

## S81 ONCOSTATIN M IS A NOVEL MEDIATOR OF HUMAN PULMONARY ENDOTHELIAL CHEMOKINE AND PROTEASE ACTIVITY IN THE LUNG IN ARDS

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**Background** In the acute respiratory distress syndrome (ARDS) predominantly neutrophil mediated inflammation causes injury to the alveolar epithelial and capillary endothelial junction. Oncostatin M (OSM) is a pleiotropic IL-6 cytokine, upregulated in the lung in ARDS. We have shown that OSM synergises with other cytokines to drive IL-8 secretion from alveolar epithelial cells, but its role in driving endothelial injury is unknown. CXCL5 is an OSM-dependent neutrophil chemokine that is implicated in driving endothelial cell-mediated neutrophil recruitment to the lung in animal models of pneumonia.

We hypothesised that OSM stimulates the production of neutrophilic chemokines and proteases by the alveolar endothelium in patients with ARDS.

**Methods** Immunocytochemistry and western blotting were performed respectively on fixed and lysed human pulmonary microvascular endothelial cells (HPMECs). The cells were stimulated with OSM  $\pm$  TNF- $\alpha$ , and chemokine (CXCL5/8) and matrix metalloproteinase (MMP) production measured by ELISA. Conditioned media from LPS-stimulated human macrophages (CoMLPS), pre-incubated with inhibitory antibodies to OSM, was used to stimulate HPMECs, and the effect on endothelial protease and chemokine production measured.

### Results

- The OSM receptor (OSMR) was detected in HPMECs at immunocytochemistry and western blot of cell lysates.
- Stimulation of HPMECs with recombinant OSM and TNF- $\alpha$  increased CXCL5 from 32 (5.6) to 739 (166) pg/ml, CXCL8 from 2.2(0.4) to 11.0 (2.9) ng/ml, and MMP-3 from 35.3 (1.8) to 57.6 (10.7) pg/ml. Concentrations of the endogenous inhibitors of MMPs, i.e. Tissue Inhibitors of Metalloproteinases (TIMPs)-2 were decreased from 25.2 (1.7) to 13.7 (3.2) ng/ml while concentrations of TIMP-1 were unchanged.
- CXCL5 levels were decreased from 13.2 (2.2) to 6.5 (1.1) ng/ml and levels of MMP-3 were decreased from 78.7 (16.6) to 41.4 (9.4) pg/ml from HPMEC stimulated with CoMLPS + inhibitory Ab to OSM. Interestingly, CXCL8 was not significantly abrogated by OSM neutralisation.
- CXCL5 was increased in BALF from 20.7 (1.9) to 392.5 (53.5) pg/ml from patients with ARDS (Figure 1).

**Conclusions** OSM drives induction of neutrophil chemokines, particularly CXCL5, and proteases by the alveolar endothelium. Targeted therapeutic inhibition of OSM may down-regulate neutrophil recruitment and MMP activity in ARDS, and will be further explored in an ex vivo human lung model.

## Pulmonary Vascular Disease

### S82 BONE MARROW TRANSPLANTATION REDUCES SUSCEPTIBILITY TO PULMONARY HYPERTENSION IN BMPR2 DEFICIENT MICE

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**Introduction** Increasing evidence suggests that patients with pulmonary arterial hypertension (PAH) have abnormalities in the bone marrow (BM). Approximately 40% of patients with PAH are reported to have evidence of myelodysplasia. Conversely PAH is often found (13–48%) in patients with myeloproliferative disease. In a bone marrow transplant (BMT) model it has been shown that mice transplanted with CD133+ cells from PAH patients developed pulmonary vascular remodelling and right ventricular hypertrophy; whereas, CD133+ cells from controls did not. CD133+ cells from PAH patients also showed greater myeloid commitment. Since mutation in the bone morphogenetic protein receptor type 2 (BMPR2) is the commonest genetic cause of PAH we questioned whether bone marrow transplantation of wild type bone marrow reduces the susceptibility to PAH in the BMPR2 heterozygous mouse.

**Methods** WT mice were transplanted with BMPR2  $\pm$  BM and BMPR2  $\pm$  mice were transplanted with WT BM. Sixteen weeks post-transplant mice were exposed to low-dose chronic LPS (0.5 mg/kg 3 times a week for 6 weeks), which we recently showed is a potent stimulus for the development of PAH in the BMPR2 heterozygous mouse. Mice underwent right heart catheterisation. Tissues were removed for histology.

**Results** We observed a significant increase in RVSP when WT mice were transplanted with BMPR2  $\pm$  bone marrow after chronic LPS dosing. There was an increase in spleen weight and circulating platelets in WT mice with BMPR2  $\pm$  bone marrow and a converse reduction in spleen weight and platelets in BMPR2  $\pm$  mice with WT BM. Bone marrow histology demonstrated reduced myeloid cells and megakaryocytes, iron deposition and fibrosis related to LPS administration. In preventative studies BMPR2  $\pm$  mice transplanted with WT bone marrow did not develop significant pulmonary hypertension after chronic LPS exposure.

**Conclusions** The susceptibility to PAH in the BMPR2 heterozygous mouse can be conferred on a wild type animal by bone marrow transplantation of BMPR2 deficient bone marrow. Transplanting WT BM into BMPR2  $\pm$  mice prevented PAH. The role of bone marrow derived cells in the pathobiology of PAH requires further elucidation, but may point to a potential future treatment