population of AEC2, the putative alveolar epithelial progenitors, is necessary to maintain the distal epithelium for efficient gas exchange as well as providing lung compliance, elastance, and innate immunity. Biomarkers of AEC2 injury are seen in the plasma of patients with acute exacerbations of IPF (7), suggesting a systemic response to AEC2 damage. This balance of soluble factors released by damaged AEC2, along with local and infiltrating cells and matrix, will determine whether the wound will be sealed by epithelial or mesenchymal cells. Well-studied soluble factors in the AEC2 damage milieu that have been shown to reduce fibrosis in animal studies include keratinocyte growth factor (KGF) (by supplementation), or transforming growth factor- β (by inhibition) (8).

PROTEOMIC ANALYSIS OF BRONCHOALVEOLAR LAVAGE CAN BE USED TO CHARACTERIZE THE ALVEOLAR MILIEU

A dynamic proteomic database for human bronchoalveolar lavage (BAL) is available on the internet (9). It was set up to hunt for new disease markers in BAL, to examine post-translational modifications of BAL proteins, and to compare BAL in health and

disease, and its database expands with advances in technology. Increased resolution and sensitivity now enable the detection of low abundance proteins and low molecular weight peptides (9). A comparison of BAL from patients with IPF, sarcoidosis, and healthy control subjects (10) versus BAL associated with inflammatory myopathies (11) and acute respiratory distress syndrome (12) showed protein patterns in the AEC2 milieu specific to each disease. The ability to identify proteins in a high-throughput manner and build “proteomic profiles” for specific diseases is a major leap in the early and most importantly minimally invasive detection of pulmonary disease via biomarkers.

CELL-BASED THERAPIES HAVE THE POTENTIAL TO LIMIT PROGRESSION OF LUNG DISEASE

Cell-based therapies (CBTs) encompass the idea that endogenous (residing within an organ) or exogenous cells (isolated from outside the organ) can be manipulated to ameliorate disease progression or repair, regenerate, or replace a diseased organ. CBTs using circulating blood cells, including those from bone marrow, have augmented repair and prevented subsequent emphysema or fibrosis in several animal lung damage models, including lipopolysaccharide (LPS), elastase, irradiation, naphthalene, and bleomycin-mediated acute lung injury (13–17). Stem cells have also been used to target therapeutic agents to the site of damage (e.g., KGF delivered by bone marrow stem cell and expressed via an inducible lentivirus (18). However, as these therapies cannot reverse scar formation or restore fibrotic alveolar microstructure to normal, our emphasis is placed on devising methods for rapid, appropriate alveolar repair. The relatively low engraftment of the donor cells in repairing damage models suggests that their protective effect is paracrine in nature, augmenting the alveolar epithelial membrane’s endogenous healing milieu. Identification of factors contributing to a prohealing, proepithelial milieu would allow preconditioning of cells pre-transplant or direct manipulation of the alveolar milieu, leading to novel therapeutic approaches to prevent progression to end-stage lung disease. If harnessed, the potential of cellular therapies extends beyond simply ameliorating symptoms of lung disease, as many pharmaceuticals do, to halting and preventing further injury or degradation.

