Double-negative αβ T cells are early responders during lung ischemic reperfusion injury

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Introduction

Lung ischemia reperfusion injury (IRI) remains one of the most common complications following lung transplantation and is often accompanied by renal insufficiency. Recent work has elucidated a novel subset of αβ T cell receptor positive CD4-CD8- (double-negative; DN) T cells in the kidneys, which have protective anti-inflammatory properties against renal IRI. We hypothesized that DN T cells are also found in the lung, are activated by lung IRI, and could be a new therapeutic target for the injured lungs

Hypothesis

Double-negative T cells are early responders in lung ischemia reperfusion injury

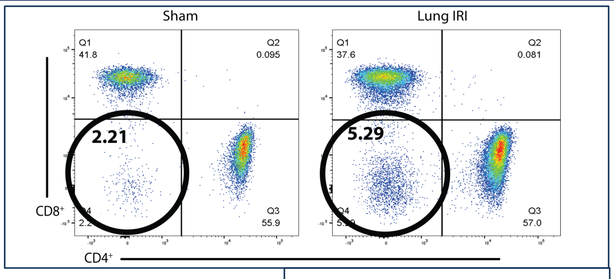
Objectives

We sought to describe this new set of T cells and the molecular changes that occur under ischemic injury

Materials and Methods

- WT male, 8-weeks old C57BL/6 mice (n=10 biological replicates) weighing 23 to 25 g underwent lung ischemic reperfusion injury
- Ischemia was induced with unilateral left pulmonary artery and vein occlusion (LPAVO) for 30 minutes then reperfused for 0.5, 1, 3, and 6 hours.
- Lymphocytes isolated from the lungs underwent FACS staining. These subsets of T cells were then assessed by flow cytometry (LSRII) and quantified.
- Lung tissue was homogenized and probed for various markers in a Western blot. Signal intensity quantification for western blots was performed using ImageJ software.
- All P-values were generated using Student's t-test with two-sides distribution and equal variance via Microsoft Excel. *denotes a P-value of <0.05, **denotes a P-value of <0.02.

Results



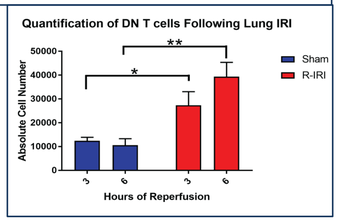
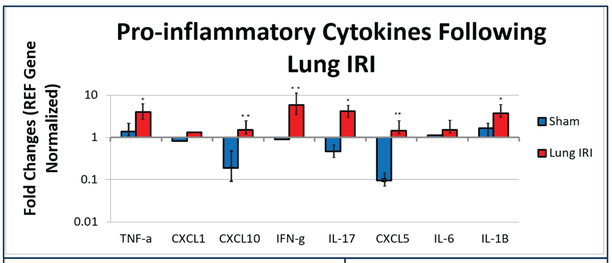
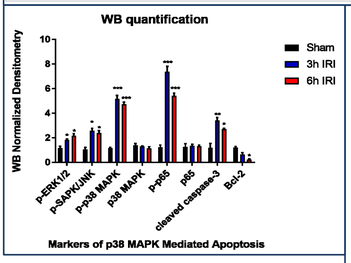


Figure 1. Rapid expansion of DN T cells following lung IRI. Lung tissues were harvested for lymphocyte isolation, and cells were sorted through flow cytometry. Higher levels of DN T cells were seen in the lungs following IRI when compared to sham subjects. Flow cytometric analysis revealed a 2-fold rise of DN T cells between the injured and control lung ($p<0.001$) at 3 and 6 hour reperfusion. Percentage of cell population and absolute number of cells were quantified.





