

REVIEW

The enigmatic role of the neutrophil in asthma: Friend, foe or indifferent?

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Summary

Whilst severe asthma has classically been categorized as a predominantly Th2-driven pathology, there has in recent years been a paradigm shift with the realization that it is a heterogeneous disease that may manifest with quite disparate underlying inflammatory and remodelling profiles. A subset of asthmatics, particularly those with a severe, corticosteroid refractory disease, present with a prominent neutrophilic component. Given the potential of neutrophils to impart extensive tissue damage and promote inflammation, it has been anticipated that these cells are closely implicated in the underlying pathophysiology of severe asthma. However, uncertainty persists as to why the neutrophil is present in the asthmatic lung and what precisely it is doing there, with evidence supporting its role as a protagonist of pathology being primarily circumstantial. Furthermore, our view of the neutrophil as a primitive, indiscriminate killer has evolved with the realization that neutrophils can exhibit a marked anti-inflammatory, pro-resolving and wound healing capacity. We suggest that the neutrophil likely exhibits pleiotropic and potentially conflicting roles in defining asthma pathophysiology—some almost certainly detrimental and some potentially beneficial—with context, timing and location all critical confounders. Accordingly, indiscriminate blockade of neutrophils with a broad sword approach is unlikely to be the answer, but rather we should first seek to understand their complex and multifaceted roles in the disease state and then target them with the same subtleties and specificity that they themselves exhibit.

1 | INTRODUCTION

The most recently revised estimates by the Global Burden Disease study suggest that as many as 340 million individuals suffer from asthma worldwide, with a substantial burden of disability and around 180 000 deaths annually attributed to the disease. This epidemic of asthma is thought to be increasing in prevalence by 50% every decade and is a global affliction affecting individuals of all ages and backgrounds. It is estimated that between 5% and 10% of asthmatics suffer from a disease that could be classified as severe,¹ though recent studies have suggested that prevalence could be yet lower still.² Despite their relatively low numbers, this subset of patients represent a particularly problematic group for the healthcare

practitioner as they exhibit near daily symptoms, persistent airflow obstruction, frequent and severe exacerbations and, most importantly, are refractory to current gold standards for asthma treatment. Whilst asthma had classically been categorized as a predominantly Th2-mediated inflammation, there has in recent years been paradigm shift, and it is becoming increasingly apparent that severe asthma represents a heterogeneous disease rather than a homogeneous pathology and presentation. Consequently, there has been the realization that it is critical to recognize severe asthma phenotypes with distinct pathogenesis and subsequently define unique therapeutic targets to provide a personalized strategy for treatment. It is now evident that a neutrophilic infiltrate is a prominent feature of the inflammation observed in a subset of asthmatics, being particularly

associated with severe, corticosteroid refractory disease. Whilst neutrophils have classically been viewed as relatively crude and primitive, it is now recognized that these cells can exhibit heterogeneity and a pronounced awareness of the localized environment and a remarkable capacity to interact with and modulate the behaviour of a multitude of cells. Thus, the precise role of the neutrophil in the pathophysiology of asthma is likely to be complex and multifactorial, and there is a clear need to dissect their exact contribution to the disease phenotype—be it non-consequential, detrimental or even beneficial—and plan specific therapeutic strategies accordingly.

2 | OUR EVOLVING VIEW OF NEUTROPHILS IN INFLAMMATION

Neutrophils are indispensable components of the host's immune response, being readily recruited to the site of infection before killing the invading micro-organisms with an array of toxic products.^{3,4} The indiscriminate action of these products, however, means that neutrophils also cause significant bystander or “collateral” tissue damage and thus an over-exuberant or persistent neutrophilic inflammation is implicated in the pathologies of an array of diseases.^{5,6} There has therefore been the conventional view of neutrophils as crude and primitive suicide killers that arrive at a site of inflammation, releasing their payload and killing all that stands in their way. However, it is becoming increasingly apparent that this view does not do justice to a cell that at times can exhibit an amazing sophistication, versatility and complexity.^{7–9}

