Spoken sessions

Blocking the functions of Siglec-7 and -9 on both primary monocytes and MDM with neutralising antibodies does not alter the *in vitro* inflammatory response to Mtb. Antibody crosslinking Siglec-9, but not Siglec-7, on MDMs induces endogenous MMP inhibitor TIMP1 (p = 0.001, see figure 1), lowering the MMP9/TIMP1 ratio which is a predictor of proteolytic damage.

**Conclusion** Crosslinking Siglec-9 on MDMs has potential to reduce their net proteolytic activity in Mtb infection and may reduce harmful tissue damage.

**Abstract S48 Figure 1** Siglec-9 activation upregulates TIMP1 release from Mtb-infected MDM

**Methods** We used a double-transgenic mouse with megakaryocyty-specific deletion of TGFβ (PF4-Cre/+/Tgfb1fl) and hence platelets lacking TGFβ. Knockout (KO) mice and wildtype (WT) littermate controls were subjected to the experimental model of lung fibrosis induced by oropharyngeal bleomycin administration. Lung tissue and broncho-alveolar lavage fluid (BALF) were investigated at 6, 21 or 28 days post-bleomycin. Complementary *in vitro* studies were performed on isolated neutrophils to investigate the effects of platelet-derived TGFβ in chemoraxis assays.

**Results** *In vitro*: Platelet-derived TGFβ was shown to be a potent neutrophil chemoattractant with maximal effect at 1ng/ml. *In vivo*: At 6 days after bleomycin treatment, neutrophils and macrophages were significantly elevated in the lung and BALF in both WT and KO animals as measured by flow cytometric analysis. No significant difference in the percentage or total cell numbers was found between WT or KO mice. At 21 days post-bleomycin, the lungs developed large fibrotic lesions when examined by micro-CT. Bleomycin-treated KO mice exhibited an attenuated fibrotic response compared with WT animals (26.9 vs. 19.6%), although not reaching statistical significance. During the wound resolution phase at 28 days post treatment, the degree of fibrosis between WT and KO animals was very similar (9.56 vs. 9.84%) as determined by micro-CT analysis.

**Conclusion** Our data suggest that despite being a potent neutrophil chemoattractant *in vitro*, platelet-derived TGFβ *in vivo* is not a major driving force during the inflammatory or resolution phases of our PF animal model, but may contribute to the development of fibrotic disease. This will be the subject of further study.

**Idiopathic Pulmonary Fibrosis: Mechanisms**

**THE ROLE OF PLATELET-DERIVED TGFβ IN PULMONARY FIBROSIS**

**Background** Pulmonary Fibrosis (PF) is characterised by abnormal wound healing involving fibroblast proliferation, myofibroblast differentiation and increased extracellular matrix deposition. TGFβ is an important driving force in fibrotic disease, however the source of this cytokine in PF is ill-defined. Platelets can release large amounts of TGFβ, and we, and others, have shown platelet deposition in the lungs of patients with idiopathic pulmonary fibrosis (IPF), although the role of these cells in PF is unknown.

**Hypothesis** We propose that platelet aggregation and release of platelet-derived TGFβ contributes to the aberrant wound healing in fibroproliferative lung disease.

**Introduction** The central mechanism in IPF is a dysfunctional alveolar epithelial-fibroblast interaction resulting in an aberrant repair process. This defect is influenced by other immune processes; one of these is the macrophage pathway. Macrophages are heterogeneous immune cells that can control all phases of the repair process. ‘M2’ or ‘reparative’ macrophages have anti-inflammatory and reparative phenotype, with high scavenger activities. We investigate how monocytes (precursors of monocyte-derived lung macrophages) might contribute to fibrogenesis in IPF.

