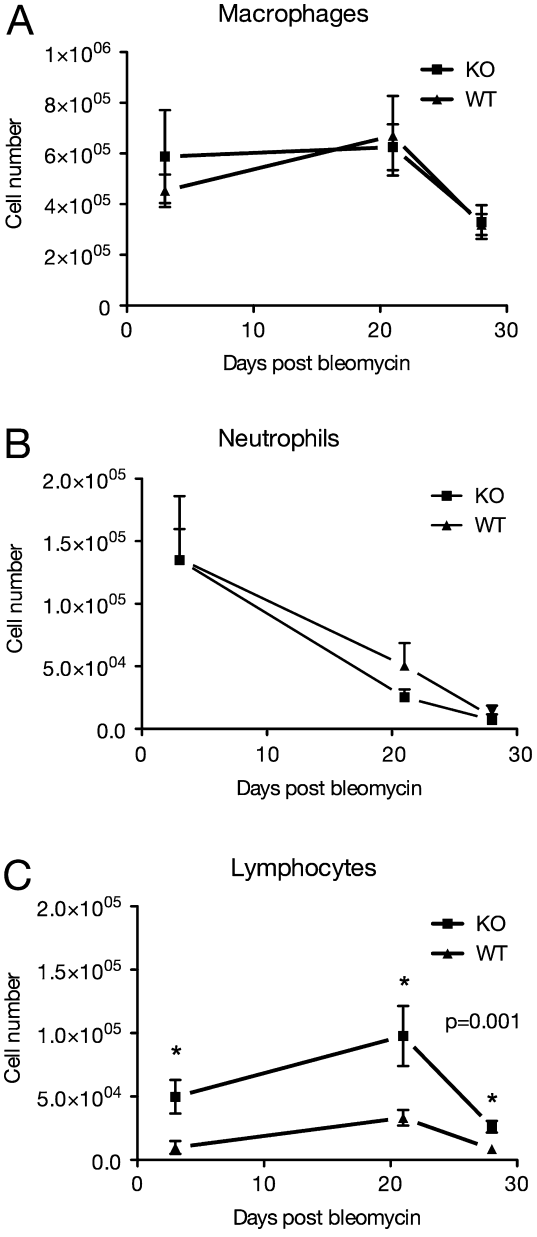
cellular behaviour both via inhibition of MMPs and unique functions including inhibition of ADAM17 mediated release of membrane-bound TNFa. TIMP3 gene polymorphisms protect against hypersensitivity pneumonitis (HP) and TIMP3$^{-/-}$ mice show spontaneous pulmonary airspace enlargement, increased inflammatory responses in models of hepatic injury and arthritis and increased neoangiogenesis. We hypothesise that TIMP3 and TIMP3 gene polymorphisms contribute to inflammatory processes in ILD. This has been investigated by TIMP3 gene SNP discovery and a case-control SNP association study in sarcoidosis. SNP function has been investigated in primary human cells from sarcoidosis patients and controls of known genotype. Finally the response of TIMP3 -/- mice to lung injury with bleomycin has been examined.

Methods *SNP discovery*: the TIMP3 gene was PCR amplified and sequenced in 22 subjects. *Association studies*: 175 UK AfroCarribean sarcoidosis patients and 284 controls were genotyped using TaqMan assays. *Functional studies*: alveolar macrophages (AM) were isolated from bronchoalveolar lavage (BAL), n=18, and monocyte-derived macrophages (MDM) from venous blood, n=14, with real-time PCR of TIMP3 mRNA via TaqMan assay. *Animal studies*: TIMP3$^{-/-}$ mice and controls were treated with oropharyngeal bleomycin (2 mg/kg) and lungs assessed at 3, 21 and 28 days for BAL, histology, RNA and protein analysis.

