# HUMAN RHINOVIRUS AND ASTHMATIC EPITHELIUM - CHANGES IN BARRIER INTEGRITY AND FUNCTION

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## Introduction

Fully differentiated airway epithelial cells (AECs) derived from adults with asthma have been shown to exhibit regions of compromised tight junction (TJ) integrity. This can result in infiltration of bacteria, macromolecules and respiratory viruses down to the basal epithelium, resulting in an immune response and ultimately inflammation. However, it remains unclear whether this is intrinsic to the asthmatic epithelium, or a consequence of asthma-specific stimuli. There are many known respiratory viral contributors to asthma exacerbations, of which human rhinovirus (HRV) is known to be a major initiator in children. Although it has been demonstrated that children with asthma have a decreased capacity for epithelial recovery following viral infection, namely in barrier integrity restitution, there remains a paucity of information as to whether these observations are directly related to the changes in TJ profile expression. Thus, we hypothesized that HRV would incite changes in TJ, resulting in reduced barrier integrity and altered permeability to differently sized molecules in children with asthma.

## Methods

AECs from children with and without asthma were investigated in this study (n=38 asthmatic; n=96 non-asthmatic). Cells were derived from tracheal brushings of patients identified as free from bacterial or viral infections, and were undergoing elective surgery for non-respiratory related conditions. Cells were cultured, fully differentiated and subsequently infected with human rhinovirus minor serotype 1B (HRV-1B) for 48hr. Assessment of mRNA and protein expression for claudin-1, occludin and ZO-1 was performed via qPCR and in-cell western respectively, and confocal microscopy performed to visualise TJ relative to infection sites. Structured Illumination Microscopy (SIM) was also performed on primary AEC cultures from children with and without asthma to investigate TJs expression and localization.

## Results

Basal gene expression of claudin-1 and occludin (1.4 and 2.6 fold respectively) was significantly higher in AECs in children with asthma compared to their non-asthmatic counterparts. In contrast, protein expression was significantly lower for claudin-1, occludin and ZO-1. Following HRV infection, a significantly sustained decrease in TJ protein expression was observed in both asthmatic and non-asthmatic AECs for occludin and ZO-1, but a recovery to basal levels by 48hr was observed only in non-asthmatic AECs. Visualisation of TJ staining at baseline, showed ZO-1 and occludin as continuous belt-like structures around the cell perimeter for control samples. Following viral infection, TJ complex prevalence was lower in asthmatic samples than in non-asthmatic samples.

## Discussion

In this study, we found evidence that gene expression in asthmatic epithelial cultures was higher for all TJ proteins was than non-asthmatic cultures, however the protein expression for these cultures was lower. This suggests a possible post translation regulation of TJs in asthmatic patients, leading to

reduced barrier integrity. Furthermore, we were able to demonstrate that epithelial barrier integrity is intrinsically different in asthmatic AECs compared to non-asthmatics, suggestive of impaired barrier function. Following HRV infection, we observed a sustained reduction in barrier integrity in AECs of children with asthma. This compromised barrier would facilitate the movement of pathogens across the epithelium, increasing inflammation and prolonging exacerbations in paediatric asthma.

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