S116
MICRONA REGULATION OF TOLL-LIKE RECEPTOR 7 FUNCTION IN SEVERE ASTHMA: RELEVANCE TO VIRAL RESPONSES

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Background and Aims Viral-induced disease exacerbation is common in asthma and studies have identified that both bronchial epithelial cells and alveolar macrophages (AM) from asthmatics have a reduced interferon (IFN) response to rhinovirus infection. The mechanism behind this defect is unclear. As asthmatic peripheral blood mononuclear cells have been reported to have defective toll-like receptor (TLR) 7 function, we investigated the effects of microRNAs (miRNAs) in AM from healthy control (HC) and severe asthma (SA) volunteers with relevance to TLR7-viral interactions. MicroRNAs are non-coding RNAs that down-regulate gene expression by suppressing translation. We identified and focused on 3 miRNAs that could target TLR7. Additionally, we investigated if manipulating the expression of these miRNAs can ameliorate the defective IFN response in AM.

Methods 26 HC and 30 patients with SA (BTS Step 4/5) were recruited for bronchoscopy. AM were isolated from bronchoalveolar lavage using the adherence to plastic technique. Expression of miRNAs and TLR7 was determined by qRT-PCR and western blotting. AM were transfected with a combination of antagonism, specifically directed against the 3 miRNAs, and then treated with imiquimod (5µg/ml), a TLR7 agonist, or human rhinovirus-16 (HRV16) and IFN-β expression was determined after 24 hours using qRT-PCR and ELISA.

Results Expression of all three miRNAs was significantly increased in SA compared to HC. TLR7 mRNA was found to be significantly reduced in AM from volunteers with SA compared to HC. Western blotting confirmed reduced expression of TLR7 protein in AM from SA compared to HC. Compared to mock transfected AM, AM transfected with the 3 antagonism showed significantly increased imiquimod-induced IFN-β mRNA and protein expression and significantly increased HRV16-induced IFN-β mRNA production.

Conclusion TLR7 expression is significantly reduced in SA compared to HC. The differential expression of the miRNAs identified may lead to impaired viral sensing by asthmatic AM and contribute to the defective IFN response to rhinovirus. Importantly, TLR7 induced IFN-β production by human AM can be significantly augmented by inhibition of these miRNAs. The identification of these miRNAs and our ability to manipulate their expression in human AM offers the potential for future miRNA-based therapies in asthma.

S117
EFFECTS OF HYPOXIA ON EOSINOPHIL APOPTOSIS, EFFEROCYTOSIS AND SENSITIVITY TO GLUCOCORTICOIDS

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Many tissues including the skin, intestinal epithelium and potentially the airway operate at ‘physiological’ levels of tissue hypoxia with normal PO2 values below 3KPa. Both sterile and non-sterile inflammation exacerbates the degree of tissue hypoxia and predicts the need for granulocytes including eosinophils to operate efficiently under hypoxia. In these experiments we have examined the effects of hypoxia on eosinophil longevity and show that a PO2 below 3KPa severely attenuates the pro-apoptotic effect of dexamethasone.

S118
IL-13 INDUCED MOUSE AIRWAY INFLAMMATION INDUCES AN INCREASE OF SOLUBLE ADAM33 IN BRONCHOALVEOLAR LAVAGE FLUID, WHICH IS ENZYMATICALLY ACTIVE AND ASSOCIATED WITH BRONCHIAL HYPERRESPONSIVENESS

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Rationale The asthma susceptibility gene ADAM33/Adam33 is associated with bronchial hyperresponsiveness (BHR) in humans and mice. Soluble ADAM33 is increased in bronchoalveolar lavage fluid (BALF) of allergic asthma patients (Lee JY et al, AJRCCM 2006 April 1;175(7):729–35). Its levels correlate with declining FEV1 %, suggesting a role in asthma remodelling in asthma. Maternal allergy orenogenous IL-13 suppresses Adam33/ADAM33 mRNA expression but enhances ADAM33 protein processing in human embryonic and juvenile mouse lungs (Hatchi HM et al, JACI 2009 Sep; 124(3):590–7, 597). We hypothesise that conditional expression of IL-13 in mouse lungs induces the enzymatically active, soluble form of ADAM33 in BALF, which is associated with BHR.

Methods IL-13 expression wasinduced using Doxycycline in C57BL/6Ta/Ta(Oet/Il-13 double-transgenic (dTG) mice. Methacholine challenge and lung function measurements were performed and lungs were harvested for mRNA extraction and immunohistochemistry (IHC). BALF was obtained for Western-blottting for Adam33 and testing of ADAM33 enzymatic activity using a fluororescenceresonance energy transfer (FRET) peptide assay.

Results There was asignificant increase in BHR to Methacholine in IL-13 expressing double transgenic mice. IHC showed an increase in bronchial smooth muscle in lungs of double transgenic mice. Similar to the RTqPCR findings in human embryonic and juvenile mouse
lungs, IL-13 suppressed Adam33 mRNA but no difference in α-smooth muscle actin (αSma) was evident. Immunoblotting for ADAM33 in BALF demonstrated a 76kDa band, consistent with the ADAM33 ectodomain and processed forms αS8/44kDa in dTg animals. ADAM33 enzymatic activity was also significantly increased.

**Conclusion** The data suggest that allergic inflammation induced by IL-13 suppresses Adam33 mRNA expression but induces the release of soluble forms of ADAM33, yielding enzymatically active forms. The release of soluble forms may play a role in airway remodelling, potentially leading to BHR. We next propose to test the effect of specific ADAM33 inhibitors on airway remodelling in this allergic mouse model to assess their potential as novel treatments for asthma.

