CD4+ T cells Modulate Macrophage Activation via IL-17 During Lung Ischemia-Reperfusion Injury

Ashish K. Sharma[1], Joel Linden[2], Irving L. Kron[1] and Victor E. Laubach[1]
Departments of [1]Surgery and [2]Medicine, University of Virginia Health System, Charlottesville, VA

Summary

Background: Our prior studies have implicated a role for CD4+ T lymphocytes and IL-17 in the initiation of lung ischemia-reperfusion (IR) injury (Yang *et al.*, *J. Thorac. Cardiovasc. Surg.* 2009). We have also demonstrated a protective role of adenosine A_{2A} receptor (A2AR) activation in lung IR injury (Gazoni *et al.*, *J. Thorac. Cardiovasc. Surg.* 2008). The role of the IL-23/IL-17 axis in T cell-mediated inflammation has been postulated in other models but remains to be elucidated in lung IR injury.
Hypothesis: This study tests the hypothesis that IL-17-producing CD4+ T cells initiate lung IR injury by modulating alveolar macrophage activation and that activation A2AR (via a specific agonist, ATL313) on CD4+ T cells attenuates lung IR injury.
Results: Lung IR injury in Rag-1 KO, IL-23 KO and IL-17 KO mice was significantly reduced compared to WT mice as demonstrated by markedly improved lung function. A significant decrease in expression of proinflammatory cytokines/chemokines after IR occurred in Rag-1 KO, IL-23 KO and IL-17 KO mice compared to WT mice which was restored by adoptive transfer of CD4+ T cells from WT but not IL-17 KO mice. Using an *in vitro* model of hypoxia/reoxygenation (HR), CD4+ T cells from WT mice had a multifold increase in IL-17, RANTES and MIP-1α compared to normoxic control, which was significantly attenuated by IL-17 antibody or A2AR activation. Conditioned media from HR-exposed CD4+ T cells transferred to RAW264.7 macrophages induced MIP-1α, TNF-α, RANTES and MCP-1 production, which was significantly attenuated by IL-17 antibody or ATL313.
Conclusions: The IL-23/IL-17 axis plays a key role in lung IR injury. IL-17-producing CD4+ T cells may initiate lung IR injury via macrophage activation and induction of proinflammatory cytokines/chemokines. A2AR activation attenuates IR injury in part by inhibiting IL-17 production from CD4+ T cells.

Methods

***In vivo* mouse lung ischemia-reperfusion (IR):** Animals 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.
***In vitro* hypoxia-reoxygenation (HR) model:** Primary CD4+ T cells from WT mice and RAW264.7 macrophages were grown in culture and exposed to hypoxia (3hr) (5% O_2) followed by reoxygenation (1hr) (21% O_2). Conditioned media from HR-exposed CD4+ T cells (with or without pretreatment with anti-IL-17 antibody, 10 ng/ml or ATL313, 10 μM) was transferred to RAW264.7 cells for 4 hrs.
Pulmonary function: Pulmonary function was evaluated using an isolated, buffer-perfused mouse lung system (Harvard Apparatus) 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 PULMODYN data acquisition system (Hugo Sachs Elektronik).
Adoptive transfer and flow cytometry: CD4+ T cells were isolated and purified from splenocytes of C57BL6 WT or IL-17 KO mice using the manufacturer's protocol (Miltenyi Biotec, Auburn, CA). $2x10^7$ cells were injected in Rag-1 KO mice via tail vein injection one week prior to lung IR. Purity of CD4+ T cell reconstitution in recipient spleen and lungs was confirmed by flow cytometry (FACS-Canto, BD Biosciences).
Cytokine/chemokine protein measurements: A mouse-specific multiplex cytokine panel assay (Bio-Rad Laboratories) was used to quantify the cytokine and chemokine protein content in BAL fluid.

