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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

doi:10.1136/thx.2010.150946.44

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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. χ^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

doi:10.1136/thx.2010.150946.45

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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.