Interleukin-13 Stimulation Reveals the Cellular and Functional Plasticity of the Airway Epithelium

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Abstract

About 50% of patients with asthma exhibit chronic airway inflammation driven by the type 2 cytokines interleukin (IL)-4, IL-5, and IL-13. These patients with type 2-high asthma experience more allergic symptoms, greater airway hyperresponsiveness, and more severe mucus obstruction than patients with type 2-low asthma. Mouse models of asthma have shown that much of the airway dysfunction in these models can be generated by IL-13 stimulation of the airway epithelium alone. Both *in vivo* mouse model studies and *in vitro* studies of human mucociliary airway epithelial cultures have shown that IL-13 induces cellular remodeling of the airway epithelium through proliferation-independent transdifferentiation processes. In both humans and mice, IL-13 stimulation of the airway epithelium results in generation of hypersecretory mucin 5AC (MUC5AC)-expressing mucus cells. Whereas club cells have been shown to be the source of these mucin 5AC-positive mucus cells in mice, the origin of these mucus cells in humans is unclear. In humans, chronic IL-13 stimulation appears to result in loss of ciliated cells. Moreover, IL-13 stimulation can block ciliated cell differentiation from human basal airway epithelial cells. Coincident with IL-13 cellular remodeling are

reported decreases in mucociliary transport and ciliary beat frequency. These IL-13-mediated changes in mucociliary function are accompanied by disorganization of cilia, a decrease in the ratio of mucin 5B (MUC5B) to mucin 5AC, and mucus gel tethering to the epithelial surface by mucin 5AC. These airway epithelial responses to IL-13 are mediated by multiple transcription factors, including signal transducer and activator of transcription-6 (STAT6), SAM pointed domain-containing Ets transcription factor (SPDEF), Forkhead box A2 (FOXA2), and Forkhead box J1 (FOXJ1). In addition, analysis of RNA-sequencing data derived from airway epithelial cells shows how IL-13 stimulation promotes broad changes in gene expression across the transcriptome. These results reveal the plastic nature of airway epithelial cells that enables the epithelium to undergo remodeling and functional shifts in response to IL-13 stimulation. With use of new technology, future studies should lead to greater understanding of how IL-13 and other stimuli of disease bring about airway epithelial remodeling, which may aid in the development of therapies that ameliorate airway dysfunction in asthma and other diseases.

Keywords: airway epithelium; asthma; interleukin-13 (IL-13); mucin 5ac (MUC5AC); type 2 inflammation

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Interleukin-13 as a Driver of Type 2-High Asthma

Asthma is a chronic airway disease characterized by reversible airway obstruction with varying amounts of airway inflammation and mucus obstruction. The centrality of the airways to disease pathology has called attention to the role of mucociliary airway epithelium dysfunction in the asthma disease process. Moreover, the airway epithelium is the interface through

which the lungs first interact with environmental factors (e.g., respiratory viruses, air pollution, cigarette smoke), exposure to which is associated with the development of asthma in genetically susceptible individuals. The number and complexity of these airway-level gene–environment interactions underlie the observation that multiple disease endotypes, or pathobiological mechanisms, drive asthma development and influence disease severity.

In 2009, a groundbreaking study led by Woodruff and colleagues established the first and most prominent of these endotypes using microarray gene expression profiling of bronchial airway epithelial brushings (1). This disease endotype, called *type 2-high asthma*, was observed in approximately 50% of the adult patients with mild asthma screened and was characterized by inflammation caused by the type 2 cytokines interleukin (IL)-4, IL-5, and IL-13. Multiple populations of airway immune cells have