Results

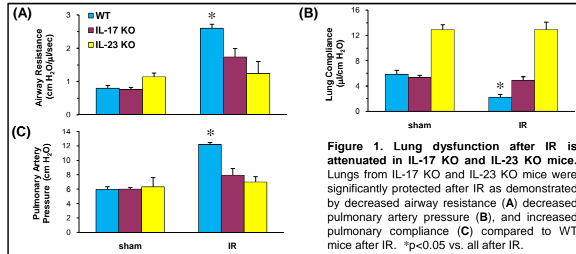

Figure 1. Lung dysfunction after IR is attenuated in IL-17 KO and IL-23 KO mice. Lungs from IL-17 KO and IL-23 KO mice were significantly protected from lung dysfunction after IR as demonstrated by decreased airway resistance (**A**) decreased pulmonary artery pressure (**B**), and increased pulmonary compliance (**C**) compared to WT mice after IR. *p<0.05 vs. all after IR.

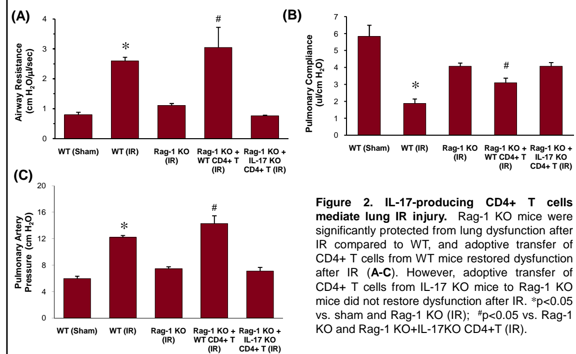

Figure 2. IL-17-producing CD4+ T cells mediate lung IR injury. Rag-1 KO mice were significantly protected from lung dysfunction after IR compared to WT, and adoptive transfer of CD4+ T cells from WT mice restored dysfunction after IR (**A-C**). However, adoptive transfer of CD4+ T cells from IL-17 KO mice to Rag-1 KO mice did not restore dysfunction after IR. *p<0.05 vs. sham and Rag-1 KO (IR); #p<0.05 vs. Rag-1 KO and Rag-1 KO+IL-17KO CD4+T (IR).

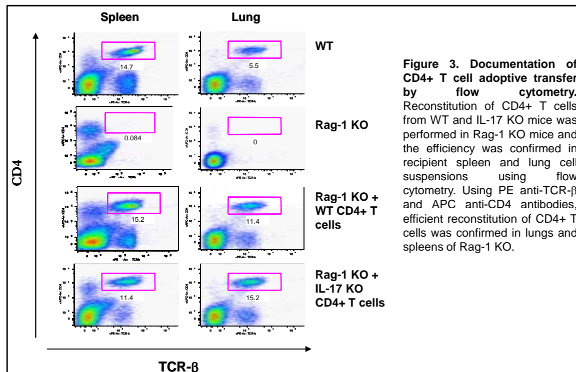

Figure 3. Documentation of CD4+ T cell adoptive transfer by flow cytometry. Reconstitution of CD4+ T cells from WT and IL-17 KO mice was performed in Rag-1 KO mice and the efficiency was confirmed in recipient spleen and lung cell suspensions using flow cytometry. Using PE anti-TCR-β and APC anti-CD4 antibodies, efficient reconstitution of CD4+ T cells was confirmed in lungs and spleens of Rag-1 KO.

Results

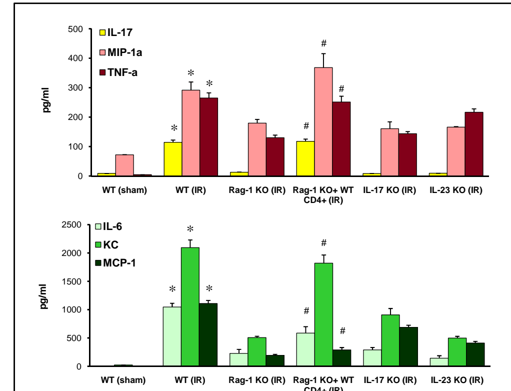

Figure 4. Proinflammatory cytokine/chemokine production after lung IR is mediated by the IL-23/IL-17 axis. Cytokines/chemokines were measured in bronchoalveolar lavage (BAL) fluid. Rag-1 KO mice have significantly decreased production of proinflammatory cytokines/chemokines compared to WT mice. Adoptive transfer of CD4+ T cells from WT mice to Rag-1 KO mice restored all cytokine/chemokine levels except MCP-1. However, IL-23 KO and IL-17 KO mice had significantly decreased cytokine/chemokine production after lung IR. *p<0.05 vs. WT (sham) and Rag-1 KO (IR); #p<0.05 vs. IL-17 KO (IR) and IL-23 KO (IR).

