



# Alveolar Resident Macrophages Play a Predominant Role in Acute Lung Ischemia-Reperfusion Injury

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## Background:

Lung ischemia-reperfusion injury (LIRI), an unavoidable consequence of lung transplantation, is a biphasic inflammatory process. Previous studies demonstrated that the later phase is dependent on neutrophil recruitment and activation, however, the early acute phase is neutrophil-independent and occurs within minutes of reperfusion (Fiser SM et al. Lung transplant reperfusion injury involves pulmonary macrophages and circulating leukocytes in a biphasic response. *J Thorac Cardiovasc Surg* 121: 1069-1075, 2001.) likely involving alveolar macrophage activation.

## Hypothesis:

The early acute phase of LIRI is dependent on resident macrophages of the lung.

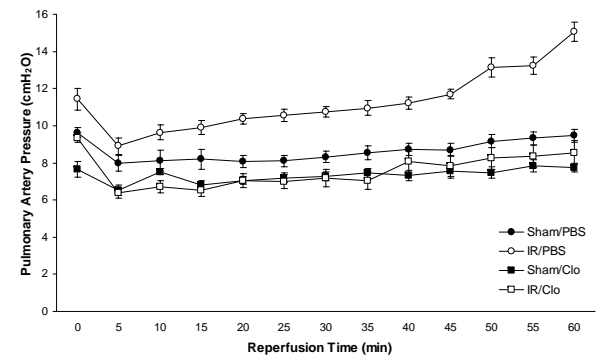
## Methods:

A buffer-perfused, *in situ*, isolated mouse model of LIRI was utilized (Maxey TS et al. Tumor necrosis factor-alpha from resident lung cells is a key initiating factor in pulmonary ischemia-reperfusion injury. *J Thorac Cardiovasc Surg* 127: 541-547, 2004). Four groups of C57Bl/6 mice were tested. In groups 1 and 2, mice were treated with liposome-encapsulated phosphate-buffered saline intratracheally 24 hours prior to experiment. In groups 3 and 4 (macrophage depleted groups), mice received liposome-encapsulated clodronate to deplete alveolar macrophage. The lungs of groups 1 and 3 underwent 60 minutes ischemia followed by 60 minutes reperfusion. Groups 2 and 4 underwent 120 minutes perfusion without ischemia. The lung pulmonary artery pressure and pulmonary compliance were recorded automatically by computer throughout the experiment. Lung tissue and bronchoalveolar lavage (BAL) underwent TNF- $\alpha$ , MCP-1 and MIP-2 assay by ELISA. The mRNAs of these cytokines/chemokines in lung tissue were evaluated by RT-PCR. The lung wet/dry index and vascular permeability were also measured.

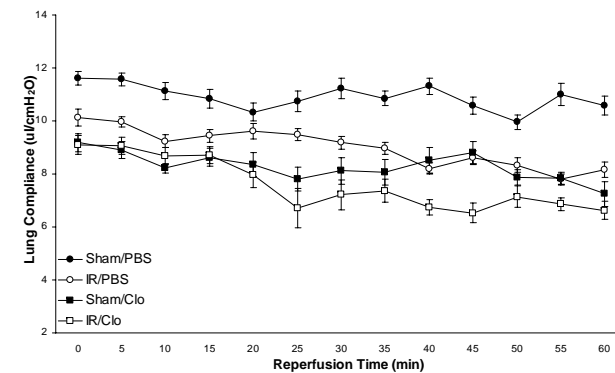
## Conclusion:

Alveolar resident macrophages are critical mediators in the early acute phase of mouse LIRI.

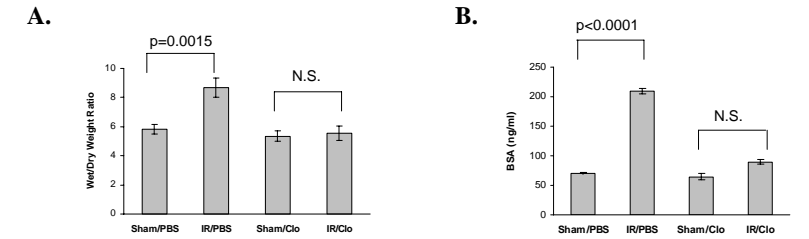
## Results:



