Hypothesis

We hypothesised that bronchial epithelial cells from normal children at an air-liquid interface for 28 days. We enumerated the presence of goblet and ciliated cells at the end of the experimental period.

Results

IL-9 stimulation alone did not alter goblet cell numbers in differentiating cultures from normal or asthmatic children. IL-9 stimulated PBECs from normal children had reduced ciliated cell numbers and increased MUC5AC secretion at the apical surface which was not seen in asthmatic cells. Combining IL-9 and IL-13 had no additional synergistic effect.

Conclusions

We conclude that IL-9 alone or in combination with IL-13 had no additional synergistic effect.

Background

IL-9 is a pleiotropic Th2 cytokine that has been implicated in the pathogenesis of asthma. IL-9 has been linked to goblet cell hyperplasia and decreased ciliogenesis in animal models and an epithelial injury model in adults. We tested the effects of IL-9 alone and in combination with IL-13, an important cytokine in allergic asthma, during differentiation of bronchial epithelial cells from normal children exposed to IL-13, IL-31 or an IL-13/31 combination would alter their phenotype towards that of an asthmatic epithelium.

Methods

Markers of differentiation, real time PCR for MUC5AC, MUC5AC ELISA and transepithelial electrical resistance (TEER) were assessed.

Results

We found that well-differentiated paediatric bronchial epithelial cells highly expressed the IL-31 receptor (IL-31RA). Transepithelial electrical resistance (TEER) indicated good formation of tight junctions which was found to be similar across all treatment groups. We found that IL-13 stimulation reduced the number of ciliated cells compared with control (IL-13 stimulation: mean = 4.8% (SD = 2.5); Control: mean = 18.1%, (SD = 5.9)). We did not find that the combination of IL-13 and IL-31 had any additional effects to that of IL-13 alone (IL-13/31 combination stimulation: mean = 5.1% (SD = 4.6); Control: mean = 18.1%, (SD = 5.9)). Stimulation with IL-13, IL-31 and the IL-13/IL-31 combination did not result in any changes of goblet cell numbers.

Conclusions

IL-31RA receptor is present in abundance in well-differentiated paediatric bronchial epithelial cells however IL-31 does not exhibit any detrimental effects on mucociliary differentiation or proliferation. In addition, IL-31 does not appear to have a synergistic effect when combined in culture with IL-13, in the differentiation process.

Background

Asthma is characterised by airway remodelling which includes smooth muscle hypertrophy, goblet cell hyperplasia and subsequent mucus hyper-secretion. Th2 cytokines including IL-13 and more recently IL-31 have been implicated in the pathogenesis of asthma.

Objectives

We aimed to examine the effects of IL-13 (20 ng/ml), IL-31 (20 ng/ml) and an IL-13/31 combination (20 ng/ml of both) on the in vitro mucociliary differentiation of paediatric bronchial epithelial cells (PBECs) from normal patients.

Methods

Markers of differentiation, real time PCR for MUC5AC, MUC5AC ELISA and transepithelial electrical resistance (TEER) were assessed.

Results

We found that well-differentiated paediatric bronchial epithelial cells highly expressed the IL-31 receptor (IL-31RA). Transepithelial electrical resistance (TEER) indicated good formation of tight junctions which was found to be similar across all treatment groups. We found that IL-13 stimulation reduced the number of ciliated cells compared with control (IL-13 stimulation: mean = 4.8% (SD = 2.5); Control: mean = 18.1%, (SD = 5.9)). We did not find that the combination of IL-13 and IL-31 had any additional effects to that of IL-13 alone (IL-13/31 combination stimulation: mean = 5.1% (SD = 4.6); Control: mean = 18.1%, (SD = 5.9)). Stimulation with IL-13, IL-31 and the IL-13/IL-31 combination did not result in any changes of goblet cell numbers.

Conclusions

IL-31RA receptor is present in abundance in well-differentiated paediatric bronchial epithelial cells however IL-31 does not exhibit any detrimental effects on mucociliary differentiation or proliferation. In addition, IL-31 does not appear to have a synergistic effect when combined in culture with IL-13, in the differentiation process.
(bronchoscopy for upper airway symptoms) were included. All underwent fiberoptic bronchoscopy with endobronchial biopsies (EB). EB were processed to paraffin, and 5 μm sections were cut and stained with haematoxylin and eosin and used to quantify RBM thickness, epithelial shedding and volume fraction (Vv) of subepithelial smooth muscle indexed to submucosa.

