

IL-17 and “T_H2-high” asthma: Adding fuel to the fire?

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Recent advances in our understanding of asthma have been propelled by the identification of distinct subsets of patients with this disease. An important goal is to identify asthma “endotypes,” which are defined as subtypes of asthma that are driven by different pathophysiologic processes. The expectation is that endotyping will improve our understanding of molecular mechanisms, suggest targeted therapies, and guide the development of appropriate biomarkers.

The best-characterized asthma endotype is “T_H2-high” asthma. The type 2 cytokines IL-4, IL-5, and IL-13 had long been implicated in asthma pathogenesis. The more recent discovery that IL-13-induced genes, including periostin, were selectively induced in airway epithelial cells of approximately 50% of patients with mild-to-moderate stable asthma led to the designation of this subset of patients as having T_H2-high asthma.¹ Patients with T_H2-high asthma had heightened airway hyperresponsiveness and eosinophilia and greater improvement in lung function in response to inhaled corticosteroids compared with patients with “T_H2-low” asthma. This approach directly led to the recognition of periostin as a serum biomarker for predicting responsiveness to the anti-IL-13 biologic agent lebrikizumab.² Although the “T_H2-high” designation is now widely used, it is important to recognize that cells other than T cells, including type 2 innate lymphoid cells, can produce IL-13.³ Furthermore, some T cells that produce IL-13 might differ from classical T_H2 cells in their production of other cytokines. Efforts to look beyond T_H2 cells are motivated by the realization that type 2 cytokine blockade does not completely reverse asthma pathology and a need to identify mechanisms, targets, and biomarkers in patients with T_H2-low asthma.

Interest in the IL-17 family has been fueled by studies showing that the closely related cytokines IL-17A and IL-17F induce mucin production, airway smooth muscle hypercontractility, and corticosteroid-resistant inflammation in mouse models.^{4,6} In human tissues IL-17A and IL-17F levels correlated with increasing asthma severity and in some studies were associated with neutrophilic inflammation, heightened eosinophilic inflammation,

or both.^{7,9} Specialized populations of IL-17-producing T_H17 cells differentiate from naive CD4⁺ T cells in the presence of IL-23, IL-6, IL-1 β , and/or TGF- β and are distinguished from other T_H cells by the expression of the transcription factor retinoic acid-related orphan receptor γ t.⁷ Reciprocal inhibitory effects of IL-17 cytokines on T_H2 cell differentiation and type 2 cytokines on T_H17 differentiation serve to reinforce distinct patterns of cytokine expression in some systems.⁷ More recently, in a challenge to the traditional discrete categorization of T_H subtypes, populations of CD4⁺ T cells that express both IL-17A/F and type 2 cytokines have been identified in the lungs of mice with allergic airway disease and blood from asthmatic patients.^{10,11} These cells were identified by cell-surface markers, and cytokine expression was determined after *ex vivo* stimulation, leaving questions about the ability of these cells to produce cytokines *in vivo*. Furthermore, these studies did not attempt to identify whether patients with T cells that produce both IL-17 and type 2 cytokines are phenotypically distinct from other asthmatic patients.

In this issue Irvin et al.¹² take a different approach to further understand the role of T_H cells that express both IL-17 and type 2 cytokines in asthmatic patients. These investigators studied patients with a range of asthma severity, including many with refractory asthma. Rather than studying blood cells, they took the more challenging (and likely more informative) approach of analyzing cells obtained by means of bronchoalveolar lavage. The analyses relied heavily on intracellular staining for IL-4 and IL-17A in cells that were not stimulated *ex vivo* or treated with inhibitors of cytokine secretion. Detecting intracellular cytokines with this approach has been challenging, but the amounts of cytokine-producing cells reported in this study were higher than those reported in previous studies, perhaps reflecting differences in severity or other differences in study populations or methods. Subjects were designated as “T_H2/T_H17 predominant” (more IL-4/IL-17 dual-positive cells than IL-4 single-positive cells), “T_H2 predominant” (more IL-4 single-positive than dual-positive cells), or “T_H2/T_H17 low” ($\leq 5\%$ of both cell types). The authors provide additional support for the existence of IL-4/IL-17 dual-positive T_H cells by demonstrating correlations between dual-cytokine expression and the combined expression of transcription factors and cell-surface markers classically associated with T_H2 (GATA3, signal transducer and activator of transcription 6, and chemoattractant receptor–homologous molecule expressed on T_H2 cells) or T_H17 (retinoic acid–related orphan receptor γ t, signal transducer and activator of transcription 3, and CCR6) cells.

