Alveolar Epithelial Cell–Derived Mediators: Potential Direct Regulators of Large Airway and Vascular Responses

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Abstract

Bronchial epithelial cells and pulmonary endothelial cells are thought to be the primary modulators of conducting airways and vessels, respectively. However, histological examination of both mouse and human lung tissue reveals that alveolar epithelial cells (AECs) line the adventitia of large airways and vessels and thus are also in a position to directly regulate these structures. The primary purpose of this perspective is to highlight the fact that AECs coat the adventitial surface of every vessel and airway in the lung parenchyma. This localization is ideal for transmitting signals that can contribute to physiologic and pathologic responses in vessels and airways. A few examples of mediators produced by AECs that may contribute to vascular and airway responses are provided to illustrate some of the potential effects that AECs may modulate.

Keywords: alveolar epithelial cell; inducible nitric oxide synthase; IL-33; receptor for advanced glycation end products; ventilation-perfusion mismatch

At a Glance Commentary

Alveolar epithelial cells are in close proximity to conducting airways and vessels. Several alveolar epithelial cell–derived molecules such as inducible nitric oxide synthase, IL-33, and receptor for advanced glycation end products provide examples of mechanisms by which alveolar epithelial cells may communicate with and modulate large airways and vessels.

The primary function of the lung is gas exchange, which occurs in the alveoli. Many studies have demonstrated how bronchial epithelial cells and pulmonary vascular endothelial cells modulate the airways and blood vessels, respectively. There are also significant data suggesting the involvement of immune cells and mesenchymal cells within and surrounding the airways and vessels. However, in general, research has not been focused on the potential role of alveolar epithelial cells (AECs) in modulating responses in conducting airways and pulmonary vessels. AECs, which line the adventitial surface of conducting airways and pulmonary vessels, are in an ideal location to directly

modulate both upper airway and vascular responses. This concept is an important one to remember as more studies identify crucial roles for AEC-derived molecules in the pathogenesis of airway diseases such as asthma.

Normal Lung Physiology

Bronchial epithelial cells and vascular endothelial cells, which line the lumen of airways and pulmonary vessels, respectively, are known to regulate airway and vascular tone. However, the potential of AEC-mediated modulation of proximal conducting airway and vessel responses has

yet to be considered fully and investigated thoroughly. The idea that AECs may play a direct role in upper airway physiology is often controversial, because AECs are thought to be distal to the bronchi, are not surrounded by smooth muscle cells, and function primarily in gas exchange. All tissues/organs are lined with epithelium or mesothelium on their outer surfaces, so it is not surprising that the adventitial surface of conducting airways and pulmonary vessels within the lung parenchyma are also lined with epithelial cells. The epithelial cells lining the adventitial surface of the conducting airways and blood vessels in both human (Figures 1 and 2) and mouse (not shown)

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Figure 1. Alveolar epithelial cells line the adventitial circumference of large airways. (A) Hematoxylin and eosin section of human lung showing bronchiole with ciliated columnar epithelium. Note that alveolar epithelium abuts the adventitia around the entire circumference of the airway, *Alveolar sac. (B) Higher-power image showing alveolar epithelial cells lining the adventitial surface of the larger airway. (C) Immunochemical staining of surfactant protein C (blue stain) highlights type II alveolar epithelial cells (arrowheads) lining the adventitial surface of a larger airway. (D) Higher power of the surfactant protein C-stained section showing the type II cells on the adventitial surface of the larger airway. (A and C) Scale bars: 20 μm . (B and D) Scale bars: 6 μm .

lungs are, in fact, AECs. Because the outer adventitial circumference is greater than the circumference of the lumen, AECs are likely to have a potential to modulate bronchial and vascular effects that is at least similar to that of the bronchial epithelial cells and vascular endothelial cells, respectively. This is a practical setup because AECs, at the forefront of gas exchange, would be logical cells to communicate with airways and vessels in the regulation of ventilation-perfusion matching.

AEC-Derived Mediators of Airways and Vessels

Ventilation-Perfusion Matching

Hypoxic pulmonary vasoconstriction is a physiological phenomenon in which blood vessels in the lung constrict to limit blood flow to areas of low ventilation (1). One mediator of this response is inducible nitric oxide synthase (iNOS), which uses

arginine as well as O_2 as substrates in nitric oxide (NO) production (2). Therefore, if PO_2 is rate limiting, oxygenating the alveolar airspace may promote NO release from AECs. The oxygen concentration in the lung ranges from 1 to 260 μM , and the Michaelis constant of oxygen of iNOS in the lung is 135 μM , indicating that iNOS activity correlates with O_2 levels in the lung as long as arginine is also available (3). Therefore, a ventilated alveolar space with increased O_2 has the potential to promote an increase in NO production and, in turn, blood flow. Conversely, an unventilated airspace with low O_2 may lead to decreases in NO and, in turn, blood flow. Thus, less NO would be expected to be produced in hypoxic environments (3, 4).