**Results**

Epithelial shedding was increased in atopic but not asthmatic subjects, (p=0.02 and p=0.37, respectively), and in children with asthma was correlated with exhaled nitric oxide (r=0.4, p=0.005). RBM thickness was increased in severe asthmatics compared to controls (p<0.0001), but a trend only to increased thickness was seen in mild asthmatics compared to controls (median [range] values: 6 (4.4–8.4) and 4 (3.1–7.5) μm, respectively; p=0.06). The Vv of subepithelial airway smooth muscle was only increased in severe asthmatics compared to controls (0.20 (0.0–0.65) and 0.09 (0–0.16), respectively; p=0.002). Interestingly, there was a positive relationship between RBM thickness and smooth muscle Vv fraction in asthmatics, but not in controls (r=0.31, p=0.02 and r=0.5, p=0.07, respectively) (Abstract S87 Figure 1).

**Discussion**

We report for the first time a direct relationship between RBM thickness and airway smooth muscle mass in paediatric asthma. It is unknown if the relationship is causal, or both are driven by a common underlying process. Combinations of components of airway remodelling, rather than single factors, may prove to be more informative when phenotyping children with severe asthma.

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**Abstract S88 Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Severe asthma</th>
<th>Mild/moderate asthma</th>
<th>Control</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL eosinophils %</td>
<td>2.7 (1–51)</td>
<td>0.7 (0–27.7)</td>
<td>0 (0–5.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BAL neutrophils %</td>
<td>3.3 (0.3–73)</td>
<td>1.7 (0–7.3)</td>
<td>2.7 (0–14)</td>
<td>NS</td>
</tr>
<tr>
<td>Mucosal eosinophils (×/mm²)</td>
<td>11.2 (0–209.3)</td>
<td>3.7 (0–14.5)</td>
<td>0 (0–25.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Mucosal neutrophils (×/mm²)</td>
<td>9.8 (0–125.6)</td>
<td>11.4 (0–22.2)</td>
<td>1.2 (0–58.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Mucosal mast cells (×/mm²)</td>
<td>45.7 (0–189)</td>
<td>63.1 (9.2–72.9)</td>
<td>60.5 (0–165.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Muscle mast cells (×/mm²)</td>
<td>12.3 (0–299)</td>
<td>18.3 (7–72.8)</td>
<td>0 (0–50)</td>
<td>NS</td>
</tr>
<tr>
<td>Vv (sm/subepithelium)</td>
<td>0.20 (0–0.65)</td>
<td>0.06 (0–0.3)</td>
<td>0.03 (0–0.16)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values are median (range). All highlighted p values denote difference between severe asthma and controls. BAL, bronchoalveolar lavage; RBM, reticular basement membrane; sm, smooth muscle; Vv, volume fraction of airway smooth muscle indexed to subepithelium.

**Conclusions**

Children with SA have increased luminal and submucosal eosinophils. However, in contrast to reports in adults of AHR being associated with mast cell myositis, we have found severe asthmatic children with mast cell myositis were more likely to have PAL. Mast cell myositis may be a feature of severe asthma in children.

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**S89 BIMODAL IRON OXIDE NANOPARTICLES FOR HYPERThERMIA THERAPY AND MR IMAGING IN CANCER**

**Introduction**

Superparamagnetic iron oxide nanoparticles (SPION) offer attractive possibilities in biomedicine. Hyperthermia treatment of cancer involves introducing SPION into tumours and applying an alternating magnetic field (AMF). The AMF causes the SPION to heat, resulting in cell death. It has been shown previously that mesenchymal stem cells (MSCs) can be labelled with SPION, with no effect on cell survival, and that they will migrate to and integrate into lung metastases in vivo, following systemic administration. Furthermore, SPION can be used to follow the fate of labelled cells in the body as they cause a marked shortening in T2* on MRI. However, this has not been studied in childhood disease.

**Hypothesis**

Children with SA have increased submucosal eosinophils and mast cells within smooth muscle compared to age-matched mild asthmatics and non-asthmatic controls.

**Methods**

75 children, mean age 11.8 (5.6–17.3) years, SA with SA, 7 with mild/moderate asthma (MA) and 15 non-asthmatic controls (bronchoscopy for upper airway symptoms) were included. All underwent spirometry and bronchodilator reversibility, fractional exhaled nitric oxide (FeNO) measurement, fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) and endobronchial biopsy (EB).