



Abstract S76 Figure 1 Proposed view of the TGF β -galectin-3 feedback loop. TGF β activates TGF β receptor leading to the phosphorylation of Smad2. Ultimately leading to the upregulation of galectin-3 through increased expression of LGALS3. The increase in levels of galectin-3 leads to the increase in galectin-3 at the cell surface. Cell surface galectin-3 prolongs TGF β receptor signalling and activity by preventing its endocytosis¹ TGF β also increases the secretion of galectin-3.² Galectin-3 promotes TGF β signalling by activating latent TGF β

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ROLE OF GALECTIN-3 IN THE DEVELOPMENT OF IDIOPATHIC PULMONARY FIBROSIS

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Introduction Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive lung disease with a poor prognosis. Activation of the profibrotic cytokine transforming growth factor β (TGF β) is crucial in IPF development. It has been shown that the β -galactoside binding lectin galectin-3 can promote fibrosis through altering cellular responses to TGF β .¹ However, the precise mechanism of this is currently unknown.

Methods Immunohistochemistry (IHC) staining for galectin-3 in lung sections from patients with IPF and mice treated with 28 day of saline or bleomycin. Transformed mink lung epithelial cells (TMLCs) which stably express firefly luciferase at the plasminogen activator inhibitor-1 promoter region were used as a measure of TGF β activity. TMLCs were treated with increasing concentrations of recombinant human galectin-3 (rhGal-3) and luciferase activity was measured. Human lung fibroblasts (HLFs) isolated from control patients (non-IPF) and patients with IPF, and immortalised human bronchial epithelial cells (IHBECS) were treated with 50 μ M TGF β receptor type 1 inhibitor (Alk5 inhibitor), or 1 μ M galectin-3 inhibitor TD139 (currently in clinical trials for IPF treatment) and 10 μ g/ml rhGal-3. Mouse embryonic fibroblasts expressing the integrin α v β 6 (MEF β 6), HLFs, and IHBECS were treated with 1 μ M TD139 and 2 ng/ml TGF β . Protein expression of phosphorylated Smad2 (pSmad2) and total Smad2 were assessed by western blot. qPCR was used to measure galectin-3 at the mRNA level in HLFs treated with 2 ng/ml TGF β .

Results IHC showed that galectin-3 is increased in patients with IPF and in the bleomycin mouse model; where expression is

increased in airway epithelial cells, fibroblasts, and macrophages. Treatment with rhGal-3 increased TGF β activity in TMLCs, and significantly increased levels of pSmad2 in HLFs but not IHBECS which was inhibited by the Alk5 inhibitor and TD139. Treatment with the galectin-3 inhibitor TD139 significantly inhibited TGF β dependent Smad2 phosphorylation in MEF β 6 and HLFs but not in IHBECS. TGF β stimulation significantly increased galectin-3 expression at the mRNA level in IPF HLFs but not control HLFs. **Conclusion** Galectin-3 is involved in TGF β signalling where it can promote TGF β activation and activity in fibroblasts. Results indicate the presence of a TGF β -galectin-3 positive feedback loop (figure 1). Thus galectin-3 can potentially be a therapeutic target for IPF treatment.

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TRANSCRIPTOMIC STUDIES REVEAL MONOCYTE-RELATED GENES AS MAJOR CONTRIBUTOR TO DISEASE ACTIVITY IN PULMONARY SARCOIDOSIS

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Despite major advances in characterising the disease pathogenesis of sarcoidosis, it is still unclear which immune pathway is

the main contributor to disease activity. The most effective treatment, corticosteroids, has a high adverse effect burden so there is a need to define more specific therapeutic agents with less side effects. In addition, for some patients with progressive active disease, corticosteroids are often unhelpful; therefore better understanding of the mechanisms of disease is needed for development new drugs. This study examines the potential immune processes involved in disease activity in sarcoidosis using gene expression profiling of peripheral blood mononuclear cells (PBMCs) (n=29) and bronchoalveolar lavage cells (BALCs) (n=12). Patients with well-defined pulmonary sarcoidosis and secure tissue-supported diagnosis were recruited from the Oxford Sarcoidosis Service during a defined 2 year period. Patients were not on treatment at the point of sampling. A CTAS¹-validated chest radiograph-blood disease activity score comprising lymphocyte, ACE and IgG levels (SCAS, scores from 0 to 12 reflecting low to high activity) was used to measure activity at the point of sampling. Gene expression profiles were derived from PBMC and BALCs using the Illumina HT-12 v4 expression chip. All RNA had a RIN \geq 8. We found a significant positive correlation between the 'immune response' gene set and SCAS for BALCs, by GSEA and Metacore functional analyses. Within this gene set, a transcriptional signature related to monocyte activity and function was shown to be the most significant gene network with an unexpected downregulation of TGF β receptor signalling pathway in low activity BALCs. In PBMCs and BALCs, the two IFN-g-inducible, monocyte-produced genes CXCL-9 and CXCL-10 were the soluble factors that most correlated with increasing activity ($r\geq 0.5$ by Spearman Correlation). In an independent cohort, SCAS levels were examined against CD14^{hi} classical monocyte levels (n=40, same inclusion criteria). This showed a marked correlation between monocyte frequency and level of activity as measured by SCAS ($r=0.67$; $p<0.001$; Spearman Rank correlation). These Results implicate monocytes as a major contributor to disease activity in sarcoidosis and propose monocyte pathways as potential specific targets for new therapeutics in sarcoidosis.

