In vivo and *Ex vivo* Characterization of the Bronchial Epithelium from Asthmatic and Normal Individuals

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Abstract

The bronchial epithelial cell is the first cell of contact and a physical barrier to the external environment. Detailed cellular examination of bronchial biopsies and lavage fluid has provided convincing evidence of epithelial damage and aberrant repair in asthma. Excessive epithelial damage and fragility could arise from an enhanced susceptibility to injury, inappropriate repair or a combination of both. The aim of this study was to compare the ability of bronchial epithelial cells from asthmatic and non-asthmatic subjects to differentiate in ALI culture and characterize their response to wounding and RSV challenge.

Methods: 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, CK-18, ZO-1, E-cadherin and p63. Specific staining was evaluated using ImagePro Plus software. For wounding studies ALI cultures were wounded in a cross-hatch manner and repair was followed using DCI images for 96 hours post wounding. For RSV challenge ALIs were infected with RSV (MOI3) for 24, 48, and 96 hours and supernatants were analyzed by ELISA. Results: 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 CK-5 and p63 and decreased number of cells expressing the ciliated cell marker CK-18 compared to controls ($p<0.001$). Asthmatic airways and ALIs also expressed less tight-junctional protein ZO-1 and adherin junctional protein E-cadherin ($p<0.001$). When wounded Asthmatic ALI cultures were unable to repair compared to ALI cultures obtained from normal individuals and released significantly elevated release of inflammatory cytokines IL-6 and IL-8 ($P<0.05$). In response to RSV infection, asthmatic ALI cultures also released greater levels of both IL-6 and IL-8 compared to controls ($P<0.05$).

Conclusion: This parallel *in vivo* and *in vitro* study of pediatric asthmatic airways demonstrates that the airway epithelium is remodeled early on in the disease and displays inappropriate repair following challenge with wounding or viral infection. Our data suggests that the asthmatic epithelium is unable to form an appropriate mucosal immune barrier. Future work is required to determine the factor/s involved in this inappropriate airway remodeling.

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 and/or an inadequate repair response 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 junctional complexes in ALI culture. 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 immuno-histochemistry for Cytokeratin (CK) 5, CK-18, ZO-1, E-cadherin and p63. Specific staining was evaluated using ImagePro Plus software.
- For wounding studies ALI cultures were wounded in a cross-hatch manner and repair was followed using DCI images for 96 hours post wounding. For RSV challenge ALIs were infected with RSV (MOI_3) 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 Norma's

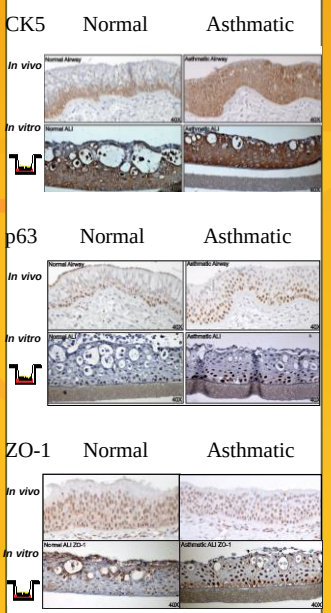

Figure 1. Bronchial sections and ALI cultures generated from the same airway from Normal and Asthmatic patients were analyzed by immuno- histochemistry for Cytokeratin (CK) 5, p63, and ZO-1.

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

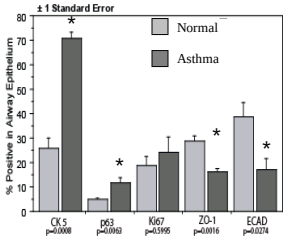

Figure 2. Entire airways of normal (n=5) and asthmatic patients were analyzed for specific staining for Cytokeratin (CK) 5, p63, Ki67, ZO-1 and E-cadherin. Values given are the mean ± SEM and P<0.05 was considered statistically significant.

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

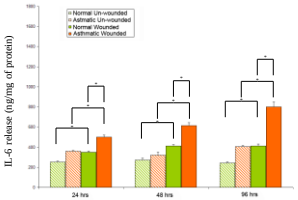

Figure 3. Normal and Asthmatic ALIs were wounded in a cross hatch manner and supernatant was removed and ALIs fixed at 24, 48, and 96 hrs. Supernatants were analyzed for IL-6 release by ELISA. Values given are mean ± SEM and P<0.05 was considered statistically significant.

Figure 4. IL-8 Release from Normal and Asthmatic ALIs following wounding

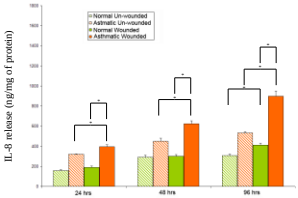

Figure 4. The supernatants from figure 3 were also analyzed for IL-8 release by ELISA. Values given are mean ± SEM and P<0.05 was considered statistically significant.

Figure 5. Wound closure rates in Normal and Asthmatic ALIs

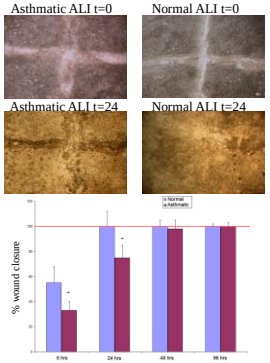

Figure 5. Normal (n=5) and asthmatic ALIs were wounding in a cross hatch manner and repair was followed using DCI images for 6, 24, 48, and 96 hrs post wounding. Values given are the mean ± SEM and P<0.05 was considered statistically significant.

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

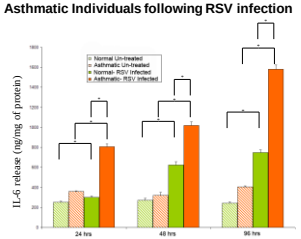

Figure 6. Normal and Asthmatic ALIs were exposed to RSV (MOI_3) or basal media for 24, 48 and 96 hrs following which supernatant was removed and ALIs fixed. Values are the mean ± SEM and P<0.05 was considered statistically significant.

Figure 7. IL-8 release from Normal and Asthmatic Individuals following RSV infection

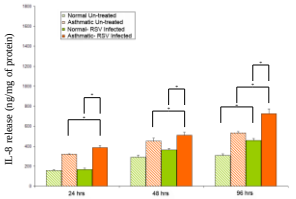

Figure 7. The supernatants from figure 6 were also analyzed for IL-8 release by ELISA. Values given are mean ± SEM and P<0.05 was considered statistically significant.

Summary

- **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 CK-5 and p63 and decreased number of cells expressing the ciliated cell marker CK-18 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 ALI cultures also released greater levels of both IL-6 and IL-8 compared to controls.**

Conclusions

This parallel *in vivo* and *in vitro* study of pediatric asthmatic airways demonstrates that the airway epithelium is remodeled early on in the disease and displays inappropriate repair following challenge with wounding or viral infection. Our data suggests that the asthmatic epithelium is unable to form an appropriate mucosal immune barrier. Future work is therefore required to determine the mechanisms involved in this inappropriate airway remodeling.

References

1. Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Panizzolo C, Zanin ME, Zuin R, Maestrelli P, Fabbri LM, Saetta M. Epithelial damage and angiogenesis in the airways of children with asthma. Am J Respir Crit Care Med. 2006 Nov 1;174(9):975-81.

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