

Repair of the Lung by Regulatory T Cells

Acute lung injury (ALI) is a significant cause of morbidity and mortality worldwide. After injury, the lungs undergo a process of repair via the regeneration of lung epithelial cells. This regeneration process is imperative to restore adequate levels of lung function, and failure to repair can result in the acceleration toward acute respiratory distress syndrome. Understanding the cell types that interact with each other and influence lung-tissue repair and function is imperative to the design of future therapeutics for lung diseases. A decade ago, a critical role of regulatory T cells (Tregs) in the resolution phase from ALI was demonstrated using the LPS model (1). $Rag1^{-/-}$ (recombination activating 1) mice that lack B and T cells showed similar ALI, but impaired recovery compared with control animals. Adoptive transfer of Tregs to $Rag1^{-/-}$ mice rescued recovery via promotion of neutrophil clearance from the lung. Furthermore, antibody depletion of Tregs in wild-type mice delayed lung repair similarly to T-cell knockout. Increased lung injury in Treg-depleted mice was shown to be effector T cell-dependent, and increased proliferation of inflammatory T cells was demonstrated (2).

Several studies have shown that Tregs are elevated in human blood and BAL from patients with ALI (1, 3, 4). ALI survivors have greater numbers of Tregs than those who succumb (3, 4). Human ALI Tregs have been shown to be capable of inhibiting the proinflammatory cytokine IFN- γ . In addition, Tregs from ALI survivors express elevated TIM-3 (T-cell immunoglobulin and mucin-domain containing-3), an immune checkpoint inhibitor, compared with samples from fatal cases. These data implicate Tregs as playing a critical prosurvival and antiinflammatory role in ALI.

In this issue of the *Journal*, Mock and colleagues (pp. 464–477) describe use of the LPS ALI model to examine cross-talk between Tregs and alveolar epithelial cells in their article entitled “Impact of Regulatory T Cells on Type II Alveolar Epithelial-Cell Transcriptomes during Resolution of Acute Lung Injury and Contributions of IFN- γ ” (5). Previous studies by this group have shown a role for Tregs in promoting tissue repair. Treg depletion resulted in decreased alveolar epithelial cell proliferation, and *in vitro* coculture showed that Tregs directly promote alveolar type II (AT2) proliferation (6). Furthermore, Tregs were shown to produce keratinocyte growth factor and amphiregulin, two epithelial proliferative cytokines, in an ALI model (7). Therefore, the main focus of the current article was to elucidate the effects that Tregs have on AT2 cells at the repair stage of lung injury. In particular, the authors were interested in studying *in vivo* changes in AT2 transcriptional profiles in the absence or presence of Tregs using $Foxp3^{EGFP}$ and $Foxp3^{DTR}$ mice during both the injury and repair stages of inflammation.

The authors hypothesized that AT2 transcription is altered by Tregs during the resolution of ALI. AT2 cells were sorted from uninjured mice and mice with or without Tregs that had been given

LPS to induce ALI. Transcriptomic analysis of AT2 cells from injured versus uninjured mice identified 49 unique genes in the Treg-depleted group. Hierarchical clustering showed that groups clustered together, and most changed genes were upregulated when Tregs were absent. Gene-set enrichment analysis after resolution with or without Tregs identified eight sets that were upregulated, five of which described IFN-response gene pathways. The authors concluded that Treg depletion has a substantial effect on AT2 cells, mainly focused on altered IFN responses.

The authors then sought to identify any changes in signaling molecules in BAL during LPS-induced injury in the presence or absence of Tregs. BAL was sampled at Days 1, 3, and 7 after LPS challenge. It was determined that on Day 3 at peak infection, IFN γ concentrations were increased in the absence of Tregs. Using flow cytometry, the authors determined that $CD4^{+}$ and $CD8^{+}$ T cells had significantly increased levels of IFN γ . It was concluded that without Tregs, IFN γ responses are intensified in AT2 cells, which was supported by the fact that 42 of the 49 genes they determined to be unique in their Treg-depleted group were regulated by type I or type II IFNs.

Lastly, the authors sought to determine how IFN γ may alter Treg function during ALI. To do this, the authors used an IFN γ -neutralizing antibody in the absence or presence of Tregs and observed changes in immune-cell subsets. IFN γ neutralization resulted in faster recovery and less weight loss independently of Tregs. This finding was consistent with a report that showed that IFN γ neutralization in an LPS and natural killer T-cell ALI model resulted in increased Treg accumulation in the lung and elevated IL-10 production resulting in decreased lung injury (8). When Tregs were absent and IFN γ was neutralized, there were more B lymphocytes and epithelial cells when compared with control animals. The increase in inflammatory macrophages that was observed when Tregs were depleted was lost after IFN γ neutralization, suggesting that these changes in cell populations are mediated by both IFN γ and Tregs. Neutralization of IFN γ also resulted in less activated $CD4^{+}$ T cells in the presence of Tregs. Regulation of Tregs by IFN γ has been suggested in cancer models in which IFN γ promoted Treg fragility (9). Lastly it was concluded that the reduction in Tregs led to a decrease in proliferation of both endothelial and epithelial cells, consistent with other recent findings (10). The study findings can be summarized in Figure 1. Although both Treg depletion and IFN γ neutralization altered repair after ALI, there were distinct effects of each, suggesting additional pathway involvement. The direct link between the two was not fully demonstrated, and the effect of IFN γ on Tregs or AT2 cells in this context remains to be investigated. The mechanism by which Tregs and/or IL-10 alter AT2 IFN responses also remains unclear.

