lymphoid tissue and alveolar macrophages. IPF patient lung section analysis revealed an absence of TRAIL expression compared to controls. IPF patients had significantly lower serum levels of TRAIL than controls which inversely correlated with TLCO (% predicted) and positively correlated with survival from diagnosis.

Conclusions We demonstrated that the neutrophilic inflammatory response to bleomycin is increased in TRAIL−/− compared with wild-type mice and that this finding is associated with increased collagen deposition. We also demonstrated reduced pulmonary and systemic expression of TRAIL in IPF, which correlates with worse pulmonary function and clinical outcome. This data suggests TRAIL may have biomarker potential and therapeutic benefit in pulmonary fibrosis.

S112 TNF-R1 UBIQUITOUS SCAFFOLDING AND SIGNALLING PROTEIN (TRUSS) IS A REGULATOR OF TNF-α INDUCED NF-κ-B ACTIVATION

doi:10.1136/thoraxjnl-2011-201054b.112

[1]A J Langton, [1]I Horan, [2]S Farrow, [3]D W Riches, [1]E R Chilvers, [1]H Parfrey. *[1]University of Cambridge, Cambridge, UK; [2]GlaxoSmithKline, Stevenage, UK; [3]National Jewish Health, Denver, USA*

The pleiotropic cytokine TNFα plays a key role in the pathogenesis of many chronic inflammatory lung diseases, particularly sarcoidosis, asthma and COPD. Due to its broad spectrum of activity however, current anti-TNFα therapies are of limited efficacy in these conditions and are associated with an increased risk of infection and malignancy. Interaction of TNFα with its cognate receptor, TNF-R1 initiates a signalling cascade that leads ultimately to the phosphorylation of the transcription factor NF-kB. This allows NF-kB to shuttle in a co-ordinated manner between the cytoplasm and the nucleus, leading to the up-regulation of genes that are key to cellular inflammatory and apoptotic responses. We propose that the novel TNF-R1 interacting protein TRUSS (TNF-R1 Ubiquitous Scaffolding and Signalling protein), which interacts with members of the TNF-R1 signalling cascade, may regulate this process. A549 cells, which express high levels of endogenous TRUSS, were transfected transiently with siRNA, which resulted in 80±14% (mean±SEM, n=16) knockdown of TRUSS mRNA. TRUSS deficient cells demonstrated a profound early (<1 h) defect in the nuclear translocation of p50/p65 subunits following TNFα stimulation ($p<0.05$, n=3). As a consequence, in the absence of TRUSS, p50, its precursor phospho-p105, and phospho-p65 were retained in the cytoplasm in these cells following TNFα stimulation. Furthermore, TRUSS depletion caused a reduction in TNFα stimulated NF-kB ($p<0.01$, n=7) and AP-1 ($p<0.01$, n=6) luciferase reporter activity; this was associated with a decrease in interleukin 6, RANTES, G-CSF and GM-CSF ($p<0.05$, n=6) mRNA and protein expression while MCP-1, CXCL5 and IL-8 were not affected. Although TRUSS deplete cells displayed impaired up-regulation of IkBa mRNA in response to TNFα stimulation, the protein response was intact. Upstream signalling molecules TNFR1, TRADD, TRAF2 and RIP were unaffected by TRUSS knockdown. In conclusion, these data suggest a novel role for TRUSS as a scaffold protein involved in the initial nuclear translocation of p50/p65 NF-kB subunits, which regulates the early pro-inflammatory response to TNFα. Hence TRUSS may represent a more selective therapeutic target for modulating TNFα functions.

