

What Are Those Neutrophils Doing in Severe Asthma Anyway?



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Airway inflammation is a characteristic feature of asthma and target of treatment to achieve disease control.¹ It is increasingly well recognized that the pattern of airway inflammation in asthma is heterogeneous in many respects (eg, cellular, molecular, and therapeutic response). Consequently, an appreciation of the heterogeneity in asthma has become important to profile this disease's underlying mechanisms and to direct decisions on eventual therapeutic interventions. Although an oversimplification of a complex process, patterns of airway inflammation in asthma have been divided into T2 (type 2) and non-T2, largely based on the dominant cell present in the airway: eosinophils versus neutrophils.¹

Our understanding and appreciation of T2 inflammation in asthma is based on the pattern and presence of the immune profile: Th2 lymphocytes and ILC2 cells, generated cytokines (IL-4, IL-5, and IL-13), and recruited cells—predominantly eosinophils. The relevance of T2 inflammation to clinical asthma has been confirmed with the use of biologics directed toward this pathway and has led to clinically significant reductions in the frequency of exacerbations, improved lung functions, and diminished asthma-related symptoms.² However, the complexity of even “well-defined” T2 severe asthma is reflected by studies in which, despite a careful recruitment and enrollment of subjects with characteristics to strongly indicate a T2 inflammatory pattern in severe asthma, for example, increased eosinophils and elevated FeNO, they do not respond uniformly or completely to T2 blockade.³

The complexity of T2 inflammation in severe asthma pales when compared with our knowledge and understanding of non-T2, or T2 low, severe asthma.⁴⁻⁷ The unknowns in this phenotype are further expanded when the primary cellular pattern of inflammation of non-T2 severe asthma is considered—increased airway neutrophils. The biology of the primary effector cell in T2 asthma, the

eosinophil, is well chronicled as well as its contributions to airway dysfunction. When the neutrophil's role in severe asthma is considered, there is the presence and characteristics of inflammation, but lacking is convincing evidence of the mechanisms by which the neutrophil participates in these processes. Moreover, in efforts to identify the role and contribution of neutrophils to asthma severity, investigators have largely explored adult populations of patients. Although severe asthma and airway inflammation occur in pediatric/adolescent populations, the frequency of this phenotype is less prevalent and the conduct of studies in younger populations poses significant limitations.

In continuing efforts to fill a knowledge void on the contribution of neutrophils to severe asthma and the role of this cell in pediatric airway disease, Grunwell et al from Emory University, Department of Pediatrics, have built on their earlier observations that led them to pursue their current research findings, “Children with neutrophil-predominant severe asthma have proinflammatory neutrophils with enhanced survival and impaired clearance,” in this issue of the *Journal of Allergy and Immunology: In Practice*.⁸ Their study represents considerable effort and, as a result, adds new information on the biology of airway neutrophils in severe asthma, and in a unique and unstudied population—children and adolescents.

Their hypothesis is designed to test whether the inflammatory environment of the airway in children with neutrophil-predominant severe asthma, which is refractory to high doses of inhaled corticosteroids (ICS), promotes neutrophil survival and disrupts innate immune defenses. This concept suggests, though not directly stated, that as leukocytes enter the airway, they encounter an environment that changes their cellular phenotype, and acts to promote airway inflammation. The participant population in this study is well defined and consists of children and adolescents, 6 to 21 years of age, who had a physician diagnosis of asthma, and, importantly, reversible airflow obstruction ($\geq 12\%$ change in forced expiratory volume in 1 second postbronchodilator) to confirm the presence of asthma. A key feature that makes this study novel is the use of a diagnostic bronchoscopy to obtain bronchoalveolar lavage (BAL) fluid to identify and quantitate the cellular inflammatory profile as well as a source for the “airway environment” that might influence neutrophil biology. A smaller population of asthmatic children and adolescents with moderate persistent asthma were included as an important comparator and had been treated with a lower dose of ICS for at least 6 months. The moderate asthma cohort also had bronchoscopy performed for nonasthma indications (suspected foreign body [$n = 3$], suspected aspiration [$n = 5$]), and an abnormal chest radiograph [$n = 5$]). As stated by the authors, the use of a normal subject population posed ethical issues and was not included.

The median percentage of BAL neutrophil was 5% in the severe asthma group. Based on this value, the severe population

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was divided into 2 groups: neutrophil “low” (<5% neutrophils) severe asthma (n = 29) and neutrophil “high” (≥5% neutrophils) severe asthma (n = 38). Although this neutrophil cut point is arbitrary, the use of the median value is a rational and helpful demarcation. Subjects with moderate asthma (n = 12) had a mean BAL neutrophil presence of $2.4\% \pm 0.3\%$. In studying the biological characteristics of airway neutrophils in severe asthma, appropriate consideration was given to the contribution of inhaled (ICS), or systemic corticosteroids, to the presence of airway neutrophils. Therefore, a distribution of the severe asthma population to neutrophil “high” and neutrophil “low” helped address the relative contribution of ICS to the presence and characteristics of BAL neutrophils. The neutrophil “high” severe group’s ICS dose was 705.2 ± 56.9 µg/day versus 749.8 ± 66.9 µg/day in the neutrophil “low” group. This similarity in ICS dose in these 2 populations minimizes a potential confounding influence of ICSs on differences of BAL neutrophils. The mean ICS dose (fluticasone equivalent) in the moderate asthma group was 300.3 ± 49.6 µg/day.

