

REVIEW

## Aberrant function of neutrophils in asthma

Meijia Wang, Jianping Zhao, Jungang Xie

Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Department of Respiratory and Critical Care Medicine, National Clinical Research Center of Respiratory Disease, Wuhan 430030, China

Correspondence: Jungang Xie

E-mail: xiejgg@hotmail.com

Received: March 10, 2018

Published: April 17, 2019

**Asthma is a chronic inflammatory airway disease, with an array of cells involved in the pathogenesis. The role of neutrophils in asthma pathogenesis is controversial. This review highlights the mechanisms of neutrophils about their aberrant function in asthma and factors contributing to impaired response to corticosteroids, which may contribute to a better understanding of asthma pathogenesis and consequently, facilitate the development of novel strategies for managing and treating neutrophilia in asthma.**

**Keywords:** Asthma; Neutrophils; Chemotaxis; Phagocytosis; Neutrophil extracellular traps; Glucocorticoids; Steroid resistant

**Abbreviations:** DCs, dendritic cells; Th2, T helper type 2; BALF, bronchoalveolar lavage fluid; PAF, platelet activating factor; fMLP, formyl-methionyl-leucyl-phenylalanine; ROS, reactive oxygen species; NETs, neutrophil extracellular traps; LPS, lipopolysaccharide; GCs, glucocorticoids; NSAIDs, non-steroidal anti-inflammatory drugs; ASA, acetylsalicylic acid; GR, glucocorticoid receptor; HDAC2, recruitment histone deacetylase-2; PMA, phorbol myristate acetate; MIF, macrophage migration inhibitory factor; MKP-1, mitogen-activated protein kinase phosphatase-1.

**To cite this article:** Meijia Wang, et al. Aberrant function of neutrophils in asthma. *Inflamm Cell Signal* 2019; 6: e1535. doi: 10.14800/ics.1535.

### Introduction

Asthma is a very common disease characterized by airway inflammation, reversible airway obstruction, airway hyperresponsiveness, and airway structural remodeling [1, 2]. Many types of cells, such as eosinophils, T lymphocytes, mast cells, neutrophils and dendritic cells (DCs) are involved in the pathophysiology of the airway inflammation in asthma. The role of T helper type 2 (Th2) lymphocytes, mast cells and eosinophils in the pathogenesis of asthma has been well established [3], but the role of neutrophils is controversial [4]. Previous studies demonstrated that severe asthma, persistent asthmatic status, acute asthma exacerbation, and corticosteroid-resistant asthma were associated with increased numbers of neutrophils in peripheral blood and bronchoalveolar lavage fluid (BALF), sputum or bronchial biopsy samples [5, 6]. There is increasing evidence

demonstrated that neutrophils may involve in the development or exacerbation, and especially be associated with the severity of the disease [7, 8]. In this review, the authors discussed the aberrant function of neutrophils in the pathogenesis of airway inflammation in asthma.

### Aberrant function of neutrophil in asthma

Neutrophils are the most abundant circulating polymorphonuclear leukocytes in human peripheral blood and play a fundamental role in the innate immunity [9]. They are recruited rapidly to sites of inflammation and initiate the inflammatory response, but they also actively participate in the resolution of inflammation. Their primary role is to kill invading bacteria and certain fungal species through phagocytosis by the release of preformed granular enzymes and proteins, and the production of a range of oxygen species.

