Results Fifty two patients completed the BCKQ questionnaire: 36 conventional PR programme [20 male, MRC 3 (IQR 2–4), age 67 (± 8.5) years, BMI 30 (± 6.6) kg/m², FEV₁ (% predicted) 53 (± 21), pre ISWT 280 m (± 163), pre HADS anxiety 6.6 (± 4.8), pre PRAISE 45 (± 8.4)] and 16 online [14 male, MRC 3 (IQR 2–4), age 67 (± 7.4) years, BMI 25 (± 5.0) kg/m², FEV₁ (% predicted) 47 (± 27), pre ISWT 365 m (± 201), pre HADS anxiety 7.5 (± 5.1), pre PRAISE 49 (± 7.9)]. There were no significant differences in baseline characteristics. A statistically significant difference was seen in knowledge within each group following either the conventional PR programme (change=5 points, $p \leq 0.001$) or the web programme (change=11 points, $p \leq 0.001$). The change in scores between the groups was also significantly different ($p \leq 0.01$) in favour of the web-based programme (Table 1).

Discussion Patients are able to gain improvements in knowledge around their condition using a website programme as an alternative to the traditional spoken sessions in a PR programme.

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Abstract S84 Table 1 Between group changes of the Bristol COPD knowledge questionnaire following either conventional PR or a web-based programme

	WEB	PR	Difference Between groups (p value)
Pre BCKQ	35 $\pm$ 10	38 $\pm$ 10	0.3
Post BCKQ	46 $\pm$ 6	43 $\pm$ 7	0.2
Change in BCKQ	11 $\pm$ 8	5 $\pm$ 7	0.009*

* $p \leq 0.01$

Mechanisms of asthma

S85 CORTICOSTEROID-RESISTANT NEUTROPHILIC AIRWAY INFLAMMATION AND HYPERRESPONSIVENESS CAUSED BY IL-13

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Rationale Severe corticosteroid refractory asthma is a significant unmet medical need. It accounts for 10% of the asthma population and 50% of the health economic burden. Recent understanding of asthma heterogeneity has evolved beyond clinical characteristics, allowing definition of distinct disease phenotypes such as those defined by levels of Type 2 inflammation (Type-2 high 'eosinophilic' disease and Type-2 low 'neutrophilic' disease). However, a recent study using dupilumab (an antibody that blocks the common IL-4 and IL-13 receptor chain, IL-4R α) as an add-on therapy in adults with uncontrolled persistent asthma showed efficacy irrespective of baseline eosinophil count (Wenzel *et al.*, Lancet 2016). The

aim of this work was to use IL-13 transgenic mice to test the hypothesis that a subset of IL-13 mediated airway responses are corticosteroid-unresponsive and contribute to ongoing airways symptoms.

Methods IL-13 expression in the lungs was induced using Doxycycline (DOX) in *Ccsp-rtTA/Otet-IL-13* double-transgenic (*Ccsp/IL-13*) mice. Littermate control single transgenic mice also received DOX. Where indicated, mice received daily intra-peritoneal injections of 3 mg/kg Dexamethasone (Dex) for 3–7 days and control mice received saline. Methacholine challenge and lung function measurements were performed and lungs harvested for mRNA analysis and immunohistochemistry (IHC). BALF was obtained for ELISA and differential cell counts.

Results Compared to controls, *Ccsp/IL-13* mice showed significantly increased airway hyperresponsiveness (AHR) to methacholine and IHC revealed increased bronchial smooth muscle and goblet cell metaplasia. The BALF of these mice contained mixed eosinophilic and neutrophilic inflammation, but neutrophils predominated. Characteristic Th2-responsive genes (*IL-13*, *Eotaxin*, *Muc5ac*, *Periostin* and *SerpineB2*) as well as genes more characteristic of Th17 responses (*Cxcl1/Kc*, *Cxcl2* and *Csf3*) were significantly elevated. Treatment with Dex did not abrogate AHR, even though eosinophilia and the 'Th2' gene signature were significantly reduced. However, neutrophils and the 'Th17' signature remained elevated.

Conclusion Although IL-13 promotes eosinophilic airways disease, it can also drive corticosteroid refractory inflammation characterised by persistent neutrophilia, Th17 cytokines and maintenance of AHR. These findings may help explain the beneficial effect of dupilumab in uncontrolled asthma. The *Ccsp/IL-13* mouse may be a useful model for dissecting the molecular pathways and mechanisms associated with predominant neutrophilic, corticosteroid refractory disease.

S86 EXTRACELLULAR MATRIX DEPOSITED BY ASTHMATIC HUMAN AIRWAY SMOOTH MUSCLE CELLS ENHANCES BASAL ACTIVATION OF TGF β

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TGF β is a widely distributed, pleiotropic cytokine implicated in tissue remodelling in many diseases including severe asthma. It is secreted in a latent form that requires activation for function. Mechanotransduction of intracellular forces through cell surface integrins can lead to TGF β activation. We have previously shown that human airway smooth muscle (HASM) cells can activate TGF β via $\alpha v \beta 5$ integrins. In the present study we have investigated the effect of extracellular matrix (ECM) on basal TGF β activation in primary asthmatic and non-asthmatic HASM cells. We assessed basal TGF β activation in non-asthmatic (n=9) and asthmatic (n=7) HASM cells. TGF β activation was assessed using a TGF β reporter cell assay and a phosphorylated Smad2 ELISA. Cell contractility was assessed using a collagen gel contraction assay and traction force microscopy. ECM was isolated from HASM cells and cross-over experiments performed where non-asthmatic cells were cultured on asthmatic ECM and vice-versa then TGF β activity determined. Expression of ECM crosslinking enzymes was

