



New insights in neutrophilic asthma

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Purpose of review

Recent advances in both murine models and clinical research of neutrophilic asthma are improving our understanding on the etiology and pathophysiology of this enigmatic endotype of asthma. We here aim at providing an overview of our current and latest insights on the pathophysiology and treatment of neutrophilic asthma.

Recent findings

Activation of the NLRP3 inflammasome pathway with increased IL-1 β has been demonstrated in various studies involving patients with asthma. It has been suggested that type 3 innate lymphoid cells are implicated in the inflammatory cascade leading to neutrophilic inflammation. The role of neutrophil extracellular traps is only at the start of being understood and might be an attractive novel therapeutic target. A diverse panel of nonallergic stimuli, such as cigarette smoke, intensive exercise, cold air or saturated fatty acids, have been linked with neutrophilic airway inflammation. Azithromycin treatment could reduce asthma exacerbations and quality of life in patients with persistent asthma.

Summary

Research of the last few years has accelerated our insights in mechanisms underlying neutrophilic asthma. This is in stark contrast with the lack of efficacy of different therapies targeting neutrophil chemotaxis and/or signalling cascade, such as IL-17A or CXCR2. Macrolide therapy might be a useful add-on therapy for patients with persistent asthma.

Keywords

biological, inflammasome, interleukin-17, neutrophil extracellular trap, neutrophil, targeted treatment

INTRODUCTION

In the twentieth century, asthma has been associated with allergy and eosinophilic airway infiltration, making allergic asthma the first and best characterized asthma phenotype [1]. Research at that time focused on treatment options that reduce eosinophilic infiltration and diminish the accompanying Th2 inflammation [2,3]. Hence, steroid treatment, optimal to fulfil that goal, became the cornerstone of asthma treatment [4]. However, some patients with severe asthma demonstrated airway neutrophilia despite inhaled corticosteroid (ICS) treatment [5,6]. These patients respond poorly to steroid treatment [7].

DEFINITION OF NEUTROPHILIC ASTHMA

Since the discovery of the asthma phenotype with predominant neutrophilic airway inflammation [5,6], several attempts to define this 'neutrophilic asthma' have been made. First, neutrophil percentages in sputum exceeding the numbers in healthy control individuals were used to define 'pathologic'

neutrophil percentages, leading to a cut-off of 61% [7,8]. Later on, researchers adjusted that cut-off to 76% because of higher 'normal' values in healthy individuals [9,10]. This is also probably related to other factors, such as age, that also influence airway neutrophil percentages in healthy individuals [11]. However, also lower cut-off values (>40%) that are based on median neutrophil proportions in patients are reported [12].

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KEY POINTS

- Neutrophilic asthma occurs in 15–25% of the asthma cases and is more prevalent amongst steroid-dependent severe asthmatics.
- Some emerging pathophysiological mechanisms contributing to neutrophilic inflammation have been identified, such as activation of the NLRP3-IL1 β pathway and innate lymphoid cell type 3.
- Several therapeutic strategies interfering with airway neutrophilia, both directly or indirectly, have been evaluated for patients with varying severity of asthma.

PREVALENCE

Estimating the prevalence of ‘neutrophilic asthma’ is dependent on the cut-off of airway neutrophils used to define this phenotype. In a small observational study, neutrophilic asthma (neutrophils >61%) was observed in 28% of stable asthmatic adults ($n=29$) and in 20% of stable asthmatic children ($n=49$) [13]. A recent large observational study in a Belgian patient cohort ($n=833$) reported that neutrophilic asthma (neutrophils >76%) can be observed in approximately 15% of the asthmatic individuals [10]. In contrast, in the above cited study using a cut-off of 40%, approximately 25% of the cohort ($n=423$) showed neutrophilic asthma. Using cluster analysis, these authors, moreover, confirmed that high sputum neutrophilia was present in two clusters of moderate-to-severe asthma patients [12]. Patients in both clusters are in need of frequent healthcare utilization, despite treatment with high doses of ICS or oral corticosteroids, but differ in their lung function impairment [12]. Additionally, total cell counts in combination with increased neutrophil proportions was suggested to identify patients with neutrophilic asthma [14].

