The Role of Apical Junctional Proteins in Epithelial Repair in Asthmatic Patients

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Abstract

Rational: In normal airway epithelium the apical junction complex (AJC) forms a barrier against inhaled environmental insults. However little is known about the role of the AJC in regulating airway epithelial functions and in the setting of asthma.

Methods: Airway sections and air-liquid interface epithelial cultures (ALI-AECs) from normal (n=7) and asthmatic (n=8) patients were embedded for transmission electron microscopy (TEM) or analysed by immunoblot or immunohistochemistry for expression of the AJC proteins, E-cadherin and Zonular Occludin-1 (ZO-1). ALI-AECs were treated for 1, 4, 6, 24 and 48 h with RSV (MOI of 3 pfu/cell) or vehicle control and trans-epithelial resistance (TEpR) measured before ALI-AECs were fixed and immunostained for E-cadherin and ZO-1.

Results: Using TEM, sections of asthmatic airway sections and ALI-AECs demonstrate decreased numbers of adhesion junctions within the AJC compared to non-asthmatics ($P<0.05$). This observation was confirmed by immunoblot and immunohistochemistry which demonstrated decreased expression of E-cadherin ($P<0.05$). When ALI-AECs were treated with RSV, asthmatic ALI-AECs showed decreased TEpR compared to non-asthmatic ALI-AECs which correlated with decreased E-cadherin expression. E-cadherin is a tumour suppressor protein and has been proposed to mediate contact inhibition of cell growth upon homophilic binding. Interestingly, *In vitro* asthmatic epithelial cells demonstrate elevated doubling times but conversely, take longer to repair scratch wounds than epithelial cells obtained from non-asthmatics.

Conclusion: These data suggest that aberrant expression of AJC proteins such as E-cadherin may have important roles in epithelial repair following environmental challenges.

Introduction

Although asthma is an inflammatory disorder of the conducting airways involving TH2-type T cells, there is increasing evidence that the airway epithelium plays an important role in orchestrating the inflammatory response following interacting with multiple environmental factors (1).

In normal airway epithelium the apical junction complex (AJC) forms a barrier against the external environment which includes aero allergens, viruses and particulate matter.

In asthma, part of the abnormal response of the airway epithelium is due to impaired barrier function caused by primary disruption of epithelial AJCs. This disordered epithelial function allows inhaled substances to pass more easily into the airway wall to interact with immune and inflammatory cells, which may also account for asthmatic susceptibility to air pollution and respiratory virus infection (2-3).

AJC formation has recently been shown to influences other aspects of epithelial function such as epithelial proliferation and differentiation, however currently nothing is known about the role of AJCs with regards to these functions in the asthmatic airways (4-5).

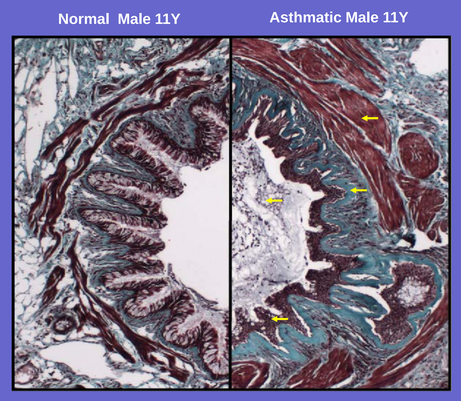

Figure 1. Representation airway sections from a 11 year old non-asthmatic male donor (left panel) and age matched male fatal asthmatic (right panel), stained with masons trichrome. Yellow arrows indicate the remodeling features associated with the disease which include increased smooth muscle mass, sub-epithelial fibrosis and thickening of the basement membrane, mucus plugging and infiltration of inflammatory cells and dysplasia of the airway epithelium.

Hypothesis

Aberrant expression of AJCs within the asthmatic epithelium leads to ineffective repair and mucosal immune barrier function following exposure to noxious environmental stimuli.

Table 1: Donor Cohort

Patient ID	Gender	Age (yrs)	Disease	Medication	Cell Source
aN1	Male	20	None	None	Donor Lung
aN2	Male	21	None	None	Donor Lung
aN3	Female	22	None	None	Donor Lung
aN4	Male	18	None	None	Donor Lung
aN5	Male	24	None	None	Donor Lung
pN6	Female	4	None	None	Donor Lung
pN7	Male	14	None	None	Donor Lung
pA1	Female	8	Asthma	Albuterol, Singulair	Donor Lung
pA2	Male	11	Asthma	Albuterol	Donor Lung
aA3	Female	21	Asthma	Albuterol, Advair	Donor Lung
pA4	Female	15	Asthma	Albuterol, Advair	Donor Lung
pA5	Male	14	Asthma	Albuterol	Donor Lung

Table 1. Patients are identified as adult (a) or pediatric (p) and by disease status denoted as either none (N) or asthmatic (A).

Methods

Airway epithelial cell isolation : Airway epithelial cells (AEC) were obtained from de-identified human lungs from asthmatic and non-asthmatic normal donors not suitable for transplantation and donated for medical research were obtained through the International Institute for the Advancement of Medicine (Edison, NJ). From the transplant tissue, AEC were isolated by protease digestion as previously described (6). The study was approved by the relevant ethics committees within each institution and the clinically relevant information for each of the subjects is listed in table1.

