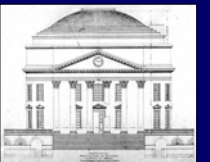




Tumor Necrosis Factor- α from Resident Alveolar Macrophages is a Key Initiating Factor in Pulmonary Ischemia-Reperfusion Injury

Thomas S. Maxey¹, Irving L. Kron¹, T. Brett Reece¹, Peter Ellman¹, Richard I. Enelow², Benjamin M. Gaston³, Victor E. Laubach¹, and Allan Doctor³

Departments of ¹Surgery, ²Pulmonary and Critical Care Medicine, and ³Pediatrics. University of Virginia Health System, Charlottesville, VA, USA



Background

Ischemia Reperfusion (IR) injury is tissue injury that occurs following reperfusion of an ischemic organ.

Pulmonary IR injury:

- Occurs in 15-20% of lung transplants
- Leads to increased ICU, hospital stay, total cost, and increased mortality¹
- Independently predicts Bronchiolitis Obliterans and Chronic Rejection²

Contributing Factors:

- Reactive oxygen species
- Circulating Neutrophils
- Pulmonary Macrophages
- Cytokines such as TNF- α

Tumor Necrosis Factor- α (TNF- α):

- Secreted mainly from activated macrophages
- Tumor growth regulation
- Inflammation & infection
- Pulmonary edema
- Enhances neutrophil accumulation

Hypothesis

TNF- α from resident alveolar macrophages is a key initiating factor in pulmonary IR injury.

Methods

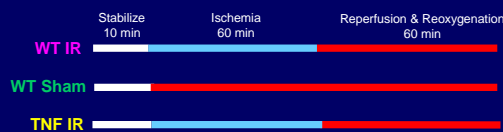
- C57BL6 adult male mice were used for wild-type (WT) IR and WT Shams (n=7).
- TNF- α gene knockout mice (on C57BL6 background) were used for TNF-/- IR group (n=7).
- Mice were anesthetized with ketamine and xylazine followed by tracheotomy and intubation, and ventilated with 40% oxygen.
- Animals are heparinized and exsanguinated.
- Using the isolated perfused mouse lung system (Hugo Sachs Elektronik, Germany, Figure 1), the pulmonary artery is cannulated through the right ventricular outflow tract and perfused with isotonic pH balanced buffer (pO₂ and pCO₂ tension was similar to mixed venous blood gas). See [Poster B18](#) for details of the isolated mouse lung IR system.
- The pulmonary circuit is closed by left atrial cannulation.
- Lungs are stabilized for 10 min and rendered ischemic by halting perfusion and ventilating with nitrogen. Reperfusion is initiated with buffer-reperfusion and oxygen ventilation.
- Parameters Measured include:
 - Pulmonary artery pressure
 - Airway resistance
 - Static Compliance
 - Pulmonary Edema (Lung weight Index)
 - Histologic Lung Injury Score graded by pathologist based on degree of septal thickening, fibrin deposits, and pulmonary edema

Study Groups

WT Sham: wild-type lungs perfused for 120 min without ischemia

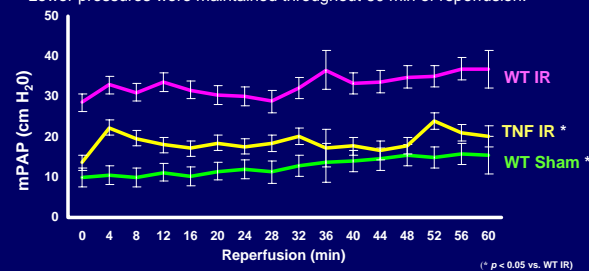
WT IR: wild-type lungs ischemic for 60 min followed by reperfusion for 60 min

TNF IR: TNF-/- lungs ischemic for 60 min followed by reperfusion for 60 min

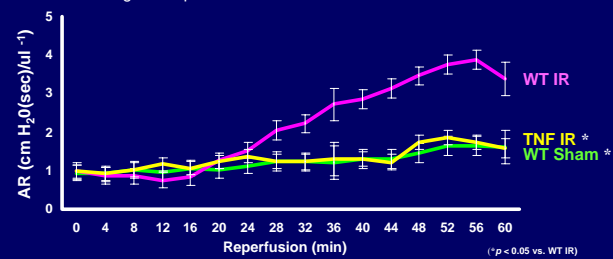


Results

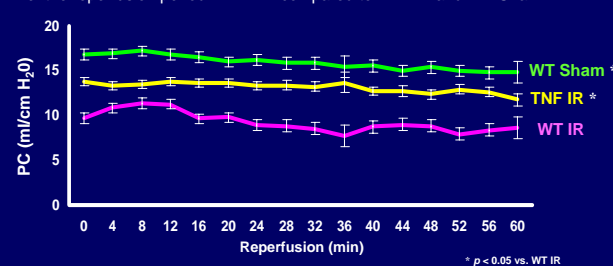
Mean Pulmonary Artery Pressure (mPAP) was significantly improved immediately upon reperfusion in TNF IR compared to WT IR. Lower pressures were maintained throughout 60 min of reperfusion.



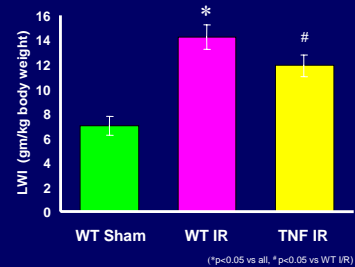
Airway Resistance (AR) remained similar among all groups during early reperfusion. Significant increases in AR occurred at 28 min and continued to increase throughout reperfusion in WT IR vs. TNF IR and WT Sham.



Pulmonary Compliance (PC) was significantly lower throughout the entire reperfusion period in WT IR compared to TNF IR and WT Sham.



Results

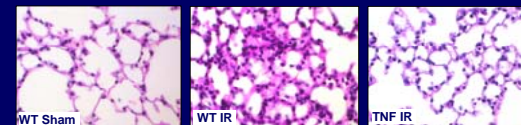
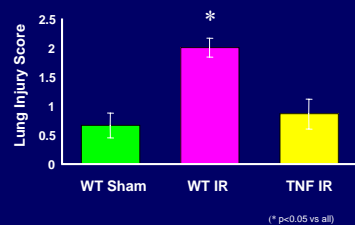


Lung Weight Index (LWI)

(a marker of capillary leak and pulmonary edema) was significantly higher in both IR groups compared to Sham. The LWI was significantly lower in TNF IR lungs compared to WT IR lungs.

Histologic Lung Injury Score

was significantly worse in WT IR lungs compared to WT Sham and TNF IR lungs. No differences were noted between TNF IR and WT Sham.



Lung histology (40x magnification, H&E staining) demonstrating fibrinous exudate and thickened septae in WT IR lungs and improved histology in TNF IR lungs.

Summary

- Circulating neutrophils are not required for the initiation of pulmonary IR injury.
- Animals deficient in TNF- α are significantly protected from IR injury.
- Because TNF- α is mainly secreted by activated macrophages, these cells likely initiate reperfusion injury.
- It is possible that cells other than macrophages can also contribute to TNF- α -mediated lung IR injury.

Conclusions

TNF- α secretion from non-circulating cells (resident lung cells) is a key initiating factor in pulmonary IR injury.

References

1. King RC et al. *Ann Thorac Surg* 69:1681-5, 2000.
2. Fiser SM et al. *Ann Thorac Surg* 73:1041-8, 2002.



Figure 1