**Chemotaxis** In the course of asthma, neutrophils infiltrate the airways and release many inflammatory mediators during early stage of asthma development [10]. That neutrophils migrating to airways is based on the reactivity of neutrophils to chemoattractive signals, known as chemotaxis which is crucial for an efficient control of pathogens [11]. The chemotactic activity of neutrophils induced by platelet activating factor (PAF) was greater in patients with asthma than in healthy subjects, which implied that neutrophils involved in the pathogenesis of asthma through their heightened recruitment into the bronchoalveolar lumen and the lung by local release of chemotactic factors [12]. Also, neutrophils from asthmatic patients exhibited increased chemotactic activity in the presence of homologous (pooled sera from healthy individuals) or autologous (patient's own serum) serum compared with non-asthmatic controls. Autologous serum-induced neutrophil chemotaxis in patients with uncontrolled asthma was greater than controlled asthma [13]. This phenomenon could be explained by increased chemotactic factors, such as IL-8, TNF $\alpha$ , LTB4 and IL-17A, in the circulation of patients suffering from asthma [6, 14-17]. Our previous study also demonstrated that neutrophils from steroid-resistant asthma released more IL-8 at the presence of asthmatics serum, which might contribute to additional recruitment and activation of neutrophils in a positive feedback manner [18]. Moreover, Lavinskiene *et al.* reported that the migration of neutrophils to the airways after allergen challenge was increased in asthmatic comparing to non-asthmatic subjects, because they were more sensitive to IL-8 stimulation [19]. Though, a previous study indicated that neutrophils from mild asthmatic subjects exhibited impaired chemotaxis velocity in the presence of IL-8 and fMLP (formyl-methionyl-leucyl-phenylalanine) compared with non-asthmatic patients suffering from allergic rhinitis. And they identified neutrophil chemotaxis velocity as a sensitive marker for the discrimination between asthmatic and non-asthmatic individuals presenting similar symptoms from airways [20]. The possibility to explain the impaired chemotactic response of neutrophils might be the different methods, microfluidic chip and automated readout method as shown in their study [20]. The increased migration of neutrophils into the airways stimulates the accumulation and abnormal activation of inflammatory cells, which are largely responsible for oxidative stress and production of proteases and inflammatory cytokines contributing to lung injury and chronic inflammation [21, 22].

**Phagocytosis** Neutrophils have long been considered as phagocytes whose main purpose is to engulf and degrade microorganisms. The reduced ability of alveolar macrophages from subjects with COPD to phagocytose

apoptotic bronchial epithelial cells contributed to secondary necrosis of the uncleared material and perpetuation of inflammation [23]. And in non-eosinophilic asthma, the persistent airway neutrophilia and chronic inflammation were due to the impaired efferocytosis of macrophage [23]. It has been demonstrated that neutrophilic phagocytosis in asthmatic children was defective either when triggered via pattern recognition receptors or mediated by complement and immunoglobulin receptors. Moreover, short-term corticosteroid treatment (3–6 months) was insufficient in restoring proper phagocytic function of neutrophils [24]. Though phagocytic activity was increased after challenge in patients with asthma, it was still not sufficiently effective [19]. The significant decrease in phagocytosis of neutrophils shown in asthma contributed to the progression of late-phase allergen-induced airway inflammation [19, 25].

**ROS Release** of the reactive oxygen species (ROS) in the mechanism of respiratory burst is one of the main functions of neutrophils. Neutrophils recruited by allergen challenge generate sustained ROS in the airways, which may promote allergic inflammation through ROS generation [26]. The effects of reactive oxygen species in asthma include decreased beta-adrenergic function in lungs, airway smooth muscle contraction, increased vascular permeability, bronchial hyperresponsiveness, increased mucus secretion, impaired ciliary activity, generation of chemotactic factors and lipid peroxidation, and secondary production of mediators with a bronchoconstrictor effect [27]. It has been demonstrated that neutrophils purified from bronchoalveolar lavage fluid and peripheral blood from patients with allergic asthma released significantly higher quantities of ROS compared to healthy individuals [13, 19, 27-29]. In mice mode of asthma, the gp91phox deficiency mice, the dominant superoxide generating enzyme in neutrophils, had decreased ROS production, and attenuated allergic inflammatory response to allergen challenge [30]. Activated neutrophils in allergic patients cause the subsequent release of intracellular toxic oxidants and enzymes, which in turn activate proteases leading to more intensive ROS production. And intensive ROS production activates redox-sensitive signaling cascades, stimulates production of proinflammatory cytokines, and promotes inflammation and lung damage [19].

**Neutrophil extracellular traps** Recently, it is discovered that neutrophils are able to release their nuclear DNA in the form of neutrophil extracellular traps, known as NETs. Neutrophil extracellular traps production results from the extracellular release of decondensed chromatin, histones and granular proteins from neutrophils activated by various stimuli including cytokines (e.g. IL-8 or TNF- $\alpha$ ), PMA,

lipopolysaccharide (LPS), or microbial proteins [31, 32]. NETs are an important component of innate immunity, but their role is ambiguous as causing severe host tissue damage in certain pathological conditions [33]. Accumulating evidences shown that NETs directly contributed to host cell death and chronic tissue damage when formed in excess or were insufficiently cleared by mechanisms that were still poorly understood [34]. Recently, several studies have detected increased NETs and NET components in neutrophils from airways or peripheral blood of patients with asthma, suggesting an involvement of NETs in asthma pathogenesis [35-37]. Accumulation of excessive NETs contributed to the persistent airway neutrophilia and enhanced asthma severity by damaging airway epithelium and triggering inflammatory responses of human airway epithelial cells and eosinophils [35]. Thus further study should emphasize on elucidating the exact role that NETs are playing in asthma, and illuminating whether regulation of neutrophil activation or NETs formation will offer therapeutic benefit to patients.