A common misconception is that neutrophils are short lived, isolated and terminally differentiated cells that arrive at the site of inflammation before throwing out their pre-formed products and being replaced by more specialized immune counterparts. In reality, neutrophils are able to sense their localized environment, being receptive to the presence of pathogens, tissue damage and signals derived from localized cells.^{10–15} This information is integrated into a central algorithm informing the neutrophil and defining its ensuing behaviour. Consequently, if deemed appropriate the survival of the neutrophil can be substantially augmented,¹⁶ efferent effector programs induced and an impressive biosynthetic programme initiated that may culminate in the production of a vast array of pro-inflammatory, anti-inflammatory, immunoregulatory, angiogenic or fibrogenic cytokines.^{8,17} The ability of neutrophils to modulate the phenotype and behaviour of disparate cells is immense and beyond the scope of this review, but it is noteworthy that neutrophils are increasingly recognized for their capacity to directly modulate facets of the adaptive immune response. Specifically, neutrophils have been demonstrated to exhibit some level of classical antigen presenting cell (APC) function,^{18–24} drive the recruitment, maturation and activation of macrophages and dendritic cells (DCs),^{25–31} and act as a source of signals that can drive the recruitment, activation and differentiation of T cells³² as well as B-cell survival, maturation and differentiation.^{33,34} Thus, the neutrophil is fundamental to the orchestration of an inflammatory response and can engage in complex-bidirectional interactions with localized immune and stromal cells at the

site of inflammation—be it through aforementioned soluble mediators or direct cell–cell contact.

The general consensus has been that neutrophils are inherently pro-inflammatory cells, but increasingly it is becoming apparent that these cells also exhibit a clear immune-regulatory or even immunosuppressive capacity. Neutrophils are able to facilitate the resolution of inflammation through production of pro-resolving lipid mediators,^{35,36} anti-inflammatory cytokines such as IL-10³⁷ (although this has become a source of conjecture³⁸) and decoy receptors that can act as potent chemokine and cytokine scavengers.^{39–42} Furthermore, whilst we have highlighted the capacity of neutrophils to promote APC function and antigen-specific T-cell responses, they are also able to inhibit these aspects of an inflammatory response in certain settings^{43–48}—thus neutrophil functionality is seemingly complex and likely context dependent.

Thus whilst neutrophils undoubtedly have the capacity to impart enormous tissue destruction, it should be stressed that this is not the norm and that the neutrophil actually makes a concerted effort to resolve its own inflammation and initiate a programme of tissue repair.⁷ Neutrophils are intrinsically wired to self-destruct via apoptosis within a defined period to prevent the unwanted release of their potent arsenal into an extracellular environment.^{49,50} They release products that function to suppress their own activation and limit the toxicity of their most potent effector mechanisms,⁵¹ as well as dictating the production of anti-inflammatory and pro-resolving lipid mediators that retard further neutrophil recruitment.^{35,36} Moreover, the neutrophil ultimately defines the recruitment and reprogramming of macrophages in a non-phlogistic manner to promote tissue repair and wound healing.^{52–54} Subsequently, when we see a disease state characterized by the presence of a robust neutrophilic infiltrate, we should not assume that their contribution is solely destructive but should instead contemplate their pleiotropic functions, adverse and even beneficial, whilst also viewing them in the context of infections, comorbidities, environmental triggers and medications that would also modulate the neutrophil decision-making algorithm.

3 | THE HETEROGENEITY OF HUMAN ASTHMA

Asthma has classically been regarded as a Th2-driven pathophysiological condition. This view originally emanated from analysis of limited patient samples and from researchers working with mouse models of allergic asthma whom reported an allergic, eosinophilic and Th2-mediated disease.^{55–57} However, the disappointing results attained from clinical trials focused at specifically targeting these Th2 pathways, the realization that some patients with severe asthma presented with an underlying pathology devoid of eosinophils and that the efficacy of corticosteroids was dependent upon the type of underlying inflammation, ultimately led to a rethink of the notion of asthma as a single disease with a Th2 aetiology.^{55,58–61} The development of more efficient and safer sampling methods has in turn

facilitated a more extensive analysis and understanding of the characteristic pathologies of human disease. Accordingly, asthma has undergone a paradigm shift, and it is becoming increasingly apparent that severe asthma represents a heterogeneous disease rather than manifesting as a homogeneous pathology and presentation.^{55,57,62} Consequently, there has been the realization that it is critical to recognize severe asthma phenotypes with distinct pathogenesis and subsequently define unique therapeutic targets to provide a personalized strategy for treatment.⁶³ The heterogeneity of the disease has made classification difficult for the medical practitioner, and this coupled with misconceptions about the relation between different aspects of severe asthma, along with underlying comorbidities and confounders, have hindered treatment strategies and novel drug development.