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INCREASED CD16BRICD62LDIMCD11B+SUBSET OF NEUTROPHILS IN BRONCHOALVEOLAR LAVAGE FROM PATIENTS WITH INTERSTITIAL LUNG DISEASE

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Introduction The Interstitial Lung Diseases (ILD) are a heterogeneous group of inflammatory and fibrotic diseases of the interstitium, with worst cases resulting in pulmonary fibrosis (PF). Increased neutrophils are found within the lung or bronchoalveolar lavage (BAL) in ILD and predict a poor prognosis. Neutrophil adhesion molecules, e.g., CD18/b2 integrins (LFA-1; CD11a, Mac-1; CD11b and CR3; CD11c) and L-Selectin (CD62L) regulate cellular recruitment and fibrosis in animal models of bleomycin-induced PF. Expression of the Fc receptor (CD16) is upregulated during neutrophil activation, whilst ICAM-1 (CD54) is a marker for neutrophil reverse-transmigration back across the endothelium.

Study Aim Investigate adhesion molecule expression profile of neutrophils in ILD patients compared to controls.

Methods BAL samples were collected from ILD and non-ILD patients undergoing bronchoscopy with informed consent. Adhesion molecule expression was studied via flow cytometry by staining cells with CD16, CD62L, CD11b, CD11c, CD11a, CD18 and CD54 antibodies.

Results Flow cytometric analysis of BAL showed significantly more neutrophils in ILD lavage express CD11b and CD18 compared to non-ILD controls ($p=0.0016$ and $p=0.0211$ respectively). No significant differences were found in CD11c or CD11a expression. Further analysis revealed ILD lavage contained a higher percentage of CD16^{bric}CD62L^{dim} neutrophil subset expressing CD11b than non-ILD lavage controls ($p<0.0001$); a subset previously associated with a suppressive phenotype.¹ In addition, ICAM-1 expression was significantly down-regulated in ILD lavage neutrophils ($p=0.0397$) and this was also reflected in the CD16^{bric}CD62L^{dim} neutrophil population ($p=0.0445$).

Conclusions From our preliminary study, we have observed an increased percentage of CD16^{bric}CD62L^{dim}CD11b⁺ subset of neutrophils in ILD lavage compared to controls. ILD lavage neutrophils express significantly less ICAM-1. This suggests that more neutrophils are entering and being retained within the lung in ILD. Further experiments will dissect whether ILD neutrophils have altered functions (such as NETosis, ROS production, adhesion or migration) to contribute to disease progression.

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LOCALISED HYPOXIA ENHANCES NEUTROPHIL EXTRAVASATION AND ACTIVATION IN INTERSTITIAL LUNG DISEASE

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Background Neutrophilic inflammation is common in various diseases and may contribute to the pathophysiology of interstitial lung disease (ILD), however the underlying mechanism are not fully understood. Localised tissue hypoxia is often accompanied with inflammation, which may alter cellular responses to drive immunopathology.

Hypothesis We propose that hypoxia modulates neutrophil functions including integrin activation, neutrophil extravasation and neutrophil extracellular trap (NET) release that may contribute to pulmonary damage in ILD

Methods Pulmonary hypoxia was assessed using both fluoromisonidazole (FMISO)-PET scanning of ILD patients and immunohistochemical HIF-1a staining in ILD and control lung sections. To examine the effects of hypoxia, isolated neutrophils were cultured under hypoxia (1% oxygen) or normoxia (21% oxygen) prior to experimentation. Neutrophil integrin expression was evaluated using flow cytometry. Neutrophil