S113 THE INFLUENZA VIRUS ACTIVATES TGFß VIA AN αVß6-INTEGRIN MEDIATED PATHWAY

doi:10.1136/thoraxjnl-2011-201054b.113

[1]A S Stavrou, [1]L J Jolly, [1]J P Porte, [2]P W Weinreb, [1]A T Tatler, [1]A J John, [1]A K Knox, [2]S V Violet, [3]T H Hussel, [1]S K Kuchipudi, [1]S D Dunham, [1]G J Jenkins. *[1]University of Nottingham, Nottingham, UK; [2]Biogen Idec Inc, Cambridge; [3]Imperial College London, London, UK*

Introduction and Objectives Idiopathic pulmonary fibrosis is a chronic progressive lung disease of unknown cause. Its pathogenesis is poorly understood but activation of latent TGFß on lung epithelium is an important factor. TGFß must be activated, as it is secreted in a latent complex with its propeptide, the latency associated peptide, and the avß6 integrin is a key activator in the lung. The *Influenza A* virus is a single-stranded segmented RNA virus that infects epithelial cells leading to cell death and injury. Toll-like receptors (TLRs) detect pathogens, such as influenza. TLR3 activation has been found to increase RhoA activity. We previously showed that RhoA is a key intermediary in avß6 integrin-mediated TGFß activation. The aim of this study is to investigate whether influenza can activate TGFß and stimulate TLR3 leading to activation of TGFß through the avß6 integrin in epithelial cells.

Materials and Methods Immortalised human bronchial epithelial cells (iHBECs) were infected with influenza A (H2N3) virus at a multiplicity of infection 1 with, or without, the avß6 blocking antibody 6.3G9. iHBECs were also stimulated with the synthetic TLR3 ligand poly(I:C). TGFß activity was determined by: (1) immunoblotting for phosphorylated (phospho-) Smad2, and (2) Transformed mink lung cells (TMLC)-iHBEC cocultures. Infection efficiency was measured by Interferon ß mRNA levels by real-time qPCR.

Results Infection with H2N3 and stimulation with poly(I:C) led to increase in phospho-smad2 and luciferase activity in coculture indicating increase in TGFß activation levels in a dose- and time-dependent manner. In both cases this was blocked with the addition of 6.3G9. qPCR data following infection showed increased IFNß1 and PAI-1, indicating the ability of the virus to infect the cells and activate TGFß.

Conclusions Influenza infection and poly(I:C) activates TGFß in iHBECs in an avß6 integrin dependent manner. The data suggests a novel mechanism by which influenza infection of epithelial cells may promote lung fibrosis.

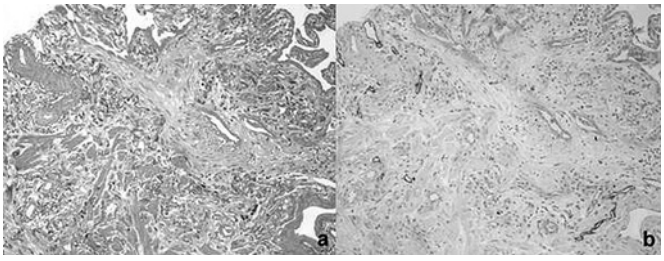
S114 LYMPHATIC VESSEL DISTRIBUTION IN FIBROTIC LUNG DISEASES

doi:10.1136/thoraxjnl-2011-201054b.114

[1]E A Renzoni, [2]E Weber, [2]F Sozio, [2]A Rossi, [1]A U Wells. *[1]Interstitial Lung Disease Unit, Royal Brompton Hospital, London, UK; [2]Molecular Medicine Section, Department of Neuroscience, University of Siena, Siena, Italy*

Relatively little is known on lymphatic vessel remodelling in lung fibrosis, and whether differences in lymphatic distribution underlie the worse survival seen in idiopathic pulmonary fibrosis, compared to the other fibrotic interstitial pneumonias. Lymphatic vessel remodelling and a deficit in lymphatic clearance could lead to prolonged exposure to pathogenic antigens and/or pro-inflammatory/pro-fibrotic mediators in the alveolar-interstitial space. In this study, we compared lymphatic and blood vessel morphology in lung biopsies of six patients with idiopathic pulmonary fibrosis (with usual interstitial pneumonia pattern-UIP), six patients with fibrotic non-specific interstitial pneumonia associated with scleroderma (NSIP) five patients with fibrotic organising pneumonia (FOP) and five controls (normal lung peripheral to resected cancer). Consecutive sections were stained with Movat's pentachrome and with double immunostaining for von Willebrand factor (blood vessels) and for podoplanin (lymphatic specific marker D2-40) (see Abstract S114 figure 1). Area, perimeter and position were recorded for all lymphatic vessels and for blood vessels with a diameter >15 µm, to be sure to exclude capillaries. In the three disease groups, blood

