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 (TNF α /IFN γ -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 TNF α /IFN γ , 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 TNF α /IFN γ 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-1 β (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-1 β , 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.