Methods Human neutrophils were purified from healthy volunteers by discontinuous percoll gradients. PAO-1 and PHD-deficient strains were grown in lysogeny broth and equivalent growth curves confirmed. Supernatants from wild-type and PHD-deficient P. aeruginosa were harvested at two hours and then co-cultured with neutrophils in normoxia and hypoxia. Neutrophil viability and apoptosis was then assessed using AnnexinV/Fluorescein isothiocyanate staining on flow cytometry at three and five hours.

Results Control neutrophils in normoxia saw a decrease in viability of 6% between three and five hours. Neutrophils treated with supernatant from the PHD-deficient strain experienced a decrease in viability from 3139 (±968) cells at three hours to 2058 (±586) at five hours – a decline of 34% (P < 0.05). Normoxic neutrophils treated with the wild-type strain, however, saw a decrease of 21% (P < 0.05).

Hypoxic conditions reversed the killing effects of wild-type P. aeruginosa: after five hours neutrophils in normoxia experienced a 21% decrease in viability, whereas the viability of hypoxic cells only decreased by 8% (P < 0.05).

Discussion These data highlight the relationship between tissue oxygen tensions and host immunity and that bacteria have evolved virulence factors with novel mechanisms of action; namely preventing neutrophil survival at sites of inflammation. Moreover, the potential oxygen-sensing capabilities of prokaryotes are intricately linked to bacterial virulence.
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, iMEP, 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/spuva 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 spuva can be used to predict disease severity.

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**PNEUMOLYSIN PROMOTES NEUTROPHIL: PLATELET AGGREGATION IN VITRO**

1G Nel, 1C Durandt, 1A Theron, 1GR Tintinger, 2TJ Mitchell, 3C Feldman, 3R Anderson.

**Methods** Neutrophil: -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 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 samples at a slow rate using a Gallios flow cytometer.

**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

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**TARGETING SIGLECS TO REDUCE PROTEASE-MEDIATED DESTRUCTION IN TUBERCULOSIS**

W Beynon, R McMullan, D McAuley, C O’Kane. Centre for Experimental Medicine, Queen’s University, Belfast, UK

**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 (MMPs) 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 cross-link 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.