

The other side of asthma: Steroid-refractory disease in the absence of T_H2-mediated inflammation

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In an accompanying article in this issue of the *Journal*, Lo et al.¹ provide yet another example of a non-TH2 steroid-insensitive cell type that might contribute to severe asthma. An increasing number of articles have challenged us to re-examine the pathobiologies of asthma to more selectively target culprit cells and mediators. A number of cell types have remained under the radar and provide a foundation for this editorial.

As the most common chronic lung disease, much attention has been directed to understanding the cells and mediators responsible for the well-characterized symptoms of asthma: reversible airway obstruction, airway inflammation, and bronchial hyper-responsiveness. Because reversible airway obstruction can occur with and without inflammation or bronchial responsiveness, this definition of asthma provides a rationale for investigating the heterogeneity of the syndrome. Attempts at cluster analysis using clinical parameters have suggested several groupings, further underscoring the clinical heterogeneity of the disease. A pressing issue is the ability to link specific clinical phenotypes to pathogenic pathways at the cellular and molecular level. This need for understanding the pathobiology of the many faces of asthma has spurred the concept of asthma endotypes, directly linking a pathologic mechanism or mechanisms or pathway to a specific disease subtype.

At present, one endotype has been defined that is associated with CD4⁺ T_H2 immune responses in the lower airways. Most prominent are increased levels of T_H2-type cytokines, including IL-4, IL-5, and IL-13, in the lung tissue and bronchoalveolar lavage fluid. Airway and peripheral blood eosinophilia, IgE directed to environmental allergens, and responsiveness to glucocorticoids are generally part of this endotype signature. Although not all patients with the T_H2 asthma endotype are recognizably atopic, this endotype does suggest, at least in part, that allergens are major triggers of the disease.

The airway inflammation seen in asthmatic patients can be eosinophilic, neutrophilic, mixed, or paucicellular. Because eosinophils dramatically undergo apoptosis in response to glucocorticoids, does the absence of airway eosinophilia simply reflect the effects of steroid therapy? As a result, do neutrophils

remain because they are steroid insensitive, or is this neutrophil signature identifying other pathways leading to the many clinical features of asthma? Does the absence of airway eosinophilia indicate a different asthma endotype or endotypes or a different pathobiological mechanism for the disease? Paucicellular (or minimal) inflammation in asthmatic patients might suggest that anti-inflammatory treatment was effective in this subgroup of patients but that asthma persists because of abnormalities of stromal cells (smooth muscle hypertrophy, goblet cell hyperplasia, and airway remodeling).

One answer might come from identification of specific biomarkers that reliably associate with disease subtypes. Studies have distinguished a T_H2 -high endotype from a T_H2 -low endotype based on gene expression studies of airway epithelial brushings.² The patients with T_H2 -high asthma and activation of airway epithelial cells were characterized by increased expression of the genes encoding periostin, serpin, and CLCA1; airway eosinophilia; and a response to glucocorticoids. The T_H2 -low group was less responsive to glucocorticoids and more commonly associated with airway neutrophilia. Whether periostin or other gene signatures prove to be sensitive or specific enough to identify T_H2 -type asthma and are predictive of a response to glucocorticoids remains to be validated. It should be noted that the genes encoding periostin, serpin, and CLCA1 are IL-13-responsive genes. Because IL-13 is produced not only by T_H2 cells but also by $CD8^+$ T cells, type 2 innate lymphoid cells, natural killer cells, natural killer T cells, and mucosal-associated invariant T cells, the assignment of an IL-13-high gene expression profile only to a T_H2 endotype is not entirely accurate. Ideally, this endotype should be labeled an IL-13-high or type 2 cytokine (IL-4, IL-5, and IL-13)-high endotype. The next challenge will be to determine the dominant immune cell type that drives this type 2 cytokine-high endotype because this might allow better understanding of the heterogeneity of this asthma phenotype.

Important questions are whether an endotype is stable and whether the underlying pathologic mechanisms remain true over time. Given exposure to changing environments, it might not be surprising that activation of underlying disease pathways can be responsive to these changes. Phenotypes determined by biomarkers might be less stable than those clusters defined by physiologic variables, and the instability of the phenotype could be even more obvious in patients with severe asthma. As a result, heterogeneity among asthmatic patients might not only reflect interpatient differences but also inpatient differences.

This notion suggests that in the same patient the underlying disease mechanism or mechanisms might be different at different disease stages. Thus, in patients with seasonal asthma, allergen is a common trigger. At other times, other triggers might be the cause of both exacerbations and disease maintenance. Viruses are common causes of asthma exacerbations, as is air quality with changes in levels of ozone and diesel exhaust particles, as well as

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exposure to cigarette smoke. Each of these environmental exposures elicits a characteristic and potentially distinct inflammatory cell signature. Although allergen exposure is often associated with an influx of CD4⁺ T_H2 cells and airway eosinophilia, exposure to viruses, ozone, diesel exhaust particles, or cigarette smoke elicits the influx of neutrophils, CD8⁺ T cells, and, in some situations, T_H17 cells. Not surprisingly, these different responses tend to be associated with a variable response to glucocorticoids.³⁻⁵ Thus allergen-triggered asthma might be steroid responsive, whereas asthma triggered by other stimuli might be less responsive to glucocorticoid therapy.

