

in pancreatic endocrine function. This may be important in CF, where glucose handling is deficient even in those without established CF-related diabetes (CFRD). To look at this further, we assessed the response to a glucose challenge throughout the day in CF patients and compared it with healthy controls.

Method We compared 20 CF patients (17 pancreatic insufficient) without known CFRD with 6 healthy age and BMI matched controls. Following an overnight fast subjects consumed a standardised mixed meal (the gold standard measure of endogenous insulin secretion³, providing an equivalent glucose load to a standard OGTT) at 0800, 1300 and 1800 hours on the same day. Blood glucose and insulin were measured over 120 minutes for each test meal and the area under the curve (AUC) calculated for the entire duration of each test. β -cell indices [β -cell function (%B), insulin sensitivity (%S), insulin resistance (IR)] were measured using the HOMA method⁴.

Results See Table (mean \pm SEM). CF subjects had greater overall glucose levels throughout the day when compared to controls for all 3 tests ($p<0.005$). β -cell function was highest in the afternoon in the CF group in keeping with a lower AUC_{glucose} at this time and there was a decrease in %S and an increase ($p=0.03$) in IR with progression of the day.

Conclusions This study demonstrates insulinopenia and reduced insulin sensitivity in the CF population resulting in glucose intolerance. Although not the primary defect in CF, there is an increase in insulin resistance as the day progresses. The clinical implications of this study are important not only for the diagnosis of CFRD but also its management in terms of the timing and profiling of exogenous insulin administration.

References

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Abstract S124 Table 1 Blood Glucose and markers of pancreatic beta-cell function

	Controls			CF		
	Morning	Afternoon	Evening	Morning	Afternoon	Evening
AUC _{Glucose} (mmol/L)	500 \pm 27	560 \pm 9	571 \pm 22	820 \pm 33	697 \pm 20	831 \pm 35
AUC _{Insulin} (μ U/ml)	4128 \pm 626	3662 \pm 548	3582 \pm 634	4005 \pm 573	3263 \pm 482	3449 \pm 555
% B	127 \pm 11	136 \pm 23	152 \pm 17	136 \pm 19	166 \pm 27	149 \pm 19
% S	109 \pm 22	112 \pm 29	71 \pm 9	91 \pm 8	78 \pm 9	77 \pm 10
IR	1.0 \pm 0.2	\pm 0.2	1.6 \pm 0.3	1.4 \pm 0.2	1.7 \pm 0.3	2.1 \pm 0.5

Novel mechanisms in lung fibrogenesis

S125 PRO-FIBROTIC EFFECTS OF MULTI-WALLED CARBON NANOTUBE EXPOSURE ON PRIMARY HUMAN ALVEOLAR TYPE II EPITHELIAL CELLS AND FIBROBLASTS

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Multi-walled carbon nanotubes (MWCNTs) are hollow fibre-like nanomaterials which are being investigated for use in drug delivery and as biosensors. However, due to their structural similarity to asbestos fibres, inhaled MWCNTs may elicit similar adverse health effects such as fibrosis and mesothelioma. Animal studies have suggested that this may be possible, however there is currently limited human data.

We hypothesised that the pro-fibrotic potential of MWCNTs would be determined by their physicochemical properties i.e length and concentration of impurities. We exposed primary human alveolar type II epithelial (ATII) cells and pulmonary fibroblasts to 30nm diameter CNTs of increasing length (0.2–2 μ m, 3–5 μ m and 10–30 μ m) and increasing purity (49%, 69% and >97%) for up to 96 hours. Oxidative stress, TGF β release, soluble collagen release, and cell proliferation were measured in fibroblasts and release of VEGF, MCP-1, TGF β and surfactant proteins (SP) A and D were measured in ATII cells.

MWCNTs induced oxidative stress in fibroblasts within 4h and a significant dose-dependent cell proliferation after 96h ($P<0.05$) that was not affected by MWCNT length or purity. Furthermore, there was a significant, dose-dependent, 4–7-fold increase in release of collagen, exceeding what could be accounted for by proliferation alone ($P<0.05$). There was a trend towards shorter and less pure CNTs inducing greater collagen release ($P<0.1$). Release of VEGF and MCP-1 from ATII cells was not induced by CNTs. However, TGF β , SP-A and SP-D were released by ATII cells and were found bound to MWCNTs. More SP-A bound to the 0.6–2 μ m MWCNTs compared to the longer MWCNTs; the converse was true for SP-D. In addition the >97% pure MWCNTs bound more surfactant protein than the lower purity MWCNTs. Significantly more TGF β was bound to the 10–30 μ m MWCNTs compared to shorter MWCNTs.