been shown to produce these type 2 cytokines, including, T-helper type 2 (Th2) cells, mast cells, basophils, eosinophils, and group 2 innate lymphoid cells. In this work, they showed that patients with type 2-high asthma exhibited higher levels of airway hyperresponsiveness and increased serum immunoglobulin E levels, blood eosinophil numbers, and airway mucin 5AC (*MUC5AC*) gene expression (1). Other studies of the bronchial epithelium have replicated this result in adult patients with asthma, including those with more severe disease (2, 3). My colleagues and I recently used nasal airway epithelial expression analysis to show that the same type 2-high signature exists in children with asthma and is associated with atopic asthma, blood eosinophils, and the recent occurrence of asthma exacerbations (4). Moreover, we were able to directly measure *IL13* expression levels using targeted RNA-sequencing analysis. The expression level of *IL13* was strongly correlated with the expression of most genes that were differentially expressed in subjects with asthma compared with control subjects, suggesting that IL-13 is a main driver of airway epithelial dysfunction in asthma. Further supporting the importance of IL-13 in the disease process, transgenic overexpression of IL-13 alone in the airways of mice has been found to trigger most asthma features, including airway hyperresponsiveness, mucus overproduction, eosinophilic inflammation, and subepithelial fibrosis (5). The effects of IL-13 on the airway result from responses taking place in multiple cell types, including eosinophils, fibroblasts, T cells, B cells, airway smooth muscle cells, and airway epithelial cells. Although all components of this multicellular response are important, the response of airway epithelial cells has been shown to be critical for the airway phenotypes triggered by IL-13 stimulation. Specifically, the occurrence of mucus metaplasia and airway hyperresponsiveness was preserved in mice that were genetically engineered to have competent IL-13 signaling only in airway epithelial cells (6). Therefore, a comprehensive understanding of human airway epithelial cell responses to IL-13 has important mechanistic and therapeutic implications for asthma. Below, I discuss how IL-13 alters the cellular composition and mucociliary function of the epithelium, what is known about the molecular

control of these changes, and how current approaches can fill gaps in the understanding of this important airway epithelial response.

Interleukin-13-induced Cellular Remodeling and Metaplasia of the Airway Epithelium

Mucociliary clearance, barrier protection, and other functions of the airway epithelium are dependent on whether the airway epithelium is composed of the correct proportions of the multiple airway epithelial cell types. For nearly two decades, researchers have used direct IL-13 challenge and IL-13-driven allergen challenge mouse models to examine *in vivo* cellular remodeling of the airway epithelium mediated by IL-13.

At baseline, the nontracheal conducting airways of mice are composed of similar proportions of ciliated and nonciliated secretory cells (7). These nonciliated secretory cells are club cells, which secrete club cell secretory protein. Absent stimulation, few mucus secretory cells are present in the mouse airway, and few basal epithelial cells occur beyond the tracheal airway. These mouse studies have established that IL-13 stimulation of the airway epithelium dramatically increases numbers of mucus-producing secretory cells, a change that has been described as goblet cell hyperplasia or, more commonly, as mucus cell metaplasia. The characteristic mucin expressed by these IL-13-induced mucus cells is *MUC5AC*. Evans and colleagues found that ovalbumin challenge in mice resulted in nearly all club cells becoming mucus-producing cells without an increase in proliferation, supporting a metaplastic process (7). In contrast, they did not observe a change in the frequency of ciliated cells, nor did they observe mucin expression in ciliated cells. Lineage-tracing studies confirmed that club cells were the source of new mucus cells in the mouse airway upon allergen stimulation and that this process did not involve cell proliferation (8). Further ovalbumin mouse model lineage-tracing studies of ciliated cells, which are generally regarded as terminally differentiated, showed no evidence of ciliated cells becoming mucus cells in the tracheal airway epithelium (9).

Despite these findings, studies by Tyner and colleagues suggested that ciliated cells were a source of newly formed mucus cells in

a virus-induced mucus metaplasia mouse model (10). Moreover, they found ciliated cells expressing *MUC5AC* in IL-13-stimulated mouse air-liquid interface (ALI) tracheal airway epithelial cultures (10).

