

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

doi:10.1136/thoraxjnl-2012-202678.121

H Rupani, P Dennison, N Jayasekera, T Sanchez-Elsner, P H Howarth. *Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom*

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 expression 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 antagomirs, specifically directed against the 3 miRNAs, and then treated with imiquimod (5ug/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 antagomirs 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 GLUCOCORTICOSTEROIDS**

doi:10.1136/thoraxjnl-2012-202678.122

L Porter, AS Cowburn, N Farahi, CA Fiddler, AM Condliffe, ER Chilvers. *University of Cambridge, Cambridge, England*

Many tissues including the skin, intestinal epithelium and potentially the airway operate at 'physiological' levels of tissue hypoxia with normal PO_2 values below 3KPa. Both sterile and non-sterile inflammation exacerbates the degree of tissue hypoxia and predicates 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 PO_2 below 3KPa severely attenuates the pro-apoptotic effect of dexamethasone.

Human blood eosinophils were prepared from healthy donors using hetastarch-sedimentation and EasySep[®]-immunomagnetic beads, and cultured in RPMI + 10% autologous serum for 6–24 h. Apoptosis and efferocytosis were quantified using standard morphology, AnV/PI staining and myeloperoxidase counter-stain methods. Hypoxic incubation (H) (media PO_2 2.9 ± 0.1 KPa) caused a marked survival response in eosinophils compared to normoxia (N) (% apoptosis at 24 h: N $16.3 \pm 3.0\%$; H $1.2 \pm 0.2\%$, $n=5$), which was of similar magnitude to that observed with IL-5 (N $4.2 \pm 0.3\%$). This hypoxic survival effect was mimicked by the iron chelator/2-oxyglutarate analogue DFO (10 mM) and DMOG (1 mM) and blocked in a concentration-dependent manner by the protein synthesis inhibitor cycloheximide. In preliminary experiments hypoxia also independently reduced the capacity of monocyte-derived macrophages to phagocytose apoptotic eosinophils as seen previously with neutrophils. Most strikingly, hypoxic incubation also reduced the normal pro-apoptotic effect of dexamethasone (1 μ M) (% apoptosis at 24 h: N + Dex $27.6 \pm 3.9\%$, H + Dex $11.2 \pm 2.9\%$). qPCR analysis of the glucocorticosteroid-dependent gene *GILZ* and the hypoxia-HIF-1 α -dependent gene *GLUT1* demonstrated that the above suppression of dex-induced eosinophil apoptosis was not a consequence of the inhibition of steroid-induced transcriptional activity (mRNA fold change at 16 h: *GILZ* N 12.89 ± 3.6 , *GILZ* H 23.9 ± 9.2 ; *GLUT1* H (control) 28.5 ± 7.2 , *GLUT1* H (Dex) 45.5 ± 13.5 , $n=3$).

These data suggest that hypoxia can cause a profound effect on eosinophil longevity and macrophage uptake, and render these cells partially resistant to the pro-apoptotic effects of dexamethasone. This may impede the effective resolution of eosinophilic inflammation *in vivo*.

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**

doi:10.1136/thoraxjnl-2012-202678.123

^{1,2}ER Davies, ²G Chen, ¹ST Holgate, ¹DE Davies, ²JA Whitsett, ^{1,2}HM Haitchi. ¹*Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK*; ²*Division of Pulmonary Biology, Cincinnati children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, USA*

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 Apr; 173(7):729–35). Its levels correlate with declining FEV₁, suggesting a role in airway remodelling in asthma. Maternal allergy oregonous IL-13 suppresses *Adam33/ADAM33* mRNA expression but enhances ADAM33 protein processing in human embryonic and juvenile mouse lungs (Haitchi 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 was induced using Doxycycline in CCSP-rtTA/Otet-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-blotting for ADAM33 and testing of ADAM33 enzymatic activity using a fluorescence resonance energy transfer (FRET) peptide assay.

Results There was a significant 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 RT-qPCR findings in human embryonic and juvenile mouse