

The Role of Neutrophils in Asthma



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KEYWORDS

- Steroid insensitivity • Irreversible airway obstruction • Severe asthma • Immunocyte
- Airway remodeling • Intrinsic asthma

KEY POINTS

- Although asthma defines a syndrome associated with airway inflammation, heterogeneity exists concerning the type of inflammation that modulates airway hyperresponsiveness.
- Common triggers of asthma exacerbations are preferentially mediated by neutrophilic airway inflammation. However, there exists no therapeutic approach that uniquely targets neutrophils in asthma.
- Targeting the activation state rather than the number of neutrophils may offer unique therapeutic approaches to improve asthma outcomes in patients with steroid-sensitive asthma.

INTRODUCTION

Asthma characterizes a complex syndrome manifested by airway obstruction, inflammation, and hyperresponsiveness. Contemporary thought suggests that patients with asthma exhibit marked heterogeneity in disease severity and in response to therapy. About 61% of adult patients with asthma exhibit allergen sensitivities or atopy, and 39% manifest little evidence of allergic disease.¹ Because the majority of patients with allergic disease and asthma demonstrate corticosteroid sensitivity, inhaled corticosteroids serves as a mainstay in asthma therapy. Despite this approach, at least 40% of patients are insensitive to inhaled corticosteroids and manifest a pathology that impugns neutrophils as important in asthma pathogenesis. To date, the mechanisms that regulate nonallergic asthma, traditionally known as intrinsic asthma, remain an unknown.

Disclosure Statement: Consultant/ad board, research grant, speaker: AstraZeneca, MedImmune. Consultant/ad board: Novartis. Research Grant: Theratrophix, Amgen, RIFM, Vertex, BMS, Gilead, Genentech, Sanofi, Regeneron, Speaker: Boston Scientific, Teva, Boehringer-Ingelheim.

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Immunol Allergy Clin N Am 38 (2018) 629–638

<https://doi.org/10.1016/j.iac.2018.06.005>

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Evidence suggests that neutrophil numbers in the bronchial wall or in sputum correlate with disease severity and are associated with asthma exacerbations.^{2,3} Asthma exacerbations substantially contribute globally to morbidity, mortality, and health care costs.⁴ Allergens, viruses, environmental tobacco smoke, and air pollution exposure commonly trigger exacerbations. Some, but not all, triggers respond to conventional therapy that includes oral glucocorticoids (GCs).^{5,6} After allergen exposure, T-helper type 2 inflammation orchestrated by trafficking CD4-positive lymphocytes and eosinophils and by activation of mast cells evokes airway obstruction that reverses on treatment with GCs. Collectively, this cellular cascade defines a type 2 inflammatory response. Environmental tobacco smoke, ozone, or viruses, however, induce neutrophilic inflammation or directly modulate airway epithelial, smooth muscle, or neural cell function to evoke airway hyperresponsiveness and obstruction that is steroid insensitive.^{4,7,8} Presumably, the direct effects of these triggers on airway structural cells may not induce typical type 2 responses but in part may be mediated by a T-helper type 1 (Th1) inflammatory response. In murine and human models of disease, studies suggest that non-type 2 responses (in the presence or absence of persistent type 2 inflammation) may be associated with severe airway obstruction that is, mediated by interferons (interferon- γ), T-helper type 17, and nitric oxide production.⁹ Typically, Th1 responses are steroid insensitive.⁹ The lack of airway inflammatory cells or biomarkers in the presence of airway obstruction and airway hyperresponsiveness can also define pauci-immune or pauci-granulocytic asthma.¹⁰ Characterization of novel therapeutic approaches targeting neutrophilic or pauci-immune asthma may have a profound impact on decreasing asthma exacerbations and improving asthma severity.

Using unbiased statistical approaches, cluster analyses organize patient data (blood samples, pulmonary function testing, and so forth) in a manner that demonstrates that some groups of patients are more similar than others according to mean vector connectivity (k-means algorithm).¹⁰ Investigators have identified asthma clusters manifested by predominant neutrophilic inflammation, pauci-immune asthma, and steroid-insensitive disease.¹⁰ Collectively, evidence suggests that some asthma exacerbations and specific asthma cohorts at baseline manifest predominantly neutrophilic phenotypes.