IL-13-mediated cellular remodeling of the human airway epithelium has been examined *in vitro* using ALI and organoid mucociliary airway epithelium culture models. In these models, donor-isolated primary basal airway epithelial cells are differentiated into a mucociliary epithelium using various culture protocols. Depending on the culture method used, the resulting mucociliary epithelium is composed of basal, ciliated, mucus secretory, (potentially) club secretory, and other nonciliated columnar cells, similar to the human *in vivo* airway epithelium. Multiple studies have demonstrated that IL-13 stimulation during the differentiation process heavily skews the basal epithelial cell differentiation process to generate significantly more *MUC5AC*-positive mucus cells and fewer ciliated cells in the resultant cultures (11, 12). Researchers in several studies have also examined the cellular effects of IL-13 stimulation on established mucociliary differentiated human ALI epithelial cultures. Similar to *in vivo* mouse studies, these studies revealed large increases in the number of *MUC5AC*-positive mucus cells with IL-13 stimulation (11, 13). However, in contrast to the mouse studies, these studies also showed decreases in the number of ciliated cells (11, 13). This raised the question whether ciliated cells were transdifferentiating into mucus secretory cells. Through lineage tracing in IL-13 stimulation experiments of mucociliary differentiated ALI cultures, Turner and colleagues observed the presence of *MUC5AC*-positive mucus cells that were also expressing the ciliated cell lineage marker Forkhead box J1 (*FOXJ1*) (11). Further support for this transdifferentiation process can be found in a human airway epithelial culture study in which researchers identified cells expressing both ciliated and mucus cell markers after IL-13 stimulation (13). However, transition of ciliated cells to mucus cells alone is unlikely to explain the rapid appearance (<48 h) of such a large number of *MUC5AC*-positive mucus cells (without ciliated cell characteristics) upon IL-13 stimulation. Furthermore, the IL-13-determined fate of the large number of mucin 5B (*MUC5B*)-expressing secretory cells, basal

cells, and other nonciliated cells in these mature human ALI epithelial cultures is unclear. Specifically, no studies to date have been done to examine the IL-13-induced fate of club cells in the human airway, cells that are clearly a dominant source of MUC5AC-producing mucus cells generated in IL-13 and allergic mouse models. This is due in part to differences in how investigators define the club cell population that for some requires ultrastructural examination of cell organelle content not attempted in many studies. In addition, others have reported that club cells are rare in the human upper airways, though these reports are subject to concerns regarding the quality and sensitivity of the club cell secretory protein antibody used. Supporting the presence of upper airway club cells, my colleagues and I found that secretoglobin, family 1A, member 1 (*SCGB1A1*) (encoding the club cell secretory protein) is among the most highly expressed genes within *in vivo* nasal airway epithelial specimens and human tracheal ALI *in vitro* cultures (4, 14). In fact, our studies of *in vivo* nasal brushings showed that *IL13* levels are strongly anticorrelated with levels of the club cell gene, secretoglobin, family 3A, member 1 (*SCGB3A1*) in patients with asthma, and bronchial expression studies revealed secretoglobin, family 3A, member 1 to be downregulated in patients with type 2-high asthma (1, 4). Moving forward, IL-13 stimulation studies of the human mucociliary airway epithelium need to be conducted to examine changes in the proportions and fates of all cells in the epithelium together, in order to clarify the mechanisms at play. In addition, both quantitative histological and RNA-sequencing studies of the airway epithelium from patients with type 2-high asthma are needed to determine whether the cellular remodeling observed in the IL-13-stimulated airway epithelium cell culture models is also observed *in vivo*.

Interleukin-13-mediated Changes in Mucociliary Clearance and Airway Mucus Properties

Impaired mucociliary clearance has been observed in patients with asthma, and mucus plugging has been reported in both severe and fatal asthma (15, 16). A role for IL-13 in modifying mucociliary clearance is supported by its ability to induce an airway

epithelial hypersecretory phenotype and to increase the ratio of mucus to ciliated cells in the airway epithelium, as discussed in the preceding section. In addition, IL-13 has been reported to reduce ciliary beat frequency in organoid cultures of human airway epithelial cells (12). In support of the hypothesis that IL-13 can directly impair ciliated cell function, multiple studies have shown that IL-13 both induces mislocalization of ciliated cell basal bodies away from the apical cell surface and drives inhibition of Forkhead box J1 expression, a critical transcription factor in ciliated cell development (13). This raises the possibility that IL-13-induced reduction in ciliary beat frequency is at least partially due to dysfunctional ciliated cells.