vessels were significantly smaller (mean diameter: FOP 18±2, NSIP 13±1, UIP 13±1 μm) compared to controls (23±2 μm). Density of lymphatic vessels was significantly reduced in NSIP and UIP (21±2 mm^{-2}), compared to controls (35±4 mm^{-2}) and their size was significantly greater (mean diameter: NSIP 111±10 μm, UIP 121±5 μm, controls: 74±9 μm). In controls, 85±6% of the parenchymal lymphatics were close (<100 μm) to a blood vessel, and only 5±4% were in proximity of bronchoalveolar spaces, while in all three disease groups they were less frequently perivascular (FOP 47±6%, NSIP 55±3%, UIP 56±2%) and more frequently associated with the bronchoalveolar lumen (FOP 52±11%, NSIP 85±3%, UIP 69±2%). Lymphatic vessels were rarely seen inside Masson bodies and never inside fibroblastic foci (Abstract S114 figure 1). These data are consistent with a substantial remodelling of lymphatic vessels in fibrotic lung disease, with a shift of lymphatics away from blood vessels.



Abstract S114 Figure 1 Two consecutive sections of a VIP biopsy. Movat's pentachrome staining (A) shows fibroblastic focus stained in light blue. Panel B corresponds to the consecutive section, lymphatic vessels stained by D2-40 (black), blood vessels (vWf) in red. Lymphatic vessels are absent within fibroblastic focus, but are seen in the fibrotic interstitium.

Respiratory muscles, exercise and ventilation

S115 THE EFFECT OF POSTURE ON THE 2ND INTERCOSTAL SPACE SURFACE PARASTERNAL ELECTROMYOGRAM (EMGPARA): VALIDATING A NOVEL CLINICAL TOOL TO MEASURE NEURAL RESPIRATORY DRIVE

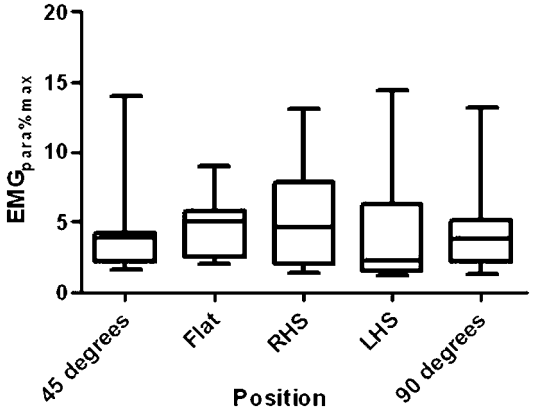
doi:10.1136/thoraxjnl-2011-201054b.115

[1]M C Ramsay, [1]E-S Suh, [2]S Mandal, [1]P Murphy, [2]J Steier, [3]A Simonds, [4]N Hart. *[1]Department of Asthma, Allergy and Respiratory Science, Division of Asthma, Allergy and Lung Biology, King's College London, London, UK; [2]Lane Fox Respiratory Unit, Guy's and St Thomas' NHS Foundation Trust, London, UK; [3]National Heart and Lung Institute, Respiratory Biomedical Research Unit, Royal Brompton Hospital and Imperial College London, London, UK; [4]Guy's and St Thomas' NHS Foundation Trust and King's College London, National Institute of Health Research Comprehensive Biomedical Research Centre, London, UK*

