following therapy with Bosentan ($n = 6$) or sildenafil ($n = 1$). Interestingly, improvement in CI at repeat RHC correlated positively with a further increase in HGF levels ($r = 0.8$, $p = 0.03$).

Abstract P157 Figure 1.

Conclusion Plasma HGF concentrations correlate with haemodynamic severity in CTD-PAH. Further work to determine whether high levels of HGF are involved in the pathogenesis of PAH or are instead elevated in a reparative response to underlying pulmonary vascular remodelling is required.

REFERENCES

1. Ono M *et al.* *Circulation* 2004;110:2896–92
2. Hiramine K *et al.* *Int J Molec Med* 2011;27:497–502

P158 ENDOTHELIAL PROGENITOR CELLS FORM BIOLOGICAL EXCLUSION BARRIERS SIMILAR TO THAT OF MATURE ENDOTHELIAL CELLS- A THERAPEUTIC POTENTIAL IN SYSTEMIC SCLEROSIS?

¹RWG Good, ¹SLT Trinder, ¹BAA Abdi, ²RY Yu, ¹CPD Denton, ¹DJA Abraham, ¹AMH Holmes; ¹Center for Rheumatology and Connective Tissue Diseases, London, United Kingdom; ²Department of Surgery, UCL Medical School, Royal Free Hospital, London, United Kingdom

10.1136/thoraxjnl-2013-204457.309

Abstract Vascular complications associated with systemic sclerosis (SSc) including pulmonary arterial hypertension (PAH-SSc), result from endothelial damage and loss of barrier function. Endothelial progenitor cells (EPCs) express endothelial (VEGFR2⁺, CD31⁺) and haematopoietic (CD133⁺) markers. They home to sites of vascular injury and differentiate into endothelial cells restoring the endothelium. In SSc patients circulating levels of EPCs are reduced. This study aimed to develop a robust method to grow EPCs from peripheral blood mononuclear cells (PBMCs) and to compare cellular functions to mature endothelial cells.

Methods EPCs and human pulmonary artery endothelial cells (hPAECs) were seeded into transwell inserts and grown to confluence. Cells were incubated with TNF α (50ng/ml), and their capacity to form biological barriers assessed using FITC-albumin (5mg/ml). FITC-albumin 'leak' was quantified by fluorescent absorbance over time. We further assessed the responses of EPCs to TNF α stimulation by ELISA to quantify pro-inflammatory cytokine release.

Results EPCs form a biological exclusion barrier with similar capabilities as mature hPAECs. TNF α significantly enhanced the permeability of EPCs ($P < 0.05$) and hPAECs ($P < 0.05$) monolayers. There is no difference in EPC colony formation between HC and SSc EPCs.

Discussion We developed a robust method for isolating EPCs from PBMCs. We have demonstrated that EPCs can maintain an endothelial barrier consistent with that observed by mature hPAECs *in vitro*. We have established that EPCs respond to TNF α in a similar manner to mature PAECs. We have shown no significant difference in the capacity of PBMCs from SSc patients to form EPC colonies compared to HCs.

P159 PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION ARE HYPORESPONSIVE TO INFLAMMATORY STIMULI

¹LC Price, ²M Paul-Clark, ²C Meng, ²D Shao, ²JA Mitchell, ²IM Adcock, ¹SJ Wort; ¹Royal Brompton Hospital, London, UK; ²National Heart & Lung Institute, Imperial College London, London, UK

10.1136/thoraxjnl-2013-204457.310

Background Inflammation is a key feature of pulmonary arterial hypertension (PAH); circulating levels of plasma cytokines and chemokines are raised, some of which (including interleukin (IL)-6 and CXCL8) are associated with increased mortality (1). We wished to determine whether it was possible to detect an altered inflammatory phenotype in circulating inflammatory cells from PAH patients compared to controls.

Methods Following ethical approval, patients with idiopathic PAH and age-matched non-smoking control subjects were selected. Peripheral blood mononuclear cells (PBMC) (10^5 cells) or citrated whole blood (WB) were plated into 96-well plates and treated with E. Coli LPS (0–1 g/ml) (Sigma) or recombinant TNF- α (0–10ng/ml) (R&D systems) for 24h at 37°C. Following centrifugation, plasma was aspirated and samples frozen at -20°C, and IL-6 and CXCL8 ELISAs later performed. Data are mean $\pm$ SEM.

Results All patients had idiopathic PAH ($n = 12$) (7 in NYHA class III-IV) with 6MWD 370 ± 18 m, mPAP 72 ± 5 mmHg. All patients were taking advanced PAH therapies but not conventional anti-inflammatory therapies. There was no age difference between patients and controls ($n = 9$) (36.5 ± 3.7 vs. 36.2 ± 2.7 years, NS).

At baseline, release of IL-6 and CXCL8 was higher from PBMC from PAH patients than controls (918.1 ± 280.5 vs. 195.2 ± 64.3 for IL-6 ($p < 0.05$) and 4203 ± 1354 vs. 1048 ± 288.4 $p < 0.05$ for CXCL8), although no difference was seen between groups from the whole blood assays. Following stimulation with LPS, however, release of both IL-6 and CXCL8 was lower from both WB and PBMC assays for PAH patients compared to controls. For example for LPS-induced IL-6 release from PBMC, both the maximal effect (E_{max}) was lower in PAH vs. controls (5148 ± 1320 vs. 9154 ± 2510 pg/ml, $p < 0.05$) and the log EC_{50} (half maximal effective concentration) was higher in PAH vs. controls (-2.70 ± 0.27 vs. -5.90 ± 1.16 g/ml, $p < 0.01$) (Figure 1). Similar results were seen following stimulation with TNF- α .

Conclusion The main findings of this study are that: (1) baseline CXCL8 and IL6 release was higher from PBMCs of patients with PAH compared to control donors; (2) PBMCs and WB from patients were hyporesponsive to LPS (and TNF- α),