However, other data suggest that IL-13-induced changes to mucus could also be indirectly altering ciliary beat frequency. The viscoelastic properties of mucus are determined in part by the mucin proteins that compose the mucus gel. The dominant mucins in human airway mucus are MUC5B and MUC5AC (17). Both *in vivo* and *in vitro* data indicate that IL-13 increases MUC5AC expression while decreasing *MUC5B* gene expression (1, 4, 18). Moreover, a recent study demonstrated that the sputum isolated from healthy subjects had a higher ratio of MUC5B to MUC5AC mucin, whereas for patients with asthma, the inverse was true (19). This study also showed that patients with asthma with the highest sputum eosinophil counts (a marker of type 2 inflammation) exhibited the highest sputum MUC5AC/MUC5B ratios. Another recent study of human ALI airway epithelial cultures revealed that IL-13 reduced mucociliary transport, but that these changes occurred without affecting ciliary beat frequency (18). Rather, this study showed that the MUC5AC protein induced by IL-13 was tethering the mucus gel to the epithelium, preventing efficient mucociliary transport. Combined, these studies make a strong case for IL-13-mediated changes to the airway epithelium decreasing mucociliary function in patients with asthma. More comprehensive studies are needed to determine how IL-13 alters ciliated cells on molecular and ultrastructural levels. In addition, IL-13 may stimulate many other changes to mucus cell function beyond those mediated by MUC5AC production and function. For example, changes to mucin folding, mucin carbohydrate modification, and mucin cross-linking in an IL-13-stimulated epithelium

may be important in the generation of pathologic mucus. Thus, studies are needed to determine how the apical protein secretome and protein composition of mucus being produced by the epithelium are altered under IL-13 stimulation.

Molecular Control of Interleukin-13-induced Changes to the Airway Epithelium

A significant amount of effort has been focused on identifying the key signaling intermediates and transcription factors that underlie airway epithelial responses to IL-13. A series of IL-13 airway stimulation and ovalbumin challenge studies in mice have identified some of the core elements in airway epithelial IL-13 signaling. Mouse knockout studies have revealed that IL-13 signaling is initiated by cytokine binding to a heterotrimeric receptor composed of the IL-4 receptor α and IL-13 receptor α 1 protein subunits (20). This receptor binding results in activation of Janus kinases that phosphorylate the signal transducer and activator of transcription 6 (STAT6) transcription factor, allowing it to enter the nucleus and drive a first wave of gene expression. Downstream of signal transducer and activator of transcription 6 activation, many transcription factors have been and continue to be identified that are important to IL-13 responses. As a potential mechanism for the IL-13-induced blockade of ciliated cell formation reported in human airway epithelial cells, researchers who performed these same experiments reported concomitant decreases in Forkhead box J1 expression, a master transcription factor regulator of ciliated cell development (13). Tyner and colleagues implicated a blockade of ciliated cell apoptosis through epidermal growth factor receptor signaling as a mechanism for ciliated to mucus cell transdifferentiation in mice (10). Little else is known about IL-13-activated mechanisms in ciliated cells.

Rather, more studies have been done to investigate IL-13-driven mechanisms of mucus metaplasia and mucin production. Activation of signal transducer and activator of transcription 6 is a critical first step because signal transducer and activator of transcription 6-knockout mice do not develop IL-13-driven mucus metaplasia (6). Downstream of signal transducer and

activator of transcription 6 (STAT6) is activation of SAM pointed domain–containing Ets transcription factor (SPDEF), which was shown to be necessary for mucus metaplasia in the ovalbumin mouse model (8). In fact, SAM pointed domain–containing Ets transcription factor expression alone has been shown to be sufficient to trigger mucus metaplasia in mice (8). SAM pointed domain–containing Ets transcription factor affects additional transcriptional cascades because it activates expression of Forkhead box A3 (FOXA3) and represses expression of the transcription factor Forkhead box A2 (FOXA2), a known repressor of mucus metaplasia (8). The genes and mechanisms by which these transcription factors drive IL-13–induced mucus metaplasia and increase MUC5AC expression are poorly understood. *In vitro* studies in human airway epithelial cells have implicated two genes that are highly induced by IL-13 in the control of MUC5AC expression. Specifically, enzymatic activity of arachidonate 15-lipoxygenase (ALOX15), in generating 15-hydroxyeicosatetraenoic acid species, was implicated in IL-13–driven MUC5AC expression, whereas extracellular signaling by chloride channel accessory 1 (CLCA1) protein was found to drive MUC5AC expression through activation of mitogen-activated protein kinase signaling (21, 22). Although these seminal studies have established critical factors in the molecular control of IL-13 signaling within the airway epithelium, a recent genomic study revealed the much broader impact of IL-13 stimulus. Nicodemus-Johnson and colleagues examined the genome-wide IL-13 expression and methylation response of cultured basal airway epithelial cells derived from the tracheas of 57 human donors (23). They found that the expression of 8,524 genes, or 63% of the airway epithelial transcriptome, was altered after 24 hours of IL-13 stimulation. Likewise, they observed 2,920 CpG motifs that were differentially methylated by IL-13 stimulus, including motifs in proximity to 21% of the differentially expressed genes. In addition, there was an enrichment of these IL-13–induced methylation changes among the methylation changes in fresh bronchial epithelial brushings that separate subjects with asthma from healthy control subjects. These exciting studies reveal the genomic breadth of IL-13 effects and suggest