NO released from cells can have a rather high diffusing and signaling capacity, dependent on the amount of NO produced in proximity to the target cells and other factors such as superoxide levels (5, 6). For example, NO released by

endothelial cells may diffuse to smooth muscle cells in vessels to cause vasodilatation, thus increasing blood flow to oxygenated areas of the lung. Type II AECs (AECII) express both NOS1 (constitutive NOS) and NOS2/iNOS, with iNOS being the primary NO producer during instances of AECII stimulation (7). Therefore, in a similar manner, NO released from AECII lining the adventitia of blood vessels may also diffuse to smooth muscle cells in the blood vessel, inducing vasodilation and increased blood flow in aerated areas of the lung.

Similarly, during inflammatory states when large amounts of immune cells are recruited to the lung tissue, blood flow to the lung may be increased via vasodilation of pulmonary vessels. Several studies have shown that iNOS expression in AECII increases and iNOS-mediated production of NO rises in response to inflammatory cytokines such as $\text{IFN-}\gamma$, IL-1 β , tumor necrosis factor- α , and endotoxin, as well as during endotoxemia *in vivo* (7–9). There is evidence that NO production by AECIIs may surpass that of other cell types within the lungs (10), suggesting that AECs may be a major regulator of iNOS-mediated ventilation perfusion matching.

Innate Immunity

Another example of how AECs can directly affect upper airways and vessels is through the release of proinflammatory cytokines and mitogenic growth factors. AECs produce a plethora of proinflammatory mediators including macrophage chemoattractants (e.g., monocyte chemoattractant protein-1), neutrophil chemoattractants (e.g., IL-8), and eosinophil chemoattractants (e.g., eotaxins) (11, 12). AECIIs also play a crucial role in initiating antiviral IFN responses and antimicrobial responses (13, 14). Recent studies have identified important roles for epithelial-derived cytokines and receptors in the pathogenesis of lung diseases such as asthma and chronic obstructive pulmonary disease (COPD) (reviewed in [15, 16]).

One of these molecules, the alarmin IL-33, has been shown to be a crucial mediator of allergic airway inflammatory responses after viral infection (17–20) or allergen exposure (21–27). IL-33 expression is most prominent in AECs, specifically AECII (28–31). When released from

Figure 2. Alveolar epithelial cells line the adventitial circumference of pulmonary vessels. (A) Hematoxylin and eosin section of human lung showing blood vessel surrounded by alveoli. Note that alveolar epithelium abuts the adventitia around the entire circumference of the vessel. *Alveolar sac. (B) Higher-power image showing alveolar epithelial cells lining the adventitial surface of the blood vessel. (C) Immunohistochemical staining of surfactant protein C (blue stain) highlights type II alveolar epithelial cells (arrowheads) lining the adventitial surface of a blood vessel. (D) Higher power of the surfactant protein C–stained section showing the type II cells on the adventitial surface of the blood vessel. (A and C) Scale bars: 20 μ m. (B and D) Scale bars: 6 μ m.

AECII, IL-33 has many direct and indirect proinflammatory downstream effects not only on the immune system, but also on airways and blood vessels. Proteomic and transcriptomic studies suggest that IL-33 directly activates signaling cascades (e.g., mitogen-activated protein kinase and nuclear factor- κ B) in a number of cell types including macrophages and endothelial cells, leading to enhanced expression of proinflammatory cytokines, chemokines, and cell adhesion molecules (32, 33). IL-33 also triggers Th2 immune responses in the lung via activation of group 2 innate lymphoid cells (ILC2s) (34–37), which are found abundantly in bronchovascular bundles (38). IL-33–induced activation of ILC2s leads to increased IL-13 levels in the lung and subsequent airway smooth muscle cell contraction, hyperreactivity, and mucus production (39). Therefore, AECII–ILC2 communication may serve

as a bridge between the alveoli and conducting airways to mediate pulmonary pathology (40). Alveoli–endothelial cross-talk may also be mediated via IL-33. IL-33 has been shown to up-regulate intercellular vascular adhesion molecule-1 and vascular cell adhesion molecule-1 expression in endothelial cells (41, 42). This function is dependent on nuclear factor- κ B signaling and promotes recruitment of immune cells from the blood to sites of inflammation.

