Linking Acute Infection to Chronic Lung Disease The Role of IL-33–Expressing Epithelial Progenitor Cells

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Abstract

Respiratory infection is a common feature of the major human airway diseases, such as asthma and chronic obstructive pulmonary disease, but the precise link between acute infection and chronic lung disease is still undefined. In a mouse model of this process, parainfluenza virus infection is followed by long-term induction of IL-33 expression and release and in turn innate immune cell generation of IL-13 and consequent airway disease signified by excess mucus formation. IL-33 induction was traceable to a subset of secretoglobin-positive airway epithelial cells linked to progenitor/stem cell function. In corresponding studies of humans with chronic obstructive pulmonary disease, an increase in IL-33 production was also detected in concert with up-regulation of IL-13 and airway mucus formation. In this case, increased IL-33 production was localized to a subset of airway basal cells that maintain an endogenous capacity for increased

pluripotency and ATP-regulated release of IL-33 even *ex vivo*. The results provide evidence of a sustainable epithelial cell population that may be activated by environmental danger signals to release IL-33 and thereby lead to IL-13–dependent disease. The progenitor nature of this IL-33–expressing ATP-responsive cell population could explain an acquired susceptibility to chronic airway disease. The findings may therefore provide a new paradigm to explain the role of viral infection and the innate immune system in chronic lung disease based on the influence of long-term epithelial progenitor cells programmed for excess IL-33 production. Further studies are needed to address the basis for this type of postviral reprogramming and the means to correct it and thereby restore airway mucosal immune function to normal.

Keywords: airway epithelial cell; IL-33; IL-13; progenitor/stem cell; innate immune response

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Common airway diseases exhibit a fundamental susceptibility to chronic inflammation driven by inhaled environmental stimuli. In the case of chronic obstructive pulmonary disease (COPD), the environmental influence is classically believed to involve smoking tobacco or burning biomass fuel (1) and, in the case of asthma, to depend on allergen sensitization and subsequent exposure (2), but a revised view suggests that respiratory viral infection of the lower airways also contributes to the disease process alone (3–17) and in synergy with other stimuli

(18, 19). However, the transient nature of respiratory viral infection and the long-term nature of chronic inflammatory disease remained difficult to reconcile. This discrepancy appears even more difficult to resolve for inflammation due to an innate immune response that is conventionally viewed as built for short- rather than long-term activation.

Therefore, it was unexpected when it was revealed that an innate immune pathway links respiratory viral infection to chronic lung disease in a newly developed mouse model of this process (12, 20, 21).

Central to this pathway was an immune axis that featured the interaction of natural killer T (NKT) cells, tissue macrophages, and perhaps innate lymphoid cells that are responsible for IL-13 production and consequent disease manifested by airway hyperreactivity and excess mucus formation (13–15, 17, 22). The basis for the persistent immune activation was attributed to long-term viral remnants and consequent participation of antigen-presenting cells as well as long-lived immune cells, but, in fact, the mechanism for chronic activation was uncertain. This

article addresses this issue of sustainability with recent advances in the postviral mouse model of chronic lung disease and in translational studies of humans with comparable lung disease. Together, the work provides evidence that viruses might program an expanded subset of long-term IL-33–high epithelial progenitor cells to drive IL-13–dependent lung disease (23, 24). This subset of sentinel epithelial cells could then respond on an ongoing basis to environmental danger signals (including those from infections) with an excessive IL-33–driven type 2 immune response that is often a disadvantage to the host.

Analysis of a Postviral Mouse Model of Chronic Lung Disease

As introduced above, initial work on a distinct mouse model showed that a single infection with a mouse parainfluenza virus known as Sendai virus (SeV) leads to long-term inflammatory lung disease in a genetically susceptible strain of mice (12, 21). Further analysis of this postviral model of chronic lung disease aimed to determine the underlying basis for the inflammatory response. Despite the conventional view that chronic inflammation would be mediated by the adaptive immune response, the analysis of this model uncovered an innate immune axis involving semiinvariant NKT cells and alternatively activated (also known as type 2 or M2) macrophages that resulted in IL-13 expression and consequent airway hyperreactivity (monitored by methacholine-induced bronchoconstriction) and mucus overproduction (signified by mucin MUC5AC expression) (14). These studies and subsequent ones indicated that transcriptionally active virus could be found long after clearance of infectious virus, and studies from other labs showed that antigen-presenting cells (e.g., dendritic cells or macrophages) were capable of activating NKT cells and surviving for prolonged times in the tissue (25, 26). Therefore, it was possible that persistent viral stimulation with ongoing activation of antigen-presenting cells might explain the long-term nature of postviral lung disease.