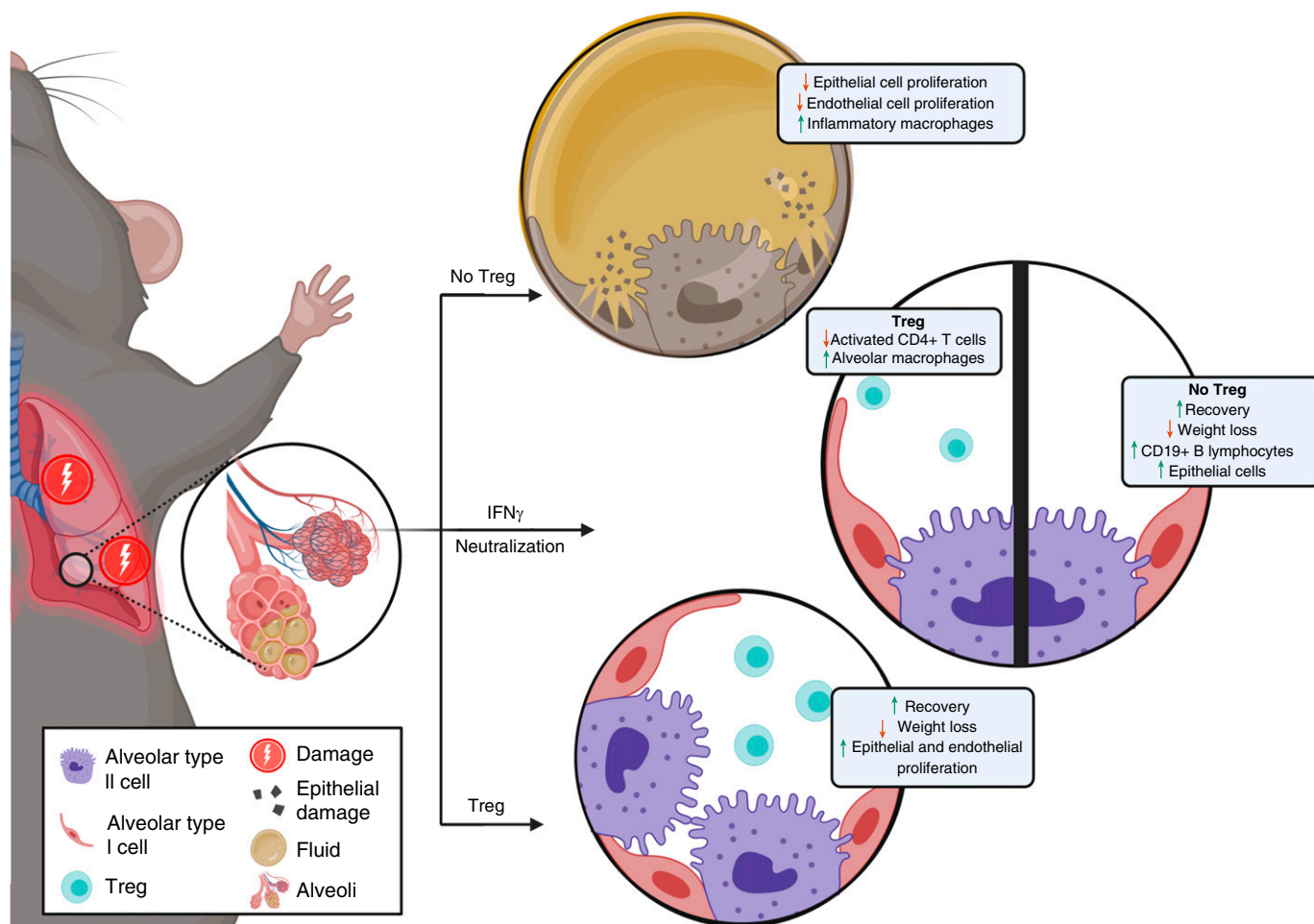


Figure 1. Regulatory T-cell (Treg) control of alveolar repair following acute lung injury (ALI). In the context of LPS-induced ALI in a preclinical animal model, recovery from inflammation and lung repair requires Tregs. In the presence of Tregs, epithelial and endothelial proliferation result in repair of alveolar structure. In the absence of Tregs, these processes are deregulated, resulting in increased inflammatory macrophage persistence, edema, and epithelial damage. The alveolar type II gene-expression signature suggests elevated IFN signaling in the absence of Tregs. IFN γ neutralization in the presence of Tregs results in increased antiinflammatory alveolar-macrophage presence and improves lung repair. Importantly, in the absence of Tregs, IFN γ plays a significant detrimental role in ALI recovery. Neutralization of IFN γ in this setting improves epithelial-cell proliferation, resolution of inflammation, and repair. The image was made in BioRender (biorender.com).

The proreparative potential of Tregs in the context of ALI and acute respiratory distress syndrome is now a topic of investigation. Immunomodulatory therapies that promote Treg proliferation and/or activity may hold significant therapeutic potential. Singer and colleagues examined the impact of targeting methylation of the *Foxp3*, a critical Treg transcription factor, locus (11). Treatment with a DNA methyltransferase inhibitor in the LPS ALI model resulted in increased Treg proliferation, suppressive function, and accelerated resolution of lung injury. Mice lacking T cells failed to improve repair when treated with the same inhibitor indicating a T cell-dependent mechanism of protection. Another study targeted Treg AKT activity using the inhibitor triciribine (12). When given 48 hours after LPS, triciribine promoted lung repair, reducing neutrophilia and edema. *In vitro*, AKT inhibition increased T-cell *Foxp3* expression, and mice with elevated Treg AKT activity (*PTEN* [phosphatase And tensin homolog] knockout) showed delayed resolution from ALI. These studies indicate that Treg modulation may be an attractive therapeutic target for ALI in

humans. Additional studies are required to determine the most efficacious approaches to boosting Treg function and to identify the timing of optimal treatments. ■

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