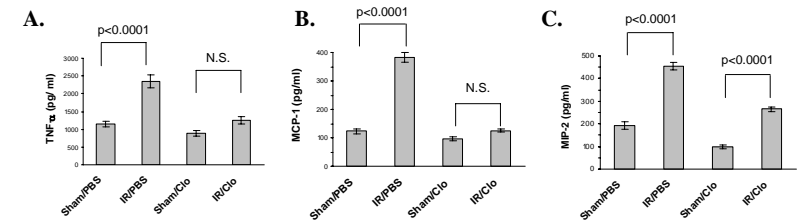
**Figure 1.** Dynamic changes in pulmonary artery pressure during 60 minutes reperfusion. The PAP in IR/Clo group is reduced to the level similar to sham group (Sham/Clo). Sham/PBS = sham lung without AM-depletion; IR/PBS = IR lung without AM-depletion; Sham/Clo = sham lung, AM-depleted; IR/Clo = IR lung, AM-depleted. \* $p < 0.001$  IR/PBS vs. Sham/PBS and IR/Clo.



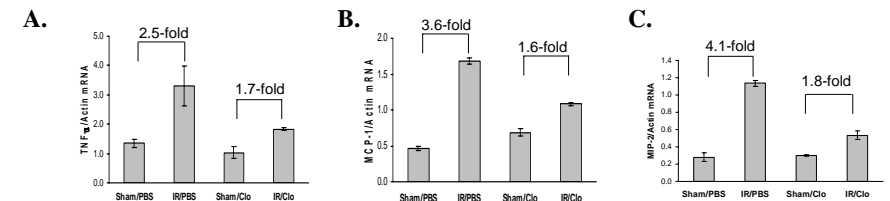
**Figure 2.** IR induced a significant change in pulmonary compliance in non-AM depleted lungs (\* $p < 0.01$  IR/PBS vs. Sham/PBS). In AM-depleted lungs, there was no significant difference between sham and IR (IR/Clo vs Sham/Clo).



**Figure 3.** Lung injury after IR is attenuated after AM depletion. (A) IR significantly increased wet/dry weight ratio in non-AM-depleted lungs (IR/PBS vs. Sham/PBS,  $p = 0.0015$ ). AM depletion abolished the IR-induced increase in wet/dry weight ratio (Sham/Clo vs. IR/Clo,  $p = 0.9936$ ). Wet/dry weight ratio was also significantly reduced in IR/Clo vs. IR/PBS ( $p = 0.0005$ ). (B) Lung vascular permeability was assessed by measuring bovine serum albumin (BSA) concentration in BAL fluid. In non-AM-depleted lungs, IR significantly increased BSA concentration by 3-fold compared to sham (IR/PBS vs. Sham/PBS,  $p < 0.0001$ ). AM depletion abolished the IR-induced increase in BAL concentration (Sham/Clo vs. IR/Clo,  $p = 0.0823$ ). BAL concentration was also significantly reduced in IR/Clo vs. IR/PBS ( $p < 0.001$ ).



**Figure 4.** ELISA analysis of TNF- $\alpha$ , MCP-1 and MIP-2 protein expression in BAL fluid. (A) TNF- $\alpha$  protein was significantly elevated over 2-fold after IR in non-AM-depleted lungs (IR/PBS vs. Sham/PBS). In AM-depleted lungs, there was no significant increase in TNF- $\alpha$  protein after IR compared to sham lungs (IR/Clo vs. Sham/Clo). (B) MCP-1 protein was significantly elevated over 3-fold after IR in non-AM-depleted lungs (Sham/PBS vs. IR/PBS). In AM-depleted lungs, there was no significant increase in MCP-1 protein after IR compared to sham lungs (IR/Clo vs. Sham/Clo). (C) MIP-2 protein was significantly elevated over 2-fold after IR in non-AM-depleted lungs (Sham/PBS vs. IR/PBS). However, MIP-2 protein level was reduced in the Sham group treated with liposome-clodronate (Sham/Clo vs. Sham/PBS,  $p = 0.0007$ ). IR also significantly elevated MIP-2 expression in AM-depleted lungs (IR/Clo vs. Sham/Clo).



**Figure 5.** Expression of mRNA for (A) TNF- $\alpha$ , (B) MCP-1 and (C) MIP-2 in lung tissue. Without AM depletion, the mRNA of each of these three cytokine/chemokines was dramatically increased by IR (IR/PBS vs Sham/PBS). AM depletion severely reduced the increase in cytokine/chemokines mRNA expression after IR (IR/Clo vs. Sham/Clo).