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Commentary

IL-33 is a well-studied cytokine that resides normally within nuclei but can be released by cell damage or stress to then signal via a single receptor widely expressed on immune cells to promote host resistance and type 2 allergic immunity. In this issue of the *JCI*, Wu et al. used a well-established model of mouse Sendai viral infection to show that IL-33 was induced in distal lung airway epithelium, specifically in cell-cycling basal cells. IL-33 induced cell-cycling basal cells to expand and migrate into the alveolar compartment, presumably to restore barrier function. However, restoring barrier function with airway-derived cells may also result in persistent alveolar metaplasia. Surprisingly, nuclear IL-33 in this system acted cell autonomously, independently of release and conventional ST2 (IL1RL1) receptor signaling. The findings uncover a signaling role for nuclear IL-33 in viral activation of mouse basal cells and add to the well-known "alarmin" function of IL-33.

Nuclear IL-33 as a growth and survival agent within basal cells

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IL-33 is a well-studied cytokine that resides normally within nuclei but can be released by cell damage or stress to then signal via a single receptor widely expressed on immune cells to promote host resistance and type 2 allergic immunity. In this issue of the *JCI*, Wu et al. used a well-established model of mouse Sendai viral infection to show that IL-33 was induced in distal lung airway epithelium, specifically in cell-cycling basal cells. IL-33 induced cell-cycling basal cells to expand and migrate into the alveolar compartment, presumably to restore barrier function. However, restoring barrier function with airway-derived cells may also result in persistent alveolar metaplasia. Surprisingly, nuclear IL-33 in this system acted cell autonomously, independently of release and conventional ST2 (IL1RL1) receptor signaling. The findings uncover a signaling role for nuclear IL-33 in viral activation of mouse basal cells and add to the well-known "alarmin" function of IL-33.

Alveolar barrier regeneration after virus-induced epithelial cell death

The adult lung epithelium is faced with repeated injuries from toxins and infectious agents, requiring coordinated cellular responses to limit damage or infection and restore healthy epithelial barriers. Pathogenic viruses such as influenza, SARS-CoV-2, and others are particularly challenging, because they frequently produce rapid cytopathic changes that lead to widespread cell death and barrier disruption. Within the alveolar compartment, these events result in acute lung injury varying from mild pneumonitis to life-threatening adult respiratory distress syndrome (ARDS). The severity of ARDS depends on the complex interplay among viral virulence, immunity, cell death, inflammation, and attempts by the epithelium to regenerate. To counter viral infection, the epithelium activates both innate immune responses to limit viral replication and severe disease, and several stem cell populations that proliferate, migrate, and differentiate to replace alveolar type 2 (AT2) cells, which are the main epithelial stem cells in the alveolar compartment (1). This rescue response is largely driven by enhanced niche factors, including Wnts and BMPs, from surrounding mesenchymal cells (2, 3). As long as a sufficient threshold of AT2 cells remain healthy, the alveolar lining layer can fully regenerate. The rescue of more disrupted alveolar barriers by stem/progenitor cells involves not only the proliferation of surviving AT2 cells but also the mobilization of several distinct distal airway stem/progenitor cells that exhibit remarkable proliferation in the face of toxic threats. These progenitors pour out of distal airways into peri-airway alveolar spaces to help restore barrier integrity and promote normal gas exchange (4–9). The mechanisms that drive this airway stem/progenitor response in the context of Sendai virus infection are the focus of the work by Wu et al. in this issue of the *JCI* (10).

Wu and colleagues in the Holtzman group previously established a model of lung infection via the Sendai virus (a mouse analog of human parainfluenza virus type 1). The system was used to study the connection between acute viral infection and subsequent chronic inflammation resembling asthma and chronic obstructive pulmonary disease (COPD) (11, 12). Recently, this group has focused on IL-33, which is mainly expressed in AT2 cells in mouse lungs, as a driver of chronic inflammation in this model (13). IL-33 resides in the nuclei and is released during cell stress or injury, giving rise to its inclusion among the danger-associated molecules known as "alarmins" (14). Once released, IL-33 signals through its only known receptor, ST2 (encoded by *IL1RL1*), which is widely expressed on immune cells. Notably, ST2 is abundantly expressed in cells associated with type 2 responses (i.e., group 2 innate lymphoid [ILC2] cells, Th2 cells, mast cells, basophils, eosinophils) to induce type 2 immunity, eosinophilia, enhanced mucous production, and other features of asthma and COPD (14–17). Indeed, current clinical trials of IL-33 inhibitors are directed primarily at allergic disorders (18). Apart from allergic inflammation, however, IL-33 release and signaling on immune cells also contributes to clearance of viruses and other pathogens (15, 19). Sendai virus is known to produce cytopathic effects and apoptosis in both airways and the alveolar epithelium, consistent with high levels of extracellular IL-33, yet the damage is less severe than the widespread epithelial necrosis and ARDS induced by influenza A or SARS-CoV-2 infection in humans (20, 21). The distribution and extent of cell death is not well documented in Wu et al. (10) but likely coincides with the sites of peri-airway basal cell accumulation. Interest-

