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Dynamics of Influenza-induced Lung-Resident Memory T Cells, Anatomically and Functionally Distinct Lung Mesenchymal Populations, and Dampening of Acute Lung Injury by Neutrophil Transfer of Micro-RNA-223 to Lung Epithelial Cells

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Recommended Reading from Northwestern University Pulmonary and Critical Care Medicine Research Fellows; Peter H. S. Sporn, M.D., Program Director

Slütter *et al.* Dynamic Equilibrium of Lung T_{RM} Dictates Waning Immunity after Influenza A Infection. *Sci Immunol* (1)

Reviewed by Keith T. Ferguson

Seasonal influenza vaccination provides protection by inducing formation of antibodies to hemagglutinin (HA), the main viral surface antigen. However, HA undergoes frequent mutation, such that the vaccine may not be well matched to the influenza A virus (IAV) strain(s) that circulate in a given year (2). As a result, vaccination can fail to elicit appropriately targeted neutralizing antibodies, in which case IAV-specific, lung-resident memory CD8 T cells (T_{RM}) may provide protective immunity (3). A problem in this regard is that lung T_{RM} are short-lived relative to T_{RM} in skin and other tissues (4). The current study, therefore, examined the mechanisms underlying the limited longevity of IAV-induced lung T_{RM}.

To address the discrepancy in persistence of lung and skin T_{RM} under conditions of a fixed T cell receptor response, the authors transferred Thy1.1 P14 transgenic T cells specific for the GP33 epitope of lymphocytic choriomeningitis virus into naive Thy1.2 mice, then infected the animals by epicutaneous inoculation with recombinant vaccinia virus or intranasal inoculation with recombinant IAV, both engineered to express GP33. Quantifying cells by flow cytometry, they showed that after an initial decline, the number of skin P14 T_{RM} plateaued and remained stable from 50 to 200 days after infection. Lung P14 T_{RM} numbers also declined initially, but in contrast to skin T_{RM}, never stabilized, dropping another 500-fold from 50 to 200 days after infection. Similarly, endogenous T_{RM} specific for IAV nucleoprotein declined 500-fold over the same time frame after infection with wild-type IAV. Moreover, the decline in lung T_{RM}

was associated with loss of CD8 T cell-mediated heterosubtypic protection against IAV growth when mice were infected with an H3N2 virus 125 days after immunization with an H1N1 strain.

Late memory splenic P14 cells (isolated from mice 100 d after infection with GP33-expressing IAV) were less able to migrate to the lungs of mice recently infected with IAV-GP33 than early memory P14 cells (isolated 20–30 d after IAV-GP33 infection). This correlated with a late memory gene expression profile for regulatory transcription factors considered nonpermissive for T_{RM} differentiation (Eomes upregulated; Blimp-1 and Hobit downregulated), as well as decreased expression of genes associated with leukocyte migration into tissues (5, 6).

Next, the authors performed experiments showing that circulating effector memory T cell are precursors for IAV-specific lung T_{RM}. Finally, they infected IAV-GP33-immune mice with recombinant GP33-expressing *L. monocytogenes*, which resulted in marked expansion of the circulating effector memory T cell pool and a large increase in the number of IAV-GP33-specific lung T_{RM}.

The paper by Slütter and colleagues is important in elucidating mechanisms that underlie the loss of IAV-specific, lung-resident T cell immunity, and in demonstrating that IAV-specific lung T_{RM} numbers can be expanded by booster vaccination. The results should be interpreted with caution, however, given that almost all of the data were generated using an engineered transgenic T cell system, recombinant influenza viruses expressing the transgenic T cell cognate epitope, and booster vaccination with recombinant bacteria expressing the same cognate epitope. Nevertheless, the study underscores the role of lung-resident T_{RM} in protecting against influenza and suggests the possibility of new approaches to vaccination. Further studies using less highly engineered systems are required to confirm that increasing influenza-specific, lung-resident memory T cells will protect against influenza infection in mice, and, ultimately, that such an approach

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might work in humans. Given the limitations of the existing HA-based seasonal influenza vaccine strategy, finding new ways to enhance immunity against influenza is certainly an important goal.

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Lee et al. Anatomically and Functionally Distinct Lung Mesenchymal Populations Marked by Lgr5 and Lgr6. *Cell* (7)

Reviewed by Alexandra C. McQuattie-Pimentel

Mesenchymal cells play key roles in lung development and homeostasis, and in regeneration of airway and alveolar structures during resolution of lung injury. These processes depend on interactions between cells of epithelial and mesenchymal lineage (8–11). The leucine-rich, repeat-containing, G protein-coupled receptor (Lgr) 5 and Lgr6 are expressed by epithelial stem cells in various tissues and in malignancies of epithelial origin, including lung cancer (12–14). The current study explored the functional role of Lgr5- and Lgr6-expressing cells in epithelial homeostasis and regeneration in the adult lung.

Using a tamoxifen-inducible lineage-tracing strategy, the authors unexpectedly found that Lgr5 and Lgr6 are expressed in the adult mouse lung in mesenchymal cells rather than in epithelial cells. The Lgr6⁺ cells were located around conducting airways and expressed α -smooth muscle actin, marking them as smooth muscle cells. In contrast, the majority of Lgr5⁺ cells were in alveolar regions and did not express α -smooth muscle actin. Single-cell RNA sequencing of FACS-sorted lung mesenchymal cells showed that lineage-labeled Lgr5⁺ and Lgr6⁺ cells segregated into nonoverlapping clusters with distinct gene expression signatures. Next, it was shown that Lgr6⁺ cells proliferate in the peribronchiolar microenvironment in steady-state conditions, and that Lgr6⁺ and airway epithelial cells undergo paired proliferative expansion after reductive genetic ablation of Lgr6⁺ cells. This was associated with increased expression of Wnt ligands in airway secretory cells and Wnt activation markers in Lgr6⁺ cells,

suggesting that airway epithelial cell-derived Wnts drive proliferation of Lgr6-expressing cells after targeted injury.

