AN *IN VITRO* REMODELING MODEL OF THE AIRWAY WALL

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INTRODUCTION

Recent studies have shown that mechanical stress on airway epithelial cells can induce upregulation of genes involved in airway remodeling in diseases such as asthma. For example, compressive stress on epithelial cells, in the range of that produced by smooth muscle cells, results in the upregulation of transformed growth factor-β, endothelin-1, and early growth response-1 [1] and can elicit unstressed, co-cultured fibroblasts to produce increased levels of collagen type III and fibronectin along with an increased matrix metalloproteinase-9/tissue inhibitor of metalloproteinase-1 (MMP-9/TIMP-1) ratio [2], all factors associated with remodeling. However, the relevance of these responses to airway wall remodeling is still unclear since (i) mechanotransduction is highly dependent on the environment (e.g. matrix and other cell types), and (ii) inflammatory mediators, which strongly affect remodeling, are also present in asthma. To assess the effects of mechanical stress on the airway wall in a relevant 3-D inflammatory context, we have established a tissue culture model of the human airway mucosa and submucosa that can be induced to undergo remodeling. Our model contains differentiated human bronchial epithelial cells expressing tight junctions, cilia formation, and mucus secretion atop a collagen gel embedded with human lung fibroblasts. We found that while inflammatory mediators (via activated eosinophils) drastically increased collagen production, the application of 50% strain in the same system inhibited this response, suggesting a possible protective function of strain in the inflammatory airway. In addition, the presence of these two factors in the same system resulted in a thicker epithelial layer compared to either condition alone, again suggesting that mechanical stress affects airway wall remodeling synergistically with inflammation. This integrated model more closely mimics airway wall remodeling than single-cell, conditioned media, or even 2-D co-culture systems and is relevant for examining the importance of mechanical stress on airway wall remodeling in an inflammatory environment, which may be crucial for understanding and treating pathologies such as asthma.

MATERIALS AND METHODS

The model of the airway wall was developed to facilitate the application of lateral strain. The culturing vessel has a built-in strain applicator made of porous polyethylene (PE) as depicted in Figure 1. The PE wells were filled with a suspension of human fetal lung fibroblasts (HLF) at $5x10^5$ cells/ml in 2.5 mg/ml rat tail tendon collagen. The surface of this was then covered with a thin layer of acellular collagen, and normal human bronchial epithelial cells (HBEC) were seeded on top at $2.5x10^5$ cells/cm^2. The model was cultured in submersion for one week and in air-liquid interface (ALI) for another week to allow differentiation of the epithelium. ALI was introduced by propping the model on 1.6 mm thick PE strips and submerging in media just below the epithelium. After 14 days of culture, strain and/or inflammation was imposed on the system. 50% strain was applied by removing ten spacers intermittently from the inner ring and placing them in between the inner and outer ring, while inflammation was induced with human eosinophils activated with calcium ionophore seeded on the apical surface.

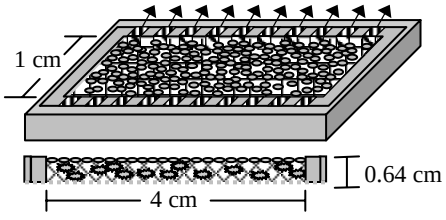

Figure 1. Schematic of the airway wall model.

RESULTS

Characterization of the Model

After one week of submerged and ten days of ALI cultures, the airway wall model was assessed for differentiation of the epithelium and cellular structures. Differentiation of the epithelial cells is apparent from (Fig. 2A) cilia growth, (Fig.2B) formation of tight junction protein, occludin, and (Fig. 2C) consistent mucus production in ALI washings of the surface over ten days. Additionally, fibroblasts are found stretched within the gel beneath the epithelium.

