

isotype control antibody. Furthermore, *itgb5*<sup>-/-</sup> mice had significantly less  $\alpha$ -SMA around their airways than wild type control mice in response to *Asp.f* treatment. However, both *itgb5*<sup>-/-</sup> and anti- $\alpha$ V $\beta$ 5 treated mice had significantly more airway inflammation and more inflammatory cells present in the bronchoalveolar lavage compared with their matched controls. These data provide evidence that airway smooth muscle cells can activate TGF- $\beta$  in vivo. Inhibition of, or genetic loss of, the  $\alpha$ V $\beta$ 5 integrin significantly reduces allergen-induced increases in airway smooth muscle mass, however, peribronchial inflammation is increased consistent with the known effects of TGF $\beta$ . Targeted inhibition of the  $\alpha$ V $\beta$ 5 integrin may reduce airway remodelling, but global inhibition is unlikely to be useful due to the enhanced inflammatory response.

### S33 VITAMIN D AND AIRWAY REMODELLING IN PAEDIATRIC SEVERE THERAPY RESISTANT ASTHMA

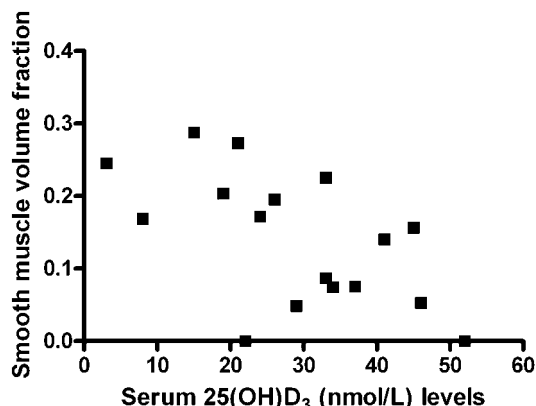
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**Background** Serum vitamin D levels have been related to asthma control and medication use in children with mild to moderate disease, but little is known about the relationship between serum vitamin D levels and airway remodelling and mucosal inflammation in asthma. We hypothesised that lower serum vitamin D levels would be associated with increased airway inflammation and remodelling and lower lung function in children with severe therapy resistant asthma (STRA).

**Methods** Nineteen children aged between 6 and 16 years with STRA underwent spirometry, fiberoptic bronchoscopy, endobronchial biopsy, and measurement of serum vitamin D (25(OH)D<sub>3</sub> nmol/l). Endobronchial biopsies stained with H&E were used to quantify airway remodelling (reticular basement membrane thickness, smooth muscle mass and epithelial shedding). Immunohistochemistry was used to quantify smooth muscle cell proliferation using proliferating cell nuclear antigen, and inflammatory cells (eosinophils, neutrophils and mast cells).

**Results** Seventeen of 19 children with STRA were vitamin D insufficient (<50 nmol/l), median (range) serum 25(OH)D<sub>3</sub> 29 (21–39) nmol/l. There was no relationship between serum 25(OH)D<sub>3</sub> and submucosal eosinophils, neutrophils or mast cells. Airway smooth muscle mass was inversely related to serum 25(OH)D<sub>3</sub> ( $r=-0.6$ ,  $p=0.007$ ) (Abstract S33 figure 1), but there was no relationship between vitamin D levels and reticular basement membrane thickness or epithelial shedding. Lung function was not related to



Abstract S33 Figure 1 Correlation between volume fraction of airway smooth muscle and serum 25(OH)D<sub>3</sub> in paediatric STRA.

serum vitamin D levels, however bronchodilator reversibility was inversely related to serum 25(OH)D<sub>3</sub> levels ( $r=-0.53$ ,  $p=0.02$ ).

**Conclusions** Vitamin D insufficiency is common in children with STRA. Lower vitamin D levels in children with STRA were associated with increased airway smooth muscle and increased bronchodilator reversibility. Randomised controlled trials of vitamin D supplementation are warranted in paediatric STRA.

### S34 THE ADHESION RECEPTOR CADM1 ON MAST CELLS MEDIATES ADHESION TO LUNG FIBROBLASTS AND SMOOTH MUSCLE

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**Introduction and Objectives** Cell adhesion molecule 1 (CADM1) contributes to cell–cell adhesion, viability and adhesion-induced degranulation in mast cells. We found that it is expressed as three alternatively spliced isoforms (major SP4, SP1 and minor SP6) in human lung mast cells (HLMCs). Here we investigated the role of CADM1 isoforms in the adhesion of mast cells to primary airway smooth muscle cells (ASM) and lung fibroblasts (LF).

**Methods** Modulation of CADM1 expression was investigated in transient transfection or adenoviral delivery in the mast cell line HMC-1 and HLMCs. Cells with overexpressed CADM1 isoforms or downregulated CADM1 were examined in cell adhesion assays.

**Results** CADM1 RNA interference in HMC-1 resulted in 60% reduction of surface CADM1 and complete loss of CADM1 determined by FACS and Western blotting, respectively, 6 days after transduction. This decrease in CADM1 expression reduced adhesion of transduced HMC-1 cells to ASM and LF by 42% and 50%, respectively. Downregulation of CADM1 in HLMCs reduced adhesion to ASM by 39%. HMC-1 cells transfected with SP1 (exon 8/9/11) and SP6 (exon 8/9/10/11) showed lower adhesion to LF compared to cells transfected with SP4 (exon 8/11). No differences in adhesion to ASM were found. When SP4 and SP1 isoforms were overexpressed up to 206% and 148% on the cell surface 6 days post transduction by viral delivery, this CADM1 overexpression did not change adhesion to ASM. However, increased levels of SP4 isoform increased mast cell adhesion to LF by 37%. Thus, CADM1 isoforms affected adhesion to LF differently. The CADM1 counter-receptors, examined by Western blotting, are nectin-3 on ASM and CADM1 + nectin-3 on LF.

**Conclusions** CADM1 contributes significantly to mast cell adhesion to ASM and LF. Mast cell adhesion to ASM is likely to be limited by the number of counter-receptors on ASM. In contrast, mast cell adhesion to LF is determined by the levels of SP4 isoform on mast cells. Increased levels of other CADM1 isoforms do not increase adhesion to LF. It is likely that increased levels of longer SP1 and SP6 isoforms in proportion to the CADM1 pool decrease mast cell adhesion to LF.

### S35 RAPAMYCIN INHIBITS IL-33-INDUCED, NUOCYTE-DRIVEN AIRWAY INFLAMMATION

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**Introduction** IL-33 is an innate cytokine that promotes Th2 responses in both the innate and the adaptive immune systems, with an established role in allergic airway inflammation.<sup>1</sup> The signalling pathway of IL-33/ ST2 is incompletely understood and the cells driving IL-33-mediated inflammation have remained elusive. Nuocytes, also known as natural helper cells, are a novel