Results The TIMP3 gene is conserved with 2 promoter SNPs and 2 synonymous SNPs in exon 3 identified. Carriage of at least one SNP showed a protective effectagainst sarcoidosis, odds ratio (OR) 0.68 (p=0.019) driven by patients <35 years, OR 0.56 (p=0.016). The protective haplotype associates with increased TIMP3 gene expression in AM and MDM (p=0.021). TIMP3$^{-/-}$ mice show increased lymphocytosis in BAL at days 21 and 28 (p=0.001) (Abstract T3 Figure 1) more diffuse injury and increased neoangiogenesis within lesions on histological assessment.

Conclusions TIMP3 promoter SNPs may increase gene expression and appear protective against granulomatous lung disease. Absence of TIMP3 leads to increased lymphocytic inflammation in the bleomycin model. Mechanisms for this are being investigated. Together these findings suggest that TIMP3 restricts lymphocytic inflammation in the lung.



Abstract T3 Figure 1 Leukocyte populations in BAL fluid of wild type and TIMP3$^{-/-}$ animals at day 3, 21 and 28 post-bleomycin treatment, showing A. Total macrophage numbers, B. Total neutrophil numbers, C. Total lymphocyte numbers. A significant lymphocytosis was seen in TIMP3$^{-/-}$ mice (p=0.001, two-way ANOVA). n=9 in both genotypes at all time points.

T4 INCREASED RETICULAR BASEMENT MEMBRANE THICKNESS BUT NOT AIRWAY SMOOTH MUSCLE IN ENDOBRONCHIAL BIOPSIES OF SEVERE PRESCHOOL WHEEZERS

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Introduction About a third of all children wheeze (Martinez, NEJM 1995), yet only half will develop asthma (Morgan, AJRCCM 2005). We know that increased reticular basement membrane (RBM) thickness, a feature of airway remodelling in older children with asthma, develops at 2–3 years of age in preschool severe recurrent wheezers (Saglani, AJRCCM 2007) but unlike older children (Regamey, AJRCCM 2008) nothing is known about any changes in airway smooth muscle (ASM) at pre-school age.

Hypothesis There is increased ASM and ASM infiltration by mast cells in preschool children.

Methods Endobronchial biopsies (EBx) were obtained from preschool children undergoing clinically indicated bronchoscopy from 2002 to 2005; severe, recurrent wheezers (n=47, median age 21.5 months) and non-wheezers (n=21, median age 19 months). Up to 12 (5 μm) sections were cut and stained with haematoxylin and eosin. ASM volume fraction was measured using point and line intersection counting (Regamey, AJRCCM 2008). A subgroup of children (n=33) had sections stained for mast cell tryptase. Area of subepithelium and smooth muscle were calculated using image analysis. Mast cells were expressed per area of subepithelium and ASM (Brightling, NEJM 2002).

Results ASM was present in EBx of 50 (73.5%) children, 17 controls (median age 17 months) and 33 wheezers (median age 21 months), with an average of 1.9 (range 1–5) sections with ASM per child. There was no difference in volume fraction of ASM, either indexed to volume of subepithelial tissue (p=0.52) or surface area of RBM (p=0.14), between wheezers and controls. There was no correlation between age and ASM in wheezers or controls. Submucosal mast cells were similar in wheezers (n=25, 139/mm^2) and controls (n=8, 121/mm^2) (p=0.5). No difference was found between wheezers and controls in the number of mast cells within ASM (p=0.17).

Conclusion Severe preschool wheezers have evidence of increased RBM thickness, but not increased ASM compared to age-matched non-wheezing controls. Since both are features of airway

remodelling in school age asthma, this suggests ASM increases only after a longer duration of symptoms. These data highlight the potential to intervene during preschool years before airway remodelling is fully established to alter the natural history of asthma.

