S74 INCREASED RESPIRATORY SYNCYTIAL VIRUS BURDEN LEADS TO MORE RAPID CELL DEATH IN PHE508DEL BRONCHIAL EPITHELIAL CELLS

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Introduction Respiratory syncytial virus (RSV) leads to serious lower respiratory tract disease and prolonged periods of symptoms in cystic fibrosis (CF) patients. This study aims to determine whether RSV viral burden and the level of cytopathic effect (CPE) is higher in CF bronchial epithelial cells.

Methods Paired immortalised bronchial epithelial cell lines, CFBE41o-, expressing either wild type (WT) or Phe508del CFTR were infected with RSV A2 at an MOI of 0, 0.01, 0.1 and 1.0. Cell viability was measured daily by Resazurin assay, and viral burden by plaque assay in HEp-2 cells and RT-PCR. Viral attachment was determined by PCR after incubating RSV with the cells for 2 hrs at 4°C. Intracellular RSV proteins were measured by western blot.

Results Phe508del CFTR cells showed significantly greater and more rapid CPE by RSV (0.1 and 1 MOI) compared to WT cells. Viral burden was increased in the Phe508del cells each day up to 7 days post infection. The levels of intracellular RSV genetic material determined by PCR were also increased by 4.0 ± 0.2 (mean of MOIs ± SD) fold at 12 hours post infection in CF cells compared to WT. It was confirmed that increased levels of viral RNA led to increased intracellular viral proteins. Virus shedding determined by PCR in the supernatant at 24 hours post infection was also increased by 10.7 ± 5.0 fold in CF cells. There was no evidence of increased RSV attachment to CF cells.

Conclusion RSV causes CPE in bronchial epithelial cells expressing Phe508del CFTR more rapidly than in WT cells. This increased CPE was associated with an increased viral burden, which occurs despite similar levels of cell attachment and is therefore likely due to increased viral replication or transcription within the cell, which led to increased levels of intracellular RSV proteins. The mechanism for this is under investigation.

S75 THE T2R38 BITTER TASTE RECEPTOR AS A MODIFIER OF HOST RESPONSE TO PSEUDOMONAS AERUGINOSA IN CYSTIC FIBROSIS: DOES T2R38 GENOTYPE IMPACT ON CLINICAL INFECTION?

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Background *Pseudomonas aeruginosa* (Pa) mediates several virulence factors through quorum sensing (QS). Intriguing *in vitro* data suggests Pa QS molecules are 'sensed' by the T2R38 receptor on airway cilia (J Clin Invest, 2012;122:4145–59), leading to changes in ciliary beat frequency and nitric oxide production, possibly enhancing bacterial clearance. Three polymorphisms occur in the gene coding this receptor, altering the amino acid sequence and receptor function; the functional allele has proline-alanine-valine (PAV); the non-functional allele has alanine-valine-isoleucine (AVI). We hypothesised that the T2R38 receptor may be important in Pa host defence in people with cystic fibrosis (CF) and that T2R38 genotype may modify infection status and clinical outcomes.

Methods Patients over 6 years with CF were genotyped for polymorphisms in the *TAS2R38* gene. Pa infection status was determined by review of all respiratory cultures during 2014 and assigned according to Leeds criteria as chronic, intermittent, Pa free or never. Only patients with ≥3 cultures/year were included in analysis. Lung function data was obtained from CF annual reviews during 2014.

Results T2R38 receptor genotypes were obtained for 271 patients: 83 (30.6%) AVI/AVI, 44 (16.2%) PAV/PAV, 116 (42.8%) AVI/PAV and 28 (10.3%) AVI/other or PAV/other. Between AVI/AVI, PAV/PAV and AVI/PAV groups there was no significant difference in median age, gender or p.Phe508del *CFTR* mutation frequency. By T2R38 genotype, there was no significant difference in the proportion of patients in each Pa infection category. In patients with intermittent or chronic Pa there was no significant difference by T2R38 genotype in mean percent predicted FEV_1 or FVC.