AEC culture: ALI-AECs were maintained in bronchial epithelial growth media (BEGM, Cambrex), containing 100u/ml penicillin and 100ug/ml streptomycin, at 37C in a Humidified 5% CO2 atmosphere. Cells were incubated with either RSV (MOI of 3 pfu/cell) or vehicle control 96hr and trans-epithelial resistance measured at 1, 4, 6, 24, 48 and 96 h. Following which ALI-AEC cultures were used for immunohistochemical analysis.

SDS-PAGE and Immunoblot: AEC monolayers and ALI-AEC cultures were lysed in protein extraction buffer with protease inhibitors. Equal concentrations of protein lysates were resolved by SDS-PAGE, transferred to nitrocellulose membranes and analyzed for E-cadherin and ZO-1.

Transmission Electron Microscopy: ALI-AEC and donor matched airways were fixed in glutaldehyde and processed for TEM and scanned using a Tecnai 12 Transmission electron microscope (FEL Inc.)

Immunohistochemical staining: ALI-AEC cultures and donor matched airways were fixed in paraformaldehyde, embedded in paraffin, stained for E-cadherin and visualized using the DAB from DAKO. Sections were analyzed using ImagePro Software.

Trans-epithelial resistance (TEpR): Was measured using an ohmometer (EVOM, World precision instruments, FL).

Results

Figure 2. Expression of E-cadherin is decreased in asthmatic airway epithelial cells *In vivo*

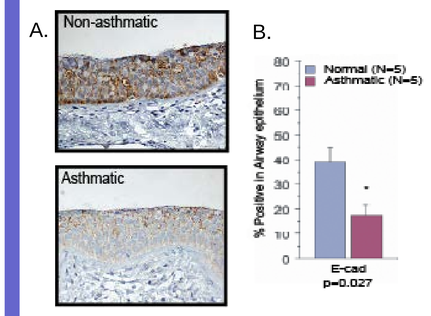

Figure 2. Expression of E-cadherin is decreased in asthmatic airway epithelial cells *n vivo*. (A) Representative airway sections for non-asthmatic and asthmatic airways stained for E-cadherin. (B) Image pro analysis of E-cadherin staining in airway sections from non-asthmatic (blue, n=5) and asthmatics donors (pink, n=5). Values given are the mean±SD, * $P<0.05$.

Figure 3. Expression of E-cadherin and ZO-1 is decreased in asthmatic airway epithelial cells *In vitro*

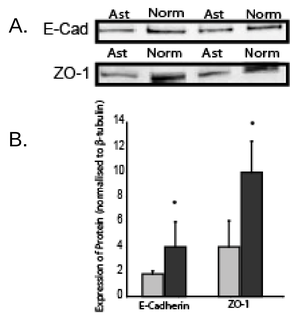

Figure 3. Expression of E-cadherin and ZO-1 is decreased in asthmatic airway epithelial cells *In vitro*. (A) Representative immunoblots probed for E-cadherin and ZO-1 in epithelial cells lysates derived from asthmatic and non-asthmatic individuals. (B) Densitometry analysis of immuno blots for E-cadherin and ZO-1 in airway epithelial cell lysates derived from asthmatic (grey, n=5) and non-asthmatic donors (dark grey, n=5).

Figure 4. Adhesion Junctions are decreased in asthmatic airways

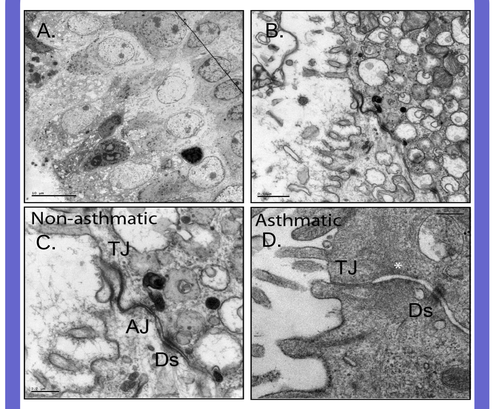

Figure 4. Adhesion Junctions are decreased in asthmatic airways. (A-B) Representative low magnification images obtained by TEM, demonstrating a stratified airway epithelium from a non-asthmatic patient. (C-D) TEM sections of airway epithelial cells within the airways of a non-asthmatic and asthmatic patients. TEM micrographs demonstrate both tight junctions (TJ) and desmosome junctions but no adherens junction (AJ, *) between epithelial cells in the asthmatic airway.

Figure 5. RSV infection decreases TEpR in differentiated airway epithelial cultures *In vitro*

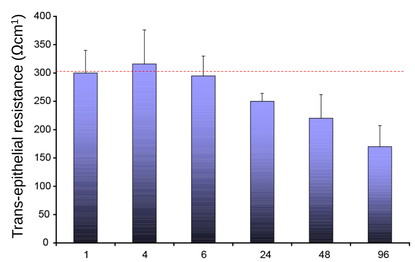

Figure 4. RSV infection decreases TEpR in differentiated airway epithelial cultures *In vitro*. Trans-epithelial resistance (RT) in air-liquid interface epithelial cultures treated with respiratory syncytial virus (MOI of 3 pfu/cell) over 96h incubation (blue bars n=3) compared to controls (red dashed line). Data given are the mean ±SD.