Non-Th2 inflammation, characterized by low eosinophilia, is observed in a substantial proportion of asthmatics, although considerably less is understood about this patient group and the underlying causes of disease. These individuals can exhibit highly variable inflammatory and physiological profiles^{57,64} and often respond poorly to corticosteroids.^{65,66} Studies aimed at dissecting the inflammatory pattern of these patients have revealed a significant innate immune component, with increased levels innate cytokines, such as TNF- α and IL-8 and airway neutrophilia having been reported.^{67,68} Confounders or triggers associated with this non-eosinophilic asthma include exercise, occupational irritants, smoking,⁶⁹ obesity⁷⁰ and recurrent infection or endotoxin exposure.⁶⁷ As early as 1988, it was identified that neutrophils may play a pathological role in asthma. Kelly et al⁷¹ identified that increased neutrophil numbers and activation within bronchoalveolar lavage fluid (BALF) correlated with increased bronchial hyper-responsiveness to methacholine. Furthermore, the same group demonstrated that isolated neutrophils from BALF of asthmatic patients produced more reactive oxygen species.^{71,72} Wenzel and colleagues subsequently reported the presence of neutrophils in BAL, endo-bronchial and trans-bronchial biopsies of patients, particularly those with severe asthma on high dose corticosteroids.⁷³ A neutrophilic component is now increasingly being recognized as a prominent feature of the pulmonary infiltrate in some subsets of asthmatic patients.^{55-57,62,73,74} Neutrophilic asthma as a phenotype has generally been described as those patients with high sputum neutrophil counts ranging from 40% to 76% of cells. However, it must be acknowledged that neutrophils represent a prominent population in blood and sputum of all asthmatics and as such may also be contributing to disease progression more broadly. Green et al⁶⁰ identified a subgroup of 60 patients with higher neutrophil counts that were less likely to be atopic within a cohort of 259 adult asthmatics. Subsequently, the same research group identified that patients with high sputum neutrophil counts but non-differential eosinophil counts had lower FEV₁ measurements post-bronchodilator administration.⁷⁵ Gupta et al⁷⁶ assessed CT scans from 99 patients with severe asthma and identified that those with limited airflow exhibited a persistent neutrophilic airway inflammation. Indeed, neutrophils are anticipated to be fundamental to the progressive airflow limitation, as they negatively correlate with FEV₁.⁶⁰ It should be acknowledged that the

majority of studies assess neutrophilia in the airway lumen of asthmatic patients, whereas many significant disease processes occur in the airway wall. Accordingly, it is important to consider the relative location of neutrophils in the context of disease pathology. Examination of post-mortem biopsies of asthmatic patients has identified neutrophil dense areas within the inner airway walls in a subgroup of patients.⁷⁷ Importantly, some studies have demonstrated that increased airway lumen neutrophils have actually been linked with improved clinical symptoms and resolution of inflammation in certain chronic lung disease.^{78,79} Therefore, disease pathology may be influenced by the location of neutrophils within the mucosal tissue and airways.

Refractory asthma that is unresponsive to inhaled corticosteroids is characterized by elevated neutrophilia and shares similar pathological features to neutrophil-driven pathology in chronic obstructive pulmonary disease (COPD), such as bronchiectasis, emphysema and fixed airflow obstruction.^{60,75,80-82} Whilst elevations in neutrophil numbers have not always been observed in patients with mild asthma,⁸³ it is now apparent that increased neutrophilia is prevalent in severe, chronic asthma and sudden severe exacerbations.^{56,60,73-75,81,84,85} Djukanovic and colleagues demonstrated that airway neutrophils in severe asthmatics displayed a pro-survival capacity with reduced numbers of apoptotic neutrophils compared to mild asthmatics and healthy control patients.⁸⁶ Subsequently, this group showed that profound EGF signalling in the epithelial cells of severe asthmatics seemingly induced neutrophil survival by inducing the release of neutrophil chemokines and survival factors such as IL-6, IL-8, GM-CSF and TNF- α .⁸⁷ Fahy and colleagues reported increased sputum neutrophils post-asthma exacerbations,⁸¹ with a subsequent study observing increased neutrophil infiltration in bronchial biopsies of patients with sudden-onset fatal asthma.⁸⁸ Additional studies have also described increased BAL neutrophils in asthmatic patients requiring ventilation due to exacerbations.⁸⁹ Likewise, sputum neutrophilia and heightened IL-8 concentrations were observed in children admitted to hospital emergency departments post-exacerbation.⁹⁰ Neutrophil infiltration has also been observed in patients with occupational asthma after being challenged with agents such as toluene diisocyanate.⁹¹ Respiratory infections have often been implicated in driving the observed neutrophilic inflammation in asthmatic patients. Respiratory syncytial virus (RSV) infection of asthmatics is associated with an increased neutrophilic infiltrate and exacerbation of disease.^{92,93} Likewise, bacterial species are frequently detected in asthmatic patients and associated with neutrophilia and exacerbations of disease.^{94,95} *Chlamydia pneumoniae* infection has been observed in asthmatic patients hospitalized with an acute exacerbation and was associated with increased neutrophil numbers.⁹⁶ Similarly, *Moraxella*, *Haemophilus* and *Streptococcus* species have been associated with elevations in neutrophilic inflammation in severe asthmatics on high dose corticosteroids.⁹⁷ Consequently, antibiotic usage has been suggested as a possible therapy for asthma.⁹⁸ A study in 52 stable asthmatics highlighted that clarithromycin treatment improved FEV₁ measurements in patients that were positive for BAL *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.⁹⁹