This new study found that the T_H2/T_H17 -predominant pattern of cytokine expression was more common in asthmatic patients than in disease control subjects. Furthermore, within the population of asthmatic patients, T_H2/T_H17 predominance was associated with more severe airway obstruction and hyperresponsiveness. A relative enrichment of IL-4/IL-17 dual-positive T cells after culture with dexamethasone, along with their

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enrichment among cells with high expression of MEK1, an endogenous glucocorticoid antagonist, offer a potential mechanism of steroid insensitivity in severe asthma. These novel findings provoke a number of important questions related to the roles of IL-17 and IL-4/IL-17 dual-positive T_H cells in patients with severe asthma.

Significant questions remain regarding the origins of IL-4/IL-17 dual-positive T_H cells and their importance relative to other IL-17-producing cells in the asthmatic lung. Irvin et al.¹² discuss the possibility that dual-positive T cells arise from conventional (IL-17-negative) T_H2 cells, perhaps in response to components of the airway microbiome or other environmental exposures; more research will be required to test this hypothesis. In addition to dual-positive T_H cells, other cell types, including IL-17 single-positive T_H17 cells and innate immune cells, also produce IL-17. One recent study suggests a critical role for IL-17-producing innate lymphoid cells,¹³ indicating the importance of considering multiple cell types as sources of this cytokine.

Another key question is how combinations of cytokines act on relevant target cells in the lung. The existence of T_H cells with the potential to release multiple cytokines, including IL-4 and IL-17A, in the same location at the same time raises questions about the possibility of additive, synergistic, or antagonistic effects. Future studies will need to characterize IL-4/IL-17 dual-positive cells more deeply, for example by analyzing production of additional type 2 cytokines (IL-5 and IL-13) and the expression of IFN- γ , IL-22, and surface IL-23 receptor, which have been implicated in the pathologic effects of T_H17 cells.¹⁴ These kinds of analyses might benefit from emerging technologies, such as single-cell gene expression profiling and multiparameter time-of-flight atomic mass cytometry. Although practical considerations have dictated a focus on studying T cells from blood or bronchoalveolar lavage fluid, it will be important to develop improved imaging methods for analyzing the spatiotemporal organization of cytokine-secreting and cytokine-responsive cells in the lung.

A critical question with major mechanistic and therapeutic implications is whether IL-17 is pathogenic in asthmatic patients. The authors found that intracellular IL-4 levels were higher in IL-4/IL-17 dual-positive than IL-4 single-positive cells. Because IL-4/IL-17 dual-positive cells contained relatively large amounts of IL-4, it is possible that the more severe disease seen in T_H2/T_H17-predominant subjects is attributable to higher production of type 2 cytokines rather than to the effects of IL-17 itself. In a recent trial, blockade of the IL-17A and IL-17F receptor IL-17R in patients with moderate-to-severe asthma did not improve symptoms or lung function.¹⁵ This might indicate that IL-17 does not have an essential role; alternatively, perhaps IL-17 is only important in a specific subset of asthmatic patients.

In support of this concept, a prespecified subset analysis suggested that patients with “high-reversibility” asthma might benefit from anti-IL-17 therapy. Therefore, measures of IL-17-producing T cells and bronchoalveolar lavage fluid IL-17 levels used by Irvin et al.,¹² perhaps combined with measures of the effects of IL-17 on target cells, might prove to be valuable for selecting subjects with appropriate IL-17-related endotypes for inclusion in future therapeutic trials.

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