PATHOPHYSIOLOGY

A clinical syndrome manifested by limited numbers of eosinophils in the airways or peripheral circulation, nonatopy, rhinosinusitis with sleep disturbances, environmental tobacco smoke exposure, and gastroesophageal reflux disease characterize neutrophilic asthma.^{11–13} Diagnostically, total sputum cell counts and neutrophil numbers are increased in comparison with those of healthy patients or patients with atopic asthma. Sputum neutrophil numbers, however, poorly correlate with methacholine responsiveness, whereas neutrophil numbers inversely correlate with pulmonary function.^{13,14} In bronchial alveolar lavage and bronchial biopsies, numbers of neutrophils increase with more severe disease.^{15–18} Evidence also suggests that sputum neutrophil numbers are associated with a progressive loss of lung function.^{13,14} Because GCs increase neutrophil numbers in tissue and peripheral blood, the diagnosis can be complicated when patients are prescribed high-dose inhaled or oral steroids.¹⁹ Given the efficacy of steroids to increase neutrophilia, this can serve as a biomarker of oral steroid adherence.

Investigators have also attempted to correlate neutrophil numbers in the sputum and peripheral blood in patients with asthma and comorbid diseases. Obesity,

gastroesophageal reflux disease, and smoking are common comorbidities associated with difficult-to-manage asthma, steroid insensitivity, and non-type 2 responses. Unfortunately, whether neutrophil-mediated airway inflammation contributes to asthma disease severity associated with comorbidity remains controversial. In the Severe Asthma Research Program (SARP) and Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes (UBIPRED) cohorts, severe asthma was associated with gastroesophageal reflux disease but sputum neutrophilia was seen in only 1 cohort.⁹ In obesity, sputum neutrophil numbers poorly correlated with asthma severity⁹ and, although smoking increases neutrophils number in sputum from patients with asthma, cause and effect relationships among these covariates remain unknown.⁹

In many research studies, sputum neutrophils were used as a biomarker for neutrophil-mediated airway inflammation. Sputum analyses unfortunately require sophisticated training and limited availability hampers their clinical use. Discovery of other novel biomarkers to identify patients with neutrophilic asthma will foster precision approaches to improve therapy. Accordingly, levels of neutrophil-derived mediators (leukotriene B₄ [LTB₄], granulocyte-macrophage colony-stimulating factor, tumor necrosis factor- α [TNF- α], IL-17A, IL-8, elastase, or matrix metalloproteinase 9) are detectable in bronchial alveolar lavage fluid and plasma in patients with severe neutrophilic asthma.^{2,16,18,20,21} Unlike eosinophilic asthma, systemic inflammation as measured by increased blood levels of IL-8, IL-6, C-reactive protein, and IL-17A was also reported in patients with severe neutrophilic asthma.²² Although such blood levels hold promise, to date there exist no reliable serum biomarkers to diagnose neutrophilic asthma.

Conventional thought suggests that neutrophils primarily traffic to inflamed sites and then secrete granular enzymes, reactive oxygen species, and proteins to eliminate invading bacteria, fungal elements, and viruses. Undoubtedly, neutrophils play pivotal roles in innate immunity.¹⁹ The 6- to 8-hour limited lifespan of the circulating neutrophil is consistent with an immune surveillance function.²³ Evidence also shows that some neutrophils survive much longer with lifespans of 3 to 5 days, especially in tissue.^{17,24,25} Further, neutrophils can reenter the circulation via lymphatics and reverse migration through vascular endothelium.^{26–28} Collectively, these observations suggest a more complex role of the neutrophil in modulating local inflammatory responses apart from host defense as shown in **Fig. 1**.

Given the differential lifespan of tissue and circulating neutrophils, these cells manifest unique functional phenotypes (**Table 1**). Mediated in part by granulocyte colony stimulating factor, granulocyte-macrophage colony-stimulating factor, interferon- γ , IL-13, LTB₄, and lipopolysaccharide,¹⁷ tissue-based neutrophils have decreased apoptosis. Pertinent to severe asthma, neutrophil subsets can express Fc γ RIII (CD16) and Fc ϵ RI and inhibit T-cell proliferation in a Mac-1- and H₂O₂-dependent manner.^{29,30} Curiously, these neutrophils retain key innate effector cell functions, such as eliminating microbes.³¹ Recruitment and retention of mucosal neutrophils, which are facilitated by T helper 17 cells (a subset of T helper lymphocytes releasing IL-17) induces secretion of IL-8 by airway epithelium.³² Epithelial-induced secretion of IL-17 and IL-8 is also insensitive to GCs, a hallmark of severe neutrophilic asthma.²⁷ Neutrophils may transdifferentiate to express CD11a, CD11 b, CD11c, HLA-DR, CD80, and CD86, acquiring an antigen-presenting cell phenotype (neutrophil/dendritic hybrid) in mice.³³ Whether such a transitional phenotype exists in severe asthma remains unknown. Collectively, current evidence suggests that neutrophils modulate adaptive responses and provide novel therapeutic targets for further exploration.