### **Involvement of neutrophils in airway inflammation of asthma**

The role of neutrophils is ambiguous in the pathology of asthma. Abundant studies reported that airway neutrophilic inflammation was associated with asthma phenotypes/endotypes, severe asthma, asthma exacerbations, and corticosteroid treatment responses [38].

*Phenotype* Neutrophils are one of the most inflammatory cells in airway. Based on the proportions of neutrophils and eosinophils in sputum are the criteria for pathologic phenotype of asthma. Subjects with increased eosinophils in sputum are eosinophilic asthma; neutrophilic asthma are based on raised sputum neutrophils above a defined cut-off level and paucigranulocytic asthma are those with normal numbers of both eosinophil and neutrophil in airways; In addition, some individuals have a mixed type of inflammation, mixed granulocytic asthma, when there is sputum neutrophilia and eosinophilia [39]. Cut-off levels used to define sputum eosinophilia were various in different researches as follows: >1.01% [39], >2% [40],  $\geq 2\%$  [41], or  $\geq 3\%$  [42], but a  $\geq 3\%$  cut-off was reported to be the most precise value to identify airway eosinophilia [43]. The cut-off for sputum neutrophilia has not been clearly established with a wide range of values: >40% [44],  $\geq 50\%$  [45], > 61% [46], or  $\geq 76\%$  [47].

*Severity and airflow obstruction* The neutrophils may contribute to the pathophysiology of severe asthma since increased neutrophilic inflammation in induced sputum and

in the bronchial submucosa has been reported in such patients [48-51]. The persistent neutrophilic airway inflammation is associated with fixed airflow obstruction [49]. The relationship between neutrophilic airway inflammation and progressive airflow obstruction is biologically plausible because neutrophils can secrete a variety of inflammatory factors including cytokines, proteases, and lung parenchymal reactive oxygen species that can cause mucus hypersecretion and airway damage [52]. Several researches shown that sputum total neutrophil counts were associated with reduced lung function and based on this finding it has been speculated that airway neutrophils might involve in the pathophysiology of irreversible airflow obstruction in asthma [52, 53]. Therefore, neutrophils are increased in the airways of severe asthma, and may contribute to refractory disease.

*Glucocorticoids use* Glucocorticoids (GCs) are the most effective drugs for the treatment of asthma. It has been proposed that airway neutrophilia seen in asthma might be due to corticosteroid treatment for the function of promoting neutrophil survival by inhibiting their apoptosis [54-56]. However, a study by Hastie *et al.* demonstrated that corticosteroid treatment had no effects on sputum cell counts suggesting minimal effects of continuous corticosteroids exposure on sputum granulocytes [57]. Another study on severe asthma subjects shown that there was no statistical difference in sputum neutrophilia between subjects on high dose inhaled or chronic oral corticosteroids even though the subjects stratified by intensity of corticosteroid exposure [44]. The persistent neutrophilia in asthma should not own to the use of glucocorticoids, because glucocorticoids exerted the same effects on the apoptosis of neutrophils from steroid-sensitive asthma and steroid-resistant asthma with increased neutrophils in airway, as shown in our study [18]. In addition, Uddin and colleagues indicated that unresolved airways neutrophilia in severe asthma were the result of their prolonged survival promoted by factors released by inflammatory and structural cells of the airways [58]. So, airway neutrophilia in asthma may be a manifestation of the disease itself rather than the result of treatment with high-dose corticosteroids.

*Exacerbation* Up to 80% of exacerbations in adults with asthma were associated with sputum neutrophilia, although the predominant sputum cell type could alter during successive exacerbations [59-62]. The causes of asthma exacerbations are numerous including viruses, allergens (dust mite, pollen, animal dander), occupational exposures (grains, flours, cleaning agents, metals, irritants, woods), hormones (menstrual asthma), drugs (ASA, NSAIDs, beta-blockers), exercise, stress, and air pollutants. But viruses were detected

in airway secretions in most of the cases [63]. It is widely recognized that the neutrophilia might be the consequence of a virus infection leading to the acute exacerbation. Some studies have confirmed that predominant neutrophils in the sputum of patients with acute asthma exacerbation were due to respiratory tract infection [64, 65]. However, neutrophils in asthma exacerbations are also due to causes other than infection. Increased neutrophil counts were observed in bronchial lavage fluid of patients with noninfectious acute exacerbations [66]. Dominant neutrophilic inflammation in adults with acute asthma cannot be explained by infection, since *C. pneumoniae*, a potential cause of asthma exacerbation and neutrophilic inflammation was not detected in almost all samples [67]. Initial increased neutrophils, interestingly, continued to increase throughout resolution of the exacerbation, which reminded that neutrophil might have roles in both the initiation and resolution of attacks [68].

