cellular matrix, resulting in loss of airway or alveolar function. Murine lineage tracing experiments have suggested that these fibroblasts may, in part, be derived from lung epithelium via epithelial to mesenchymal transition (EMT). Transforming Growth Factor-β1 (TGF-β1) has been shown to drive EMT both in vitro and in vivo. Previous work in our lab has shown that inflammatory stimuli such as Tumour Necrosis Factor-α (TNFα) can accentuate TGF-β1 driven EMT in primary bronchial epithelial cells (PBEC). This crosstalk between inflammatory and TGF-β1 signalling may have implications in the pathogenesis of fibrotic disorders of the lung including Bronchiolitis Obliterans Syndrome and Idiopathic Pulmonary Fibrosis. We hypothesised that TGF-β activated kinase-1 (TAK-1), a protein phosphorylated in response to both TGF-β1 and TNFα, may be a key convergent point and assessed its role in inflammatory accentuation of EMT.

**Methods** PBEC isolated from stable lung transplant recipients were treated with TGF-β1 (10 ng/ml) and/or TNFα (20 ng/ml) and the phosphorylation and localisation of signalling molecules SMAD-3 and TAK-1 assessed (western blot, confocal microscopy). TAK-1 function was blocked using a selective inhibitor and by siRNA knockdown; effects on EMT marker expression were assessed (western blot, confocal microscopy).

**Results** TAK-1 is phosphorylated in response to both TGF-β1 (161% *increase*) and TNFα (145%) with an accentuated phosphorylation upon co-treatment (516%, *n=3*). Co-treatment also resulted in pronounced nuclear localisation of phosphorylated TAK-1, which has not previously been reported. Chemical Inhibition of TAK-1 phosphorylation significantly reduced TGF-β1 driven down-regulation of E-cadherin (22% *inhibition*) and up-regulation of Vimentin (78%) and Fibronectin (39%, $p<0.05$, n=4). Furthermore, TAK-1 inhibition significantly reduced TNFα accentuated TGF-β1 driven down-regulation of E-cadherin (39%) and up-regulation of Fibronectin (87%) and Vimentin (258%, $p<0.05$, n=4). These findings were independently validated via siRNA knockdown of TAK-1.

**Conclusions** These findings suggest that TAK-1 is an important convergent point for inflammatory and TGF-β1 signalling, controlling the accentuation of EMT in primary human airway epithelium. Examination of TAK-1 activation and function in animal models of lung fibrosis may provide information on the potential for TAK-1 inhibition as a therapeutic target.

## S141 A DISINTEGRIN AND METALLOPROTEASE (ADAM) 33 PROTEIN IN PATIENTS WITH PULMONARY SARCOIDOSIS

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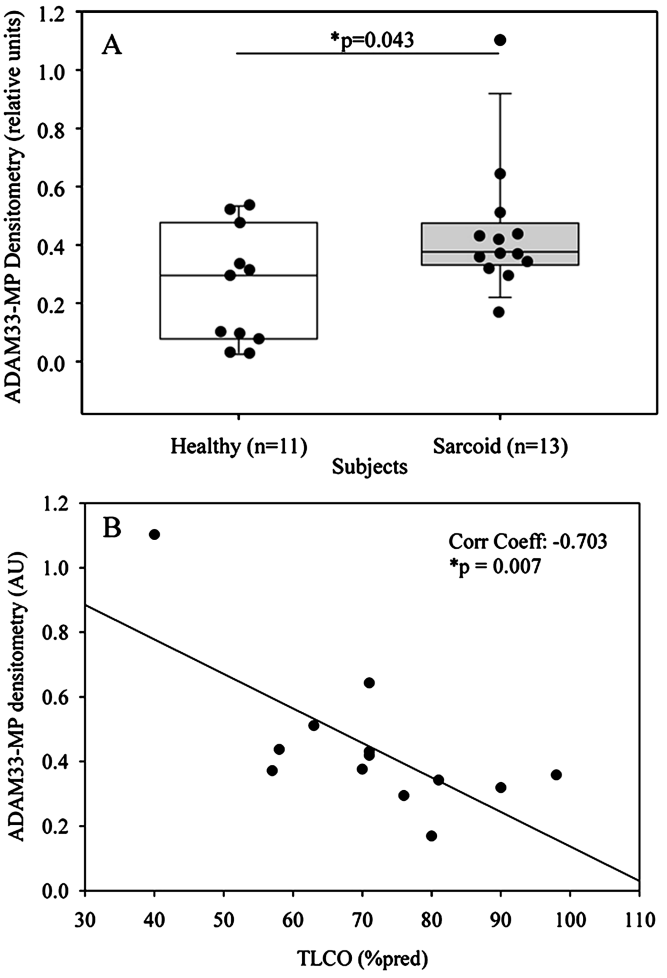
**Background** The asthma and chronic obstructive pulmonary disease (COPD) gene, *A Disintegrin And Metalloproteinase (ADAM)33*, is selectively expressed in mesenchymal cells and its metalloprotease activity has been linked to angiogenesis and airway remodelling. A soluble form of ADAM33 (sADAM33) has been identified in the bronchoalveolar lavage fluid (BALF) of asthmatic patients and its levels inversely correlate with lung function and disease severity. Because tissue remodelling also occurs in pulmonary sarcoid, we hypothesised that sADAM33 is elevated in BALF of patients with this disease which, like asthma, is heterogeneous.