With it now apparent that there is a need to tailor therapies to specific sub-phenotypes of patients with severe asthma,⁶³ there has become a sharper focus on those patients presenting with severe persistent, steroid-resistant, neutrophilic inflammation for which treatment options are completely inadequate. However, this is a population that is complex and incompletely understood with little concept of the exact role of the neutrophil in the asthmatic lung. Accordingly, it is critical that we define and dissect in what way the neutrophil may contribute to asthma pathophysiology and subsequently if and how we should target this cell therapeutically.

4 | A PATHOLOGICAL ROLE FOR NEUTROPHILS IN ASTHMA?

Despite the clear indication that neutrophils can be a prominent component of the allergic response, their significance and relative contribution to the development and pathophysiology of asthma remain unclear. Nonetheless, the general consensus is that neutrophils are detrimental and indicate a worse prognosis: neutrophilic inflammation in the asthmatic lung is generally associated with enhanced severity of disease, diminished responsiveness to corticosteroids, lower lung function and is a feature of rapid onset asthmatic deaths. Direct interrogation of the role of neutrophils in animal models of allergic airways disease has been surprisingly lacking. Neutrophil depletion with an anti-Gr-1 monoclonal antibody in an OVA murine model significantly reduced the magnitude of late asthmatic response (LAR).¹⁰⁰ Additionally, in a mouse model of chemical induced asthma, it was demonstrated that neutrophil depletion prevented airway hyperresponsiveness (AHR), lung epithelial damage and reduced airway inflammation.¹⁰¹ Studies in dogs have also supported the notion that neutrophils may contribute to the pathology of asthma, with ozone-induced AHR associated with increased numbers of intraepithelial neutrophils¹⁰² and the depletion of neutrophils reducing AHR in this model.¹⁰³

IL-17 is a cytokine that is consistently linked with asthma and particularly the severe forms of the disease,^{104,105} with several studies reporting correlations between IL-17 levels and the number or neutrophils retrieved from sputum, BAL and blood.^{104,106,107} Whilst IL-17 has no direct effect on neutrophil function, it is able to indirectly promote neutrophil recruitment by triggering the release of neutrophil chemokines from structural cells^{108,109} and is also capable of promoting neutrophil survival and activation.^{110,111} Animal models of allergic airway disease have highlighted the importance of IL-17 producing cells in the recruitment of neutrophils and induction of AHR.^{108,112} In a steroid-insensitive Th-17 cell adoptive transfer model of allergic airways disease, neutrophil depletion reduced airway inflammation and rescued lung compliance but did not improve AHR to methacholine.¹¹³ Whilst the physiological relevance of some of these models to human disease and the specificity by which neutrophils are targeted can be debated, there is enough to argue that neutrophils can contribute

to asthma pathophysiology. Nonetheless, detailed mechanisms underlying any detrimental effects of neutrophils in asthma are predominantly speculative.

As already noted, products released by neutrophils, such as proteases and reactive oxygen species, possess a marked capacity to cause direct non-specific tissue damage and thus it is easy to rationalize the pathological contribution of these cells when deregulated in the asthmatic lung. This likely represents an oversimplified view, however, of the probable multifaceted and complex potential of neutrophils in allergic airways disease. As previously discussed, neutrophils release a plethora of mediators that, directly or indirectly, drive the migration, activation, maturation, differentiation and survival of both stromal cells and leucocytes.⁷⁻⁹ Furthermore, as neutrophil derived products are capable of eliciting significant tissue damage, this may result in cell death and release of "alarmins" that may further activate the immune system. Given the capacity of neutrophils to interact with localized stromal cells as well as both innate and adaptive arms of the immune response, as discussed in a previous section, it is logical that neutrophils could contribute to both sensitization and elicitation phases of allergic airways disease. It is worth considering briefly how some of the most prominent neutrophil-derived products may directly participate in the development of some of the hallmark pathological features of asthma—although some of this interpretation and conclusions are extrapolated from basic biological studies or those in other disease states, and as such their significance may still be somewhat speculative with regards to asthma.