Repetitive injury of the airways from allergens, viruses, and toxicants in asthma evokes remodeling that increases matrix deposition, airway smooth muscle mass,

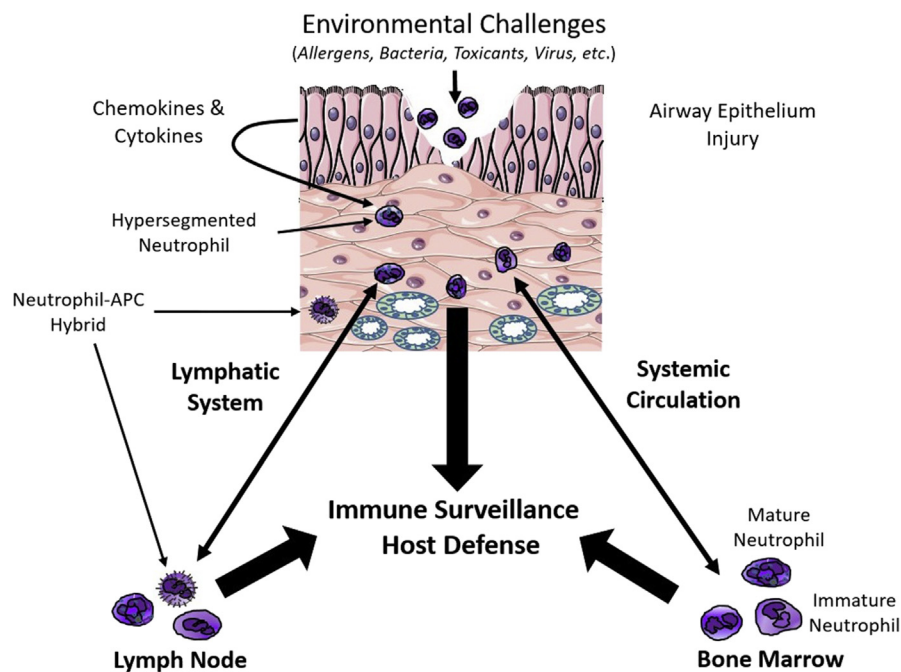


Fig. 1. Neutrophil subsets mediate immune surveillance and host defense. Evidence suggests that neutrophil subsets may mediate differential effects on immune surveillance and microbial killing. A variety of epithelial insults (ozone, bacteria, and viruses) induce secretion of chemokines and cytokines that promote neutrophil trafficking. Most circulating neutrophils are a mature phenotype (neu-2). On injury other phenotypes appear, namely neu-1, the immature or band neutrophil, and neu-3, the hypersegmented neutrophil. During asthma exacerbations, the presence of chemokines and cytokines (IL-8, IL-17A, and so forth) prolongs the lifespan of the neutrophil and modulates T-helper type 2 and innate immune responses. The prolonged lifespan also enables neutrophils to migrate from tissue to the systemic circulation or to lymph nodes to modulate adaptive immune responses. (Adapted from Panettieri RA. Neutrophilic and pauciimmune phenotypes in severe asthma. *Immunol Allergy Clin North Am* 2016;36(3):572; with permission.)

Table 1 Neutrophil subsets manifest specific phenotypes			
Neutrophil Attributes	Immature	Mature	Hypersegmented
Nuclei morphology	Band	Segmented	Multilobed
L-selectin (CD62 L) ^a	+	+	—
FcγRIII (CD16) ^a	—	+	+
T-cell suppression	—	—	+
Lifespan	Hours	Hours	Days
Neutrophil-APC hybrid	—	—	+
Activated ^b	—	—	+
Microbial killing	—	+	+

Abbreviation: APC, antigen-presenting cell.
^a Cell surface expression.
^b Activated: Capable of endocytosis, neutrophil extracellular net formation, and MPO and matrix metalloproteinase production.
From Panettieri RA. Neutrophilic and pauciimmune phenotypes in severe asthma. *Immunol Allergy Clin North Am* 2016;36(3):573; with permission.

mucus gland hypertrophy, and angiogenesis.^{4,7,8} The physiologic consequences of remodeling events in the pathogenesis of irreversible airway obstruction remains unclear.⁸ Activated neutrophils secrete transforming growth factor- β , matrix metalloproteinase 9, elastase, and myeloperoxidase.¹⁴ In bronchial alveolar lavage, matrix metalloproteinase 9 levels correlate with neutrophil counts in asthma.³⁴ Taken together, neutrophils may play a role in promoting airway remodeling, but this notion remains controversial.

