from BR patients (n = 115), categorised by bronchiectasis severity index (BSI) scores and sera samples from HV controls (n = 26)

Results Endobronchial biopsies from BR airways had a significantly ($p < 0.05$) higher number of blood vessels per mm of basement membrane than HV samples (18 and 9 blood vessels/mm basement membrane respectively). Stimulation of HV neutrophils with a variety of molecules (PMA, fMLP, LPS, TNF-α etc.) resulted in a significant increase in VEGF secretion compared to unstimulated ($p < 0.05$). Although elevated VEGF was found in some patient samples there was no significant correlation between sera/sputa VEGF and individual patient BSI scores.

Conclusion The increased presence of vascular tissue seen in BR could indicate a pro-angiogenic airway environment in BR. The in vitro data collected also show that a variety of stimulants can initiate secretion of VEGF by neutrophils. However, our data does not suggest that VEGF levels in sera or sputa can be used to predict disease severity.

S47 PNEUMOLYSIN PROMOTES NEUTROPHIL: PLATELET AGGREGATION IN VITRO

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Introduction and objectives The pneumococcal cholesterol-binding, pore-forming toxin, pneumolysin (Ply), appears to be a key mediator not only of the acute lung injury, but also myocardial damage, associated with severe pneumococcal disease. Although direct Ply-mediated cardiopulmonary toxicity has been implicated, the neutrophil- and platelet-targeted pro-inflammatory activities of the toxin are also believed to contribute to the pathogenesis of these adverse events, albeit by poorly characterised mechanisms. To test the hypothesis that Ply promotes neutrophil: platelet networking, we have investigated the effects of the toxin on the induction of heterotypic aggregation of these cells *in vitro*.

Methods Neutrophil: platelet-enriched buffy coat suspensions were prepared from the heparinised blood of healthy, adult humans by sedimentation (at 37°C) and diluted 1:50 in Hanks' balanced salt solution. Following 5 min of preincubation, recombinant Ply (10–80 ng/ml), or the pneumolysoid, delta 6Ply (attenuated with respect to pore-forming activity, negative control), or adenosine 5'-diphosphate (ADP, 100 µM, positive control) were added to the cell suspensions. After a further 5 min period of incubation at 37°C, samples were stained with 5 µl of each of the following murine, anti-human, fluorochrome-labelled monoclonal antibodies: CD16-APC (neutrophils), CD42a-PE (platelets), and CD45-Krome Orange, and incubated for 15 min at room temperature in the dark. This was followed by analysis of samples at a slow rate using a Gallios flow cytometer. The relative numbers of platelets interacting with a single neutrophil were determined using the relative mean fluorescence intensities of CD16+/CD42a+/CD45+ neutrophils.

Results These are shown in the accompanying table. Addition of Ply to the mixed cell suspension resulted in statistically significant dose-related formation of neutrophil:platelet aggregates which was maximal at 80 ng/ml and greater in magnitude to that observed with ADP, while delta6Ply was ineffective.

Conclusion Ply, at pathologically-relevant concentrations, promotes neutrophil:platelet aggregation *in vitro*, an activity which is dependent on the pore-forming properties of the toxin. Given the increasing recognition of the role played by platelets in driving neutrophilic inflammation, this activity of Ply may exacerbate pulmonary and myocardial injury in severe pneumococcal disease.

Abstract S47 Table 1 Pneumolysin-mediated formation of neutrophil: platelet aggregates

System	Neutrophil: platelet aggregates (mean fluorescence intensity ± SDs)
Background	11.24 ± 5.0
ADP (100 µM)	20.63 ± 5.6*
Ply (10 ng/ml)	18.90 ± 10.7*
Ply (20 ng/ml)	27.92 ± 16.08*
Ply (40 ng/ml)	33.21 ± 17.09*
Ply (80 ng/ml)	42.09 ± 23.05*
Delta6Ply (80 ng/ml)	14.86 ± 7.49

*$p < 0.05 - p < 0.0009$

S48 TARGETING SIGLECS TO REDUCE PROTEASE-MEDIATED DESTRUCTION IN TUBERCULOSIS

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Background Tuberculosis (TB) is an inflammatory disease caused by infection with *Mycobacterium tuberculosis* (Mtb). The disease is often characterised by destructive pulmonary pathology, which itself aids transmission, and many who complete otherwise successful treatment are left with lasting respiratory impairment following TB-driven tissue damage and remodelling. This is largely mediated by matrix metalloproteinases (MMP) induced in the inflammatory response.

The CD33-related siglecs are transmembrane receptors that bind sialic acid. They are selectively expressed on immune cells, where they mediate inhibitory signalling. Murine Siglec-E is upregulated on macrophages by LPS. Its activation by crosslinking with sialylated nanoparticles reduces inflammatory cytokine release and mortality in murine models of sepsis and lung injury. Siglecs −5, −7 and −9 are candidate human orthologs of Siglec-E, known to inhibit inflammatory cell activation and proliferation. We hypothesised that the human orthologs of Siglec-E are upregulated in response to Mtb, which like LPS is a TLR-4 ligand, and that their activation would reduce TB-driven inflammation.

Methods Siglec expression at gene and protein level on primary monocytes isolated from blood donation, and in a monocyte derived macrophage (MDM) model was investigated by qPCR, flow cytometry and western blotting in both unstimulated and Mtb-infected cells. Monocytes and MDMs were infected with Mtb and incubated with antibodies to either neutralise or crosslink siglecs -7 and -9. The effect on their secretion of inflammatory cytokines and MMPs or their inhibitors (Tissue Inhibitors of Metalloproteinases – TIMPs) was measured by ELISA.

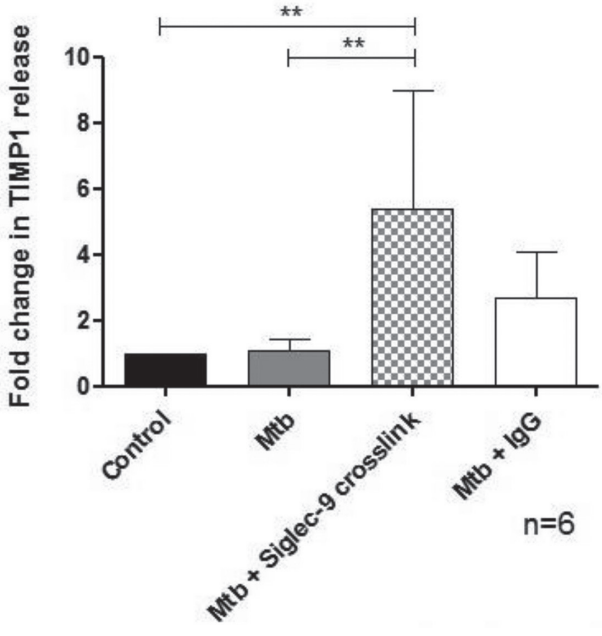
Results

Siglecs -5, -7 and -9 are constitutively expressed on human monocytes and MDMs.

Unlike Siglec-E in mice, these siglecs are not upregulated by LPS stimulation, nor by infection with Mtb.

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-infested MDM

Idiopathic Pulmonary Fibrosis: Mechanisms

S49 THE ROLE OF PLATELET-DERIVED TGFβ IN PULMONARY FIBROSIS

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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.
Methods We used a double-transgenic mouse with megakaryocytic-specific deletion of TGFβ (PF4-Cre^{+}/Tgfb1^{fl}) 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 chemotaxis 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.

S50 MONOCYTES FROM IPF PATIENTS SHOW PRE-CONDITIONED PRO-REPAIR FEATURES

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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