Neutrophils are a rich source of matrix metalloproteinases (MMPs), especially MMP-8 and MMP-9, which contribute to extracellular matrix degradation in asthma. MMP-8 has been reported to be elevated in BAL and biopsy samples of asthmatics, exhibiting a significant inverse correlation with FEV₁ and a greater level of activation in severe, poorly controlled asthmatics.¹¹⁴ An abundance of literature highlights the augmented levels of MMP-9 in the asthmatic lung, and although this protease can be derived from a multitude of cells, it has been found to be almost entirely neutrophil derived.¹¹⁵ BAL, sputum, exhaled breath condensates and tissue biopsy levels of MMP-9 have all been described to be elevated in asthmatics, with levels being reportedly refractory to corticosteroids and correlating with the extent of cellular infiltrate and the severity of disease.¹¹⁵⁻¹²¹ MMP-9 is capable of degrading extracellular matrix components including type IV collagen of the basement membrane that contributes significantly to endothelial/epithelial integrity. The endothelium forms a selective barrier between the vasculature and the lung interstitium preventing the accumulation of fluid (oedema), which contributes to clinical symptoms of asthma.^{122,123} For example, oedematous airways are narrowed, they exhibit increased airflow resistance and altered mechanical properties, and it has been suggested that their hyperreactivity is enhanced. The airway epithelium forms the first structural barrier to inhaled allergens and disruption of this barrier may facilitate access of allergens to submucosal cells and promote inflammation.^{124,125} The potential of MMP-9 to drive asthma related pathophysiology is reinforced by the finding that

MMP-9 deficient mice exhibit impaired mononuclear cellular infiltration and bronchial hyperresponsiveness in a model of allergen-induced airway inflammation.¹²⁶

Neutrophil-derived serine proteases, such as neutrophil elastase (NE) and proteinase 3 (PR3), are capable of degrading elastin of the extracellular matrix, and elastic fibres are disrupted in the bronchi of asthmatic patients owing to a protease imbalance.¹²⁷ Accordingly, NE is reportedly elevated in induced sputum of asthmatics where it negatively correlated with FEV₁ and in bronchial secretions during asthma exacerbations.^{56,128,129} Furthermore, neutrophils isolated from asthmatics released NE by an IgE-dependent mechanism following challenge with specific allergens that evoke symptoms in the respective patients, and levels of NE release inversely correlated with FEV₁.¹²⁸ NE possesses the potential to promote many of the pathophysiological features of asthma, with a purported capacity to promote epithelial damage, vascular permeability, altered cilia beat frequency, mucus gland metaplasia and mucus hypersecretion, smooth muscle cell proliferation, collagen deposition and airway wall thickening and ultimately bronchoconstriction and bronchial hyperactivity.^{56,84,129-134}

Neutrophils are a prominent source of reactive oxygen species (ROS), being capable of producing quantities of superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and hypochlorous acid (HOCl). Whilst these species are indispensable component of host defence against invading micro-organisms, they are also capable of immense tissue damage in the asthmatic lung. Peripheral blood neutrophils isolated from adult asthmatics with clinically stable disease have been demonstrated to produce more O₂⁻ in response to PMA or fMLP stimulation relative to healthy controls.^{135,136} Furthermore, in those subjects with asthma, the level of O₂⁻ generation correlated with the degree of AHR to methacholine¹³⁵ and inversely with FEV₁.¹³⁶ Additionally, it was increased with progression of disease and disease duration and was greater when patients were undergoing an exacerbation.¹³⁶ Furthermore, reports have suggested a relationship between ROS and airway narrowing, with ROS shown to be capable of increasing the contractile response of airway smooth muscle,¹³⁷ enhancing endothelial barrier dysfunction with subsequent plasma leakage via up-regulation of VEGF¹³⁸ and promoting goblet cell metaplasia and mucus hypersecretion via activation of epidermal growth factor receptor.^{132,133,139}

