Smooth muscle proliferation is stimulated by the airway epithelium

Reynold A Panettieri*

Department of Chemical, Biochemical Engineering and Material Science, 3120 Natural Sciences II, University of California, Irvine, Irvine, CA 92697-2715, USA

Abstract

Chronic tissue injury and inflammation triggered by exogenous stimuli can cause acute bronchoconstriction. Over time, structural changes such as goblet cell hyperplasia, subepithelial fibrosis, smooth muscle cell hypertrophy and hyperplasia, and increased vascularity and edema occur in the airway wall (airway remodeling). An intensive effort is aimed at understanding the mechanisms that lead to airway remodeling, in hopes of not only slowing, but perhaps reversing, the structural alterations.

Airway epithelial cells play a critical role in the defense system of the lungs by providing an important barrier function to potentially toxic environmental agents that can promote epithelial damage, or induce bronchoconstriction in susceptible individuals. Not surprisingly, epithelial shedding, marked by increased numbers of epithelial cell clumps (creola bodies) in sputum and bronchial epithelium desquamation, are features of airway injury. The sequelae is an airway epithelium in a chronic state of wound repair, which secretes soluble mediators (e.g., IGF) necessary for cell proliferation, migration, and extracellular matrix synthesis consistent with a healing wound environment.

Communication between the epithelium and the underlying fibroblast in the lamina propria is prevalent and normal during fetal lung development. In contrast, the airway smooth muscle lies adjacent to the lamina propria, and therefore a further distance from the epithelium. For the epithelium to influence smooth muscle cell behavior, soluble mediators would need to diffuse through the lamina propria. Hence, the vast majority of investigation has focused on the role of the epithelium in subepithelial fibrosis. Although progress has been made in identifying mitogenic stimuli of airway smooth muscle, the potential role of epithelium-derived mediators in smooth muscle proliferation remains unexplored.

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Introduction

We hypothesized that an injured airway epithelium secretes soluble mediators at biologically relevant concentrations to stimulate smooth muscle cell hyperplasia. To address this hypothesis, we used a co-culture model of primary Normal Bronchial Epithelial Cells (NHBE) and primary normal Human Airway Smooth Muscle cells (HASM), as well as an in vivo model of epithelial injury in the rabbit trachea. Our results demonstrate that an injured airway epithelium promotes HASM cell proliferation. Furthermore, proliferation in the smooth muscle (trachealis) region was observed in the rabbit trachea after repeated epithelial injury. To our knowledge, this is the first report demonstrating the role of the epithelium in airway smooth muscle cell proliferation. The results are consistent with an increasing interest in epithelial dysfunction as a target of therapeutic intervention in airway diseases.

Discussion

Tissue injury along with inflammation is associated with remodeling as observed in several airway diseases including asthma, chronic obstructive pulmonary disease, and fibrosing alveolitis. One feature of airway remodeling is smooth muscle cell hyperplasia, which impacts airway caliber and decreases lung function. Current anti-inflammatory strategies do not reverse smooth muscle hyperplasia, creating a need for alternative therapies. Using both a novel in vitro co-culture model as well as an in vivo model of epithelial injury in the trachea, our study demonstrates that epithelial-derived mediators stimulate airway smooth muscle proliferation at baseline and after injury. We identified IL-6, IL-8, and MCP-1 as mediators, which contribute significantly to proliferation after injury, and MMP-9 as a novel mediator involved in both baseline and injury-induced proliferation. IL-6, IL-8, and MMP-9 levels are affected by the co-culture of NHBE and HASM, while MCP-1 is mainly produced by HASM. While not all diseases with epithelial injury manifest airway smooth muscle proliferation, our results provide potentially new targets to limit smooth muscle hyperplasia in the airways, and are consistent with a growing interest in the health of the airway epithelium.

IL-6, IL-8, and MCP-1 expression and release from airway epithelial cells has been demonstrated by numerous groups with interest in the response to infectious diseases. MMP-9 expression in NHBE cells has also been reported in airways after injury and in infectious diseases. Airway epithelium–derived bFGF (FGF-2), IGF-1, PDGF-BB, VEGF, HB-EGF, MMP-2, and ET-1 have demonstrated a role in cell proliferation, myofibroblast induction, angiogenesis migration, matrix degradation, and the immune response, however, a role

in airway smooth muscle proliferation has not been demonstrated. During epithelial injury, the absence of neighboring cells at the wound edge and hence loss of tight junctions can activate mechanosensors present on the cell surface. The mechanical signals are converted into a chemical response via site-specific integration with signal-transducing molecules (e.g., MAPK) that initiate a cascade of signaling events within the cells to release cytokines and chemokines.