What did Grunwell et al⁸ find about the neutrophil high versus neutrophil low groups? Concentrations of a wide variety of cytokines and chemokines were increased in the BAL fluid of severe asthma versus moderate asthma. Moreover, a large number of proteins were found in greater concentrations in the neutrophil high subjects and included IL-1β, IL-6, IL-33, CXCL8 (IL-8), CXCL10, CCL2, CCL3, and CCL4. Furthermore, BAL levels of myeloperoxidase and elastase, but not lactoferrin, were increased in the neutrophil high group, likely reflecting a greater number of neutrophils and neutrophil activation in the airway lumen. These increased markers of inflammation did not translate into features of more severe clinical asthma, such as lower lung function or greater frequency of exacerbations. Therefore, the significance of these findings to a clinical pattern of asthma remains unclear.

The investigators also attempted to “model” the effects of the airway environment. They did this by incubating primary neutrophils, for example, neutrophils from normal subjects, with BAL fluid from both high and low severe neutrophilic asthma. Although there are obvious limits to this approach, the BAL fluid from a neutrophil high subject enhanced the phagocytic index of neutrophils, but not measures of oxidation. These findings suggest that the airway environment may promote neutrophil inflammatory activities. The causative mediator(s) were not identified.

From my perspective, there are a number of “take home” messages from these studies that are novel and relevant to neutrophilic inflammation, for example, the study of airway cells and the sources of study—children and adolescents, BAL fluid immune parameter profiles, and neutrophil function, in a modeled *in vitro* setting. First, the detection of increased concentrations of BAL fluid myeloperoxidase and elastase (see Figure 1⁸) raises the possibility that neutrophil activation has occurred and may cause greater airway injury. Second, key, or anticipated, cytokines/chemokines are increased in the BAL in the neutrophil “high” severe asthma group: IL-6, IL-33, CXCL1, CXCL8, and CXCL10, along with macrophage activating factors: CCL2, CCL3, and CCL4. In contrast, IL-17, a primary cytokine considered to participate in neutrophil inflammation and severe asthma, was not mentioned, and it is unknown if IL-17 was absent or not part of their assay detection system.⁹ Their detected pattern of mediators, particularly IL-8, also provides potential explanations for the presence of increased neutrophils

and a regulation of this cell’s inflammatory capacity.^{10,11} However, the relationship of these BAL mediators to clinically existing asthma severity and the resulting pathophysiology was not established. Even though such explanations may be correlative, but not causative, a relationship of these airway environmental products with neutrophil biology and clinical characteristics would point to a relevance of neutrophil-derived inflammation.

Of additional interest, but a complex aspect of their study, was the *in vitro* incubation of primary neutrophils with BAL fluid from neutrophil “low” and neutrophil “high” severe asthma. Grunwell et al⁸ showed that BAL fluid from neutrophil “high” severe asthma enhanced this cell’s phagocytic function but not its oxidative metabolism. In addition, BAL fluid from neutrophil high severe asthma led to increased expression of CD62L and CC11b on normal neutrophils, providing indirect evidence for a greater likelihood of cell adherence and migration.¹² Again, the translation of these findings to distinct clinical characteristics in neutrophil high asthma is not apparent.

The authors conclude that airway neutrophils in neutrophil “high” severe asthma are not “benign.” To a large extent, their data address this assumption and indicate that these airway cells are unlikely to just “be there,” or a bystander cell. This knowledge advances our understanding of neutrophilic inflammation in asthma. The BAL fluid cytokine/chemokine profile was also informative as it identified what may be key mediators to regulate the neutrophil’s presence and inflammatory function in severe asthma. From their findings, it is possible to consider potential causes in the resulting disease expression and eventual therapeutic targets.

There are many remaining questions. What is the function of airway neutrophils in severe disease?¹³ There are logistic limits to such studies, as it is difficult to isolate sufficient airway neutrophils for study. Peripheral blood leukocytes in some phenotypes of asthma are normal both in number and function; however, it would have been interesting to use peripheral blood neutrophils from the neutrophil high population of severe asthma to determine if these cells are more responsive to the BAL factors detected in severe disease. Perhaps circulating cells in severe asthma are “primed” and would be more susceptible to further activation. Nonetheless, Grunwell et al⁸ add important information on how and under what conditions neutrophils may contribute to severe disease, and that these effects are not ICS dependent. Their story, and that of the neutrophil in non-T2 asthma, has yet to be fully told, but their work provides a new chapter in this quest, and importantly, extends efforts toward airway events in children and adolescents.

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