Conclusion T2R38 genotype does not appear to modify Pa infection in people with CF, or to modify lung disease severity in people with CF and intermittent or chronic Pa infection. Further work is underway to investigate T2R38-dependant responses to Pa *in vitro*.

Abstract S75 Table 1 Pa infection status by T2R38 genotype (Chi squared p = 0.422)

	Never n (%)	Pa free n (%)	Intermittent n (%)	Chronic n (%)
AVI/AVI	4 (4.8)	20 (24.1)	11 (13.2)	38 (45.8)
PAV/PAV	3 (6.8)	16 (36.4)	8 (18.8)	14 (31.8)
AVI/PAV	4 (3.5)	36 (31.0)	26 (22.4)	44 (37.9)

Acute Lung Injury and Interstitial Lung Disease

S76 ENDOPLASMIC RETICULUM STRESS CORRELATES WITH FIBROSIS IN INTERSTITIAL LUNG DISEASE

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In interstitial lung disease (ILD), pulmonary fibrosis is associated with a poor prognosis. Distinct histological features differentiate between the ILDs, however it is unknown if there are shared pathogenic mechanisms for the development of fibrosis. Endoplasmic reticulum (ER) stress has been implicated in the pathogenesis of familial and sporadic idiopathic pulmonary fibrosis (IPF). In response to ER stress, cells trigger the integrated stress response and upregulate chaperones, such as BiP, and the phosphatase GADD34, which can regulate EMT, cell proliferation and survival.

AIMS We hypothesise that ER stress may be involved in the pathogenesis of fibrosis in all interstitial lung diseases.

Paraffin embedded lung biopsy sections from 8 patients with familial pulmonary fibrosis, 11 sporadic idiopathic pulmonary fibrosis (IPF), 12 non-specific interstitial pneumonia (NSIP) and 10 hypersensitivity pneumonitis (HP) were evaluated for BiP and GADD34 by immunohistochemistry. Using light microscopy, 6

high power fields were scored for fibrosis, inflammation, BiP and GADD34 using semi-quantitative analysis by 2 blinded, independent investigators. Data were analysed by linear regression using Prism software.

BiP and GADD34 were localised to reactive type II pneumocytes and columnar epithelium within areas of fibrosis. GADD34 was also evident in the endothelium. No staining was detected in fibroblasts. Epithelial GADD34 correlated with extent of fibrosis in familial pulmonary fibrosis ($r^2 = 0.72$, $p < 0.001$), IPF ($r^2 = 0.51$, $p < 0.0001$) and NSIP ($r^2 = 0.46$, $p < 0.0001$). In contrast, BiP was associated with fibrosis in IPF ($r^2 = 0.49$, $p < 0.0001$) and HP ($r^2 = 0.59$, $p < 0.0001$).

These data show that ER stress and the unfolded protein response are associated with fibrosis in ILD. Hence targeting ER stress may be a novel therapeutic option for pulmonary fibrosis.

S77 MODULATORY EFFECTS OF RHEUMATOID ARTHRITIS IgG ON NEUTROPHIL ACTIVATION: A POTENTIAL ROLE IN RA-ILD

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Background Rheumatoid arthritis (RA) is an autoimmune rheumatic disease (ARD) characterised by circulating autoantibodies, including anti-citrullinated protein antibodies (ACPA). Many RA patients have extra-articular disease, including ~10% with interstitial lung disease (ILD). Risk factors for RA-ILD include: male sex; age; smoking history and ACPA positivity.

Hypothesis We propose that RA-IgG, including ACPA, modulate neutrophil functions including: generation of reactive oxygen species (ROS), neutrophil adhesion and generation of neutrophil extracellular traps (NETs) that may contribute to joint and extra-articular tissue damage, including ILD.