Lactoferrin, derived from the secondary granules of neutrophils, has been reported to be elevated in sputum of asthmatics that readily expectorate relative to healthy controls.¹⁴⁰ Furthermore, augmented lactoferrin expression is seen in lung tissue sections from individuals with fatal slow-onset and sudden-onset asthma relative to non-asthmatic controls.¹⁴¹ Lactoferrin functions as an important mediator of host defence against bacterial and fungal pathogens by sequestering iron. However, it is now acknowledged that lactoferrin is a multifunctional protein with an array of immunoregulatory functions pertinent to the establishment of an inflammatory response, with roles ascribed in antibody synthesis, pro-inflammatory cytokine production, complement activation, T-cell proliferation and NK cell cytotoxicity.¹⁴² It has been postulated that lactoferrin in the asthmatic

airways may have a role in eosinophil activation, with studies highlighting its capacity to promote O₂⁻ production, degranulation and cysteinyl leukotriene release.¹⁴³ Although this list of neutrophil products is not meant to be exhaustive, it highlights how neutrophil-specific products can directly drive tissue damage and many of the hallmark features of asthma pathophysiology.

5 | NEUTROPHILS IN ASTHMA: NECESSARILY AN EVIL?

Whilst a strong case can certainly be made for a pathological role for neutrophils in asthma, the evidence is by no means conclusive. Although clinical data highlight the presence of neutrophils in severe, poorly controlled asthma, the presence does not naturally equate to causality. Indeed the majority of the evidence is largely circumstantial, generally representing a snapshot of a complex chronic disease with variable origins and aetiologies—consequently studies clearly dissecting the contribution of neutrophils in asthma are limited and predominantly speculative. Manipulation of neutrophilic inflammation in a clinical setting has done little to support the notion that they may underlie the observed pathophysiology and represent a viable therapeutic target in asthma. In a small phase 2 study, administration of an anti-CXCR2 receptor antagonist to asthmatics reduced sputum and peripheral neutrophil counts, with some reduction in mild exacerbations but no improvement in FEV₁, symptoms or asthma control.¹⁴⁴ Consequently, the CXCR2 antagonist AZD5069 also proved ineffective in reducing the frequency of severe exacerbations or improving asthma symptoms or lung function when tested in a phase 2b trial in patients with uncontrolled, severe asthma.¹⁴⁵ Similarly, various strategies that have targeted the potent neutrophil chemoattractant LTB₄ in asthmatics have yielded disappointing results,¹⁴⁶⁻¹⁴⁹ and treatment with an anti-IL-17 receptor antibody in poorly controlled moderate-to-severe asthmatics failed to show patient benefit.¹⁵⁰ However, it is important to recognize that these clinical studies alone do not fully support the assertion that neutrophils are non-pathogenic in asthma as many of the trials failed to selectively enrol patients with a heightened neutrophilia and the inclusion of subjects of a non-neutrophil endotype may mask efficacy of the treatments. Furthermore, several of the studies failed to demonstrate a reduction in airway neutrophils thus precluding any conclusions being drawn as to their pathological involvement.

It could be argued, however, that the presence of neutrophils is inconsequential and has little impact on the outcome of disease, with perhaps the lungs of severe asthmatics being more conducive to neutrophil recruitment or survival. Many of the patients with severe asthma are on high doses of corticosteroids, which function to inhibit neutrophil apoptosis via several molecular mechanisms¹⁵¹—suggesting that the steroid treatment itself actually accounts for the elevated neutrophilia.^{152,153} For example, Cowan et al¹⁵⁴ observed a reduction in sputum neutrophil counts post-steroid withdrawal, which eliminated a subgroup of patients that were previously