There have been limited effective methods to specifically detect whether neutrophils in airways were a distinct pathophysiological characteristic or the result of other factors, such as glucocorticoids, infection.

#### Factors of insensitive to corticosteroids in neutrophilia

Neutrophils are generally considered to be less responsive to GCs, since events involved in neutrophil activation, including adherence, chemotaxis, degranulation, and arachidonic acid metabolite release are not effectively inhibited by glucocorticoids [69]. In the course of asthma, neutrophilic inflammation is associated with an impaired therapeutic response to corticosteroids [70, 71]. It is unknown if the neutrophils themselves contribute to the relative steroid insensitivity or if this is determined by the mechanisms that lead to the recruitment of neutrophils in asthma [72]. Previous studies about the mechanisms of glucocorticoid insensitivity were focused on decreased binding affinity of the glucocorticoid receptor (GR) and translocation, increased expression of the alternatively-spliced variant GR- $\beta$ , increased binding of the activated GR receptor to pro-inflammatory transcription factors such as AP-1 and reduced recruitment of histone deacetylase and so on [73, 74]. And those in their researches are mostly other cell types, such as peripheral blood mononuclear cells [75-77], alveolar macrophages [78-80], or monocytes [81, 82]. These factors also exerted critical effect on the impaired response to glucocorticoid in neutrophilia.

*Increased GR- $\beta$  expression* The effects of GC are mediated by the GC receptor (GR) -  $\alpha$ , which represses expression of various genes encoding inflammatory

mediators [83, 84]. In addition to GR- $\alpha$ , GR- $\beta$  functions as a putative dominant negative form of GR- $\alpha$  by forming heterodimers with it to impair steroid sensitivity [85-88]. A variety of pro-inflammatory cytokines were found to enhance the expression of GR- $\beta$  such as TNF- $\alpha$ , IFN- $\gamma$ , interleukin 1 (IL-1), IL-2 and IL-4 in combination, IL-17/IL-23 and IL-13 [85-87, 89, 90]. High levels of GR- $\beta$  have been reported in neutrophils, and these may be upregulated by IL-8. These data suggests that the inflammatory environment in itself may increase neutrophil insensitivity by increasing the ratio of GR- $\beta$  to GR- $\alpha$ , and promoting further heterodimer formation [91]. It has not been proved whether other cytokines could improve the expression of GR- $\beta$  or not. The intensive expression of GR- $\beta$  contributed to neutrophil insensitivity. And strategies aimed at reducing expression of GR- $\beta$  in neutrophils may provide a starting point for the development of novel anti-inflammatory treatments for neutrophil-associated disease.

*Abnormal histone acetylation* Histone acetylation plays a critical role in the regulation of inflammatory genes and the mechanism of action of glucocorticoids [92]. The anti-inflammatory actions of glucocorticoids are partly related to the recruitment histone deacetylase-2 (HDAC2) activated by glucocorticoid receptors (GR), which inhibits the activation of inflammatory genes by the transcription factor nuclear factor- $\kappa$  B (NF- $\kappa$  B) [93]. Reduced HDAC2 activity has been reported in numerous glucocorticoid-insensitive diseases [94]. A recent research focused on abnormal histone acetylation of neutrophils shown that impaired nuclear recruitment of histone deacetylase-2 (HDAC2) could be an important mechanism of steroid resistance of the neutrophilic inflammation in exacerbations of asthma, which led to continuing enhanced expression of neutrophil chemoattractants and survival factors [95]. Additionally, oxidants in the airways of asthmatic patients generated by oxidative stress were due to the formation of peroxynitrite, tyrosine nitration, and lipid peroxidation, which could decrease the activity of HDAC2 via the nitration of tyrosine residues in HDAC2 [96]. Tyrosine nitration of HDAC2 is an important mechanism of steroid resistance [97]. These studies suggest that abnormal histone acetylation is an important mechanism of glucocorticoid resistance in asthma.