Methods Neutrophils and IgG were isolated from RA patients or healthy controls (HC). Bronchoalveolar lavage (BAL) was performed on ILD patients and controls underdoing bronchoscopy for other reasons. ROS production was measured using an enzymatic assay to assess hydrogen peroxide (H2O2) generation. Neutrophil integrins were quantified by flow cytometry. Effects of purified IgG upon neutrophil adhesion to immobilised fibrinogen (Mac-1/aMß2-dependent) and fibronectin (VLA-4/a4ß1-dependent) were determined using a fluorescent adhesion assay. NETosis was measured using a novel capture ELISA.

Results We demonstrated increased NETs in the BAL of patients with active ILD (n = 3; ARD-ILD) compared to those with inactive disease (n = 2; IPF and ARD-ILD) or controls (n = 1; CLL). In addition, we showed binding of RA-IgG to control neutrophils, which increased with neutrophil activation. Stimulation of HC (n = 12) and RA (n = 7) neutrophils with PMA produced similar rates of H2O2 generation (p = 0.9939). Exposure of HC neutrophils to RA-IgG (n = 9) however, increased H2O2 production compared to HC-IgG (n = 9) ($p < 0.0001$), which was not blocked by FcR blockade. RA-IgG also enhanced PMA-stimulated adhesion of HC neutrophils to fibrinogen (p = 0.0028) and fibronectin (p = 0.0024), which was inhibited by aMß2 or ß1 integrin blockade respectively. RA-IgG increased both spontaneous (p = 0.0248) and PMA-induced (p = 0.0200) NETosis of HC neutrophils compared to HC-IgG. Immunofluorescence studies demonstrate that aMß2 activation induces NETosis. Further examination found that NETosis could be suppressed by p38 MAPK inhibition (p = 0.0034).

Conclusion We have shown that RA-IgG modulates several aspects of neutrophil activation and function. In addition, we found increased neutrophil activation in the lungs of patients with active ARD-ILD. Further work is underway to evaluate the contribution of these processes to the pathogenesis of RA-ILD.

S78 LYMPHOPAENIA AND INCREASED ACE LEVELS STRATIFY SARCOIDOSIS PATIENTS TO UNDERLYING INCREASE IN IFN-γ + LYMPHOCYTE AND TNF-α+ MONOCYTES RESPECTIVELY

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Introduction Sarcoidosis is a heterogeneous disease, and different mechanisms may contribute to disease activity at any one time. Both TH1 lymphocyte (IFN-γ+, IL-2+ CD4 T cells) and activated tissue macrophages contribute to the formation of granuloma and are established disease pathways. More recently, monocytes (precursors of tissue macrophages) have also been implicated. We reasoned that if we could identify a commonly used clinical test as a biomarker for these disease pathways, it could be used as a disease activity marker, and to guide evaluation of new therapies and repositioning of current drugs. In this study, we question whether serum ACE and circulating lymphocyte count correlated with different cellular immune function.

Methods 44 consecutive patients fulfilling the ATS-WASOG diagnostic criteria for sarcoidosis within a 2-year period were recruited. Patients on treatment, current cigarette smokers, intercurrent immune disease and malignancies were excluded. Blood samples from all patients were processed contemporaneously for ACE, lymphocyte counts and CD4 T cell intra-cellular cytokine staining (ICS) for IFN-γ, IL-2 and IL-17 and CD14hi monocytes ICS for TNF-α.

Results and discussion We found no correlation between lymphocyte count and ACE ($r = -0.2$; $p = 0.86$) suggesting that these two abnormalities reflected independent processes. Lymphopaenia was significantly correlated with markers of activated CD4 T cells (IFN-γ, IL2+ and TNF-α+) ($r = -0.50$, 0.50 and 0.60 respectively; all $p < 0.001$; Spearman Rank Sum test); while ACE only correlated with level of TNF-α+ monocytes ($r = 0.60$; $p < 0.0001$).

Conclusions These results suggest that high ACE and lymphopaenia (i) reflect disease activity (ii) are likely to be endpoints of two different mechanistic pathways, and (iii) that they could potentially stratify patients into those with lymphocytic dominant and monocyte dominant disease processes. Thus a monocyte pathway inhibitor could be used specifically in patients with high ACE levels while drugs that target T cell activity may be targeted to those with lymphopaenia.