long-term consequences of IL-13 exposure through epigenetic reprogramming. Nicodemus-Johnson and colleagues did not explore the cellular mechanisms and network organization of the entire set of differentially expressed genes, because they focused on whether these expression changes were driven by DNA methylation changes. Nonetheless, these results suggest that transcriptome network analyses represent an excellent opportunity to gain better understanding of the details of IL-13 molecular responses in airway epithelial cells.

The Exciting Challenges Ahead

Although many questions remain in the quest to fully understand IL-13 effects on the airway epithelium, some fundamental insights about airway epithelial biology can be gleaned from the evidence collected up to this point. Foremost is the remarkable plasticity of the airway epithelium that, in response to stimulation, can quickly change cellular composition without cellular division through activation of transcriptional programs, leading to transdifferentiation of cell types. Moreover, this alteration of cellular composition is due not just to shifting proportions of cell types residing in the airway epithelium under baseline conditions but also to the generation of novel cell types that appear qualitatively different, such as the mucus cell generated by IL-13 stimulation. These changes in cellular composition allow the airway epithelium to dramatically alter its secretory characteristics and mucociliary function. This plasticity will likely extend to other immune and external environmental exposures (e.g., air pollution, respiratory viruses, allergens, cigarette smoke) that contribute to the development of asthma and other airway diseases.

Future human airway epithelial studies of these exposures, as well as continued studies of IL-13, will benefit from the advantages offered by recent technological developments, which will allow for much deeper, more precise, and increasingly comprehensive analyses. Foremost among these technological developments is single-cell RNA-sequencing, which enables investigation of the full transcriptomic responses to exposures at the level of individual cell types rather than as average responses within a pool of mixed cell types. Moreover, this technology can enable researchers to identify and characterize

novel cell states that may emerge for a given cell type in response to exposures. One can also use single-cell RNA-sequencing to measure more comprehensively and precisely how cell-type frequencies change over time and in the face of perturbation. Another important advance has been the development of more robust and consistent protocols for the establishment of primary airway epithelial cultures from collected donor cells (24). These protocols make feasible the generation of primary airway cultures on enough donors to enable population-scale experiments. As shown in the previous section, these population scale datasets can be used to carry out powerful genome-wide response analyses. Experiments involving cells from many donors can also be done to measure genetically informed exposure responses as a way to identify gene–environment interactions that are important to asthma and airway disease, as my colleagues and I have recently shown (14). Last, novel clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated endonuclease 9 (CRISPR-Cas9) methods can be used to generate gene knockouts in human mucociliary epithelial cells, permitting one to examine the influence of candidate genes in an exposure response. Showing the feasibility of this approach, our laboratory adapted a CRISPR-Cas9 system, together with novel culture protocols to generate *MUC18* gene-knockout human ALI airway epithelial cultures, which were used to characterize the role of *MUC18* in inflammatory responses to Toll-like receptor stimuli (25, 26).

Studies that apply these technologies will help to close the remaining gaps in our knowledge of airway epithelial IL-13 response. These future studies will also likely precipitate rapid advances in the understanding of how the airway epithelium responds to and is modified by other environmental or immunity-related stimuli that are relevant to airway disease. I believe that the detailed pathophysiological knowledge emerging from this future work will spur the development of novel therapeutic interventions, enabling researchers to reverse remodel a diseased airway epithelium, reestablishing the cellular composition and behavior inherent to healthy airway function. ■

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

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