Title: Role of T lymphocyte location in *Schistosoma*-induced pulmonary hypertension

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Background: Schistosomiasis is a parasitic infection that can cause pulmonary hypertension (PH), a disease of thickened lung blood vessels. We previously found Th2 CD4 T cells are required for *Schistosoma*-PH in mice. However, it is unknown if CD4 T cells need to be located in the lung to initiate the localized inflammation that leads to vascular remodeling. Here, we tested the hypothesis that *Schistosoma*-PH requires activated Th2 CD4 T cells to migrate to the lung parenchyma by administering FTY720, which blocks lymphocyte egress from lymph nodes.

Methods: Female C57Bl6 mice (Jackson Labs) were sensitized with 240 *S. mansoni* eggs/gram intraperitoneally (IP) and were challenged intravenously (IV) 2 weeks later with 170 eggs/gram by tail vein injection. Daily FTY720 (0.5mg/kg IP) or PBS (equivalent volume IP) was started the day before IV egg administration. 3 days after IV eggs, N=3 mice per group were sacrificed, and the lungs digested for flow cytometry. 7 days after IV eggs, N=3-4/group underwent right heart catheterization to measure right ventricle systolic pressure (RVSP).

Results: FTY720 treatment decreased the number of circulating CD3 and CD4 T cells in FTY720-treated mice compared to vehicle-treated mice. In the mediastinal lymph nodes, there were more CD3 and CD4 T cells, whereas in the lungs, there were fewer CD3 and CD4 T cells in the FTY720-treated mice. The percentage of CD44+CD69+ (i.e., educated and activated) CD4 T cells was greater in the lungs of the FTY720-treated mice, but the absolute number was decreased. FTY720 treatment caused no change in RVSP and or any change in right ventricle hypertrophy compared to vehicle-treated mice.

Conclusions: Blocking T cell migration into the lungs was insufficient to suppress PH following IV *Schistosoma* egg challenge. Pre-positioned CD4 T cells in the lung may be sufficient to cause Type 2 inflammation and PH.