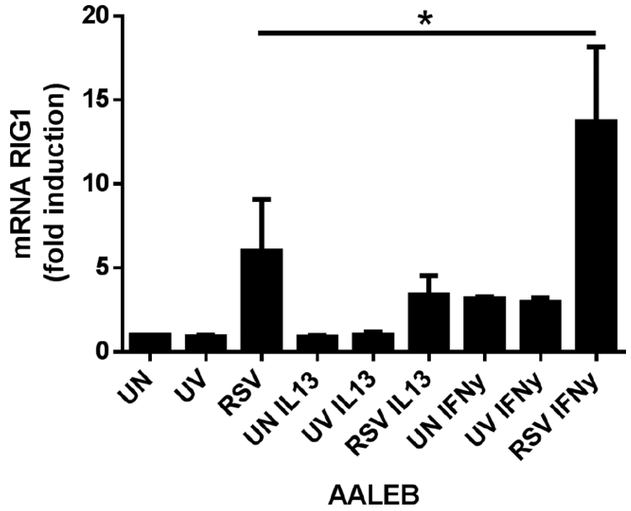

Abstract S129 Figure 1 RIG1 expression in RSV infected and primed AALEB by RTPCR. AALEB were pre-treated for 24 h with 10 ng/ml IFNy or IL-13, 10 ng/ml. RNA was extracted and reverse transcribed
UN: no infected, UV: inactivated RSV, RSV: Respiratory Syncytial Virus, IL13: Interleukine 13, IFNy: Interferon gamma. Data analysed by one-tailed paired t test. * $p < 0.05$, ($n = 4$).

development of a long term abnormality which is a hallmark of asthma.

We optimised an *in-vitro* model using AALEB, human immortalised bronchial epithelial-derived cells which were pre-treated for 24 h with cytokines that mimic Th1 environment (IFNy, 10 ng/ml) and Th2 (IL-13, 10 ng/ml) before being infected with RSV A, MOI=2 for 48 h. Quantitative real-time PCR with Taqman primers was used to assess expression of innate genes. Cells were collected after 48 h and stored in Trizol. Chromatin Immuno Precipitation (ChIP) with antibodies against histone modifications was used to assess epigenetic controls. In order to confirm epigenetic regulation of innate genes we used a panel of HAT, HDAC and histone demethylase inhibitors.

We initially studied the impact of cytokines on a range of innate anti-viral genes. RIG1 was differentially expressed and reductions in expression associated with higher viral titres. IFNy priming induced increases in RIG1 mRNA at 48 h that correlated at the promoter with enrichment of H3K9ac and RNApolII (active-promoters) and reduction of H3K9me3 (repressive-promoters). We observed a statistically significant increase of RIG1 expression by IFNγ when co-incubated with SAHA (HDAC I and II inhibitors) and JIB-04 (Pan-Jumanji histone demethylase inhibitor). No effects of Th2 priming were seen at the level of antiviral responses.

This *in-vitro* study suggests the inflammatory environment of naive epithelial cells can induce epigenetic modulation of innate immune responses at the level of histone methylation and acetylation and hence potentially lead to long term impacts on antiviral immunity. The presence of a Th1 milieu appears key to the development of effective anti-viral responses.

S130 TNFα DRIVEN CAR PHOSPHORYLATION PROMOTES TRANS EPITHELIAL MIGRATION OF LEUKOCYTES

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Transepithelial migration (TEpM) of leukocytes during the inflammatory process requires engagement with receptors expressed on the basolateral surface of the epithelium. One such receptor is Coxsackie and Adenovirus Receptor (CAR) which binds to Junction Adhesion Molecule – L (JAM-L) on leukocytes during TEpM. Here we provide the first evidence that TEpM of THP1 cells requires, and is controlled by phosphorylation of the cytoplasmic tail of CAR. Our *in-vitro* data shows that these leukocyte cells can adhere to an epithelial layer but where the cytoplasmic tail of CAR is prevented from undergoing phosphorylation the leukocytes are unable to transmigrate. Furthermore we show that this CAR phosphorylation step is driven by TNFα signalling via a TNFR1-PI3K-PKCδ dependent signalling pathway. Interestingly our work demonstrates that THP1 cells can secrete TNFα thereby activating the CAR phosphorylation pathway leading to TEpM without addition of exogenous TNFα but where TNFα is added this process is augmented. We also use a mouse model to confirm that CAR phosphorylation in response to inflammatory stimuli occurs in-vivo. Both acute (a 24 h inhaled TNFα challenge) and chronic (a 34 day ovalbumin challenge) inflammatory conditions are studied. Using confocal microscopy techniques we show that the cytoplasmic tail of CAR is phosphorylated. Specifically this is seen at the cell membrane of epithelial cells of bronchioles with associated inflammatory cells in the interstitium. Taken together these data describe a novel method for the control of TEpM by transmigrating leukocytes that can also be heightened by the presence of pro-inflammatory cytokines during inflammation. This provides a novel target for controlling inflammation at the epithelium, a key component of the pathogenesis of many diseases including asthma.

S131 PERIPHERAL BLOOD MONONUCLEAR CELLS FROM CHILDREN WITH SEVERE ASTHMA EXHIBIT AN IMPAIRED CORTICOSTEROID SENSITIVITY, WHICH ALSO CORRELATES WITH INCREASING BODY MASS INDEX

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Introduction Corticosteroid (CS) insensitivity contributes to the difficulty in managing children with severe asthma.[1] A better understanding of the molecular mechanisms driving this defective response could provide novel therapeutic options for these patients. Peripheral blood mononuclear cells (PBMCs) from adults with severe asthma have been used to demonstrate an impaired sensitivity to CS, enabling the delineation of potential underlying mechanisms. Whether CS insensitivity exists in PBMCs from severely asthmatic children, however, requires further validation.

Objective To determine whether PBMCs from children with severe asthma have an impaired *in vitro* responsiveness to corticosteroids.

Methods We conducted an observational feasibility study comparing the corticosteroid sensitivity of PBMCs from asthmatic children on British Thoracic Society treatment step 4–5 ($n = 7$) with healthy controls ($n = 5$). PBMCs from 5 ml of venous blood were plated in the presence of 100 ng/ml of lipopolysaccharide (LPS), and in the absence or presence of either 10^{-8} M or 10^{-6} M of dexamethasone (DEX). ELISA assays were used to determine the levels of TNF-α and IL-8, and the% suppression of these by DEX. Pearson product-moment correlation tests