To determine if this was the case in a relatively unbiased approach, an analysis of whole genome expression was performed

using lungs from mice inoculated with SeV versus ultraviolet-inactivated SeV. This method identified expression of the IL-33 cytokine gene as the most highly induced of all of the cytokine and cytokine-receptor encoding genes represented on the genome-wide gene expression array. Subsequent experiments using treatment with anti-IL-33 receptor (IL-33R) blocking monoclonal antibodies (directed against IL1RL1/ST2 protein) as well as work on IL-33R–deficient and IL-33–deficient mice indicated that IL-33–IL-33 receptor signaling was required for induction of IL-13 gene expression and consequent airway disease as manifested by excess airway mucus production. In contrast, IL-33R signaling was not required for airway hyperreactivity. This finding highlights again the relatively potent control of the IL-33 to IL-13 axis over airway mucus production and suggests that additional factors may influence airway hyperreactivity after viral infection.

The postviral model was next used to address the question of the cellular source of IL-33 expression, given that a previous report suggested that alveolar macrophages might be the primary site of IL-33 production based on immunostaining and fluorescence-activated cell sorter analysis (27, 28). However, it was found that existing anti-mouse IL-33 antibodies demonstrated inconsistent specificity in mouse tissues. Therefore, approaches were developed and pursued to detect the corresponding IL-33 mRNA expression using *in situ* hybridization and to localize IL-33 protein expression using a transgenic mouse engineered to produce a β -galactosidase reporter in place of endogenous IL-33 (29). These approaches consistently localized IL-33 induction to a subset of secretoglobin 1A1- and secretoglobin 3A1-positive airway epithelial cells along with the previously recognized constitutive expression of IL-33 in surfactant protein C–positive alveolar type 2 cells. Thus, IL-33 expression marks an airway cell population linked in other injury models to repair, renewal, and remodeling in the distal airway epithelium (13, 30–32) and an alveolar type 2 cell population responsible for the same progenitor functions in the alveolar compartment (33, 34). Further studies are required to identify the precise progenitor characteristics of the IL-33–high subset of airway epithelial cells. Nonetheless, the

findings suggested that an IL-33–expressing progenitor cell population might be a feature of chronic obstructive lung disease, so it was natural to pursue this possibility in humans with comparable lung disease.

Analysis of Humans with Chronic Lung Disease

Our previous work had detected the initial evidence of IL-13 expression along with M2 monocyte/macrophage accumulation and MUC5AC production in the lungs of patients with very severe COPD (13–15, 17). In addition, we had obtained preliminary evidence of synergy between tobacco smoking and viral infection in producing chronic airway disease in the postviral mouse model (19). Therefore, we continued to pursue the issue of IL-33–high progenitor cells in further studies of humans with very severe COPD. This strategy was enabled by the availability of whole lung explants from recipients undergoing lung transplantation for very severe (Global Initiative for Chronic Obstructive Lung Disease stage IV) COPD and from donors without COPD as controls. This analysis demonstrated significantly increased *IL33* mRNA and protein levels in lung samples from subjects with COPD compared with subjects without COPD. Increased *IL33* mRNA was particularly found in airway-enriched samples (guided by computed tomography–based imaging), in which increased levels were associated with increased levels of *IL13*, *IL13Ra1*, and *MUC5AC* mRNA and were significantly correlated with *IL13Ra1* and *MUC5AC* mRNA. Similar to our findings in mice, the increase in *IL13Ra1* expression suggested a mechanism for autoamplification of IL-13 actions in COPD, and the relative increase in *IL33* expression was not accompanied by a significant increase in lung levels of *IL25* or *TSLP* expression in subjects with COPD compared with subjects without COPD.

