Influenza infected wild-type mice contains elevated levels of IL-1α 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-1α 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-1α 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-1α 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 Vβ6-INTEGRIN-MEDIATED TGFβ ACTIVITY IN HUMAN EPITHELIAL CELLS VIA STIMULATION OF TLR3

doi:10.1136/thoraxjnl-2012-202678.132

[1]L Jolly, [1]A Stavrou, [2]S Violette, [2]P Weinreb, [3]T Hussell, [1]G Jenkins. *[1]University of Nottingham, Nottingham, United Kingdom; [2]Biogen Idec, Cambridge, MA, USA; [3]Imperial College, London, United Kingdom*

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 (TGFβ) 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 TGFβ by the αVβ6-integrin. Therefore, we hypothesised that influenza infection could stimulate TLR3 leading to activation of latent TGFβ 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) (20μg/ml) were able to activate TGFβ the following TGFβ activation assays were used; detection of phospho-smad2/3 in nuclear extracts of cell lysates by ELISA; analysis of TGFβ activity in cells transiently transfected with a TGFβ-sensitive reporterconstruct; and a co-culture of iHBECS with a TGFβ reporter cell line (TMLCs). To confirm the involvement of TLR3, cells were dual transfected with a TGFβ-sensitive reporter and a dominant negative TLR3 construct designed to prevent TLR3 signalling. The role of the RhoA-ROCK pathway, and αVβ6-integrin were investigated using the ROCK inhibitor H1152, and the αVβ6-integrin blocking antibody 6.3G9, respectively.

H1N1 infection and poly(I:C) caused an increase in luciferase in iHBECs transiently transfected with a TGFβ 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 TGFβ as measured by a co-culture assay and this could be blocked by H1152 and 6.3G9 suggesting the involvement of ROCK, αVβ6-integrin and the requirement for cell-to-cell contact. Finally, arole for TLR3 in this process was confirmed in cells transfected with a dnTLR3 construct which lost the ability to activate TGFβ in response to poly(I:C) orH1N1.

In conclusion, these data show that both influenza A and poly (I:C) lead to increased TGFβ activity in iHBECs. This supports the hypothesis that influenza A infection activates TGFβ via TLR3 and the αVβ6 integrin. These data suggest anovel mechanism by which influenza infection of epithelial cells may promoteairway and lung fibrosis.

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

doi:10.1136/thoraxjnl-2012-202678.133

[1]N Smoktunowicz, [1]R Alexander, [1]L Franklin, [1]AE Williams, [2]G Jarai, [1]CJ Scotton, [1]PF Mercer, [1]RC Chambers. *[1]Centre for InflammationTissue Repair, UCL, London, UK; [2]Novartis Horsham Research Centre, Horsham, UK*

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 (γHV68) 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-γHV68 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-γHV68 infected animals at 14 days p.i. ($p<0.01$) compared to bleomycin alone treated mice.

Conclusions γHV68 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

doi:10.1136/thoraxjnl-2012-202678.134

RL Simms, WR Coward, AJ Knox, L Pang. *The University of Nottingham, Nottingham, UK*

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 (PGE_2) 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-β1 (2ng/ml 3d) was used to induce myofibroblast differentiation. The effect of PGE_2, β_2-agonists Salmeterol and Formoterol, the direct adenylyl cyclase