T5 KCa3.1 ION CHANNEL BLOCKERS RESTORE CORTICOSTEROID SENSITIVITY IN CYTOKINE-TREATED AIRWAY SMOOTH MUSCLE (ASM) CELLS FROM BOTH COPD AND ASTHMATIC PATIENTS

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Background The K^+ channel KCa3.1 is expressed by several inflammatory and structural airway cells including mast cells and airway smooth muscle (ASM). We have proposed that this channel may play roles in the development of both airway inflammation and remodelling in asthma and COPD. The role of KCa3.1 channels in chemokine secretion by ASM is not known.
Aims To investigate the expression of KCa3.1 in ASM in the airways of healthy and asthmatic subjects, and its function in *ex vivo* cultured primary human ASM cells.
Methods Tissue was collected at bronchoscopy from subjects with asthma and healthy controls, and either processed into GMA for immunohistochemistry, or dissected for the culture of ASM. Further ASM samples were cultured from patients with COPD undergoing lung resection for carcinoma. To examine ASM chemokine production, we used our well-established cellular model of corticosteroid resistance (TNFα/IFNgamma-treated ASM cells).
Results KCa3.1 immunostaining was evident in the ASM in healthy subjects and patients with asthma. There was no difference in the level of expression between healthy subjects (n=7), and those with moderate (n=5) and severe (n=6) asthma. In cultured ASM cells exposed to TNFα/IFNgamma, both ELISA and RT-PCR demonstrated expression of CX3CL1 or CCL5 which were (1) synergistically produced at 24 h and (2) completely resistant to fluticasone pre-treatment (100 nM). We found that KCa3.1 block alone did not inhibit the secretion of CX3CL1 or CCL5. Interestingly, the failure of fluticasone to suppress CX3CL1 and CCL5 expression in response to TNFα/IFNgamma combination was reversed by TRAM-34, a selective inhibitor of KCa3.1 channels. The increased anti-inflammatory action induced by the TRAM-34-fluticasone combination was observed in cells derived from healthy (n=3), asthmatic (n=3) and COPD (n=3) patients. In addition, restoration of corticosteroid sensitivity by KCa3.1 blockers was associated with an increased GR phosphorylation on serine 211 residues.
Conclusions Together, these data suggest that targeting KCa3.1 channels could serve as a novel approach to enhancing/restoring steroid sensitivity in pulmonary disease.

T6 ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: IDENTIFICATION OF PHENOTYPE-SPECIFIC BIOMARKERS AND BIOLOGICAL CLUSTERS

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Background Exacerbations of chronic obstructive pulmonary disease (COPD) are heterogeneous in their aetiology and inflammatory response. We investigated the presence of biological phenotypes of COPD exacerbations and biomarker associations with pathogens and inflammation.
Method Patients with COPD were observed for 1 year at stable and exacerbation visits. Spirometry, health quality assessments, blood and sputum (for differential cell counts) were collected at each visit. A large panel of biomarkers were measured in sputum and serum. Viruses and selected bacteria were assessed in sputum by polymerase chain reaction and routine diagnostic bacterial culture. Biomarkers that differentiated exacerbations associated with bacteria, viruses or eosinophilic airway inflammation were investigated. Biological phenotypes were explored using cluster analysis.
Result 145 patients (101 men, 44 women) entered the study. 182 exacerbations were captured from 86 patients. 55%, 26% and 28% met our definitions for bacteria, virus or sputum eosinophil associated exacerbations. Respectively each of the associated exacerbations were best identified by sputum IL-1β (area under receiver operator curve 0.89 (95% CI 0.83 to 0.95), serum CXCL10 (IP-10) 0.83 (0.70 to 0.96), and percentage peripheral blood eosinophils 0.85 (0.78 to 0.93). The odds ratio (95% CI) of an eosinophil or bacteria associated exacerbation was 2.7 (1.3 to 5.7) and 4.9 (2.4 to 9.9) if a sputum eosinophilia or bacterial pathogen was detected on ≥1 occasion at stable state. Four biological clusters were identified which validated the subgroups of exacerbations associated with bacteria, virus or sputum eosinophilia.
Conclusion COPD exacerbation heterogeneity can be defined. Sputum IL-1β, serum CXCL10 (IP-10) and percentage peripheral blood eosinophils could be used to identify bacteria, virus or eosinophil associated exacerbations of COPD. Whether these biomarkers can be applied to direct therapy warrants further investigation.