

Abstract

Objective: Post-ischemic reperfusion of the lung triggers pro-inflammatory responses that stimulate injurious neutrophil chemotaxis. We hypothesized that T lymphocytes are recruited and activated during reperfusion and mediate subsequent neutrophil-induced lung ischemia-reperfusion injury (IRI).

Methods: An *in vivo* mouse model of lung IRI was employed. C57BL/6 mice were assigned to either sham group (left thoracotomy) or study groups which underwent one hour left hilar occlusion followed by 1-24 hr reperfusion. Following *in vivo* reperfusion, the lungs were perfused *ex vivo* with Krebs Henseleit buffer whereby pulmonary function was assessed. Lung vascular permeability, edema, neutrophil accumulation and cytokine/chemokine production (TNF- α , IL-17, CCL3 and CXCL1) were assessed by Evans blue dye assay, wet-to-dry weight ratio, myeloperoxidase assay and Bioplex bead array, respectively.

Results: Our study demonstrated that 2-hour reperfusion resulted in greater pulmonary dysfunction than 1-hr or 24-hr reperfusion, hence 2-hr reperfusion period was used for the remaining experiments. Comparable and significant protection from IR-induced lung dysfunction and injury occurred after antibody-depletion of neutrophils or CD4+ T cells, but not CD8+ T cells ($\#p < 0.05$ vs. IgG control). Lung IRI was proportional to the infiltration of neutrophils but not T cells. Moreover, pulmonary neutrophil infiltration and the production of CXCL1 (KC) were significantly diminished by CD4+ T cell depletion, but not vice versa.

Conclusions: CD4+ T lymphocytes and neutrophils accumulate during reperfusion injury and contribute sequentially to lung IRI. This study suggests that CD4+ T lymphocytes play a critical role in stimulating chemokine production and neutrophil chemotaxis during lung IRI.

Methods

***In vivo* lung IRI.** C57BL6 mice underwent left lung ischemia by occlusion of the left hilum for 1 hr after left thoracotomy. Five minutes before reperfusion animals were re-intubated and the suture occlusion was removed. Mice were extubated, allowed to awaken, and returned to their cage for 2 hrs. After 2 hrs reperfusion the animals were re-anesthetized and pulmonary function was measured. Sham animals received thoracotomy without hilar occlusion.

Isolated, buffer-perfused mouse lung system: Pulmonary function was evaluated using an isolated, buffer-perfused mouse lung system at the end of scheduled reperfusion. Isolated lungs were perfused with Krebs-Henseleit buffer for 10 min. Hemodynamic and pulmonary function parameters were recorded throughout this period by the PULMODYD data acquisition system (Hugo Sachs Elektronik).

***In vivo* depletion of neutrophils, CD4+ or CD8+ T lymphocytes.** Rat anti-mouse Gr-1 mAb (10 μ g) (eBioscience, San Diego, CA) was used to deplete circulating neutrophils by injecting via tail vein 24 hrs prior to lung ischemia. Depletion of CD4+ or CD8+ T cells was achieved by anti-CD4 mAb (GK1.5) or anti-CD8a mAb (53-6.7) injected i.p. for two days at a dose of 0.2 mg/day.

Bronchoalveolar (BAL) fluid collection: After pulmonary function measurements, the lungs were lavaged with 0.5 ml saline and the BAL fluid was collected and centrifuged (1500g for 15 min at 4°C).

Cytokine and chemokine protein analysis: A mouse-specific multiplex cytokine panel assay (Bio-Rad Laboratories) was used to quantify the cytokine and chemokine protein content in BAL fluid.

Lung wet-to-dry ratio: Lung wet-to-dry ratio was used as an indicator of pulmonary edema. The lower lobe of the right lung was harvested, weighed and placed in a vacuum oven (at 58°C) until a stable, dry weight was achieved. The ratio of lung wet weight to dry weight was then calculated.

Myeloperoxidase (MPO) measurement: MPO was measured in BAL fluid as an indicator of neutrophil infiltration into alveolar spaces. The quantitative measurement of MPO was calculated by the mean absorbance values of each sample at 450 nm, and the final concentrations are expressed as ng/ml.

Results

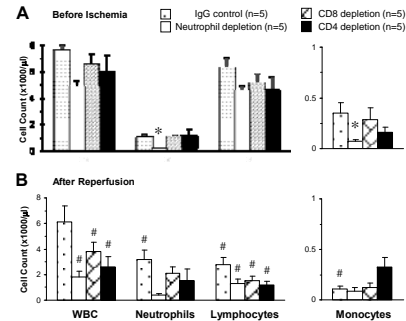


Figure 1. Complete blood cell counts in antibody-treated mice. (A) Prior to hilar ligation, there was an 80% reduction in neutrophils in neutrophil-depleted mice compared to IgG control mice, a 19% reduction in lymphocytes in CD8-depleted mice, and a 26% reduction of lymphocytes in CD4-depleted mice. * $p < 0.05$ vs. IgG control. (B) Reperfusion caused a significant decrease in total circulating leukocytes and lymphocytes in all-antibody-treated mice. # $p < 0.05$ vs. corresponding group in (A).