**Methods** BALF was obtained from healthy controls (n=11) and patients with sarcoid (n=13) using fibre optic bronchoscopy according to current guidelines. After removal of immunoglobulins using Protein A/G and enrichment using Concanavalin A beads, sADAM33 was identified in BALF by Western blotting. A FRET peptide cleavage assay was used to assess ADAM33-like activity in BALF. Lung function (FVC%) and gas transfer (TLCO%) were measured at time of first diagnostic workup.




Abstract S141 Figure 1

**Results** sADAM33 protein in BALF was detected as a 25kDa fragment and levels were significantly increased in samples from sarcoid patients when compared to healthy controls ($p<0.05$) (Abstract S141 Figure 1A). Levels of sADAM33 were inversely correlated with lung function (FVC % predicted) ($p<0.05$) and gas transfer (TLCO% predicted) ($p<0.01$) (Abstract S141 Figure 1B). No difference in sADAM33 enzymatic activity was observed between healthy and sarcoid BALF samples.

**Conclusion** Release of sADAM33 is increased in sarcoid in association with abnormal lung function. Further studies will be required to determine whether the release of sADAM33 results in dysregulated metalloprotease activity, leading to angiogenesis and pulmonary parenchymal remodelling in pulmonary sarcoid. Since *ADAM33* polymorphism is related to reduced lung function in asthma and COPD, this study raises the possibility that there may also be genetic associations between *ADAM33* and some forms of pulmonary sarcoid. Finally, the occurrence of sADAM33 in asthma and sarcoidosis and its relation to reduced lung function suggests that it may be a biomarker of pulmonary remodelling in these diseases.

## S142 THE $K^+$ CHANNEL KCa3.1 IS EXPRESSED IN HUMAN LUNG FIBROBLASTS

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**Introduction** Idiopathic pulmonary fibrosis (IPF) is a common, progressive interstitial lung disease. Current treatments are ineffective. Ion channels are emerging as attractive therapeutic targets and in particular, the $Ca^{2+}$-activated $K^+$ channel $K_{Ca}3.1$ has been shown to modulate the activity of several structural and inflammatory cells which play important roles in model diseases characterised by tissue remodelling and fibrosis. We hypothesise that $K_{Ca}3.1$-dependant cell processes are a common denominator in IPF.
**Aims** The aim of the study was to determine whether the $K_{Ca}3.1$ channel is expressed in human lung derived (myo)fibroblasts, key effector cells in IPF.
**Methods** Human lung (myo)fibroblasts derived from non-fibrotic lobectomy specimens were grown in vitro, and characterised by immunofluorescence. RT-PCR was used to examine $K_{Ca}3.1$ mRNA expression. Western blot was used to confirm the presence of $K_{Ca}3.1$ channel protein. Patch clamp electrophysiology was performed to demonstrate the presence of functional $K_{Ca}3.1$ channels. To elicit $K_{Ca}3.1$ currents the $K_{Ca}3.1$ opener 1-EBIO (0.1 mM) was used.
**Results** Human lung (myo)fibroblasts, express the $K_{Ca}3.1$ channel at the mRNA level. Western blot demonstrated that the $K_{Ca}3.1$ protein is also present in human lung (myo)fibroblasts. (Myo)fibroblast cell lysates contained immunoreactive protein of approximately 48kD molecular weight, consistent with the reported size of the $K_{Ca}3.1$ channel. Patch clamp electrophysiology demonstrated the presence of ion currents typical of those carried by $K_{Ca}3.1$ channels. These increased in magnitude from passage two through passage seven. Overall, $K_{Ca}3.1$ currents were elicited in 62% of cells tested. In those cells, baseline currents of (mean±SEM) 53.76±7.95 pA at +40 mV increased to 1375±195.1 pA following addition of 1-EBIO (n=40 cells, $p<0.0001$), and were blocked by the selective $K_{Ca}3.1$ blocker, TRAM-34 (200 nM). There was an accompanying negative shift in cell reversal potential from −13.11±2.011 to −42.60±2.061 with addition of 1-EBIO ($p<0.0001$), which was reversed by TRAM-34.
**Conclusion** Human lung-derived (myo)fibroblasts express functional $K_{Ca}3.1$, $K^+$ channels. These findings raise the possibility that blocking the $K_{Ca}3.1$ channel may inhibit pathological myo(fibroblast) function in IPF, and thus offer a novel approach to therapy.