Introduction Although neural respiratory drive (NRD), as measured by diaphragm EMG, has been shown to reflect the balance between the respiratory muscle load and capacity providing a marker of disease severity, it requires insertion of an oesophageal catheter which limits its clinical utility. 2nd intercostal space surface EMGpara has been shown to be a useful alternative non-invasive monitoring tool in acute COPD (Murphy *et al. Thorax* 2011;**66**:602–8) and overnight in asthmatic patients (Steier *et al. Thorax* 2011;**66**:609–14). Previous data has suggested that there is reduced activation of the chest wall muscles in normal subjects in the supine posture as a consequence of a change in chest wall configuration. To assess the clinical utility and validity of EMGpara to continuously monitor changes in NRD, we investigated the effect of different posture on the EMGpara in normal subjects.

Methods Wet gel electrodes were placed at the parasternal edge of the 2nd intercostal space following skin preparation. Signals were amplified and filtered before analogue to digital conversion and digital processing providing the raw signal and root mean squared data. Five positions included sitting at 45 degrees, lying flat, lying on the right and left hand side and sitting at 90 degrees. EMGpara was measured during 2 min of tidal breathing in each posture. Resting EMG signal was normalised to the maximal inspiratory manoeuvres performed in each position (EMGpara%max).

Results Eight healthy subjects were recruited with a mean age 32 years ±2 years; 4 male; BMI 23±2 kg/m^2. Mean EMGpara%max was 4.60±3.93% sitting at 45 degrees, 4.82±2.27% lying flat, 5.32±3.91% lying on the right hand side, 4.47±4.47% lying on the left hand side, 4.58±3.75% sitting at 90 degrees. A repeated measures ANOVA showed there was no significant difference in $EMG^{para\%max}$ between the different postures (p= 0.97; Abstract S115 figure 1).



Abstract S115 Figure 1 Box and whisker Plot. Reference position is 45 degrees. There was no difference lying flat (p=0.9), lying on the right hand side (RHS; p=0.7), lying on the left hand side (LHS; p=0.9) and with 90 degrees upright (p=1.0).

Conclusion NRD, as measured by $EMG_{para\%max}$, is stable across a range of different postures. It provides comparable information independent of body position and could be considered as a monitoring tool in clinical practice, including for overnight monitoring.

S116 PARASTERNAL MUSCLE ELECTROMYELOGRAPHY (EMGpara) REFLECTS OBSERVED CHANGES IN DYNAMIC HYPERINFLATION DURING ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (AECOPD)

doi:10.1136/thoraxjnl-2011-201054b.116

[1]E Suh, [1]M Ramsay, [2]S Mandal, [2]E Boleat, [3]B Christian, [3]K Henderson, [1]P Murphy, [1]J Moxham, [1,2,4]N Hart. *[1]Department of Asthma, Allergy and Respiratory Science, Division of Asthma, Allergy and Lung Biology, King's College London, London, UK; [2]Lane Fox Respiratory Unit, Guy's and St Thomas' NHS Foundation Trust, London, UK; [3]Emergency Department, Guy's and St Thomas' NHS Foundation Trust, London, UK; [4]Guy's and St Thomas' NHS Foundation Trust and King's College London, National Institute of Health Research Comprehensive Biomedical Research Centre, London, UK*

Background During AECOPD, expiratory flow limitation results in dynamic hyperinflation (DH), respiratory neuromechanical uncoupling, and increased work of breathing causing breathlessness. We have previously demonstrated that 2nd intercostal space EMG_{para}, as a direct marker of neural respiratory drive (NRD), is able to detect clinical change in hospitalised AECOPD patients. We hypothesised that EMG_{para} has an indirect relationship with DH.

Method Patients admitted with AECOPD at a metropolitan teaching hospital were recruited. Inspiratory capacity (IC) was used as a measure of DH. EMG_{para}, spirometry and IC manoeuvres were measured twice daily from admission until the patient was fit for discharge. Dyspnoea scores (modified Borg score, visual analogue