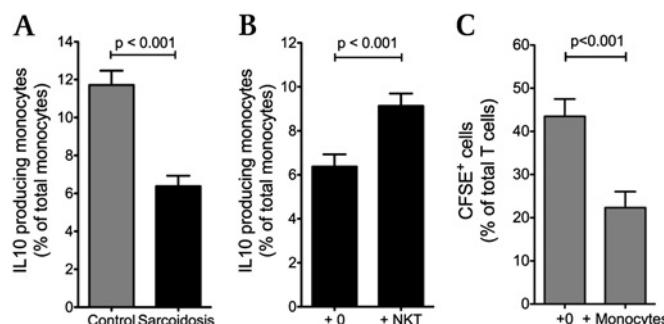


that NKT cell deficiency in sarcoidosis results in abnormal monocyte activity.

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. NKT cells 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 $\text{G}\alpha\text{Q}/\text{G}\alpha\text{11}$ IN SURFACTANT PROTEIN C-POSITIVE EPITHELIAL CELLS REDUCES TGF β ACTIVATION AND RESULTS IN INFLAMMATION AND ALVEOLAR AIRSPACE ENLARGEMENT

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Activation of latent TGF β by the epithelially-restricted avb6 integrin is critical in the pathogenesis of lung injury and fibrosis, and disruption of this pathway promotes emphysema development. We have previously shown that G α q and RhoA signalling pathways are central to avb6 integrin induced TGF β activation in vitro. To assess the role of the G α q/G α 11 signalling pathway in the lungs, we generated mice with deletion of the G α q and G α 11 α -subunits in Surfactant protein C (SftpC)-positive epithelial cells (G α q/G α 11DKO). SftpC-Cre mice were crossed with constitutive G α 11-deficient animals (Gna11 $^{−/−}$; G11KO) carrying floxed alleles of the G α q 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 G α q/G α 11DKO lungs as early as 2 weeks, but became larger and more widespread at later timepoints. G α q/G α 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 G α q/G α 11DKO mice contained significantly higher cells numbers ($12.5 \pm 2.5 \times 10^5$) than G11KO mice ($0.96 \pm 0.2 \times 10^5$) with increases in the percentage of neutrophils, lymphocytes and enlarged and vacuolated alveolar macrophages. mRNA analysis of G α q/G α 11DKO BAL cells showed significantly increased MMP12, RELM α and Arginase 1 suggesting an increase in the number of alternatively activated macrophages. To assess levels of active TGF β in the lungs, phosphorylated SMAD2 (pSMAD2), a component of the TGF β signalling pathway, was measured by ELISA of nuclear extracts from BAL cells. G α q/G α 11DKO BAL cells contained significantly lower levels of pSMAD2 than those from G11KO mice, suggesting decreased levels of active TGF β in the lungs of G α q/G α 11DKO mice. These data suggest that the G α q/G α 11 signalling pathway in SftpC-positive epithelial cells regulates TGF β 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

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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 cytopsin 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