**Methods** 35 IPF patients (25 sampled while stable and 10 with AE-IPF) diagnosed according to the 2011 ATS/ERS/JRS/ALAT guidelines, with ‘definite’ or ‘probable’ IPF and age and gender-matched healthy controls were recruited over a one-year period. Those with emphysema greater than 25%, current smokers and malignancy were excluded. Lung function and CT fibrosis score1 were performed. Phenotype and function of purified monocytes and monocyte-derived macrophages (MDMs) were determined using qPCR and multi-flow cytometry for selected M1 and M2 genes and proteins (M1 – CD64 M2 – CD163 and CD200R by FACS; and 26 M1 and M2 macrophage markers against three house keeping genes). The ability of MDMs to phagocytose
(using pHrodo method) and ROS content (using H2DCFDA assays) ex vivo were also examined.

**Results and discussion** Circulating monocyte levels were significantly higher in IPF compared to healthy controls (p < 0.001) and correlated negatively with lung function (FVC r = −0.6, p = 0.003) and CT fibrosis score (r = 0.45, p = 0.007). IPF monocytes displayed higher M2:M1 ratio profile compared to healthy controls – with higher IL10, CD163, IL1R2, FGL2 and lower TNFa and CXCL10 gene expression. When these monocytes were differentiated to macrophages (MDMs) ex vivo, IPF macrophages showed a significantly higher level of M2 markers and CD14 expression, reduced phagocytosis and produce lower levels of ROS –supporting M2 and pro-repair phenotype.

**Conclusions** Our data show that circulating monocytes in IPF are elevated compared to age-matched controls, correlate positively with disease severity, and are different from those found in age-matched healthy controls. They have pro-repair, M2-like features and differentiate to pro-repair monocyte-derived macrophages and may contribute to the aberrant repair process in IPF.

**REFERENCE**


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**MTOR REGULATES TGF-β INDUCED PRO-FIBROTIC GENE EXPRESSION IN PRIMARY HUMAN LUNG FIBROBLASTS**


10.1136/thoraxjnl-2016-209333.57

**Introduction** TGF-β is a major pro-fibrotic cytokine with a critical role in the pathogenesis of idiopathic pulmonary fibrosis (IPF). TGF-β drives fibroblast to myofibroblast differentiation and extracellular matrix synthesis. mTOR plays a critical role in regulating protein translation and is the catalytic subunit of two functionally distinct complexes, mTORC1 and mTORC2, which regulates protein translation and is the catalytic subunit of two functionally distinct complexes, mTORC1 and mTORC2, which have differential sensitivities to rapamycin. The aim of this study was to delineate mTOR signalling in response to TGF-β in human lung fibroblasts and investigate the role of the mTOR pathway in TGF-β mediated myofibroblast differentiation and collagen synthesis.

**Methods** All human samples were obtained with informed, signed consent and with research ethics committee approval. Primary human lung fibroblasts (pHLFs) were grown from explant cultures. Cells were pre-incubated with varying concentrations of inhibitor before stimulation with TGF-β 1 ng/ml. Collagen biosynthesis and αSMA expression were measured by a high-content imaging based molecular crowding assay. Gene expression was assessed by qPCR. Western blots were performed to assess mTOR substrate phosphorylation.

**Results** TGF-β was found to stimulate the delayed and sustained induction of mTOR signalling in pHLFs and this signalling pathway was critical for mediating the late peak in TGF-β induced pro-fibrotic gene expression. Accordingly, active-site mTOR inhibition exerted pronounced inhibitory effects on pHLF collagen biosynthesis and myofibroblast differentiation. The induction of mTOR signalling in response to TGF-β was dependent on the canonical Smad pathway. In addition, potent and selective pharmacological agents demonstrated that TGF-β induced mTOR signalling was independent of PI3K/Akt activity, suggesting that mTOR is not activated through the prototypical linear PI3K/Akt axis downstream of TGF-β. Moreover, rapamycin-resistant mTOR signalling was found to be critical for TGF-β induced pro-fibrotic gene expression in pHLFs.

**Conclusion** mTOR is an important pro-fibrotic signalling node downstream of TGF-β and a potential target for therapeutic intervention in IPF.

**REFERENCE**