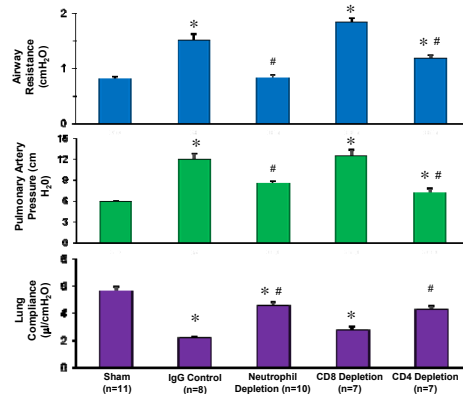


Figure 3. Effect of leukocyte subtype depletion on pulmonary function after reperfusion. Airway resistance, pulmonary artery pressure and lung compliance were significantly improved in neutrophil-depleted and CD4+ T cell-depleted mice. * $p < 0.05$ vs. sham, # $p < 0.05$ vs. IgG control and CD8-depleted mice.

Results

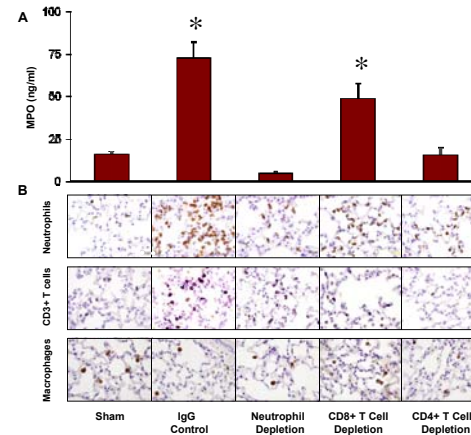


Figure 2. MPO levels and leukocyte subtype infiltration into peripheral lung after reperfusion. (A) MPO in BAL fluid after reperfusion. * $p < 0.05$ vs. sham. (B) Immunohistochemical staining for neutrophils, CD3+ T cells and macrophages in the study groups

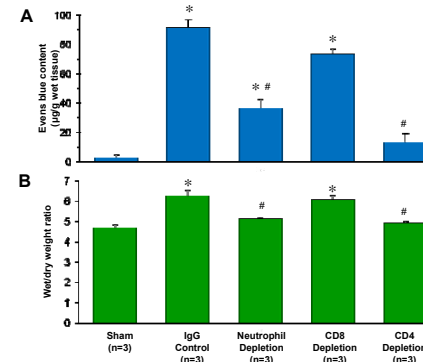


Figure 4. Microvascular permeability and pulmonary edema. Evans blue content (A) and lung wet-to-dry weight ratio (B) were significantly increased in IgG control and CD8-depleted mice than in neutrophil-depleted or CD4-depleted mice. * $p < 0.05$ vs. sham, # $p < 0.05$ vs. IgG control and CD8-depleted mice.

Results

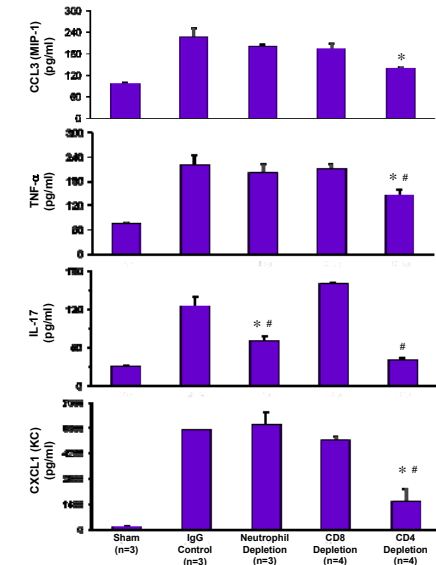


Figure 5. Expression of cytokines/chemokines in BAL fluid. CCL3 (MIP-1), TNF- α , IL-17 and CXCL1 (KC) were all significantly elevated in IgG control, neutrophil-depleted and CD8-depleted mice compared to sham. All cytokines and chemokines were significantly reduced by depletion of CD4+ T cells. * $p < 0.05$ vs. IgG control, # $p < 0.05$ vs. CD8-depleted mice.

Summary

- Lung ischemia reperfusion injury entails sequential activation of CD4+ T lymphocytes and neutrophils, resulting in a pro-inflammatory cascade leading to IR injury.
- CD4+ T lymphocytes orchestrate the chemotaxis of circulating neutrophils to the lung, wherein the neutrophils act as end-effectors causing IR injury.
- This study underscores the importance of CD4+, and not CD8+, T lymphocytes as mediators of lung IR injury.