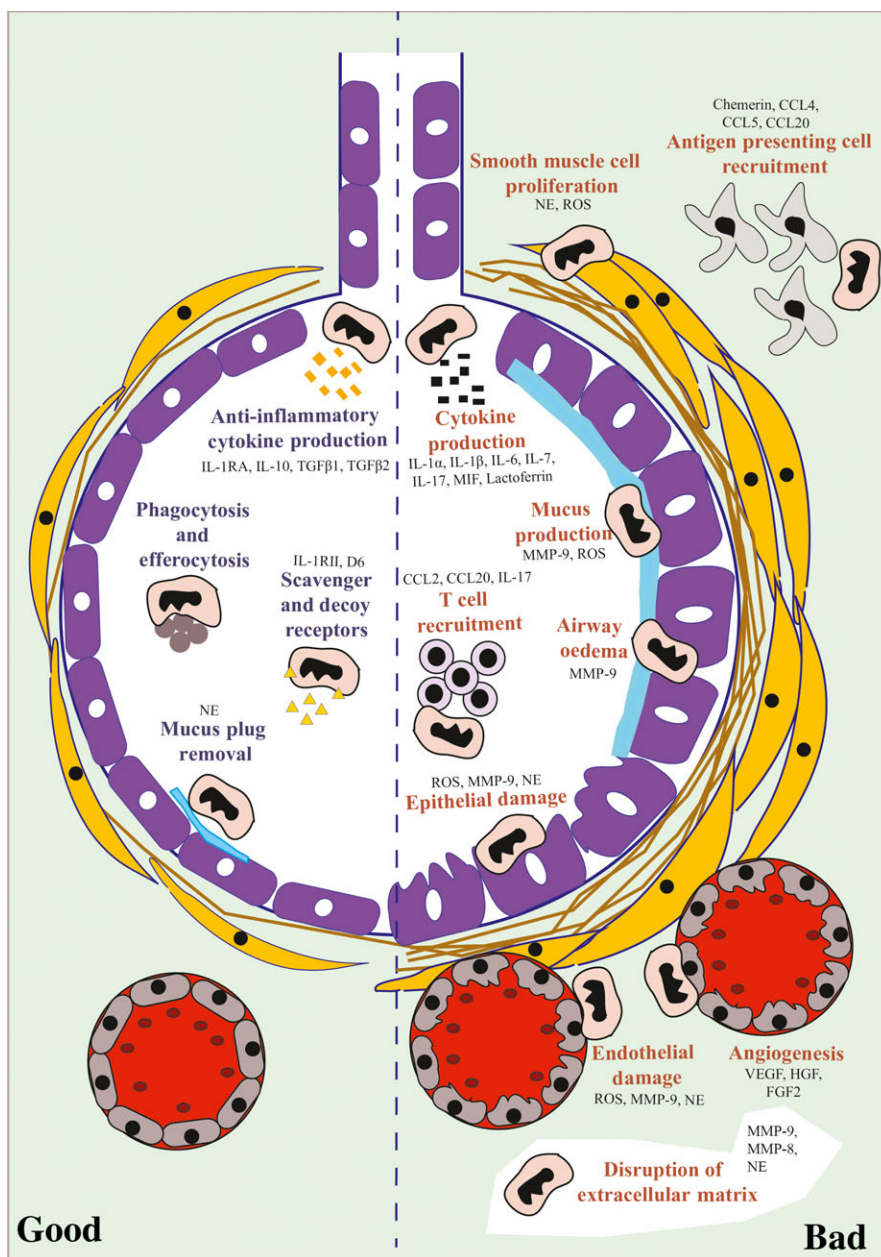


FIGURE 1 The contrasting roles of the neutrophil in asthma. There is now little doubt that a subset of patients with severe asthma present with an underlying pulmonary infiltrate with a significant neutrophilic component. However, why the neutrophil is present in the asthmatic lung and what precisely it is doing there remains largely speculative, with circumstantial and conflicting reports. Corticosteroids, cigarette smoke, infection and various other intrinsic and extrinsic factors potentiate neutrophilic inflammation and thus it is important to consider this neutrophilic phenotype in the context of comorbidities and confounders. It is generally anticipated that neutrophils fulfil a pathological role in the context of asthma. However, increasingly it is being acknowledged that neutrophils may not be entirely pathological, and may even serve positive roles in defining asthma pathophysiology. Evidence cited comes from circumstantial clinical observations, limited animal studies and cell based approaches as well as speculative roles of neutrophils based upon findings in other disease settings. Ultimately, it seems plausible that the neutrophil exhibits pleiotropic and potentially conflicting roles in defining asthma pathophysiology, with context, timing and location of the neutrophil all critical in defining the overall outcome

defined as “neutrophilic”. As such, it is important to consider that neutrophilia may be present in severe asthmatic patients as a consequence of corticosteroid administration rather than the severity of the disease alone. The relationship between neutrophilic asthma and smoking status is also unclear, and previous studies have suggested that if data are controlled for corticosteroid use and smoking then a neutrophilic asthma phenotype does not exist.^{154,155}

Conversely, is it feasible that neutrophils may in some way be beneficial and play a positive role in disease outcome? Could their presence in the more severe forms of asthma represent an effort to control inflammation, restore homeostasis and promote tissue repair? Whilst this concept may seem unlikely, it is not beyond the realms of possibility that neutrophils may fulfil some favourable role. Neutrophils have the capacity to phagocytose and clear apoptotic cells and debris and are a source of pro-resolving lipid mediators, anti-