#### Immune mechanisms

Neutrophils play a critical role in the inflammatory and immune responses by the virtue of their ability to produce a variety of cytokines. Though, Hirsch and colleagues reported that glucocorticoids exerted similar genomic effects on

neutrophils and on other blood leukocytes by quantifying the effect of glucocorticoids on LPS-induced pro-inflammatory mRNA expression in neutrophils and neutrophil-depleted leukocytes [98]. In our recent study, dexamethasone exerted impaired anti-inflammatory action on neutrophils from steroid-resistant asthma at the present of atopic asthmatic serum, a mimic of asthmatic milieu, by showing that the inhibition of IL-8 production by dexamethasone in steroid-resistant asthma was less when compared to steroid-sensitive asthma [18]. Similarly, a previous study on blood neutrophils in severe asthma shown that oxidative burst stimulated by phorbol myristate acetate (PMA), and the release of IL-8 which represented the activation of neutrophils, were not inhibited by oral corticosteroids [99]. Those data indicated that impaired function of glucocorticoids on neutrophils to repress pro-inflammation cytokine in some condition might be important in the pathogenesis of severe steroid-resistant asthma.

The importance of TH17 cells in neutrophilic inflammation lies in the ability of IL-17 to induce granulopoiesis, neutrophil chemotaxis, and the anti-apoptotic property of G-CSF [100, 101]. Accumulating evidences suggest a correlation between high levels of IL-17 and steroid-resistant disease [102]. In mice, TH17 cell-mediated airway inflammation and AHR are steroid resistant [103]. IL-17A, a crucial inflammatory cytokine produced by Th17 cells, induces a glucocorticoid resistance in human airway epithelial cells, which is mediated by PI3K activation and subsequent reduction of HDAC2 activity [104]. The role of IL-17 in glucocorticoid resistance is also associated with the expression of GR- $\beta$  in airway epithelial cells, which is not suppressed by glucocorticoids in-vitro [105]. Recently, a research by Murcia and colleagues demonstrated that activation and increased survival of equine neutrophils induced by IL-17 was insensitive to glucocorticoids [106]. So, glucocorticoid resistance induced by Th17 cells may be an important pathogenesis of steroid-resistance in airway neutrophilia.

#### *Other factors*

As mentioned above in this review, NETs are associated with inflammation and disease severity in chronic airway diseases [34, 35]. While glucocorticoid had no effect on the NETs formation in peripheral blood neutrophils induced by activation, phorbol myristate acetate (PMA), in vitro [107]. Decreased phagocytosis in neutrophils did not return to normal after corticosteroid treatment [24], because corticosteroids decrease the phagocytosis which has been seen in monocytes with decreased phagocytosis and

repression the release of inflammatory cytokines by corticosteroids [108, 109]. Asthmatic milieu impedes the anti-inflammatory of glucocorticoid in steroid resistant asthma [18]. Macrophage migration inhibitory factor (MIF) is an immunologic regulator that has anti-glucocorticoid effects [110]. It is induced by glucocorticoids and inhibits their anti-inflammatory effects, by inhibiting the induction of MKP-1. MIF has been implicated in the pathogenesis of allergic inflammation in mouse models of asthma and in severe asthma [111, 112].

#### **Conclusion and future directions**

No matter whether neutrophilia in asthma is due to corticosteroids, chronic pulmonary infection, delayed neutrophil apoptosis, or the alternative pathology of asthma. The role of neutrophils in the development of asthma is crucial. And their presence may be associated with increased asthma severity. Alterations in neutrophil functions lead to ineffective removal of pathogens and increased inflammation [19]. Hence, novel targeted medications must be developed that could control neutrophilic inflammation and still maintain their antibacterial/anti-fungal properties, thus allowing individuals to maintain effective innate immune responses to invading pathogens. Further development of strategies that emphasize on factors leading to insensitive responsiveness to glucocorticoid in neutrophilia may increase the efficacy of glucocorticoid therapy in asthmatic patients with neutrophilia and ultimately improve therapeutic benefits.

#### **Conflicting interests**

The authors have declared that no conflict of interests exist.

#### **Acknowledgments**

This work was supported by the National Natural Science Foundation of China (No. 81570033, 81570047, 81470227, 81370145, 81370156), National key basic research and development program (973 Program, No. 2015CB553403) and Changjiang Scholars and Innovative Research Team in University (No. IRT\_14R20).

#### **Author contributions**

M.W. contributed to the conception, drafting, editing of the manuscript. J.Z. and J.X. contributed to the conception, provided overall supervision and critically revised the manuscript. All authors read and approved the final manuscript.

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