As suggested above, different cell types exhibit different sensitivities to corticosteroids. To a large extent, eosinophils readily undergo apoptosis when exposed to corticosteroids, whereas neutrophils are insensitive. The presence of persistent eosinophilia in some patients despite corticosteroid therapy might reflect levels of proeosinophilic cytokines (eg, IL-5 and GM-CSF) that are so high that they cannot be suppressed or are associated with delayed apoptosis of eosinophils (GM-CSF).⁶ T-cell subsets also display a differential steroid sensitivity. CD4⁺ T lymphocytes are largely sensitive to corticosteroids. However, they can become steroid insensitive when exposed to high levels of certain cytokines. In contrast, CD8⁺ T cells are innately insensitive to corticosteroids.^{3,4} T_H17 cells, which are producers of proneutrophilic cytokines, such as IL-17, have also been shown to be steroid insensitive.⁵ Likewise, airway dual-positive T_H2/T_H17 cells are steroid insensitive.⁷ The responses triggered by mast cell or basophil activation are also steroid insensitive. Because viruses, ozone, diesel exhaust particles, and cigarette smoke can evoke neutrophil, CD8⁺ cell, and T_H17 T-cell inflammation in the lung, it might not be surprising that glucocorticoids have limited efficacy after these exposures.

The longstanding concept that CD4⁺ T cells release T_H2 cytokines and CD8⁺ T cells secrete interferons and stimulate type 1 protective immunity has been a framework in asthma thinking for several decades. However, CD8⁺ T cells are heterogeneous, with at least 2 well-defined subsets: a T_C1 subset secreting type 1 cytokines and a T_C2 subset capable of secreting type 2 cytokines, including IL-5 and, in particular, IL-13. In animal models we have shown a plasticity of CD8⁺ T cells, with the ability of these cells to convert from IFN- γ producers to IL-13 producers.⁸ At the molecular level, this conversion to IL-13 production has been associated with a major shift in transcription factor expression (eg, T-bet to GATA-3) and histone acetylation at the *GATA3* and *IL13* cytokine gene loci. In fact, the subset most sensitive to conversion appears to display high glucocorticoid insensitivity.⁴ In the mouse the conversion process has been shown to be initiated by IL-4/IL-13 receptor interactions at the CD8⁺ T-cell surface, followed by changes in levels of gene expression (interferon regulatory factor 4 [*IRF4*], *GATA3*, and *CYP11A1*) and after ligation of the T-cell receptor, cytokine gene transcription, and translation. In the lungs of sensitized and challenged mice, these CD8⁺ T cells have been identified as major IL-13 producers, which are necessary for the full development of methacholine-induced airway hyperresponsiveness and mucus hyperproduction.⁸

A parallel story is emerging with suggestions of CD8⁺ T-cell participation in asthma pathogenesis. Decreases in lung function over a 2-year period were shown to be associated with bronchial wall CD8⁺ T-cell infiltrates and not airway eosinophilia or reticular basement membrane thickness.⁹ In smoking versus

nonsmoking asthmatic patients, a significant increase in numbers of intraepithelial CD8⁺ T lymphocytes was detected.¹⁰ In peripheral blood increases in percentages of CD8⁺IL-13⁺ T cells were observed in patients with childhood asthma compared with control subjects.¹¹ Comparison of mRNA expression levels showed widespread changes in circulating CD8⁺ T cells in patients with severe asthma.¹² We have shown that the number of IL-13-producing CD8⁺ T cells in peripheral blood of patients with steroid-insensitive asthma was higher than in patients with steroid-sensitive asthma.¹³ Taken together, the data suggest that a subgroup of patients, in particular those with steroid-refractory asthma, is associated with IL-13-producing CD8⁺ T cells.

Recognition of asthma as a syndrome rather than a single disease has prompted intense investigation of the pathobiology of the many forms of the disease at a cellular and molecular level. Despite clinical manifestations appearing similar, the pathways leading to symptoms can vary considerably. Discovery of several different T-cell subsets and intricate interactions between the adaptive and innate immune systems has revealed the many faces of asthma pathobiology. The inflammatory infiltrate in asthmatic patients is complex and can consist of an obvious predominant cell type or a mixture of several cell types. Determination of the dominant inflammatory pathway might be based on identification of the cell type that represents the major source of the inflammation-inducing cytokines. There is now great interest in gene-environment interactions and epigenetic modifications of critical gene loci. What this implies is that as the environment is ever changing, so is the host response. Our perspective is that exposure to different triggers profoundly contributes to asthma heterogeneity both between patients and in the same patient at different times. Importantly, it is the nature of the trigger that might be the major driver of a predominant pathologic pathway at a given point in time. Whether it is CD4⁺ T cell, CD8⁺ T cell, innate lymphoid cell, or airway cell mediated is dictated by the trigger. In turn, this dictates whether the pathway can be blocked by corticosteroids. Severe asthma is by definition steroid unresponsive. We suggest that severe asthma is driven by steroid-insensitive pathways and that pathways involving non-T_H2 cells are a major factor in this insensitivity. Pathways driven by steroid-insensitive type 2 CD8⁺ T cells, T_H17 cells, T_H2/T_H17 cells, or neutrophils likely contribute to this insensitivity. In accepting this notion, a focus must be on defining valid targets in steroid-insensitive cells for intervention.

What is needed is a comprehensive understanding of inflammatory and pauci-inflammatory mechanisms of asthma. Identification of these differing endotypes of asthma will require integrated approaches performed in a multidisciplinary manner. With the incorporation of newer technologies, especially single-cell genomics and transcriptomics, the future for truly personalized approaches to the patient as an individual will hopefully be realized.

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