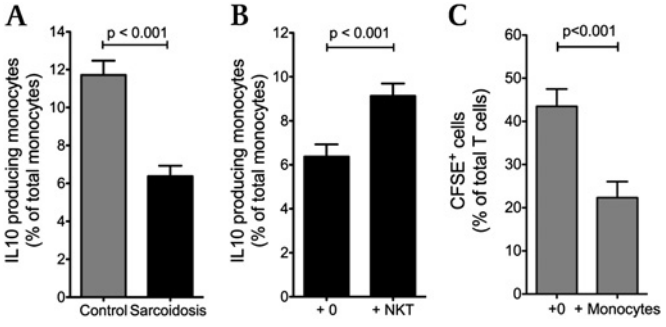
that NKT cell deficiency in sarcoidosis results in abnormal monocyte activity.

Methods Twenty-five steroid-naïve non-smoking patients with histological confirmation of sarcoidosis were recruited from the Sarcoidosis-ILD service. Circulating monocyte numbers and phenotype were first characterised using multi-colour flow cytometry. We then isolated monocytes from blood using magnetic microbeads, examined cytokine production after LPS stimulation with intracellular cytokine FACS staining and ELISA; and using monocyte-NKT cell co-culture assays, questioned whether NKT cells affected these monocytic functions.

Results We found an increase in circulating CD14CD16 inflammatory monocytes in patients with sarcoidosis, and identified a population of interleukin 10 producing monocytes in patients and controls after LPS stimulation. Monocytes from sarcoidosis patients have reduced capacity to produce IL-10 after LPS stimulation compared to control (6.37% vs 11.71% of total monocytes, p<0.001, Abstract S109 figure 1A); but addition of NKT cells improved this capacity (6.37% to 9.13%, p<0.001, Abstract S109 figure 1B). We then questioned the role of IL10-producing monocytes and show (with mixed lymphocyte reaction and CFSE assays) that these cells suppress T cell proliferation (p<0.001, Abstract S109 figure 1C).

Conclusions Our data show that sarcoidosis patients have increased inflammatory monocytes but a reduced IL-10-producing, T cell suppressing subset. NKTcells were able to interact with monocytes in vitro and increased IL-10 production by monocytes. These previously unrecognised findings, both in monocyte-NKT cross talk and in sarcoidosis immunobiology, suggest that one consequence of NKT deficiency in sarcoidosis is abnormal monocyte function with resultant loss in control of T cell proliferation. This reveals a potential new pathway of pathogenesis in sarcoidosis.



Abstract S109 Figure 1

S110 TARGETED DELETION OF GαQ/Gα11 IN SURFACTANT PROTEIN C-POSITIVE EPITHELIAL CELLS REDUCES TGFß ACTIVATION AND RESULTS IN INFLAMMATION AND ALVEOLAR AIRSPACE ENLARGEMENT

doi:10.1136/thoraxjnl-2011-201054b.110

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Activation of latent TGFß by the epithelially-restricted αvß6 integrin is critical in the pathogenesis of lung injury and fibrosis, and disruption of this pathway promotes emphysema development. We have previously shown that Gαq and RhoA signalling pathways are central to avb6 integrin induced TGFß activation in vitro. To assess the role of the Gq/11 signalling pathway in the lungs, we generated mice with deletion of the Gq and G11 α-subunits in Surfactant protein C (SftpC)-positive epithelial cells (Gq/11DKO). SftpC-Cre mice were crossed with constitutive Gα11-deficient animals (*Gna11*$^{-/-}$; G11KO) carrying floxed alleles of the Gaq gene (*Gnaq*$^{fl/fl}$) and then backcrossed onto appropriate null mice. Lungs were perfused, inflated and fixed prior to processing for histological and immunohistochemical analysis at 2, 4, 6 and 8 weeks. Bronchoalveolar lavage (BAL) cells were collected at 6 weeks for mRNA, nuclear protein extraction or histological analysis. Focal inflammatory infiltrates were visible in the Gq/11DKO lungs as early as 2 weeks, but became larger and more widespread at later timepoints. Gq/11DKO mice also exhibited significant age-related airspace enlargement compared with G11KO mice from 4 weeks onwards. From 6 weeks, inflammation was closely associated with localised disruption of the alveolar architecture and the appearance of enlarged and vacuolated macrophages within the airspaces. BAL fluid from Gq/11DKO mice contained significantly higher cells numbers ($12.5\pm2.5\times10^5$) than G11KO mice ($0.96\pm0.2\times10^5$) with increases in the percentage of neutrophils, lymphocytes and enlarged and vacuolated alveolar macrophages. mRNA analysis of Gq/11DKO BAL cells showed significantly increased MMP12, RELMα and Arginase 1 suggesting an increase in the number of alternatively activated macrophages. To assess levels of active TGFß in the lungs, phosphorylated SMAD2 (pSMAD2), a component of the TGFß signalling pathway, was measured by ELISA of nuclear extracts from BAL cells. Gq/11DKO BAL cells contained significantly lower levels of pSMAD2 than those from G11KO mice, suggesting decreased levels of active TGFß in the lungs of Gq/11DKO mice. These data suggest that the Gαq/11 signalling pathway in SftpC-positive epithelial cells regulates TGFß activation in the lungs and that deficiency in this pathway results in pulmonary inflammation and disruption of the alveolar architecture of the lung.

S111 THE ROLE OF TNF-RELATED APOPTOSIS INDUCING LIGAND (TRAIL) IN PULMONARY FIBROSIS

doi:10.1136/thoraxjnl-2011-201054b.111

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Introduction The concept of driving cellular apoptosis as a potential therapy for diseases characterised by inappropriate cellular persistence or proliferation is of widespread interest. We previously showed a death receptor ligand, TRAIL, accelerates neutrophil apoptosis without associated cell activation (*J Immunol* **170**:1027–33) and other work revealed TRAIL-induced apoptosis of human lung fibroblasts. The aims of this project were to study the role of TRAIL in a bleomycin lung injury model in wild-type and TRAIL−/− mice and in patients with idiopathic pulmonary fibrosis (IPF).

Methods Mice received intratracheal bleomycin or saline control. Bronchoalveolar lavage (BAL) at 3, 7, 16 and 23 days was analysed by cytospin morphology and haemocytometer count for % neutrophils, % neutrophil apoptosis, total number of neutrophils and total number of apoptotic cells. Flow cytometry was also used to analyse apoptosis. Collagen deposition in whole lung samples was analysed using a hydroxyproline assay. TRAIL expression and TUNEL positive events were also analysed. Serum and lung tissue from IPF patients/controls were examined for TRAIL expression and concentration. Lung function and survival data were retrieved from patient charts.

Results BAL analysis revealed statistically significant differences between TRAIL−/− and wild-type mice, with TRAIL−/− mice showing increased neutrophil numbers and reduced neutrophil apoptosis as absolute count or as % total cell count. Collagen deposition was statistically greater in TRAIL−/− mice at 16 days. At day 23, TRAIL−/− mice had decreased TUNEL positive events compared to wild-type mice. Histological analysis of murine lung sections revealed specific TRAIL expression in bronchus associated

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

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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

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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

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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