Opening the Regulatory T Cell Toolbox

Regulatory T (Treg) cells, a CD4⁺ T lymphocyte subset that expresses the lineage-specifying transcription factor Foxp3, play a critical role in suppressing overexuberant immune system activation and maintaining immune homeostasis (1, 2). Treg cells exert their immune regulatory activities via myriad mechanisms, including ligand-receptor interactions, metabolic disruption, cytolysis, and elaboration of soluble mediators that suppress the activation and proliferation of multiple immune cell types such as other T lymphocytes and antigen-presenting cells. These suppressor functions require continuous signaling through the Treg T cell receptor (TCR) (3). Beyond limiting collateral damage associated with unrestrained immune-mediated inflammation (4, 5), Treg cells also exert recently understood actions that directly repair injured tissues. These pro-repair functions appear to manifest independently of their suppressor functions. For example, Treg cells accumulate after muscle injury and elaborate factors such as amphiregulin, an epidermal growth factor receptor ligand that acts on muscle satellite cells to promote repair (6). Treg cell-derived amphiregulin also protects murine lung tissue against influenza A infection in a TCR-independent manner (7).

Alveolar epithelial cell proliferation represents a hallmark feature of active repair after lung injury (8). Mock and colleagues established that Treg cells serve as a critical determinant of alveolar type II epithelial (AT2) cell proliferation after an inflammatory lung injury (intratracheal administration of LPS) as well as in a noninflammatory model of compensatory lung growth/regenerative alveologenesis (unilateral pneumectomy) (9). In that study they also determined that contact-independent (soluble) factors account, in part, for the proliferative effect of Treg cells on AT2 cells. However, these factors' identities remained unknown.

Keratinocyte growth factor (kgf, also known as fibroblast growth factor 7 [Fgf7]) signals through the Fgfr2b receptor and leads to cell proliferation (10). In this issue of the *Journal*, Dial and colleagues (pp. 162–173) report the results of elegant experiments demonstrating that Treg cell-derived kgf promotes AT2 cell proliferation (11). The authors employed powerful multiparameter flow cytometry to define lung epithelial cell populations, including ciliated, club, AT1, and AT2 cells. They did not evaluate lineage-negative epithelial stem/progenitor cells, which the lung deploys to regenerate the epithelium after severe injury (12). Nevertheless, their data show that global kgf deficiency causes a decrease in overall epithelial as well as AT2 cell proliferation during resolution of intratracheal LPS-induced acute lung injury. kgf was not necessary for recovery of weight loss after LPS; thus, although they were not explored in this article, other mediators appear to ensure

resolution of LPS-induced inflammation even in the absence of kgf-directed epithelial proliferation.

Adoptive transfer of wild-type Treg cells into Treg cell-deficient animals after LPS treatment increased overall epithelial and AT2 cell proliferation to near-control levels; in contrast, adoptive transfer of kgf-null Treg cells failed to restore proliferation. Of note, adoptive transfer of neither wild-type nor kgf-null Treg cells rescued lung Treg cell numbers to control levels. However, the overall lung CD3⁺ T cell numbers increased in Treg cell-deficient animals despite adoptive transfer of either Treg cell genotype. Although it was not specifically evaluated in this study, these findings suggest that Treg cell suppressor effects may require higher numbers of local Treg cells than are needed for their pro-repair functions. Experiments that quantitatively tune the Treg cell response would help clarify whether the number of local Treg cells serves as a determinant of their suppressor versus pro-repair functions during lung injury resolution. An informative experiment would be to deliver varying numbers of Treg cells to lung-injured, Treg cell-deficient animals and evaluate epithelial proliferation and lymphoproliferative pathology in parallel.

The authors also examined a noninflammatory model of regenerative alveologenesis involving unilateral pneumectomy in Treg cell-deficient mice followed by adoptive transfer of either wild-type or kgf-null Treg cells. Similar to their findings during the resolution phase of LPS-induced acute lung injury, mice that received wild-type Treg cells displayed restored overall epithelial and AT2 cell proliferation, whereas kgf-null Treg cell recipients failed to increase proliferation. These experiments, performed in a noninflammatory model, show a clear distinction between Treg cell immune suppressor (anti-inflammatory) and pro-repair functions, although the specific switches that control these functions remain obscure. IL-18 and IL-33 signal Treg cells to secrete amphiregulin independently of TCR stimulation (7). However, Dial and colleagues did not identify upstream factors other than TCR stimulation that cue Treg cells to elaborate kgf. Nonetheless, their work with a noninflammatory stimulus for epithelial proliferation adds to the growing literature supporting a role for Treg cells as a direct pro-repair cell type that functions independently of anti-inflammatory effects. The use of inducible TCR ablation restricted to Treg cells (3) may help elucidate the complex signaling pathways that determine Treg cell pro-repair versus immune-suppressor programs in the lung.

Treg cell culture studies revealed that lung-origin Treg cells produced significantly more kgf and amphiregulin than spleen-origin Treg cells. Although this study stopped short of addressing the mechanisms that explain tissue-specific differences among Treg

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cells, this finding raises interesting questions. Specifically, the signals and epigenetic basis for lung-specific Treg cell phenotype and function remain unclear. An intriguing possibility is that some of the signals for lung specificity might overlap with those directing a pro-epithelial functional state. Finally, Dial and colleagues isolated Treg cells from human transplant donor lungs. Upon stimulation *in vitro*, these cells augmented production of kgf and amphiregulin by ~8-fold and ~5-fold, respectively. These results confirm that human lung Treg cells produce pro-epithelial factors that could represent therapeutic targets. Indeed, we await the results of the randomized controlled KARE trial of intravenous kgf in acute respiratory distress syndrome (trial registration ISRCTN95690673) (13).

The work by Dial and colleagues conclusively demonstrates that regulatory T cells utilize kgf as a tool to repair damaged alveolar epithelium. Future bench work should focus on defining the molecular and cellular network components that underlie lung-tissue specificity and direct Treg cell pro-repair versus immune regulatory functions. Multi-omics data sets incorporating chromatin immunoprecipitation and sequencing, chromatin accessibility assays, DNA methylation sequencing, and RNA sequencing of lung T cells responding to injury could help define the pathways and chromatin landscapes responsible for tissue specificity and differential T cell responses. Examining the contact-dependent and -independent interactions of Treg cells with putative lung stem/progenitor cells after injury will also further our understanding of the cellular networks involved in lung repair. From a translational perspective, their paper can inform translational studies of Treg cellular therapy (14) as well as trials of pro-repair factors administered to help repair the damaged lung. Ultimately, ascertaining the functional properties of lung Treg cells and their pro-repair mechanisms will help investigators design and interpret these trials, and could lead to a true reparative therapy for acute lung injury. ■

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Benjamin D. Singer, M.D.
Division of Pulmonary and Critical Care Medicine
Northwestern University Feinberg School of Medicine
Chicago, Illinois

ORCID ID: 0000-0001-5775-8427 (B.D.S.).

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