## S143 SERUM MANNOSE BINDING LECTIN DEFICIENCY IS PRESENT IN PATIENTS WITH EARLY ONSET INTERSTITIAL PULMONARY FIBROSIS AND THOSE WITH AFFECTED RELATIVES SUGGESTING A GENETIC RISK FACTOR FOR DEFECTS IN THE INNATE IMMUNE SYSTEM

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**Background** Idiopathic pulmonary fibrosis (IPF) is a serious progressive lung disease with likely environmental and genetic risk factors that are thought to contribute to the disease even though their exact nature is unknown. It is increasingly recognised that siblings and close blood relatives can develop the same condition suggesting an unknown genetic predisposition.
**Method** We have examined the serum mannose binding lectin levels (MBL) in healthy controls (HC), frequently exacerbating COPD, pulmonary TB & Sarcoidosis along with IPF patients.
**Results** Mean serum MBL levels were not statistically different in HC, COPD or TB using an unpaired t test. Cases with sarcoid had higher levels. Those with IPF onset at <50 years old and those with affected blood relatives (FH) had significantly reduced levels compared with IPF onset >50 years without a family history. Abstract S143 Table 1 shows means, SEM and p values, and the per cent of each patient group with normal >650, moderate 100–600 or severe deficiency levels <100.

Abstract S143 Table 1

| Parameter | Mean MBL±SEM pg/ml | p Value v's HC | p Value v's IPF>50 years | p Value v's IPF <50 years | p Value v's IPF & FH | % >650 MBL | % 100–600 MBL | % <100 MBL |
|---|---|---|---|---|---|---|---|---|
| HC n=111 | 1315±136 | – | 0.48 | 0.035 | 0.01 | 53 | 32 | 15 |
| COPD n=33 | 1492±257 | 0.58 | 0.90 | 0.05 | 0.022 | 55 | 27 | 18 |
| TB n=47 | 1945±268 | 0.98 | 0.24 | 0.004 | 0.004 | 72 | 11 | 17 |
| Sarcoid n=38 | 2040±275 | 0.02 | 0.12 | 0.002 | 0.0012 | 68 | 16 | 16 |
| IPF >50 n=60 | 1475±203 | 0.48 | – | 0.012 | 0.007 | 58 | 32 | 10 |
| IPF <50 n=19 | 632±213 | 0.03 | 0.012 | – | 0.59 | 26 | 42 | 32 |
| IPF & FH n=18 | 688±279 | 0.01 | 0.007 | 0.59 | – | 27 | 33 | 40 |

**Discussion** Serum MBL forms part of the complement activation and innate immune system and protects the lung from infection by organisms that bind mannose sugar (eg, strep, staph, yeasts, P. Jiveci). MBL deficiency gives an opsonisation defect with reduced phagocytosis by alveolar macrophages. Blood levels are genetically determined, with UK population data showing:

- 12% severe deficiency <100 pg/ml,
- 34% moderate deficiency 100–600 pg/ml,
- 54% normal < 650 pg/ml.

Serum levels relate to polymorphisms of the MBL2 genes. $\chi^2$ analysis of frequency distribution showed no differences for HC, COPD & IPF>50 years. TB&Sarcoid had higher frequencies of normal MBL levels compared with HC (p=0.001 and 0.024 respectively). IPF <50 & IPF& FH showed higher frequencies of moderate and severe deficiency compared with HC and all other groups (p=0.001 and 0.001 respectively).

The literature shows MBL to consistently have interesting and important central roles in lung defenses via effects on complement, apoptosis and cytokines. Its observed deficiency in young IPF and those with a FH could be a genetic risk factor of relevance, explaining its early occurrence in deficiency and later occurrence in 'sufficiency', where it gives protection from insult or injury to the lung.

# Smoke and pollution in COPD mechanisms

## S144 CIGARETTE SMOKE DYSREGULATES PRO-INFLAMMATORY CYTOKINE RELEASE FROM AIRWAY EPITHELIAL CELLS AND MACROPHAGES IN RESPONSE TO HAEMOPHILUS INFLUENZAE

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**Background** Chronic obstructive pulmonary disease (COPD) is characterised by repeated viral or bacterial exacerbations which increase morbidity, mortality and accelerate lung function decline. Chronic bacterial colonisation, frequently recognised in stable COPD patients, may contribute to airway inflammation and promote disease progression. Cigarette smoke has previously been shown to alter responses to LPS via the TLR-4 receptor in cell lines. We hypothesised that cigarette smoke would suppress the innate immune responses of airway epithelial cells and macrophages to *Haemophilus influenzae* (HI) favouring persistence.