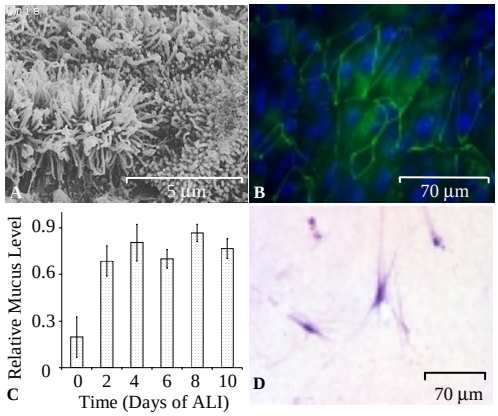

Figure 2. The airway wall model has a differentiated epithelium characterized by (A) cilia growth, (B) occludin formation, and (C) consistent mucus production. Beneath the epithelium, (D) fibroblasts are seen stretched and dispersed within the collagen matrix.

Effects of Mechanical Strain and Inflammation on the Model

Remodeling of the model was assessed for changes in structure and net collagen production after two days of stimulation. Sections of paraffin embedded models were stained with Van Gieson's and hematoxylin solutions to qualitatively view changes in structure and collagen density. In addition, net matrix production was determined by measuring [^{3}H]hydroxyproline produced from hydroxylation of [^{3}H]proline, which is a building block of fibrous ECM proteins such as collagen and elastin. [^{3}H]proline incorporation was measured using modified TCA-precipitaion methods from Swartz et al. [2]. Histological sections show that strain resulted in folds or buckling of the epithelium (Fig. 3B) when compared to a (Fig. 3A) static model. The intensity of the collagen staining appears the least in the presence of (Fig. 3C) eosinophils and retained in (Fig. 3D) strained models with eosinophils. Also, (Fig. 3D) strain in the presence of inflammation resulted in thickening of the epithelium and changes in folding pattern. Quantitative analysis of net matrix production show that models with eosinophils resulted in significant matrix synthesis while strain in the same system inhibited this process (Fig. 4).

DISCUSSION/CONCLUSION

We have developed a model of the airway wall that has physiologically relevant cellular structures and ECM components and that can allow investigations of remodeling due to mechanical stress and/or inflammatory mediators. In the model, buckling of the mucosal surface is observed with strain, introducing compressive, bending, and shear forces within the folds. In result, the deformation of the matrix directly affects the HBECs and HLFs and further communication between the two cells types facilitated. It is responsive to inflammatory and mechanical changes in its environment that appears

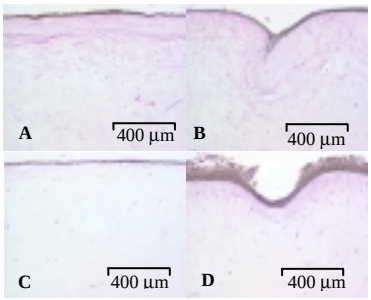

Figure 3. Van Gieson' s and hematoxylin stained sections of models under (A) static, (B) strained, (C) eosinophil added, and (D) strained with eosinophil added conditions.

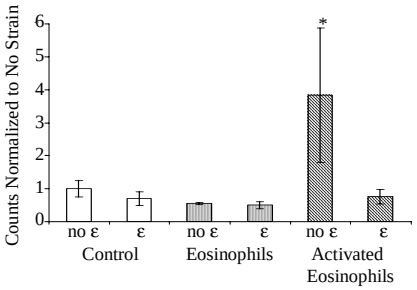

Figure 4. Net matrix synthesis (total hydroxyproline production minus degradation) was measured for static and strained MAMs with and without eosinophils. Significant net production was found in static models with activated eosinophils (*$P < 0.05$).

to work in synergy. Strain and inflammation together seem to result in structural changes that are not observed by either factor alone. Furthermore, strain appears to inhibit net matrix production in the presence of inflammation. In summary, our new model addresses the need for a more comprehensive *in vitro* model of the airway mucosa as a dynamic system and is relevant in studying remodeling associated with asthma. It is capable of supporting multiple cell types without contraction of the matrix to observe and elucidate the long-term remodeling effects of factors such as inflammation and mechanical stress.

REFERENCES

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