**S119 ROLES OF TLR3, TLR4- AND TLR5-79 IN INTERFERON INDUCTION IN BRONCHIAL EPITHELIAL CELLS AND PERIPHERAL BLOOD MONONUCLEAR CELLS FROM ASTHMATIC AND NON-ASTHOMATIC SUBJECTS**

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**Introduction** Defective rhinovirus (RV) induced interferon (IFN)-β and IFN-α production has been reported in primary human bronchial epithelial cells (HBECs) and peripheral blood mononuclear cells (PBMCs) from asthmatics. The mechanisms of defective IFN induction in asthma are unknown. Virus infection can induce IFNs through Toll like Receptors (TLR)3, TLR4 and TLRs7–9 and TLR agonists have been identified as potential therapeutic options for asthma. The role of these TLRs in IFN induction in asthma is unclear.

**Objective** To investigate IFN responses to TLR stimulation in HBECs and PBMCs from atopic asthmatic and non-asthmatic individuals.

**Methods** HBECs and PBMCs from atopic asthmatic and non-asthmatic subjects were stimulated with agonists to TLR3, TLR4 & TLRs7–9 and type I and III IFN responses assessed by qPCR and ELISA.

**Results** TLR3 and TLR7, but not TLR4, 8 or 9, stimulation induced IFN protein and mRNA expression in HBECs and PBMCs. IFNs induced were IFN-β and predominantly type III IFN-λ in HBECs and type I (–α and –β) with no IFN-α in PBMCs. TLR function was not defective in asthmatic compared to non-asthmatic subjects.

**Conclusions** TLR3 & TLR7 were the predominant TLRs involved in IFN induction in HBECs and PBMCs. Defective IFN induction to TLR agonists was not observed in these well controlled asthmatic subjects. TLR3/7 agonists could be effective in inducing IFNs in more severe/less well controlled asthmatics who may have deficient virus induced IFN production.

**Evaluation and treatment of Cystic Fibrosis**

**S120 UPDATE ON THE UK CF GENE THERAPY CONSORTIUM MULTIDOSE, NON-VIRAL, GENE THERAPY TRIAL**

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The UK CF GTC has been working for several years to determine the clinical benefit of CFTR gene therapy. Our premise was that for such a therapy to achieve clinical benefit, repeated administration would be required, and that therefore a non-viral approach was needed. We demonstrated in laboratory and preclinical models that GL67A (Genzyme Corp) was the optimal gene transfer agent, and designed a plasmid, pGM169, completely depleted of pro-inflammatory CpG motifs and driven by the novel hCEFI promoter, designed for prolonged expression. In a longitudinal observational study (the Run-in) we measured the variability of multiple outcome measures, both conventional and novel. These data have allowed us to perform power calculations and a) choose our primary outcome (FEV1), b) secondary efficacy outcomes (lung clearance index, various parameters on CT scan, Quality of life questionnaire [CFQ-R], exercise capacity and activity, and selected sputum and serum inflammatory markers), and c) safety measures (clinical findings, exacerbation rate, gas transfer, sputum culture, serum inflammatory markers, renal and hepatic markers). We have recently completed a single-dose safety and dose ranging study.

In this trial, 150 patients, aged 12 years and above are being randomised in a 1:1 fashion to active treatment or placebo and will receive the nebulised agent at monthly intervals for 12 doses. The group size was determined on the basis of a 6% relative improvement in FEV1. An adaptive design will be used for additional safety: the first 20 patients will receive 3 doses ahead of the rest of the cohort. Patients will be invited to participate in either one or two substudies, being conducted to explore mechanisms; a) nasal administration followed by nasal potential difference (PD) and brushings for mRNA expression and b) pre and post-treatment bronchoscopies for lower airway PD, gene expression and histology. The double-blinded nature of the trial means that final outcome data will only be available upon completion of the study. The trial was initiated in April 2012; here we will update on recruitment, projected time-lines and progress.

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**S121 LUNG CLEARANCE INDEX TO EVALUATE THE EFFECT OF IVACAFTOR ON LUNG FUNCTION IN SUBJECTS WITH CF WHO HAVE THE G551D-CFTR MUTATION AND MILD LUNG DISEASE**

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**Background** Ivacaftor has been shown to lead to significant improvement in lung function, exacerbation rate, weight gain and quality of life in adolescents and adults with CF and the G551D-CFTR mutation.

**Objectives** Drugs targeting the basic defect of CF may hold potential for patients with early stage disease, but establishing benefit is more difficult. If FEV1 is normal, a more sensitive test may be useful. LCI has been shown to become abnormal at an earlier stage of disease than FEV1, and thus may be a more sensitive outcome measure in this group of patients.

**Methods** This Phase 2, randomised, double-blind, placebo-controlled, multicenter, crossover study evaluated the effect of ivacaftor on LCI derived from multibreath washout of SF6 using an Innocor device. Subjects were ≥6 years with the G551D-CFTR mutation, FEV1 >90% predicted, and LCI >7.4 (upper limit of normal). Ivacaftor 150 mg or placebo was administered twice daily for two 4-week periods with a 4-week washout in between.

**Results** Twenty-one subjects were randomised and 20 received a dose of ivacaftor. Seventeen subjects completed both periods. Mean (SD) age was 16.6 (10.9) years. Mean (SD) baseline LCI was 9.0 (1.5). The treatment effect of ivacaftor for adjusted mean change from baseline in LCI at Day 29 was –2.1 (P=0.0004). Mean