Online Data Supplement

Perivascular T Cell Infiltration Leads To Sustained Pulmonary Artery Remodeling After Endothelial Cell Damage

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RV pressure measurements and assessment of RV hypertrophy

Mice were anesthetized with inhaled isoflorane delivered via nosecone. The neck was dissected exposing the internal jugular vein and carotid artery. A Millar catheter transducer (Millar Instruments, USA) was inserted via the internal jugular vein directly into the right ventricle (RV) and via the carotid into the aorta and left ventricle (LV). Pressure values were monitored, recorded, and analyzed using LabChart Software (ADInstruments). The right ventricular systolic pressure (RVSP) was reported.

Cytokine measurements

The right lung was isolated, sectioned, snap frozen in liquid nitrogen, and stored at -80ºC until further analysis. Individual lung pieces were thawed, homogenized in PBS containing a protease inhibitor cocktail (Roche, USA), and centrifuged at 10,000xg for 10 minutes at 4ºC. The concentration of cytokines was measured in the supernatant using a mouse SearchLight proteome array (Pierce Biotechnology, USA).

Serum ACE level measurements

Blood was collected from the LV into untreated tubes and allowed to clot for 2-4 hours at room temperature. Serum was collected after centrifugation and stored at -80ºC until further analysis. The serum activity of angiotensin converting enzyme (ACE) was measured using an ACE colorimetric assay according to the manufactures protocol (Alpco, USA), which is based on the cleavage of a synthetic substrate to hippuric acid. Briefly, 100 µl of a 1:1 dilution of serum were added to 300 µl of incubation buffer and 200 µl ACE substrate, and incubated for 30 minutes at 37ºC. The reaction was stopped by adding 500 µl HCl and neutralized by adding the same volume NaOH. The reaction was diluted by adding 2.0 ml of dilution buffer and developed by adding 1.5 ml color reagent. After 5 minutes of incubation at room temperature, the reaction mix was centrifuged at 1500xg for 10 minutes, and the absorbances measured at 382 nm. The concentration of hippuric acid in the reaction mix was calculated from a standard curve, and the results presented as ACE units (µmol hippuric acid / l /min).

Assessment of perivascular inflammation

Perivascular inflammation was characterized by staining for T cells, B cells, neutrophils, and macrophages and assessed by the same examiner blinded to treatment group. Ten vessels were examined from each section under light microscopy and inflammatory cells were quantified by counting total number of cells and stain positive cells. The percent positive cells were calculated as (stain positive cells/ total perivascular cells) x100.

Figure 1 supp:
H&E staining (column 1) at various time points showing inflammatory response and progressive histologic changes of vascular remodeling. CD3 (column 2), F4/80 (column 3), and neutrophil (column 4) staining at various time points showing the progression of inflammatory response in small vessels. (scale bar 50 µm)