Our results demonstrate that MWCNTs can induce pro-fibrotic responses in primary human fibroblasts. Furthermore, our unique discovery of binding of TGF β and surfactant proteins to MWCNTs suggests that this could exacerbate the fibrotic response if MWCNTs translocate across the epithelial barrier, due to the “Trojan horse” effect of MWCNTs delivering these mediators to the interstitium.

S126 INTERLEUKIN-1 ALPHA (IL-1 α) RELEASED FROM INJURED LUNG EPITHELIUM IS A CRITICAL ALARMIN DRIVING ACTIVATION OF A POTENT INFLAMMATORY PHENOTYPE IN LUNG FIBROBLASTS

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Background Activation of the innate immune system plays a key role in exacerbations of chronic lung disease. Myeloid cells are classically considered to drive innate immune responses yet the potential of fibroblasts to act as immune cells has been postulated. We hypothesized that alarmins released from lung epithelium during environmental insults such as oxidant injury and viral infection might induce innate immune responses in lung fibroblasts.

Methods Human bronchial epithelial cells (BECs) and human lung fibroblasts (HLFs) were cultured from brushings taken from lung transplant recipients and resected lung tissue respectively. Cytokine concentrations were measured by ELISA or multiplex platform (MSD). Gene expression was assessed by qRT-PCR. Wild-type and *Il1a*^{-/-} mice were infected with Influenza (PR8). Data were analysed using t-Student or Mann-Whitney U Test. Correlations were assessed using Spearman rank correlation coefficient.

Results Conditioned media from PBECs subjected to oxidant injury contained elevated levels of alarmins. Treatment of HLFs with conditioned media significantly upregulated proinflammatory cytokine expression. Anti-IL-1 α or IL-1Ra significantly reduced induction of IL-8 (93% and 95%), IL-6 (90% and 91%), MCP-1 (92% and 93%) and GM-CSF (95% and 94%). Anti-IL-1 β had no effect. Co-stimulation with Poly I:C significantly accentuated the IL-1 α induced inflammatory phenotype in HLFs. Bronchoalveolar lavage (BAL) form

Influenza infected wild-type mice contains elevated levels of IL-1 α and activates innate immune signalling in wild-type murine lung fibroblasts (MLFs) but not *Il1r1*^{-/-} MLFs. BAL from *Il1a*^{-/-} mice had no effect on MLFs and demonstrated a blunted neutrophilic response to Influenza. Clinically we show that IL-1 α is increased in BAL of lung transplant recipients with infections and within 3 months of developing bronchiolitis obliterans syndrome (BOS) ($p < 0.001$) and that IL-1 α levels positively correlated with elevated IL-8 ($p < 0.001$) and neutrophil counts ($p < 0.001$).

Conclusions We propose a new paradigm of innate immune signalling in exacerbations of lung disease, where epithelial damage triggers a potent inflammatory phenotype in resident fibroblasts. The pivotal role of IL-1 α in this process is accentuated in the presence of viral infection. This novel pathway warrants further evaluation of its therapeutic potential to limit the repeated cycles of injury and exacerbation in chronic lung diseases.

S127 INFLUENZA A AND POLY(I:C) INDUCE α V β 6-INTEGRIN-MEDIATED TGF β ACTIVITY IN HUMAN EPITHELIAL CELLS VIA STIMULATION OF TLR3

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People with chronic lung disease are more susceptible to influenza infection which may lead to exacerbation of pre-existing conditions such as fibrosis. Transforming growth factor- β (TGF β) is a profibrotic cytokine, but its role during influenza infection remains unclear. Toll-like-receptor 3 is located on the endosomal membrane and binds dsRNA, an intermediate product from replicating ssRNA-viruses such as influenza. TLR3 activation has been shown to increase RhoA activity, and we have previously shown that RhoA is a key intermediary inactivation of TGF β by the α V β 6-integrin. Therefore, we hypothesised that influenza infection could stimulate TLR3 leading to activation of latent TGF β via this integrin in epithelial cells.

Immortalised human bronchial epithelial cells (iHBECS) were used in all experiments. To determine whether influenza virus (A/PR/8/34 H1N1), or poly (I:C) (20 μ g/ml) were able to activate TGF β the following TGF β activation assays were used; detection of phospho-smad2/3 in nuclear extracts of cell lysates by ELISA; analysis of TGF β activity in cells transiently transfected with a TGF β -sensitive reporterconstruct; and a co-culture of iHBECS with a TGF β reporter cell line (TMLCs). To confirm the involvement of TLR3, cells were dual transfected with a TGF β -sensitive reporter and a dominant negative TLR3 construct designed to prevent TLR3 signalling. The role of the RhoA-ROCK pathway, and α V β 6-integrin were investigated using the ROCK inhibitor H1152, and the α V β 6-integrin blocking antibody 6.3G9, respectively.

