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 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<0.001) or the web programme (change=11 points, p<0.001). The change in scores between the groups was also significantly different (p<0.001) 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.

REFERENCE

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

<table>
<thead>
<tr>
<th>WEB</th>
<th>PR</th>
<th>Difference Between groups (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre BCKQ</td>
<td>35±10</td>
<td>38±10</td>
</tr>
<tr>
<td>Post BCKQ</td>
<td>46±6</td>
<td>43±7</td>
</tr>
<tr>
<td>Change in BCKQ</td>
<td>11±8</td>
<td>5±7</td>
</tr>
</tbody>
</table>

*p<0.01

Mechanisms of asthma

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-rTα/OrtIl-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 (Cxcl1/Kc, Cxcl2, Eotaxin, Muc5ac, Periostin and SerpinB2) as well as genes more characteristic of Th17 responses (Cxcl11/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.

EXTRACELLULAR MATRIX DEPOSITED BY ASTHMATIC HUMAN AIRWAY SMOOTH MUSCLE CELLS ENHANCES BASAL ACTIVATION OF TGFβ

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TGFβ 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 TGFβ activation. We have previously shown that human airway smooth muscle (HASM) cells can activate TGFβ via αvβ5 integrins. In the present study we have investigated the effect of extracellular matrix (ECM) on basal TGFβ activation in primary asthmatic and non-asthmatic HASM cells. We assessed basal TGFβ activation in non-asthmatic (n=9) and asthmatic (n=7) HASM cells. TGFβ activation was assessed using a TGFβ 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 TGFβ activity determined. Expression of ECM crosslinking enzymes was...
REGULATION OF TYPE 2 CYTOKINE RELEASE BY MICRORNAS REGULATE GENOME-WIDE TRANSLATION

Thorax was cell number dependent. Inhibition was not due to scavenging by epithelial cells and whether this was mediated by direct cell contact.

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+/−SD) was 509.95+/−84.95 and was reduced to 37.3+/−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+/−SD) in the presence of 50% v/v supernatant from healthy HBEC 49 +/-7 or asthma HBEC 90 +/-21 p=0.0023. IL-5: HBECB 14 +/-5 AHBEC 26 +/-12 not significant. Splitting the HBE 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 factors of translation in airways epithelium and offer potential as future therapeutic targets.

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 (subcellular 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.