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Conflict of interest: As co-founder, HAC has equity in Pliant Therapeutics and receives consulting fees from the company.

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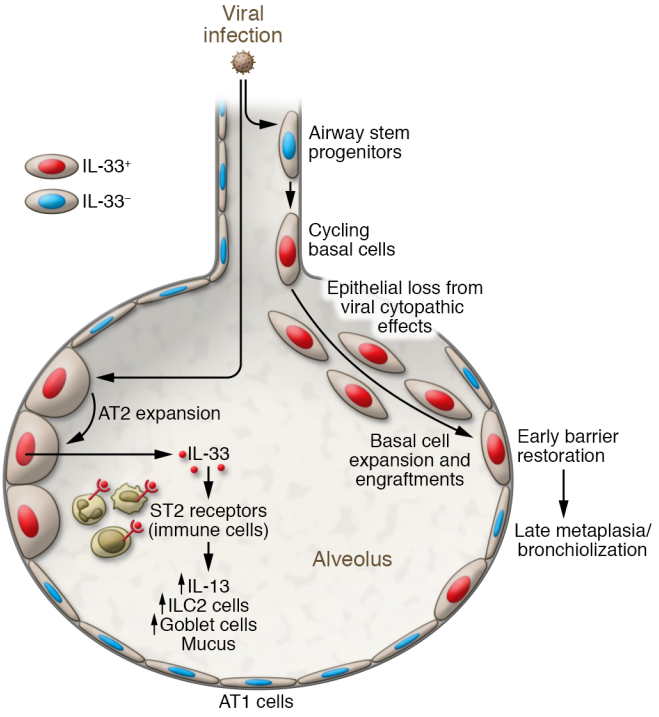

Figure 1. Dual actions of mouse IL-33 in virus-induced alveolar injury. The schematic highlights the parallel pathways by which IL-33 orchestrates epithelial responses to Sendai virus infection. Responding to cell stress or death created by viral infection, AT2 cells release IL-33 from its nuclear localization (red nuclei) to the extracellular space, where signaling through its ST2 receptors activates an allergic type 2 immune response. AT2 proliferation and expansion seen after viral infections is independent of IL-33 presence or ST2 (IL1RL1) signaling. In contrast, viral infection also induces nuclear IL-33 release in a subpopulation of cycling basal cells, where it is critical for expansion and migration from distal airways and helps restore barrier function in engraftments within denuded alveolar regions. As basal cells have never been shown to transdifferentiate into AT2 cells, reconstituting the early barrier may occur at the expense of longer-term dysplastic changes, thus perpetuating inflammation and impairing a complete return to normalcy. Similar responses in human diseases such as COVID-19 and ARDS could help explain the bronchiolization and metaplastic basal cells widely observed by pathologists during these major injuries.

ingly, AT2 cells in the Sendai virus model showed proliferation and expansion at every time point measured by Wu et al. (10), whereas AT2 cells in bleomycin- or influenza-injured mouse lungs were lost early and then followed with a regenerative response (7, 22). Early restoration of AT2 cells probably reflects less severe injury by the Sendai virus. Nonetheless, the regenerative epithelial response to Sendai virus included both AT2 cell expansion and robust mobilization and expansion of Krt5$^+$ distal airway stem/progenitor cells that persisted for at least 49 days after infection within the alveolar compartment (10).

