B cells mediate lung ischemia reperfusion injury via synergistic BCR-TLR4 signaling

Louisa Yun Zhu Bai, MD, Khashayar Farahnak, MD, Alejandro De Filippis Falcon, MD, Yuriko Terada, MD, Yuhei Yokoyama, MD, Fuyi Liao, MD, Wenjun Li, MD, Hailey M. Shepherd, MD, Daniel Kreisel, MD, PhD, Ruben Nava, MD

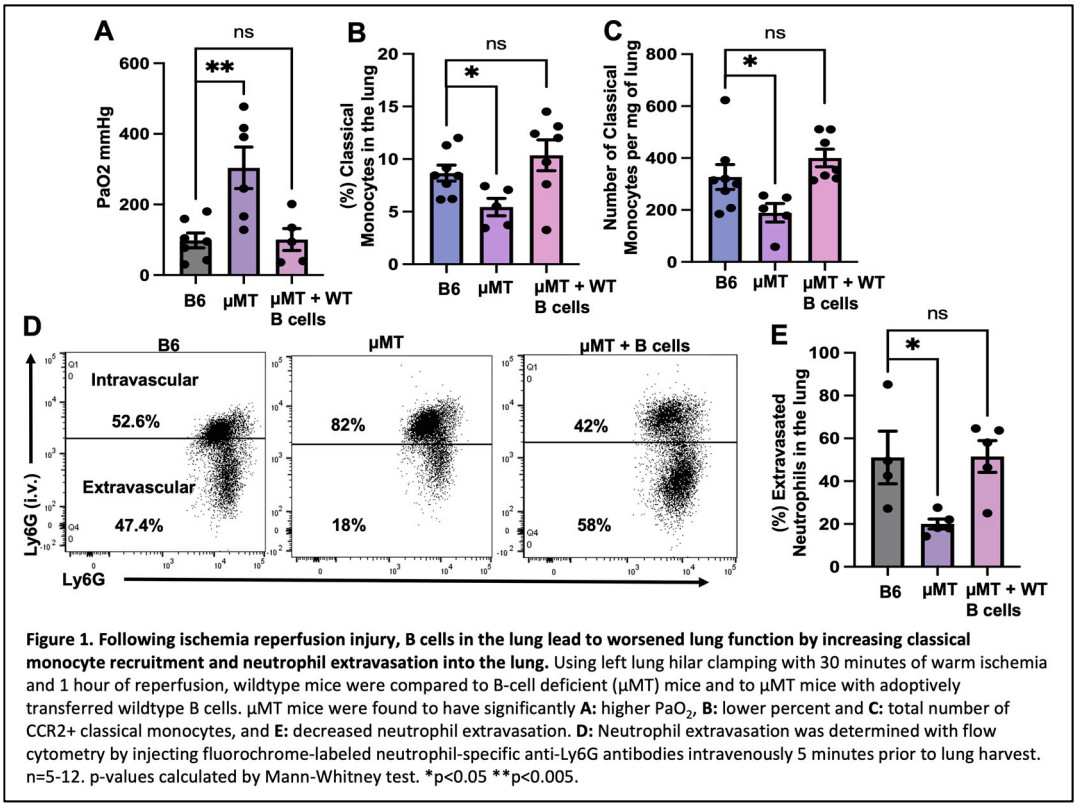

Figure 1. Following ischemia reperfusion injury, B cells in the lung lead to worsened lung function by increasing classical monocyte recruitment and neutrophil extravasation into the lung. Using left lung hilar clamping with 30 minutes of warm ischemia and 1 hour of reperfusion, wildtype mice were compared to B-cell deficient (µMT) mice and to µMT mice with adoptively transferred wildtype B cells. µMT mice were found to have significantly **A:** higher PaO_2, **B:** lower percent and **C:** total number of CCR2+ classical monocytes, and **E:** decreased neutrophil extravasation. **D:** Neutrophil extravasation was determined with flow cytometry by injecting fluorochrome-labeled neutrophil-specific anti-Ly6G antibodies intravenously 5 minutes prior to lung harvest. n=5-12. p-values calculated by Mann-Whitney test. *p<0.05 **p<0.005.

Background: Ischemia-reperfusion injury (IRI)-mediated primary graft dysfunction (PGD) after lung transplantation is a risk factor for adverse short- and long-term outcomes. Our group has previously reported that recipient-derived $CCR2^+$ classical monocytes (CMs) orchestrate the extravasation of neutrophils into the lung following transplantation, which worsens lung dysfunction. Recent work shows that B cells may play a role in kidney and heart IRI, but studies in lung are lacking. This study aims to investigate the role of B cells in the pathogenesis of lung IRI.

Hypothesis: We posit that damage-associated molecular patterns (DAMPs) or other auto-antigens released following lung IRI activate B cells, which results in the recruitment of CMs, subsequent neutrophil extravasation, and further lung damage.

Methods: We used a murine left lung hilar clamping model where 30 minutes of warm ischemia was followed by 60 minutes of reperfusion. Arterial blood gases were obtained to assess lung function, and the left lung was harvested for flow cytometric analysis. A syngeneic murine orthotopic lung transplant model (B6 CD45.1 → B6 CD45.2) was used to distinguish the role of donor (CD45.1) versus recipient (CD45.2) B cells. Samples from human donor lungs were also obtained prior to and 2 hours after transplant and analyzed.

Results: When hilar clamping is performed in B cell-deficient (µMT) mice compared to B6 mice, we observed that the lung function is preserved, as evidenced by higher PaO_2 values (p=0.02). This improvement in oxygenation is associated with a decreased proportion (p=0.02) and total number (p=0.0007) of CMs and a reduction in neutrophil extravasation (p=0.03). These results are abrogated with adoptive transfer of wildtype B cells into µMT mice prior to IRI (Figure 1). Adoptive transfer experiments of B cells from selective knockout mouse strains into µMT mice revealed that B cell Toll-like receptor 4 (TLR4) activation promotes the production of the monocyte chemokine CCL7, in a Trif-dependent fashion, which is critical for initiating CM recruitment into the lung. Importantly, TLR4-driven B cell recruitment of CMs is contingent upon simultaneous B cell receptor (BCR) activation; and lack of BCR engagement precludes CM recruitment. Live intravital two-photon microscopy showed infiltration of recipient B cells into the graft early after reperfusion and mouse lung transplantation confirmed that most B cells (>80%) in the lung are of recipient origin. µMT recipients of syngeneic lungs showed improved oxygenation compared to B6 recipients (p=0.009). Finally, the number of B cells in the graft early after human transplantation correlates positively with the number of CMs and with neutrophil extravasation.

Conclusions: This study provides novel evidence that B cells play an early causative role in lung IRI. Synergistic TLR4 and BCR activation on lung-infiltrating B cells after IRI promotes Trif-dependent CCL7 production, which causes CM infiltration and subsequent neutrophil extravasation into the lung, and ultimately decreased lung function. These findings present an exciting new avenue by which B cell-targeting therapies could improve post-lung transplant outcomes.