Unlike the situation for studies of mice, valid anti-IL-33 antibodies had been generated for analysis of lung tissue samples. Based on immunostaining of lung tissues with anti-IL-33 antibody, the cellular source of increased IL-33 production in the lungs of subjects with COPD was traced to a subset of airway basal cells (and not ciliated, serous/

secretory, or mucous cells). This subset of airway basal cells was also marked by expression of KRT5⁺, KRT14⁺, and TRP63⁺ and was particularly prominent in areas of epithelial hyperplasia and mucous cell remodeling in COPD. However, similar to the case for the mouse model, IL-33 expression did not overlap completely with KRT5, KRT14, or TRP63 immunostaining, suggesting that IL-33-expressing cells represent a distinct airway basal cell niche. Nonetheless, given the role of TRP63 in suppression of apoptosis and prolongation of epithelial cell survival (35), the findings again suggested that IL-33 expression may mark a progenitor cell population that was up-regulated as a component of chronic airway disease.

To take advantage of the tools available for analysis of lung progenitor cells, we next examined whether the IL-33 expression program continued *ex vivo* in basal cells isolated from subjects with and without COPD (24). Under submerged culture conditions (that preserved progenitor cells and IL-33 expression), we found again that levels of IL-33 expression were significantly increased in basal cells from subjects with COPD compared with subjects without COPD, even after 5 days in culture. Using fluorescence-activated cell sorter analysis, IL-33 expression was localized to an ITGA6^{hi}NGFR^{lo} subpopulation of basal cells in subjects with and without COPD, again with increased levels of IL-33 expression in cells from subjects with COPD compared with subjects without COPD. In addition, IL-33⁺ basal cells placed into an enriched three-dimensional cell culture system were fully capable of forming structures (designated tracheobronchospheres) with fidelity to original airway morphology. Moreover, a significantly increased efficiency of sphere formation was found if cultures were enriched for IL-33-expressing (ITGA6^{hi}NGFR^{lo}) cells or if cells were obtained from subjects with COPD versus subjects without COPD. These results provide evidence of a subset of airway basal cells that is marked by higher IL-33 expression and progenitor capacity and is found at increased levels in COPD.

The results from the postviral mouse model indicated that the active form of IL-33 must reach the extracellular space to bind to IL-33R for the development of chronic lung disease (because the disease

was attenuated with antibody blockade or genetic deficiency of IL-33R). It was therefore useful to determine the capacity for IL-33 release from basal cells, especially in cells from subjects with COPD versus subjects without COPD. Previous work indicated that extracellular ATP may act as a danger signal that activates purinergic receptor P2Y₂R signaling and thereby drives IL-33 release (36). In agreement with these findings, ATP administration to basal cells did indeed cause significant increases in the levels of IL-33 release from basal cells cultured from both subjects with COPD and subjects without COPD. The results implicated a regulated secretory process to direct IL-33 release under conditions of cellular stress, because there was no evidence of cytotoxicity under these experimental conditions. Taken together with the data from *in vivo* studies, the findings with cultured cells demonstrate that a subset of the airway basal cells is programmed for IL-33 expression and release. Thus, an increase in this progenitor cell population could serve as a renewable source of increased extracellular levels of IL-33 and the consequent production of IL-13 and development of IL-13-dependent lung disease.

Conclusions and Future Directions

Despite frequent proposals that acute infections might lead to chronic inflammatory disease, the relationship has been difficult to establish, at least in part because of the lack of representative animal models of this process. We were therefore able to gain significant insight into the infection-to-disease link with studies of paramyxovirus infection in mice aimed at modeling the initiation, exacerbation, and progression of chronic lung diseases such as COPD and asthma. Initial studies of the postviral mouse model revealed an innate immune axis involving semiinvariant NKT cells and M2 macrophages that interact to drive IL-13 production and consequent airway mucus production and hyperreactivity that are characteristic of chronic obstructive lung disease in humans (2, 12–15). However, this initial mechanistic work did not identify the upstream factors that might explain how acute viral infection could translate to a sustained activation of immune effector cells. Here we review the subsequent advances that identify IL-33 as a principal driving influence on the innate immune