inflammatory and immunomodulatory cytokines, decoy receptors and cytokine scavengers.^{8,35–37,39,41,42,52–54} Although the significance has not been explored in the context of asthma, it is recognized that neutrophils can facilitate the resolution of inflammation through scavenging or blocking the action of pro-inflammatory cytokines or chemokines. Specifically, apoptotic neutrophils up-regulate CCR5 on their surface in response to specific pro-resolving lipid mediators to function as a decoy receptor for CCL3 and CCL5 and terminate chemokine signalling.³⁹ Similarly, neutrophils have been reported to express the CC-chemokine receptor D6 that functions as a decoy receptor to virtually all CC-chemokines.¹⁵⁶ Furthermore, neutrophils are a source of both membrane expressed and released forms of the IL-1 decoy receptor IL-1R2, which binds IL-1β and prevents it interacting with its signal transducing receptor IL-1R1.⁴¹ Furthermore, neutrophils release IL-1RA, particularly in response to anti-

inflammatory IL-10, which acts to bind IL-1R1 but not induce any intracellular signalling.⁴² Neutrophils have themselves also been described to be a source of anti-inflammatory cytokines IL-10³⁷ and TGF- β ⁸ and so can potentially be immunosuppressive. Indeed, higher numbers of TGF- β -positive neutrophils have been reported in the airway tissue of asthmatic patients relative to healthy controls and higher levels of this cytokine are spontaneously released from peripheral blood neutrophils derived from asthmatics¹⁵⁷—potentially with an immunomodulatory role or alternatively implicated in the pathological tissue remodelling. Neutrophils also actively facilitate the resolution of inflammation through the production of pro-resolving lipid mediators that limit further neutrophil influx.¹⁵⁸ During resolution of inflammation, neutrophils switch eicosanoid biosynthesis from pro-inflammatory leukotriene B₄ production to that of pro-resolving lipoxin A₄, which acts to inhibit further neutrophil recruitment. Furthermore, neutrophils support the generation of resolvins and protectin molecules from omega-3-essential polyunsaturated fatty acids, which then act to inhibit neutrophil transendothelial migration. Furthermore, the phagocytosis of apoptotic neutrophils by tissue macrophages during the resolution of inflammation induces an anti-inflammatory IL-10^{hi} IL-12^{lo} M2-like phenotype in the macrophage.⁵³ Thus, perhaps the traditional misconception that neutrophils can only be pathological should be disregarded.

As discussed above, there is strong circumstantial evidence that MMP-9 is elevated in asthma and may contribute to pathology of disease; however, two studies in animal models have suggested that MMP-9 may actually serve a positive role in limiting and resolving allergic airways disease.^{159,160} Another study has demonstrated a positive role for neutrophil derived proteases in the destruction of mucus plugs that are such an important cause of airway obstruction in severe asthma.¹⁶¹ In keeping with these studies, it has recently been shown that the presence of neutrophil chemokines was actually associated with better clinical outcomes in a subset of patients with severe neutrophilic asthma.¹¹³ Furthermore, we have shown that a proportion of children with severe therapy resistant asthma (STRA) have increased intraepithelial neutrophils within their lungs and higher IL-17RA, which correlated with better lung function.¹⁶² Together these studies suggest that it is possible that neutrophils may play a beneficial role in the pathophysiology of asthma and that context and location may be important in defining their contribution to the disease state.

6 | CONCLUSIONS

Given that neutrophils are armed with a toxic arsenal capable of eliciting profound tissue destruction and the clear indication that neutrophils are a prominent inflammatory cell in a subset of asthmatics with an often severe and corticosteroid refractory phenotype; then, it is unsurprising that the neutrophil has been cast as the villain that drives disease pathology and that should be therapeutically targeted at all costs. Whilst this viewpoint is not without merit, we should discard the outdated misconception of neutrophils as primitive, primarily pathological cells. Instead, we should acknowledge a cell that at times

can exhibit a pronounced awareness and plasticity, an impressive and broad biosynthetic capacity, and a marked potential to influence and shape the inflammatory environment to which it enters. Furthermore, we should consider the neutrophil in the asthmatic lung as a potentially heterogeneous population and in the context of location, comorbidities and intrinsic and extrinsic confounders. In this complex setting, there is the clear need to define and dissect precisely what role the neutrophil is playing rather than infer its role from circumstantial evidence and often obsolete misgivings of the neutrophil as a harbinger of doom. It is feasible, and perhaps probable, that the neutrophil exhibits pleiotropic and potentially conflicting roles in defining asthma pathophysiology (Figure 1)—some almost certainly detrimental and some potentially beneficial—that may be variable in a context and patient specific setting. Accordingly, therapeutic manipulation of neutrophils with a broad sword approach may not be the answer and may indeed cause serious adverse effects. Rather we should first seek to understand their complex and multifaceted roles in the disease state and then target them with the same subtleties and specificity that they themselves exhibit.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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