healed relative to the control (30.88 ± 4.13%, n = 8)18 h post-wound. Similarly N/OFQ significantly promoted HASM wound closure ($p < 0.01$). Our findings showed that SCF-stimulated IL-8 release (4.43 ± 0.69 fold over control, $p < 0.01$,n = 7) was significantly inhibited by N/OFQ (3.32 ± 0.56 fold over control, $p < 0.01$, n = 7). *Ex vivo* human studies demonstrate significantly ($p < 0.01$) increased endogenous N/OFQ in asthmatic airways relative (sputum N/OFQ:59.02 ± 2.57 pg/ml,n = 26) to healthy airways (sputum N/OFQ:44.69 ± 0.43,n = 10) and identifies eosinophils as a potential source for these. Pre-treatment with N/OFQ was shown to significantly reduce agonist-induced bronchial hyper-responsiveness using *in vitro* ($p < 0.01$) and *in vivo* models ($p < 0.001$). *Ex vivo* animal studies show that N/OFQ significantly inhibits release of inflammatory mediators including IL4, IL5, IL12, IL13 and inflammatory cell recruitment including mast cells and eosinophils within the airways. Further mucus hyper-secretion was also reduced following N/OFQ pre-treatment in these models.

This is the first study to perform a comprehensive and complementary *in vivo* and *in vitro* study of the expression and actions of the N/OFQ-NOP system in the airways and provide evidence for a role of NOP activation in the management of asthma.

S19 INCREASED CRTH2 EXPRESSION IN ASTHMATIC BRONCHIAL EPITHELIUM

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Introduction and Objectives Prostaglandin-D2 (PGD2) mediates chemotaxis of Th2 cells, basophils and eosinophils through the chemoattractant receptor homologous-molecule expressed on T-helper-type-2 cells (CRTh2). Pulmonary PGD2 levels are increased in patients with more severe asthma and higher levels of Th2 inflammation. CRTh2 antagonists, designed to block PDG2 signalling, are in clinical development for the treatment of asthma. We investigated whether pulmonary CRTh2 expression is upregulated in patients with asthma compared to controls; we focused on the bronchial epithelium and cells within the submucosa.

Methods Bronchial biopsies were obtained from asthmatic patients (n = 20) and healthy subjects (n = 10). Sections were probed with an anti-human CRTh2 antibody, a secondary biotinylated goat anti-rabbit antibody and the position of the CRTh2 receptors were visualised with 3,3'-Diaminobenzidine (DAB) and counter-staining with haemotoxylin. Digital micrographs were taken and analysed using the ImageProPlus 6.0 software. The percentage positive area of epithelium was calculated along with the intensity of staining. These values were multiplied together to give an overall immunohistochemical (IHC) score for each subject. The number of positive cells/mm^2 within the submucosa was also counted.

Results CRTh2 expression was predominantly within the epithelium rather than the submucosa. There was a statistically significant (p = 0.04) increase the immunohistochemical score in the epithelium of asthmatics (74.7) compared to healthy non-smokers (38.8). There was no significant difference (p = 0.64) in the number of positive cells expressing CRTh2 within the submucosa between asthma patients (473/mm^2) and healthy controls (353/mm^2).

Conclusion CRTh2 expression is upregulated in the epithelium of asthmatic patients compared to healthy controls. This is a novel finding, suggesting that CRTh2 antagonists may exert therapeutic effects on the bronchial epithelium as well as blocking inflammatory cell chemotaxis in asthma.

Abstract S19 Table 1.

	% +ve Area of Epithelium	Intensity of Epithelial Staining	Overall IHC Score	% Positive Cells in Submucosa/mm2
Asthma	30.3* (12.6)	2.3 (4.4)	74.7* (45.9)	473.1 (509.0)
Healthy	16.6 (16.3)	1.7 (4.1)	38.8 (47.7)	353.3 (296.0

*Statistical analysis by unpaired T-test between Asthma and Healthy controls *p<0.05.*

S20 CRTH2 IS EXPRESSED BY THE BRONCHIAL EPITHELIUM AND ITS ACTIVATION DRIVES EPITHELIAL DIFFERENTIATION

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Background The chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTh2) is implicated in the pathogenesis of asthma, but its expression in the bronchial epithelium and potential role in airway remodelling is unknown.

Methods CRTh2 protein expression was assessed in bronchial biopsies (n = 24) and primary epithelial cells (n = 16) using immunohistochemistry, and using flow cytometry, immunofluorescence, and quantitative RT-PCR (QT-PCR) respectively. The effects of 13, 14-dihydro-15-keto Prostaglandin D2 (DK-PGD2) on epithelial cell migration and differentiation was determined.

Results The number of submucosal CRTh2 positive inflammatory cells was increased in asthma compared to healthy controls 27.57 per mm^2 sub-mucosal area (9.82) versus 48.17 (14.04) (p = 0.0049). CRTh2 expression was identified on normal and asthmatic epithelial cells, but its expression was decreased in bronchial biopsies from asthmatics 21.43 per 10mm^2 epithelial area (7.85) versus healthy controls 62.34 (36.41) (p = 0.0071) and similar findings were observed in primary epithelial cells. Squamous metaplasia of the bronchial epithelium was increased in asthma and related to decreased CRTh2 expression. DK-PGD2 promoted epithelial cell migration 12-fold increase (p = <0.0001) and in air-liquid interface cultures increased the number of MUC5AC$^+$ and involucrin$^+$ cells, which were blocked with a CRTh2 specific antagonist.

Conclusions CRTh2 is expressed by the bronchial epithelium and its activation drives epithelial differentiation suggesting that in addition to its well characterised role on inflammatory cell migration CRTh2 might contribute to airway remodelling in asthma.

S21 TYPE-2 INNATE LYMPHOID CELLS INDUCE CD4 T HELPER CELL TYPE-2 IMMUNE FUNCTIONS

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Introduction Type-2 innate lymphoid cells (ILC2) are a novel subset of immune cells characterised by their responsiveness to