Figure 1. Scheme for IL-33 to IL-13 immune axis in translating acute infection to chronic lung disease. Respiratory viral infection (perhaps enhanced by tobacco smoke and/or allergen exposure) in susceptible individuals leads to an increase in lung epithelial progenitor cells (subsets of airway basal cells in humans and airway secretoglobin-positive [Scgb⁺] secretory cells and alveolar type 2 cells in mice) that are reprogrammed for increased IL-33 expression and hyperplasia. Subsequent environmental danger signals (including those from additional infections) then can stimulate ATP-regulated release of IL-33, which acts on immune cells in the lung (e.g., CD4⁺ Th2 cells, type 2 innate lymphoid cells [ILC2], and semiinvariant natural killer T cells [NKT]) with interacting monocytes and macrophages (Mono) to stimulate IL-13 production. The consequent actions of IL-13 on airway epithelial cells can drive CLCA1 to MAPK13 signaling to cause airway mucous cell and mucus formation. Modified with permission from Reference 24.

axis that leads to IL-13-dependent lung disease. The evidence for a primary role for IL-33 in experimental (mouse) and clinical (human) forms of chronic lung disease consists of the observations that: (1) IL-33 expression is selectively induced relative to IL-25 and TSLP, and IL-33–IL-33R signaling is required for increased IL-13 and airway mucus production in the postviral mouse model; (2) induction of IL-33 expression is traceable to secretoglobin-expressing airway epithelial cells along with constitutive expression in alveolar type 2 cells, suggesting that progenitor cell populations might account for long-term IL-33 expression and IL-13-dependent disease in this same mouse model; (3) IL-33 expression is also selectively increased in lung tissue and is associated with an IL-13–IL-13 receptor to MUC5AC mucin gene signature in concert with excess airway mucus production in humans with very severe COPD; (4) IL-33 expression is selectively localized to nuclei in a subset of airway basal cells in human lungs, and this IL-33–high cell population is increased in lungs from humans with very severe COPD; and (5) increased nuclear expression of IL-33 is preserved in basal cells cultured from subjects with COPD and marks a cell population with increased progenitor cell capacity and ATP-mediated apically directed release of IL-33.

Together with previous results that define immune cell production of IL-13 and IL-13 signaling to mucin gene expression (12, 14, 17, 21), the latest results

provide for a new scheme to link acute infection to chronic lung disease. This scheme (depicted in Figure 1) allows for viral infection (perhaps enhanced by tobacco smoking and/or allergen reactions) to reprogram the airway with a long-term IL-33–high population of epithelial progenitor/stem cells that can respond to environmental danger signals with exaggerated IL-33 release that in turn drives downstream inflammation. In this case, the inflammatory process is characterized at least in part by induction of IL-13 expression from innate immune cells (e.g., NKT cell–macrophage interaction and innate lymphoid cells). The interaction of IL-13 with IL-13 receptor can then signal via CLCA 1 and MAPK13 to drive epithelial mucin gene expression and consequent production of inflammatory airway mucus (17, 21) that is linked to the morbidity and mortality associated with common airway diseases, such as COPD and asthma, and perhaps other hypersecretory airway diseases (37–43).

Future studies of the link between acute infection and chronic lung disease need to address ongoing and critical questions. These include: (1) more precise definition of the mouse and human progenitor/stem cell niche within the airway epithelial cell population and the basis for possible viral reprogramming of this cell population; (2) the relationship of the epithelial-cell IL-33 to immune-cell IL-13 pathway with other environmental risk factors for chronic lung disease, such as tobacco smoking and allergen exposure, as well as underlying

genetic risk factors for this type of disease; (3) the precise nature of the immune cell populations activated in this same pathway; (4) the molecular basis for translating these epithelial and immune cell signals to end-organ dysfunction, including production and processing of IL-33 as well as expression and signal transduction of IL-33 and IL-13 receptors; and (5) the unresolved issue of whether viral remnants have any role in the development of chronic lung disease. Nonetheless, ongoing research progress already indicates that viral infection is a suitable candidate to drive acute exacerbations with long-term consequences for chronic lung disease. In fact, viral infection appears significantly more potent than tobacco smoke exposure as a stimulus of airway inflammatory disease under experimental conditions (44, 45). The role of viral and epithelial actions will be better settled as key immune components (e.g., IL-33 and IL-13) are effectively blocked in patients with chronic lung disease (46, 47), and, perhaps, better antiviral agents are developed and applied to patients at risk for postviral disease (48, 49). The present findings provide a rational basis for this therapeutic strategy and for how acute infectious agents (such as common respiratory viruses) can reprogram long-term progenitor cells (such as airway basal cells) to cause chronic inflammatory disease. ■

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

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