Are There Targeted Therapies for Neutrophilic Asthma?

There currently exist no therapies approved by the US Food and Drug Administration for neutrophilic asthma. Clinicians typically prescribe combined therapies of inhaled GCs and long-acting bronchodilators as a cornerstone of medical management. Disappointingly, this approach often fails to improve pulmonary function or functional status in patients with neutrophilic asthma.³⁵ The presence of submucosal or airway neutrophils suggests that the trafficking or activation state of the neutrophil may serve as a therapeutic target. Strategies to alter neutrophil function can include blockade of chemokine receptors (CXCR2 antagonists); inhibition of the effects of TNF- α , IL-6, or IL-17; kinase inhibition (p38MAPK, PI3K-d/g, JAK, or PDE4); or macrolides. However, such approaches remain investigational.

Chemokines (CXCL1 [GRO- α], CXCL5 [ENA-78], CXCL8 [IL-8], and LTB₄) modulate neutrophil migration by activating G-protein-coupled receptors CXCR2 or, in the case of LTB₄, the LTB₄ receptor 2.¹⁹ Accordingly, blockade of these receptors would decrease neutrophil trafficking and in turn inflammatory responses. CXCR2 inhibition significantly decreased sputum neutrophils, but only modestly decreased mild exacerbations and had no effect on pulmonary function in patients with chronic obstructive pulmonary disease.³⁶ Significant decreases in circulating neutrophils induced by CXCR2 blockade but had little effect on pulmonary function or functional status in patients with severe asthma.³⁷ Further, blockade of 5-lipoxygenase that decreases LTB₄ and leukotriene D4 levels offers therapeutic benefit in asthma associated with aspirin sensitivity and nasal polyps; the relative contribution of LTB₄ and leukotriene D4 in improving patient outcomes in neutrophilic asthma remains unclear. Despite the targeting inhibition of neutrophil chemotaxis, the lack of clinical improvements and the possibility for neutropenia with immunosuppression limit the therapeutic value of this target in severe asthma.

Neutrophilic asthma is associated with increased levels of TNF- α , IL-6, or IL-17 in the blood or sputum of patients.^{14,19} Investigators have posited that binding free circulating cytokines or inhibition of their cognate receptor may improve clinical outcomes. Inhibiting the actions of TNF- α or IL-17 with monoclonal antibodies provided marginal therapeutic advantages in severe asthma.^{38–40} Whether such agents would be effective in neutrophilic asthma remains unclear because the clinical trials were not designed to assess efficacy in this cohort.

Extracellular signals via activation of receptors stimulate intracellular kinases (p38MAPK, JNK, PI3K- δ/γ , and JAK) and phosphodiesterases (PDEs) that modulate cell proliferation, chemokine and cytokine production, apoptosis, and steroid sensitivity. Each of these kinases or PDEs selectively modulates neutrophil secretion, diapedesis, and apoptosis.^{14,19} Despite intracellular kinase inhibitors being developed for chronic obstructive pulmonary disease, such agents may also have usefulness in neutrophilic asthma.³⁵ Interestingly, p38MAPK and JNK1 activation also phosphorylates the GC receptor that inhibits its translocation into the nucleus, which is necessary to mediate GCs' antiinflammatory effects.³⁵ Using cell culture models, blockade of p38MAPK or JNK1 reverses steroid insensitivity.³⁵ Whether such mechanisms are

physiologically relevant in neutrophilic asthma remains unknown. Unfortunately, given the ubiquitous expression of intracellular kinases, their inhibition may evoke undesirable adverse effects that include immunosuppression or malignancy.