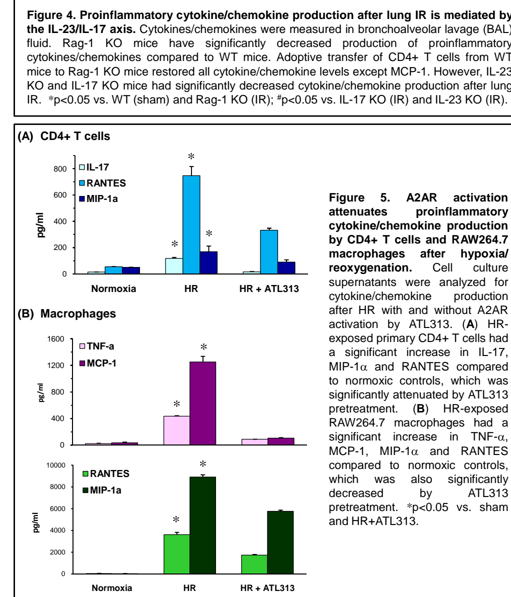

Figure 5. A2AR activation attenuates proinflammatory cytokine/chemokine production by CD4+ T cells and RAW264.7 macrophages after hypoxia/reoxygenation. Cell culture supernatants were analyzed for cytokine/chemokine production after HR with and without A2AR activation by ATL313. (**A**) HR-exposed primary CD4+ T cells had a significant increase in IL-17, MIP-1α and RANTES compared to normoxic controls, which was significantly attenuated by ATL313 pretreatment. (**B**) HR-exposed RAW264.7 macrophages had a significant increase in TNF-α, MCP-1, MIP-1α and RANTES compared to normoxic controls, which was also significantly decreased by ATL313 pretreatment. *p<0.05 vs. sham and HR+ATL313.

Results

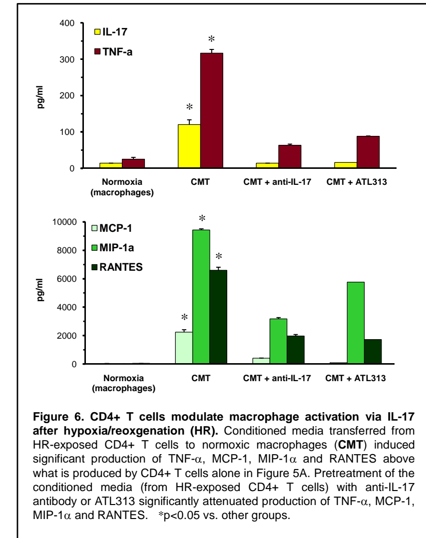

Figure 6. CD4+ T cells modulate macrophage activation via IL-17 after hypoxia/reoxygenation (HR). Conditioned media transferred from HR-exposed CD4+ T cells to normoxic macrophages (**CMT**) induced significant production of TNF-α, MCP-1, MIP-1α and RANTES above what is produced by CD4+ T cells alone in Figure 5A. Pretreatment of the conditioned media (from HR-exposed CD4+ T cells) with anti-IL-17 antibody or ATL313 significantly attenuated production of TNF-α, MCP-1, MIP-1α and RANTES. *p<0.05 vs. other groups.

Conclusions

- The IL-23/IL-17 axis plays a key role in initiation of lung IR injury.
- Lung IR injury is mediated by IL-17-producing CD4+ T cells.
- IL-17-producing CD4+ T cells modulate alveolar macrophage activation after IR by inducing TNF-α and other proinflammatory cytokines/ chemokines.
- A2AR activation attenuates IR-induced IL-17 production by CD4+ T cells and subsequent macrophage activation, thereby offering a potent, novel therapeutic target for treatment or prevention of lung IR injury.

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