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

activator Forskolin and the phosphodiesterase 4 (PDE4) inhibitor Roflumilast (all 1 μ m, 3d) was evaluated. IL-1 β (2ng/ml, 24hr) was used for cyclooxygenase 2 (COX-2) induction. α -smooth muscle actin (α -SMA, a myofibroblast marker), COX-2, EP2, EP4 and β_2 -receptor expression was analysed by Western blotting and immunocytochemistry, respectively. Adenylyl cyclase mRNA was measured by qPCR and cAMP was measured by radioimmunoassay.

Results F-IPF showed increased α -SMA and collagen expression and repressed COX-2 expression compared to F-NL. PGE₂ treatment prevented TGF- β 1-induced α -SMA expression and COX-2 repression in F-NL, which was mimicked by β_2 -agonists and Forskolin. PGE₂ also reduced α -SMA expression and increased COX-2 expression in F-IPF despite that it induced significantly less cAMP than in F-NL. But this effect on F-IPF was not mimicked by β_2 -agonists and Forskolin as they induced even less cAMP than PGE₂ in these cells. TGF- β -treated F-NL also produced less cAMP than untreated cells in response to these cAMP stimulants. However, the expression of EP2, EP4, β_2 -adrenoceptors and adenylyl cyclase isoforms was similar in F-NL and F-IPF. Furthermore, combination of PGE₂ with Roflumilast showed greater effect than PGE₂ alone on α -SMA reduction and COX-2 expression in F-IPF and F-NL, whereas Roflumilast alone had no effect.

Conclusions cAMP is a key anti-fibrotic regulator of myofibroblast differentiation. However, cAMP production in myofibroblasts is defective, probably due to increased degradation by PDE4.

S130 EXOGENOUS MACROPHAGES ARE RETAINED IN MOUSE LUNGS AFTER INJURY AND TARGET THERAPEUTIC TRANSGENES TO THE INJURED LUNG PARENCHYMA

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Introduction Pulmonary fibrosis evolves in response to epithelial injury in a number of lung diseases, and carries a poor prognosis; novel therapies are urgently needed. The epithelial mitogen keratinocyte growth factor (KGF) has been shown to prevent fibrosis in a number of animal models however its therapeutic utility is limited by its short half-life. There is a growing interest in cell therapy approaches, and we hypothesised that macrophages could be used as vehicles to target KGF therapy to injured lung.

Methods Lentiviral vectors expressing luciferase, KGF and GFP (control) were generated and used to transduce the IC-21 macrophage cell line. Appropriate transgene expression was confirmed. KGF macrophages were co-cultured with primary mouse tracheal epithelial cells in a proliferation bioassay. Luciferase-expressing macrophages were tracked longitudinally using bioluminescence imaging after oropharyngeal delivery to the lungs of mice given bleomycin to induce injury, or saline control. Immunostaining was used to localise macrophages within lung sections. KGF and GFP-macrophages were delivered during bleomycin-induced lung injury; endpoint measures included lung histology, micro-CT analysis, and quantification of inflammatory cell infiltrates, vascular leak, lung collagen by HPLC, and inflammatory and fibrotic mediators by ELISA and qPCR.

Results Exogenously delivered macrophages were retained in the lungs of bleomycin-injured mice, but not uninjured controls, when given during either the inflammatory or fibrotic phases of injury, and localised to injured lung parenchyma. KGF-transduced macrophages induced proliferation of mouse tracheal epithelial cells during co-culture, but delivery to bleomycin-injured mice was not associated with overall improvements in endpoints when delivered during either the inflammatory or fibrotic phases of injury. Delivery of macrophages *per se* was associated with an increase in inflammatory mediators consistent with classical M1 macrophage activation, which may have off-set any beneficial effects of KGF-transduced macrophages.

Conclusions Exogenously delivered macrophages are preferentially retained in injured lung and show potential as vehicles to target therapeutic transgenes by localising to damaged areas. Whilst KGF-transduced macrophages induced epithelial proliferation *in vitro*, any protective effects *in vivo* may have been negated by the exacerbatory effects of macrophage delivery. Future work will determine whether *ex vivo* manipulation of macrophage phenotype can confer therapeutic benefit.

Occupational lung disease

S131 IDENTIFYING OCCUPATIONAL ASTHMA AMONG A COHORT OF CLEANERS IN THE NORTH EAST ENGLAND

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Introduction We have demonstrated a prevalence of asthma of 14% in a survey of 1400 UK hospital and university cleaners, and an estimated incidence of asthma of 3.3/1000 person-years. 26% of cleaners reported work-related symptoms. We have explored the possibility that these cleaners have occupational asthma using serial measurements peak expiratory flow (PEF) and airway responsiveness.

Objectives To identify occupational asthma in a cohort of cleaners.

Methods A respiratory symptom questionnaire was distributed among 1400 cleaners working in three local hospital trusts and two universities. Airway responsiveness (PD20) was measured in those with asthma symptoms using a methacholine challenge test. Those with measurable airway responsiveness (PD20 <1600ug) were invited to undergo a repeat measurement away from work and to carry out serial PEF measurements that were analysed for a work-related effect using OASYS (Burge, Pantin et al. 1999).

Results 557 (40%) returned the questionnaire and 167 reported respiratory symptoms. Of these, 56 (33.5%) attended for methacholine challenge testing. 26 (46%) had quantifiable results.

12 subjects underwent serial PD20 measurements at and away from work. Overall, there were no significant changes in airway responsiveness. Geometric mean PD20 at work was 193 ug and away from work was 254 ug (t=0.6; p=0.5). 5 cleaners showed a 3-fold or more increase in PD20 away from work raising the possibility of significant changes in those individuals.

10 subjects completed serial peak expiratory flow measurements. The mean OASYS score was 1.97. One subject had a score of > 2.5 suggesting a work related effect.

Conclusion Although the prevalence of asthma symptoms in our cohort is consistent with other epidemiological evidence showing a 1.5 to 2.0 fold risk of asthma, we found little evidence of occupational asthma using conventional clinical diagnostic tests in this group. The findings are consistent with the hypothesis that cleaners develop their asthma in an unusual way, possibly through a low dose irritant mechanism.

Burge, P.S., C.F.A. Pantin, et al. (1999). "Development of an expert system for the interpretation of serial peak expiratory flow measurements in the diagnosis of occupational asthma." *Occupational and Environmental Medicine* 56(11): 758-764.

S132 CHRONIC BRONCHITIS, PULMONARY FUNCTION, AND OCCUPATIONAL EXPOSURE IN FRAMINGHAM HEART STUDY

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Corrections

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