TABLE 1. KEY SIGNALING MOLECULES WITHIN THE PROFIBROTIC ALVEOLAR MILIEU

Signaling Molecule	Origin	Target
TNF- α	Leukocytes, endothelial cells, epithelial cells, lymphocytes, macrophages	Fibroblasts, myofibroblasts
TGF- β	Macrophages, leukocytes, lymphocytes, epithelial cells, endothelial cells, mesenchymal cells	Fibroblasts, myofibroblasts, epithelial cells, T cells, monocytes
Reactive oxygen species (ROS)	Macrophages, neutrophils	Fibroblasts, myofibroblasts
PDGF	Epithelial cells, endothelial cells, macrophages, fibroblasts	Fibroblasts
MIP-1 α	Neutrophils, smooth muscle cells	Neutrophils, activated macrophages, basophils, eosinophils, T cells, B cells
IL-1	Epithelial cells, leukocytes, macrophages	Fibroblasts, myofibroblasts
IL-4	T cells	Myofibroblasts
IL-8	Macrophages, epithelial cells	Neutrophils, endothelial cells, macrophages
IL-10	Monocytes, lymphocytes	Fibrocytes, fibroblasts
IL-13	Leukocytes, T cells	Fibroblasts, myofibroblasts, T cells
IL-17A	CD4 ⁺ T cells	Neutrophils
IL-21	T cells	T cells
IL-25	Epithelial cells, T cells	T cells
IL-33	Epithelial cells	T cells
CXCL5, CXCL8, CXCL12	Macrophages, epithelial cells, fibroblasts	Fibrocytes
CCL22	Macrophages, monocytes	Monocytes, lymphocytes
CCL2/MCP-1, CCL3, CCL5, CCL12, CCL19, CCL21b	Fibroblasts, endothelial cells, macrophages, lymphocytes, smooth muscle cells, epithelial cells	Monocytes, lymphocytes, basophils, fibrocytes, fibroblasts
CCL17	Epithelial cells, endothelial cells, macrophages,	Monocytes, lymphocytes

AMNIOTIC FLUID STEM CELLS AUGMENT ENDOGENOUS ALVEOLAR EPITHELIAL HEALING

In our hands, amniotic fluid stem cells (AFSC) are particularly efficient for CBT (19). The AFSC, an intermediate type of stem or progenitor cell between embryonic stem cells and adult stem cells resident in differentiated organs, has been well characterized (20). AFSC satisfy the requirements of a stem cell potentially suitable for CBT, because they are derived from a noncontroversial source and are readily available, scalable, pluripotent, and nonteratogenic. We have chosen the human amniotic fluid stem cell AFSC to test the concept of stem cell-augmented alveolar epithelial repair, rather than mesenchymal stem cells, based on our preliminary unpublished studies, which show that the AFSC are significantly less immunomodulatory than mesenchymal stem cells.

We have shown that AFSC track *in vivo* to damaged alveolar epithelium, where they can differentiate into epithelial cells expressing markers of AEC2 (21). Furthermore, AFSC are capable of responding to various types of *in vivo* alveolar injury, including naphthalene, hyperoxia, and bleomycin-induced fibrosis. This tracking to the site of damaged AEC2 and damage-induced differentiation can be recapitulated *in vitro*, where AFSC are attracted to damaged AEC2 milieu *per se*, and expedite wound healing by contributing healing cytokines, such as the inhibition of macrophage migration inhibitory factor and plasminogen activator inhibitor 1, to the milieu (6). Our unpublished observations indicate that AFSC can also ameliorate bleomycin-induced fibrosis in a rodent model, demonstrating their potential functionality in preventing fibrotic alveolar epithelial repair. Identification of the proteins in the damaged AEC2 milieu that attract and promote differentiation of AFSC into cells expressing markers of AEC2 (surfactant protein-C, thyroid transcription factor-1, ATP-binding cassette transporter A3), and the proteins secreted by AFSC into the damage milieu that promote wound healing, would enable the design of therapeutic strategies to promote nonfibrotic reepithelialization.

SUMMARY STATEMENT

The alveolar milieu contains key signaling molecules during both homeostasis and injurious states. These cytokines and regulatory molecules play a key role in either pathogenesis or normal wound healing. The elucidation of the factors within the alveolar milieu that dictate repair processes is an important target for therapies aimed at diseases that affect the alveoli. The milieu of damaged alveolar epithelium provides a niche for inflammatory cells and innate bone marrow stem cells as well as for systemically delivered stem cells. A critical, but as-yet undefined balance of soluble factors will dictate not only which cells are attracted to the site of damage but also the *in situ* response of the infiltrating cells. Whether these cells engraft, contribute cytokines/growth factors, and/or differentiate to expedite appropriate epithelial repair, or further compromise the epithelium through inflammation and/or induction of fibrosis, will be dependent on the specific contents of the damage milieu. Thus, a detailed knowledge of the soluble protein constituents within the AEC2 milieu that maintain homeostasis in health and restore it after damage would facilitate the design of therapeutic interventions to avert inappropriate repair mechanisms after epithelial damage.

Author disclosures are available with the text of this article at www.atsjournals.org.

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