Figure 6. RSV infection decreases expression of E-cadherin in differentiated airway epithelial cultures *In vitro*

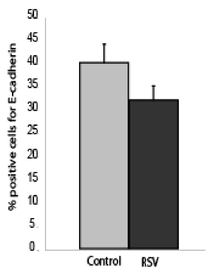

Figure 6. RSV infection decreases expression of E-cadherin in differentiated airway epithelial cultures *In vitro*. Differentiated ALI-AEC cultures derived from non-asthmatic patients were incubated with RSV (MOI of 3 pfu/cell), fixed with paraformaldehyde, embedded and then stained by immunohistochemistry for E-cadherin. Image pro analysis of air-liquid interface epithelial culture sections (n=3) demonstrated that following RSV exposure the number of cells positive for E-cadherin decreased. Data given are the mean ±SD.

Figure 7. Asthmatic airway epithelial cells *In vitro* have elevated rates of proliferation

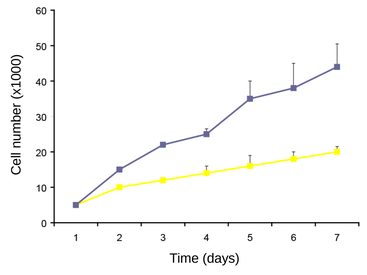

Figure 7. Asthmatic airway epithelial cells *In vitro* have elevated rates of proliferation. Doubling times expressed as cell number, of airway epithelial monolayer cultures derived from asthmatic (blue, n=5) and non-asthmatic (yellow, n=5) donors. Date given are the mean ± SD

Figure 8. Asthmatic airway epithelial cells *In vitro* do not repair following mechanical damage

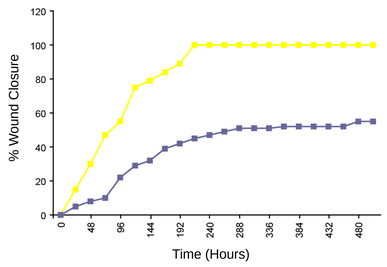

Figure 8. Asthmatic airway epithelial cell *In vitro* do not repair following mechanical damage. Wound closure rates following a mechanical scratch wound assay in airway epithelial cells derived from asthmatic (blue, n=5) and non-asthmatics (yellow, n=5) donors. Data given are the mean ± SD.

Figure 9. Asthmatic airways contain increased numbers of basal cells which only form demosomal junctions

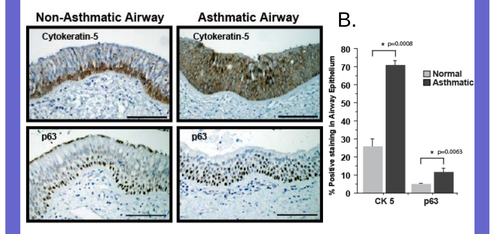

Figure 9. Asthmatic airways contain increased numbers of basal cells which only form demosomal junctions. Airway sections were immuno stained for cytokeratin-5 and p63 (brown staining) in airway epithelium of donor matched non-asthmatic and asthmatic airways. (B) The expression of cytokeratin-5 and p63 within the epithelium was quantified using Image pro plus software. Scale bar is equal to 100 µM. Data is expressed as % of positive staining in airway epithelium ±SEM. * indicates $P<0.01$ compared to % positive staining in non-asthmatic airways (n=12).

Summary

- We demonstrate that asthmatic airway epithelial cells *In vivo* and *In vitro* have decreased expression of adherens junction protein E-cadherin. Additionally that loss of this integral protein potentially leads to the lack of adherens junctions in asthmatic airway epithelium determined by TEM.
- We also show that environmental insults such as RSV infection decrease expression of E-cadherin in airway epithelial cells derived form non-asthmatic patients, leading to decreased resistance in differentiated epithelial cultures.
- Importantly, we demonstrate that asthmatic airway epithelial cells *In vitro* have elevated doubling times but, conversely are unable to repair mechanically induced scratch wounds. However future studies are required to determine if E-cadherin expression could be responsible for these changes in cellular responses which are important in normal epithelial repair.
- Finally, we demonstrate that in airway sections, the expression of basal cell markers cytokeratin-5 and p63 are increased in asthmatic airways, demonstrating that the cell phenotype is less differentiated in asthmatic airway epithelium *in vivo*. As basal cells primarily only form desmosomal contacts this alteration in epithelial phenotype could potentially provide a structural mechanism for the decreased expression of E-cadherin observed within the asthmatic airways.

Conclusions

These data suggest that aberrant expression of AJC proteins such as E-cadherin may have important roles in epithelial repair following environmental challenges.

References

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None of the authors of this abstract presentation have any financial relationships to disclose