Mitogenic stimuli for human airway smooth muscle include polypeptide growth factors (e.g., EGF), plasma-derived or inflammatory cell–derived mediators (e.g., spingosine-1-phosphate), reactive oxygen species, and components of the extracellular matrix (e.g., collagen), stretch, as well as contractile agents. Growth factors activate intrinsic Receptor Tyrosine Kinase (RTK) activity, whereas contractile agonists are linked to heterotrimeric guanosine triphosphate–binding proteins (GPCR proteins), and proinflammatory cytokines signal through glyocoprotein complexes to stimulate airway smooth muscle proliferation. PI3K and MAPK are the major transduction pathways for the RTK, GPCR, or cytokine-stimulated proliferation of HASM. Since specific inhibitors to MAPK, PI3K, and Gi pathways significantly reduced proliferation of HASM in EUS and EIS, we infer that the epithelium stimulates smooth muscle cell proliferation through multiple pathways used by growth factors, contractile agonists, and/or inflammatory cytokines. Furthermore, the proliferation of HASM on co-culture with the NHBE is sensitive to dexamethasone. Previous studies have demonstrated that selective growth factor–induced proliferation of HASM is not dexamethasone sensitive. This is consistent with our observations that MMP-9, IL-6, IL-8, and MCP-1 from the epithelium contribute to the proliferative response of the smooth muscle cells.

Methods

Levels of IL-6 and IL-8 are increased in asthmatic bronchoalveolar lavage (BAL) fluid, and an increase in MCP-1 expression in the bronchial epithelium of individuals with asthma has also been noted. IL-6, IL-8, and MCP-1 have been shown to play a role in vascular smooth muscle proliferation; however, there are limited and variable data on their effects on HASM growth. Our study suggests a role of the above inflammatory cytokines in HASM proliferation after epithelial injury.

MMPs are proteolytic enzymes believed to be essential for development, turnover, and degradation of ECM proteins and denatured collagens (also known as gelatins). A role for MMP-9 has been implicated in the re-epithelization (i.e., regrowth of denuded epithelium at the wound site) of wounds. This requires the epithelial cells at the wound edge to lose their cell–cell contacts and migrates across the wound thus, increased MMP-9 levels secreted by the epithelium after injury could impact new tissue growth and cell proliferation.

Elevated levels of MMP-2 and MMP-9 have been observed in BAL fluid and induced sputum of subjects with asthma. A role for MMPs in smooth muscle proliferation remains unclear. Evidence suggests that HASM require MMP-2 for the proliferative response to mitogens; however, although MMP-2 was present in our system and thus may contribute to smooth muscle proliferation in our model, there were no significant changes in levels of MMP-2 after co-culture or epithelial injury (Figure E4). In other words, MMP-2 may be necessary but not sufficient to stimulate HASM in our in vitro system. For example, MMP-9 can be activated by MMP-2.

We also demonstrated that chemical inhibition and silencing of MMP-9 expression significantly abrogated the epithelium-dependent increase in smooth muscle proliferation both at baseline and after injury. The silencing as observed in is transient and the MMP-9 levels start creeping back to normal after 8 days, as assessed by zymography. However, there is significant decrease in HASM proliferation at Day 8 in the silenced versus unsilenced co-culture. Interestingly, we did not detect any active MMP-9 by gelatin zymography. This observation may be due to rapid consumption; however, enzyme activity is also possible before the propeptide is cleaved using chaotropes.

The exact mechanism by which MMP-9 stimulates smooth muscle proliferation remains unclear. One possible pathway could include the activation of mitogenic signaling pathways by activating latent or matrix-bound growth factors. Alternatively, MMPs may cleave and thus directly activate GPCRs such as the protease activated receptors (PARs). GPCR agonists (e.g., thromboxane A2, prostaglandin D2) have been shown to influence HASM proliferation; however, their levels did not change significantly in our co-culture model (data not shown).

Epithelial-derived mediators must diffuse through the lamina propria to reach the airway smooth muscle. The extracellular matrix of the lamina propria contains numerous binding sites for epithelial-derived proteins including proteoglycans, fibrin, fibronectin, and collagen. These features of the diffusion pathway are not part of our in vitro model. In addition, our in vitro model does not incorporate inflammatory cells, which can migrate to the source of injury, or other stromal cells (e.g., fibroblasts), both of which are potential sources of mediators such as MMP-9. Thus, we developed an in vivo model of epithelial injury in the rabbit trachea. We mimicked the injury pattern by repeatedly denuding the epithelium over an 8-day period. We observed significant proliferation in the smooth muscle region, as indicated by increased Ki67 staining, and also increased MMP-9 levels within the lamina propria as determined by immunohistochemical staining. Our in vivo model does not allow us to identify the specific cellular source of MMP-9 (e.g., epithelium, inflammatory cells such as neutrophils, or stromal cells), or directly link epithelial release of MMP-9 to airway smooth muscle cell proliferation. In fact, the airway smooth muscle has recently been shown to be a significant source of MMP-9 in fatal asthma. Thus, our results do not allow us to quantitatively predict the contribution of epithelial-derived MMP-9 toward airway smooth muscle proliferation in vivo. Nonetheless, our in vivo observations are consistent with our in vitro results that repetitive epithelial injury can increase the release of biologically relevant levels of

MMP-9, and stimulate airway smooth muscle cell proliferation. Future studies might consider developing an in vitro model of the extracellular matrix to investigate its role in modulating the transport of epithelial-derived mediators.

Conclusion

Our results implicate the airway epithelium as a source of soluble mediators capable of stimulating airway smooth muscle cell proliferation. The soluble mediators include cytokines/chemokines IL-6, IL-8, and MCP-1 as well as the matrix metalloproteinase MMP. Our results suggest new avenues to develop therapeutic agents targeting airway smooth muscle cell proliferation via altering airway epithelial function.