To study mesenchymal–epithelial cell interactions, a three-dimensional organoid coculture system (15) was used. In these experiments, cocultures of Lgr6⁺ cells and airway epithelial stem cells (club cells) (16) gave rise to bronchiolar-type organoids containing secretory and ciliated epithelial cells, whereas Lgr5⁺ and club cell cocultures produced predominantly alveolar-type organoids containing type I and II alveolar epithelial cells. Furthermore, airway injury-induced club cell proliferation was suppressed *in vivo* when Lgr6⁺ cells were specifically ablated by an inducible genetic strategy. Bronchial epithelial differentiation was mediated by Fgf10 and Wnt signaling, and alveolar epithelial differentiation was shown to require Wnt signaling as well.

This study provides important new information demonstrating the existence of region-specific Lgr6⁺ and Lgr5⁺ mesenchymal cell populations in the lung that respectively direct growth and differentiation of bronchiolar and alveolar epithelial cells, mediated at least in part by Fgf10 and Wnt signaling. Additional studies are needed to clarify the specific Wnts and other possible signals produced by Lgr5⁺ and Lgr6⁺ cells that are necessary and sufficient to drive bronchial versus alveolar epithelial differentiation. It will also be critical to further elucidate the upstream signals that regulate Lgr6⁺ and Lgr5⁺ cells, as well as the downstream signaling events activated in epithelial progenitors that direct differentiation to one phenotype or the other. With this study, Lee and colleagues have opened the door to such future investigation, the results of which should reveal new targets for potential therapy of airway and parenchymal lung diseases.

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Neudecker *et al.* Neutrophil Transfer of *miR-223* to Lung Epithelial Cells Dampens Acute Lung Injury in Mice. *Sci Transl Med* (17)

Reviewed by Elizabeth S. Malsin

During acute lung injury, neutrophils transmigrate and enter the pulmonary parenchyma, allowing for communication between activated neutrophils and alveolar epithelial cells. In previous work, the authors showed that ATP released from myeloid cells is converted to adenosine, which dampens pulmonary inflammation and signals the alveolar epithelium to increase alveolar fluid clearance (18). Activated neutrophils can also communicate with other cells through release of microparticles, plasma membrane-limited cellular fragments that can transfer RNA, proteins, and other mediators from one cell to another (19). In this context, Neudecker and colleagues investigated whether neutrophil-epithelial cross-talk during acute lung inflammation could involve the exchange of miRNAs and miRNA-mediated alterations in gene transcription.

First, using a neutrophil-epithelial cell coculture system, the authors showed that activated human and mouse neutrophils release microparticles that transfer *miR-223* to pulmonary epithelial cells. Next, they showed that ventilator-induced lung injury (VILI) increased the level of *miR-223* in the mouse lung, and that the *miR-223* was neutrophil derived. Interestingly, monocytes and alveolar macrophages did not transfer *miR-223* to epithelial cells in the mouse lung, a finding in contrast with an earlier report (20). In addition, patients with acute respiratory distress syndrome had increased levels of *miR-223* in BAL fluid compared with healthy controls. Lentiviral-mediated overexpression of *miR-223* in pulmonary epithelial cells robustly repressed expression of poly-ADP-ribose polymerase (PARP-1), which is known to facilitate NF- κ B- and activator protein 1-driven expression of inflammatory genes associated with injury of the lung and other tissues (21, 22).

Additional experiments demonstrated worsened lung inflammation and injury associated with VILI and methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia in *miR-223*-deficient mice. The increased susceptibility of *miR-223*-deficient mice to VILI and MRSA pneumonia was reversed by repression of PARP-1 using a lentiviral shRNA. Finally, nanoparticle-mediated overexpression of *miR-223* attenuated VILI and MRSA-induced lung injury, and reduced the mortality of MRSA pneumonia in wild-type mice. Notably, this occurred without a change in bacterial clearance from the lungs of *miR-223*-treated mice.

Taken together, the results presented suggest that neutrophil-dependent transfer of *miR-223* to pulmonary epithelial cells is an endogenous mechanism that limits excessive lung inflammation and injury caused by mechanical ventilation or infection, mediated at least in part by repression of PARP-1. Of note, previous studies have shown that *miR-223* negatively regulates granulopoiesis and neutrophil activation, and that *miR-223*-deficient mice

spontaneously develop lung inflammation and exaggerated inflammatory tissue injury after endotoxin challenge (23, 24). The current paper highlights a dual role for the neutrophil in lung injury, as an effector of inflammation and tissue damage on the one hand, and as the source of a key miRNA signal to epithelial cells that dampens inflammation and improves alveolar fluid clearance on the other. The paper also reveals that the neutrophil-*miR-223*-alveolar epithelial cell-PARP-1 axis is an important pathway that could be targeted therapeutically to promote resolution of lung injury in patients with acute respiratory distress syndrome. However, this approach is not without potential pitfalls, given evidence that *miR-223* may function as a tumor promoter and play a role in pathogenesis of type II diabetes, rheumatoid arthritis, and other diseases (25, 26). In addition, PARP-1 inhibitors, which are currently in use as antineoplastic agents, could be problematic owing to their ability to interrupt DNA damage repair, interfere with mitochondrial homeostasis, and activate viral replication (27). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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