Nuclear IL-33 signals basal cell proliferation and survival

Following initial studies of the cellular changes and proliferative responses in Sendai virus–infected lungs that peaked on postinfection day 12, Wu and authors focused on the obvious expansion of Krt5$^+$ basal stem cells and their known mature progeny (goblet cells, club cells, ciliated cells) within the alveolar compartment (10). It is important to note that in the normal mouse, there are no basal cells in the alveolar compartment and virtually none in the distal airways. Although the authors did not explore the cells of origin for basal cell expansion in this study, the histological features were consistent with prior studies demonstrating that basal cell expansion after influenza viral infection arises from rare p63$^+$Krt5$^-$ cells or distinct scgb1a1$^+$ airway stem/progenitor cells (10). While cell types have been demonstrated to expand as Krt5$^+$ cells and mobilize to alveoli after viral infection and bleomycin challenge (4, 6, 7), the underlying signaling mechanisms remain unclear. Wu and authors confirmed mobilization of distal airway stem/progenitor cells reminiscent of influenza infection by lineage tracing in *Krt5-Cre-ER2* reporter mice. The researchers then moved into uncharted territory by asking whether IL-33, already known to be released mainly by AT2 cells during Sendai virus infection, could also be important to basal cell responses following infection. In a series of innovative and state-of-the-art approaches, the authors discovered that IL-33 was induced mainly in cell-cycling basal cells, along with cell-cycle genes, specifically during the period of rapid basal cell proliferation. Moreover, expansion by proliferation was attenuated in IL-33–null mice. Importantly, expansion was not affected by deletion of the IL-33 receptor (ST2), implying that nuclear IL-33 with intracellular signaling is a distinct pathway of IL-33–mediated viral responses. The authors confirmed this conclusion by crossing a conditional (*fl/fl*) IL-33 mouse with a *Krt5-Cre-ER2* driver mouse to selectively delete IL-33 in basal cells. Deletion of IL-33 specifically in basal cells greatly attenuated basal cell mobilization and expansion without impacting AT2 IL-33 release and immune signaling, or attenuating the proliferative response of AT2 cells after viral infection. As noted by the authors, these findings support two distinct arms of IL-33 signaling in viral responses (Figure 1): (a) noncanonical, IL-1–like secretion of IL-33 that acts via its well-known receptor to promote type 2 immunity and host defense; and (b) signaling via nuclear IL-33 that is critical to support basal cell survival, expansion, and alveolar migration after major lung injury. Signaling functions of nuclear IL-33 have been postulated for years, but the high technical level of single-cell transcriptional analysis, cell-specific lineage tracing, and targeted gene deletion by Wu et al. has allowed for a more granular and revealing analysis (10).

Concluding thoughts

Although the study by Wu et al. (10) is thorough, the findings evoke some questions. The authors found that attenuation of basal cell expansion and alveolar accumulation (via deletion of IL-33 specifically in basal cells) actually accelerated the

recovery of overall oxygenation and airway resistance after viral infection. This result is consistent with prior studies in mice showing that attenuation of metaplastic alveolar basal cell accumulation in influenza by selective deletion of basal cell *HIF-1* also improved oxygenation and alveolar regeneration (22). To what advantage, then, is the striking alveolar accumulation of basal cells after viral infection, as shown in the lungs by Wu and colleagues (10)? It is likely that the rapid peri-airway basal cell engraftments contributed early to improved alveolar barrier function at the expense of subsequent, potentially long-term dysplastic changes (Figure 1). This possibility was suggested by Wu et al. (10) and in previous reports (4, 5). But another possibility is raised by an interesting finding of Wu et al. (10): global, specific deletion of IL-33 in basal cells resulted in hair loss and esophageal ulceration but no phenotype in normal lungs, suggesting that basal cell nuclear IL-33 has important homeostatic functions in the skin and gut. Therefore, the overexuberant basal cell responses seen after viral infection in the lung could result from the unavoidable consequence of a fundamental nuclear signaling system occurring in other tissues. As recognized by the authors, a critical next step will be to better elucidate the actual intranuclear signaling mechanisms by IL-33 and to determine whether the mechanism or mechanisms are unique to viral infection, apply to basal cells only activated after alveolar injury, or operate in all basal cells. There are some clues based on recent findings that nuclear IL-33 associates with the transcription factor RUNX2 and alters chromatin structure, but much more insight is needed (23, 24).

Finally, it should be remembered that mouse and human distal airway anatomy and physiology are quite different. Human distal airways end in respiratory bronchioles that have their own set of stem/progenitor cells including a few basal cells (25). These basal cells are absent in mice. Moreover, human AT2 cells, unlike those in mice, do not normally express IL-33, which instead appears constitutively expressed in airway basal cells (15). Thus, the paradigms established by Wu and colleagues from studies in mice (10) are likely substantially different from those for humans with viral infections. But there are also strong common features between mice and humans. Alveolar basal cells and bronchiolization are prominent in fatal influenza and COVID-19 ARDS as well as in most fibrotic lung diseases (21, 22). The finding that nuclear IL-33 appeared to have important functions in basal cells independent of release and immune signaling should provide impetus to pursue the underlying mechanisms in humans.

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