A second molecule, the receptor for advanced glycation end products (RAGE), is a proinflammatory, immunoglobulin-like receptor that is highly expressed in the lung. Studies have localized RAGE expression to the basal membrane of type I AECs (AECI), and RAGE has been defined as a specific marker of AECI (43–46). Some researchers have also shown RAGE messenger RNA in AECII (47). Although located primarily on AECs in mice,

pulmonary RAGE has been shown recently to be necessary for the development of asthma (a disease with primary pathology in conducting airways) in mouse models of allergic airway disease (48, 49). Mice lacking RAGE do not develop airway hyperresponsiveness, mucus hypersecretion, or eosinophilic inflammation in response to allergen exposure. RAGE knockout mice also had attenuated IL-33 levels in response to allergens, suggesting that RAGE is important for IL-33 production and/or release. More work is required in the field, but it is possible that RAGE on AECI triggers the release of IL-33 from AECII. RAGE, therefore, is another alveolar-associated molecule that appears to have important effects on upper airway physiology and disease pathogenesis.

Although the focus of this perspective is to highlight the proximity of highly active AECs to conducting airways and vessels, it should be noted that the resident alveolar macrophages may also contribute significantly to such cellular communications. Alveolar macrophages produce a plethora of signaling mediators that can regulate air and blood flow as well as inflammation. Cross-talk between two cell types has been shown to mediate alveolar macrophage adherence to AECs (50), AEC proliferation (51), pulmonary inflammation, phagocytosis, alveolar macrophage activation, and epithelial plasticity (52, 53).

Potential Future Approaches

AEC-derived mediators have extensive effects on conducting airways and pulmonary vessels under a variety of conditions and are implicated in a number of pulmonary diseases including asthma, COPD, pulmonary fibrosis, acute lung injury/adult respiratory distress syndrome, pneumonia, and others (16, 54, 55). The potential mechanisms suggested here should inspire researchers throughout the spectrum of pulmonary disease to begin investigating alveolar effects on conducting airways and vessels.

A number of *in vivo* and *in vitro* models are available to aid in these studies. Cell-type-specific transgenic mice targeting AECII (56) or AECI (57) may be used to

Figure 3. Potential roles of alveolar-derived mediators in airway and vessel pathophysiological responses. Schematic representation of potential mechanisms of alveolar regulation of airways and vessels during physiologic and pathogenic processes. In the alveolar sac, environmental insults (e.g., endotoxin, α -toxin, and allergens), damage-associated molecular patterns (DAMPs), and inflammatory stimuli such as $\text{TNF}\alpha$, $\text{IL-1}\beta$, or $\text{IFN}\gamma$ stimulate the activation and response of type I and type II alveolar epithelial cells (AECI and AECII). Inflammatory mediators induce inducible nitric oxide synthase (iNOS) activity and nitric oxide (NO) release from AECII (as well as other vasodilators and vasoconstrictors), which have the potential to induce pulmonary vessel contraction or dilation. During this process, damaging reactive oxygen and nitrogen species (ROS and RNS) are produced, which can potentially lead to oxidation of DNA, lipids, and proteins. Receptor for advanced glycation end products (RAGE) mediates responses to environmental stimuli as well as DAMPs, which leads to downstream signaling via various mechanisms, including IL-33. RAGE also contributes to epithelial cell spreading and repair. Release of IL-33 into the adventitial space (or alveolar sac) has several effects on the alveolar, vascular, and conducting airway compartments. IL-33 increases expression of cell adhesion molecules on pulmonary endothelial cells (PEC), induces smooth muscle cell (SMC) contraction and type 2 inflammation in the conducting airways and respiratory airspace (alveolar sac). Type 2 inflammation surrounding bronchial epithelial cells (BEC) leads to mucus hypersecretion and airway hyperreactivity, as seen in asthma. Alveolar epithelial cells, which also interact with and communicate with alveolar macrophages (AM), also release a number of other inflammatory mediators (e.g., CXCL and CCL chemokines, IFN), which may regulate other inflammatory processes in both the alveolar region as well as the adventitia of airways and vessels.

determine the role of the specific alveolar-derived mediators in question. In addition, methods such as flow automated cell sorting or magnetic bead separation allow

for the isolation and culture of specific cell types from the lungs; such cells can be used in complex coculture models that mimic physiologic conditions to determine the

effects of alveolar epithelial cultures on endothelial, bronchial epithelial, smooth muscle, or inflammatory cells.

Final Thoughts

It is important to recognize the potential contributions of AECs to conducting airway and pulmonary vascular physiology and immune responses. Because gas exchange occurs in the alveolar parenchyma, it makes sense that AECs should contribute in modulating airway and vascular tone. In addition to highlighting the anatomic proximity of AECs to conducting airways and vessels, we have also illustrated the potential importance of AEC-derived signals on the conducting airways and vessels through a discussion of iNOS, IL-33, and RAGE signaling (summarized in Figure 3). The goal of this short perspective is to serve as a reminder to both lung biologists and clinical pulmonologists not to disregard AECs as important regulators of airways and vessels in studies of pulmonary diseases such as asthma, pulmonary hypertension, COPD, respiratory infection, pulmonary fibrosis, and lung cancer, and hopefully to inspire new studies that make discoveries concerning the importance of AECs in central airway and vascular physiology and pathology. ■

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