In some cell types, cAMP or cGMP work intracellularly as antiinflammatory signaling molecules; blockade of PDEs, which increase cAMP and cGMP levels, would amplify these antiinflammatory responses.³⁵ PDEs decrease cAMP or cGMP levels and diminishes activation of protein kinase A or G, respectively. In circulating immunocytes, PDE4 is the dominant isoform. Roflumilast, a PDE4 inhibitor, improved neutrophil-mediated airway inflammation and decreased exacerbations⁴¹ in chronic obstructive pulmonary disease; whether such effects would have therapeutic benefit in neutrophilic asthma remains unknown.

Macrolide antibiotics used to treat chronic indolent infections with atypical bacteria (*Mycoplasma pneumonia* or *Chlamydia pneumonia*) improved patient-reported outcomes in patients with severe asthma.^{42,43} The role of chronic infection in the pathogenesis of severe asthma remains controversial. Arguably, macrolide antibiotics can also act as antiinflammatory agents with a mechanism of action disparate from any antiinfective activity. Macrolides inhibit nuclear factor- κ B activation and secretion of CXCL8 and promote phagocytosis.^{44,45} In severe asthma, azithromycin reduced sputum neutrophil counts and CXCL8 levels with improved patient-reported outcomes in patients with neutrophilic inflammation.^{46,47} Taken together, the current evidence suggests that macrolide antibiotics may have value in neutrophilic asthma; the low risk of complications justifies a 6-month clinical trial in some patients prone to exacerbations.

The therapeutic benefit of combined long-acting beta agonists and anticholinergic agents is well-established in chronic obstructive pulmonary disease. Further, the addition of antimuscarinic agents in the treatment of severe asthma is a therapy approved by the US Food and Drug Administration.³⁵ Whether such approaches would have preferential effects in neutrophilic asthma as compared with those in eosinophilic asthma remains unclear. Few studies have focused on therapeutic approaches of bronchial thermoplasty in neutrophilic asthma. Bronchial thermoplasty delivers thermal energy to the airway in a manner that ultimately remodels the airway epithelium and decreases airway smooth muscle mass in the proximal airways.⁴⁸ The therapeutic improvement in exacerbations did not extinguish over 5 years of observation.⁴⁹ Other studies now show that bronchial thermoplasty also modulates mucosal inflammatory responses and collagen deposition.^{50–52} Because patients who undergo bronchial thermoplasty are poor candidates for T-helper type 2 or IgE monoclonal antibody therapies or have failed such approaches, bronchial thermoplasty may become the treatment of choice for neutrophilic severe asthma. Further studies, however, are needed to definitively show efficacy in these cohorts.

Neutrophilic Asthma: Many Unanswered Questions

Despite considerable efforts to therapeutically target neutrophilic asthma, few approaches have improved patient outcomes.^{14,19,35} Health care providers, when faced with a patient refractory to treatment, typically prescribe oral GCs. Unfortunately, oral GCs promote neutrophil survival and increase neutrophil numbers, providing a diagnostic dilemma. Given the failure of current approaches to improve outcomes in neutrophilic asthma, a fresh approach is needed. Perhaps the global inhibition of neutrophils in the circulation or airways may not be an effective therapeutic target. Compelling evidence suggests that some subsets of neutrophils provide a negative homeostatic function to inhibit inflammation, whereas others may amplify antigen presentation and T-helper type 2 inflammation.^{14,19} A greater understanding of the

heterogeneity of neutrophil responses and subsets may improve awareness that global targeting of neutrophil activation or trafficking will be uninformative. Improved phenotyping of patients with neutrophilic asthma will identify specific neutrophil endotypes that will predict therapeutic responses.

SUMMARY AND FUTURE CONSIDERATIONS

Asthma represents as a complex syndrome defined in part by reversible airway obstruction and inflammation. Although our understanding of eosinophil- and type 2-mediated inflammation in asthma has improved, our knowledge regarding neutrophilic disease remains rudimentary. Because the neutrophil may play an important role in mediating the asthma diathesis in some cohorts, we need novel therapeutic targets to modulate neutrophil activation or migration. Although drugs are early in development, the proposed targets may not uniformly offer therapeutic efficacy. Given the heterogeneity among neutrophil subsets, research paradigms should consider that global inhibition of neutrophil activation and migration may be inappropriate and may induce unwanted adverse effects. Improved characterization of neutrophil subsets and the development of accurate biomarkers to predict therapeutic responses may improve clinical outcomes. Because neutrophilic and pauci-granulocytic asthma are steroid insensitive, our current approaches of using oral steroids in refractory asthma patients with neutrophil inflammation may offer little therapeutic benefit while increasing adverse effects.

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