**Introduction**

The bronchial epithelial cell is the first cell of contact and a physical barrier to the external environment. These cells are continuously exposed to, and injured by, pollutants, allergens and viruses as part of their normal function.

- Detailed cellular examination of bronchial biopsies and BAL fluid has provided convincing evidence of epithelial damage and aberrant repair in asthma. This excessive epithelial damage and fragility can arise from an enhanced susceptibility to injury, inappropriate repair or a combination of both.
- It is therefore important to understand the regulatory mechanisms; migration, differentiation, and formation of tight junctions which are essential for normal mucosal repair.

**Specific Aim**

The aim of this study was to compare the ability of bronchial epithelial cells from asthmatic and non-asthmatic subjects to differentiate and form functional co-cultures and ALI cultures. In addition we characterized their response to wounding or respiratory syncytial virus infection.

**Methods & Materials**

- Differentiated air-liquid interface (ALI) cultures were generated from primary human bronchial epithelial cells obtained from non-transplantable lungs of normal (n=5) and asthmatic donors (n=3).
- Bronchial sections and ALI cultures generated from the same airway were analyzed by immunohistochemistry for Cytokeratin (CK) 5, p63, and ZO-1. Basal cell markers CK5, CK18, E-cadherin and p63. Specific staining was evaluated using ImagePro Plus software.
- For wounding studies ALI cultures were wounded in a crosshatch manner and repair was followed using DCI images for 96 hours post wounding. For RSV challenge ALI cultures were infected with RSV (MOI 500) for 24, 48, and 96 hours and supernatants were analyzed by ELISA.

**Results**

**Figure 1.** Asthmatic Airways In vivo and in vitro are less differentiated compared to Normal’s

**Figure 2.** Markers of epithelial differentiation and tight Junctional proteins are altered in asthmatic airways compared to normals.

**Figure 3.** IL-6 release from Normal and Asthmatic ALIs following wounding

**Figure 4.** IL-6 release from Normal and Asthmatic ALIs following RSV infection

**Figure 5.** Wound closure rates in Normal and Asthmatic ALIs

**Figure 6.** IL-6 release from Normal and Asthmatic Individuals following RSV infection

**Figure 7.** The supernatants from figure 6 were also analyzed for IL-6 release by ELISA. Values given are mean ± SEM and P<0.05 was considered statistically significant.

**Conclusions**

- The airway epithelium of asthmatics in vivo and in ALI culture demonstrated a less differentiated epithelium characterized by significantly elevated number of cells expressing the basal cell markers CK5 and p63 and decreased number of cells expressing the ciliated cell marker CK18 compared to controls.
- Asthmatic airways and ALIs also expressed less tight junctional protein ZO-1 and adherin junctional protein E-cadherin.
- When wounded Asthmatic ALI cultures take longer to repair compared to normals and released significantly elevated levels of inflammatory cytokines IL-6 and IL-8.
- In response to RSV infection, asthmatic ALIs also released greater levels of both IL-6 and IL-8 compared to controls (P<0.05).

**References**