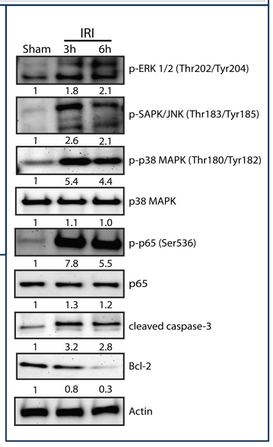


Figure 2. Lung IRI results in apoptosis and inflammation. (Right) Immunoblotting analysis of lung tissue subjected to IRI or sham conditions. The p38 MAPK signaling pathway is activated in lungs subjected to IRI as indicated by increased levels of phospho-p38. Increased levels of caspase-3 cleavage indicates cell death. Sham lungs were unable to induce a robust p-38 signaling and caspase-3 cleavage. Actin serves as a loading control. Immunoblotting was quantified with FlowJo and Excel. (Middle) Lung tissue subjected to IRI or sham conditions with 3 hour reperfusion were processed for qPCR analysis. Higher pro-inflammatory fold changes were observed In lung subjected to IRI and reduced in sham conditions.

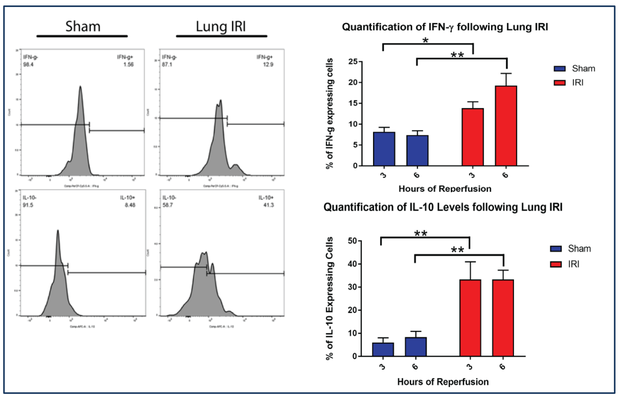
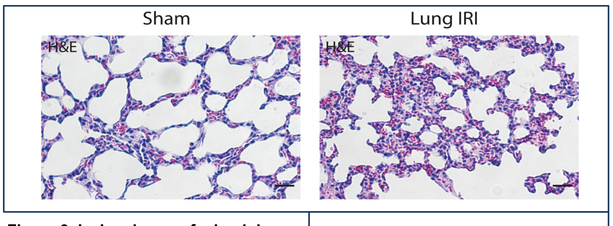


Figure 4. Ischemic reperfusion injury induces anti-inflammatory cytokine release from DN T cells. Lung that underwent IRI or sham were harvested. Lymphocytes were isolated and T cells were treated with Brefeldin A and stained for IL-10, IFN-γ, and CD45+ cells. Flow cytometry analysis revealed IL-10 and IFN-γ cytokine levels were higher in lungs that underwent IRI (3-fold). Percentage of cytokine expressing cells were quantified with FlowJo and Excel.



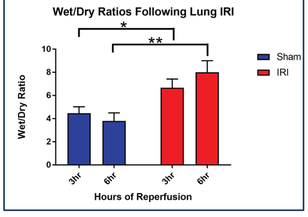


Figure 3. Ischemic reperfusion injury causes lung damage. 8 um tissue sections were immunostained with hematoxylin and eosin for histopathology analysis. Lung that were subjected to IRI exhibited thickened interstitium and infiltration of neutrophils when compared to the sham. Wet/Dry ratio was calculated by dividing the wet by the dry wet. Lungs that underwent IRI had a higher W/D ratio, indicative of more fluid and lung permeability. All scale bars= 20 um.

Key Findings

- LPAVO induced IRI results in early expansion of DN T cells in the lungs
- DN T cell expansion results in expression of higher levels of IL-10 and IFN-γ in the lungs compared to surgical controls
- Lung IRI activates the p38-MAPK signaling pathway resulting in inflammation and cell death
- Histological analysis and W/D ratio showed damage to the lung insterstitium and fluid accumulation
- Ischemic reperfusion injury to the lungs causes inflammation and cell death, as well as tissue damage. DN T cells may play a protective role by secreting high levels of IL-10

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