mice were assessed for right ventricular systolic pressure (RVSP) and right ventricular hypertrophy.
Results Reduced caveolae and depth of invaginations were observed in idiopathic PAH patients and individuals with a BMPR-II mutation when compared to controls. Interestingly, an individual with a BMPR-II mutation without disease had similar levels to controls. Furthermore, cavin-2 protein expression was decreased in cells from individuals with pulmonary hypertension, but other caveolae components appeared unaffected. In the absence of a disease stimulus caveolin-1 and cavin-2 knockout mice did not develop pulmonary hypertension although slightly elevated RVSP was observed.
Conclusions Our preliminary data suggests that caveolae formation is dysregulated in cells from individuals with pulmonary hypertension. In addition, reduced levels of cavin-2 could play a significant role in the decreased number of caveolae. Cavin-2 and caveolae generation could therefore be novel therapeutic targets for pulmonary hypertension.

S139 THE ROLE OF ENDOTHELIN RECEPTORS (ETRA/B) IN FIBROCYTE DIFFERENTIATION

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Introduction Scleroderma (SSc) is an autoimmune connective tissue disease of unknown aetiology. Pulmonary involvement including the development of pulmonary arterial hypertension (PAH) is characterised by vascular remodelling, collagen deposition and expression of connective tissue growth factor (CTGF). $CD14^+$ monocytes can differentiate into spindle shaped cells termed 'fibrocytes'. Fibrocytes express haematopoietic and mesenchymal markers including collagen, and amplify inflammatory/immune responses via antigen presentation and chemokine secretion. Fibrocyte differentiation is enhanced by fibrogenic cytokines including PDGF. The role fibrocytes play in promoting PAH in SSc is unknown.
Methods $CD14^+$ PBMCs were isolated from SSc and healthy donor blood. Fibrocyte differentiation in the presence of MCSF and/or ET-1 was assessed after 14 days. The effect of endothelin receptor (ETR) antagonists (selective/dual) on fibrocyte differentiation ($n = 6$) was investigated. SSc and control fibrocyte secretomes were assessed by ELISA ($n = 6$), and the effects on fibroblast-mediated gel contraction determined.
Results MCSF and ET-1 alone and in combination induced fibrocyte differentiation ($P < 0.05$). SSc fibrocytes exhibited enhanced differentiation from $CD14^+$ PBMCs than healthy control donors in response to MCSF ($P < 0.05$), ET-1 ($P < 0.05$) and in combination ($P < 0.01$). ETR antagonists BQ123 (ETR_A), BQ788 (ETR_B) and Bosentan ($ETR_{A/B}$) inhibited MCSF induced fibrocyte differentiation. CTGF secretion was elevated in SSc compared to control fibrocytes ($P < 0.05$) cultured with MCSF. Conditioned media from SSc fibrocytes promoted gel contraction by control pulmonary fibroblasts ($P < 0.05$).
Discussion $CD14^+$ SSc PBMCs readily differentiate into fibrocytes in response to ET-1 and MCSF via ETR_A and ETR_B. Our data suggests fibrocytes contribute to the development of PAH in SSc via a paracrine mechanism modulating the functional activities of resident tissue fibroblasts.

S140 BMPR-II DEFICIENCY LEADS TO AN INCREASE IN EGG DEPOSITION AND CYTOKINE RELEASE IN THE LUNGS OF MICE CHRONICALLY INFECTED WITH SCHISTOSOMIASIS

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Rationale and Objectives Schistosomiasis is the world-wide leading cause of pulmonary arterial hypertension (PAH) and is particularly prevalent in the third-world. More than 80% of patients with PAH in the western world have a mutation in bone morphogenetic protein type-II receptor (BMPR-II), which is a member of the transforming growth receptor-beta (TGF-b) superfamily and is important in cell proliferation and differentiation. The aim of the study was to determine if mice with a heterozygous null mutation in BMPR-II are more susceptible to schistosomiasis–induced PAH, compared to wild-type littermates.
Methods Wild-type and mutant C56/BL6 mice were infected percutaneously with a low dose of *S.mansoni*. Non-infected WT and MUT mice were also studied. At 17 weeks post-infection right ventricular systolic pressure (RVSP) and right ventricular (RV) hypertrophy, liver and lung egg counts and body weight were measured. Pulmonary vascular remodelling and liver histology were assessed by morphometry, following immunohistochemistry. Micro-CT was performed to determine egg deposition. A macrophage phagocytosis assay was also performed.
Measurements and Main results At 17 weeks post-infection there was no significant difference in RVSP, the degree of RV hypertrophy, mean area of liver vasculature, mean number of liver vessels or liver weight between infected BMPR-II + / + and BMPR-II +/- mice. However, there was a significant reduction in body weight, a significant increase in lung egg deposition and lung cytokine expression in the BMPR-II +/- mice compared to the wild-type mice 17 weeks post-infection. There was no significant difference in serum or liver cytokine levels. We saw a significant increase in pulmonary vessel wall thickness in both BMPR-II + / + and BMPR-II +/- mice infected mice, compared to their respective non-infected controls. There was no difference in the ability of macrophages from BMPR-II + / + and BMPR-II +/- mice to phagocytose fluorescently tagged beads.
Conclusions This study has shown that BMPR-II mutations do not predispose to schistosomiasis-induced PAH, but that there is an increased ability of the eggs to gain access into the lungs and a subsequent heightened inflammatory response. This appears not to be due to an innate difference in the liver vasculature or a defect in egg clearance by macrophages.

S141 BMP9 IS REQUIRED FOR LPS-MEDIATED NEUTROPHIL RECRUITMENT TO PAH-PATIENT DERIVED BLOOD OUTGROWTH ENDOTHELIAL CELLS WITH BMPR-II MUTATIONS

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Introduction Heterozygous mutations in the gene encoding bone morphogenetic protein (BMP) receptor II (BMPR-II) are present in >70% of patients with heritable pulmonary arterial hypertension (hPAH) and 15–26% of idiopathic PAH (iPAH)