H1N1 infection and poly(I:C) caused an increase in luciferase in iHBECS transiently transfected with a TGF β reporter construct. Similarly, both H1N1 and poly(I:C) caused an increase in nuclear phospho-smad2/3 which could be blocked by 6.3G9 peaking at 4h. Both agents caused an increase in TGF β as measured by a co-culture assay and this could be blocked by H1152 and 6.3G9 suggesting the involvement of ROCK, α V β 6-integrin and the requirement for cell-to-cell contact. Finally, a role for TLR3 in this process was confirmed in cells transfected with a dnTLR3 construct which lost the ability to activate TGF β in response to poly(I:C) or H1N1.

In conclusion, these data show that both influenza A and poly (I:C) lead to increased TGF β activity in iHBECS. This supports the hypothesis that influenza A infection activates TGF β via TLR3 and the α V β 6 integrin. These data suggest a novel mechanism by which influenza infection of epithelial cells may promote airway and lung fibrosis.

S128 THE EXTRINSIC COAGULATION PATHWAY IS LOCALLY UPREGULATED IN AN EXPERIMENTAL MODEL OF VIRAL EXACERBATION OF PULMONARY FIBROSIS

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Introduction Acute exacerbation (AE) of idiopathic pulmonary fibrosis (IPF) is defined as an episode of acute respiratory worsening without an identifiable aetiology. Herpes viruses infections have been implicated as a possible cause of AE in IPF. Moreover, herpes viruses have been shown to act as developmental cofactors and exacerbating agents in experimental pulmonary fibrosis. There is growing evidence that the local activation of the coagulation cascade mediates potent profibrotic effects via the activation of proteinase activated receptors (PARs) and thereby contributes to the development of pulmonary fibrosis (Scotton et al, *J Clin Invest*. 2009, 119). We hypothesised that viral infections promote the local activation of the coagulation cascade and influence the progression of established experimental pulmonary fibrosis.

Methods C57BL/6 mice were infected with γ -herpesvirus (γ HV68) or given saline 14 days after oropharyngeal bleomycin (1mg/kg) instillation. The mRNA and protein levels of coagulation factors in lung tissue homogenates were assessed by qPCR and immunohistochemistry, respectively. Total lung collagen was quantified by assessing lung hydroxyproline levels by HPLC at 7 and 14 days post inoculation (p.i.).

Results Tissue factor (TF) and factor X (FX) mRNA levels were increased in the lungs of bleomycin- γ HV68 infected mice at day 7 p.i. compared with bleomycin alone treated animals. This upregulation was associated with increased TF and FX protein immunoreactivity, which was localised to bronchial and hyperplastic alveolar epithelium and appeared to persist at 14 days p.i. Total lung collagen levels were also increased in bleomycin- γ HV68 infected animals at 14 days p.i. ($p < 0.01$) compared to bleomycin alone treated mice.

Conclusions γ HV68 infection in established pulmonary fibrosis exacerbates the fibrotic response as evidenced by the increased deposition of total lung collagen. This is preceded by an amplification of the local activation of the extrinsic coagulation cascade. A recent clinical trial suggests that systemic anticoagulant therapy (warfarin) increases mortality in IPF (Noth et al, *Am J Respir Crit Care Med*. 2012, 186). The coagulation cascade may therefore play both protective and deleterious roles in pulmonary fibrosis. We propose that future anticoagulant interventions may need to be directed at selectively targeting local profibrotic signalling responses.

S129 DECREASED cAMP PRODUCTION IN LUNG FIBROBLASTS FROM PATIENTS WITH IDIOPATHIC PULMONARY FIBROSIS

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Rationale Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease with unknown aetiology and no effective therapy. Myofibroblasts are the primary effector cells in the pathogenesis of IPF and differentiation from fibroblasts is a major source of myofibroblasts. Prostaglandin E2 (PGE₂) inhibits fibroblast to myofibroblast differentiation via the E Prostanoid 2 (EP2) receptor and cAMP, suggesting cAMP is a key regulator of myofibroblast differentiation. The aim of the present study was to evaluate the effect of different cAMP elevating agents on myofibroblast differentiation.

Methods Fibroblasts from lungs of patients with IPF (F-IPF) and from non-fibrotic lungs (F-NL) were used. TGF- β 1 (2ng/ml 3d) was used to induce myofibroblast differentiation. The effect of PGE₂, β_2 -agonists Salmeterol and Formoterol, the direct adenylyl cyclase