determined at the mRNA and protein levels. Finally, expression of LOXL2 was determined in an *Aspergillus fumigatus* model of asthma. Asthmatic HASM cells activated 3-fold higher levels of TGF β basally than non-asthmatic cells ($p < 0.01$). Both diseased and control HASM cells increased TGF β activation in response to methacholine confirming our previous data (Tatler et al 2011). A collagen gel contraction assay demonstrated that asthmatic HASM cells were hypercontractile compared with non-asthmatic cells under basal conditions ($p < 0.05$) and that contractility weakly correlated with amount of TGF β activated ($p < 0.05$). Importantly, culturing non-asthmatic HASM cells on asthmatic ECM led to increased TGF β activation ($p < 0.05$) and culturing asthmatic HASM cells on non-asthmatic ECM decreased TGF β activation ($p < 0.05$). mRNA Expression of the ECM crosslinking enzymes LOXL2 and LOXL3 was significantly increased in asthmatic HASMs ($p < 0.05$). Finally, LOXL2 protein was increased in asthmatic HASMs cells, and increased in the airway smooth muscle layer of animals challenged repeatedly with *Asp. f* compared with control challenged animals. In conclusion HASM cells derived from asthmatic patients exhibit enhanced activation of TGF β compared with non-asthmatic HASM cells. This may be driven by the diseased ECM since asthmatic HASMs cells exhibit aberrant expression of ECM crosslinking enzymes.

S87

REGULATION OF TYPE 2 CYTOKINE RELEASE BY EPITHELIAL CELLS: CHARACTERISATION OF SOLUBLE FACTOR PATHWAYS AND CHARACTERISATION OF POTENTIAL MEDIATORS

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Introduction Type 2 cytokines such as IL-13, IL-4 and IL-5 have been shown to play important roles in the pathogenesis of asthma. One source of these cytokines is type 2 CD4⁺ or CD8⁺ T cells. We have shown that epithelial cells have inhibitory effects on these T cells and that this regulatory effect could be defective in asthma. We have previously shown that type 2 T cell lines release less IL-13 in the presence of epithelial cells and others have shown that epithelial cells are able to reduce division of CD4⁺ T cells. We wished to extend these studies to determine whether bulk cultures of PBMC were able to release IL-13 and whether this IL-13 was regulated by epithelial cells and whether this was mediated by direct cell contact.

Methods We used PBMC from healthy donors and cultured cells in the presence and absence of epithelial cells with titrated doses of IL-2. We used transwells and epithelial cell supernatants to determine whether supernatants were also able to reduce type 2 cytokine secretion. We used size exclusion centrifugation to split supernatants into different fractions.

Results After culture of PBMC for 5 days in IL-2, IL-13 release (pg/10⁶ cells $\pm$ SD) was 509.95 $\pm$ 84.95 and was reduced to 37.3 $\pm$ 7.4 by A549 epithelial cells separated by a transwell. Titration of A549 cells established that inhibition was cell number dependent. Inhibition was not due to scavenging of IL-13 by epithelial cells during co-culture. Less IL-

13 was secreted by IL-2 treated PBMC (pg/10⁶ cells $\pm$ SD) in the presence of 50% v/v supernatant from healthy HBEC 49 $\pm$ 7 or asthma HBEC 90 $\pm$ 21 $p=0.0023$. IL-5: HHBEC 14 $\pm$ 5 AHBEC 26 $\pm$ 12 not significant. Splitting the HBEC supernatant into different size fractions showed that the fraction over 3 kD was less inhibitory than the fraction under 3 kD.

Conclusion There may be a soluble mediator secreted by epithelial cells that is less than 3 kDa in mass that is able to inhibit type 2 cytokine release from PBMC. These inhibitory factor(s) could contribute to the regulation of type 2 cells that and affect asthma pathogenesis.

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MICRORNAS REGULATE GENOME-WIDE TRANSLATION IN SEVERE ASTHMA BRONCHIAL EPITHELIAL CELLS AS REVEALED BY FRAC-SEQ

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Severe asthma represents a significant unmet clinical need and the molecular basis for disease persistence remains inadequately understood. Bronchial epithelial cells, at the interface of environment/tissue, are central to asthma pathogenesis. There is thus a need to evaluate genome-wide changes between health and asthma to better understand the molecular mechanisms underlying disease. The vast majority of genome-wide measurements have focused on determining changes at the DNA or mRNA levels, with little attention paid to how and which mRNAs are actually translated into protein. This may not disclose changes happening at the protein level, since mRNA and protein expression correlate poorly. To determine translation and its regulation in bronchial epithelial cells in severe asthma patients we analysed paired genome-wide expression of *transcriptional* (cytoplasmic) and *translational* (polyribosome-bound) mRNA levels employing *frac-seq* (sub-cellular fractionation and RNA-sequencing) in primary bronchoepithelium in health and severe asthma patients. We also integrated those data with genome-wide profiling of microRNAs to understand their role in gene expression and impact on the pathophysiology of severe asthma bronchial epithelium. We found both genes (=all isoforms of a gene) and mRNA isoforms differentially expressed in severe asthma airways cells, with dysregulated *transcriptional* mRNA levels (194 genes) showing little overlap with dysregulated *translational* mRNA (243 genes) expression. We determined novel inflammatory and remodelling pathophysiological mechanisms disclosed solely by polyribosome-bound mRNAs, centred in epithelium remodelling and repair pathways. We also reveal six dysregulated microRNAs accounting for ~90% of all cellular microRNA targeting, displaying preferential targeting of ~50% of mRNAs undergoing translation in severe asthma airways cells. Thus, microRNAs in human severe asthma are major regulators of translation in airways epithelium and offer potential as future therapeutic targets.