PATHOPHYSIOLOGY

Neutrophil function in asthma is only partly unravelled and perhaps neutrophils are not more than innocent bystanders [15]. However, because of release of their granules, neutrophils might contribute to airway gland hypersecretion, bronchial hyperreactivity and to airway wall remodeling, by producing matrix metalloproteinase-9 (MMP-9) observed in bronchoalveolar lavage fluid (BAL) from moderate-to-severe asthma patients [15]. Elevated MMP-9 and neutrophil elastase have also been reported in sputum of patients with neutrophilic asthma which was absent in those with eosinophilic asthma [16]. Both of these proteolytic enzymes (neutrophil elastase and MMP-9) are known to cause

structural damage to airways and promote further secretion of potent chemoattractants such as CXCL-8 by airway epithelial cells, resulting in a development of a self-sustained vicious cycle of continuous neutrophilic recruitment in airways [17]. Although neutrophils were always presumed as a homogeneous population of phagocytes, recent finding suggests that different neutrophil populations with different functions may be present in the airways and neutrophils may even differentiate towards antigen-presenting cells locally [15]. A study by Pillay *et al.* identified three subsets of neutrophils (normal, banded and hypersegmented) in the circulation following an inflammatory challenge. Each subset had a distinct nuclear morphology and pattern of surface adhesion molecule expression, with hypersegmented neutrophils showing unique ability to suppress T lymphocytes by their increased oxidative burst capacity [18]. We have recently also reported the presence of these same subsets in bronchial lavage from adults with asthma [19]. The hypersegmented nucleus can be an attribute of older neutrophils, which may have gained hypersegmentation because of long survival in inflamed airways under the presence of cytokine-rich environment [20,21] and other factors such as ICS [22].

The most classic hypothesis to explain why neutrophils are associated with severe ICS-treated asthma, is that ICS induce apoptosis in eosinophils but fail to do so in neutrophils or even prolong their survival [23–25]. However, even if neutrophil survival would be prolonged in ICS-treated asthma, one could wonder how neutrophils could be recruited to the area of inflammation in asthmatic patients in the first place?

Recent evidence suggests that interleukin (IL)-17A, either directly or indirectly, might be an important mediator to recruit neutrophils. Indeed, IL-17A is known to significantly stimulate neutrophil maturation, migration, and function, documented by the fact that overexpression of IL-17A in murine livers results in massive peripheral neutrophilia associated with enhanced granulopoiesis [26]. On the other hand, impaired IL-17 receptor signaling leads to delayed bronchial recruitment of neutrophils [27]. Along the same line, in a model of contact dermatitis, skin infiltrating activated neutrophils, measured by their myeloperoxidase (MPO) levels, were strongly reduced in IL-17A knock-out compared with wild type mice, suggesting that 17A is involved in neutrophil recruitment as well as activation [28]. Similarly, injecting anti-IL-17 monoclonal antibodies (mAb) in ovalbumin (OVA)-sensitized mice during the OVA challenge phase, leads to decreased airway neutrophilia as well as decreased BAL MPO levels [29]. To rule out the

possibility that systemic effects (induced by peritoneal sensitization) would cause the IL-17A-mediated neutrophilia in the cited mouse model, Wilson *et al.* repeated the experiments with OVA sensitization through the airways. They similarly observed IL-17A-dependent airway neutrophilia in their model and demonstrated that IL-17A may furthermore be sufficient to induce airway hyperreactivity [30]. Neutrophils, however, do not express IL-17 receptors [31] so they most likely do not directly respond to IL-17A. The mechanism of neutrophil recruitment by IL-17 is still not completely clear, but the induction of several chemokines by IL-17A-responsive cells, most likely epithelial cells, certainly plays a role [32]. Overexpression of IL-17A in lung epithelial cells, on the other hand, has been shown to induce several chemokines, but without subsequent neutrophil influx [33]. Therefore, also other IL-17A responsive mediator cells, in addition to epithelial cells, might be involved.

Several years after the discovery that IL-17A may contribute to asthma in murine models, data obtained by induced sputum from human suggested that IL-17A could also be relevant for human neutrophilic asthma. Indeed, sputum IL-17A mRNA levels were reported to be higher in asthmatic patients than in healthy control individuals and to correlate with neutrophil percentages in those patients [34]. Moreover, a clear correlation between IL-17A mRNA and IL-8 mRNA levels was also observed, suggesting that IL-17A could exert its neutrophil recruitment function by stimulating IL-8 production in humans. Increased IL-8 levels secreted by T lymphocytes, epithelial cells, smooth muscle cells and macrophages in the airways of asthmatics in relation to neutrophilia, was also observed earlier by other authors [35–37] and therefore, was suggested to be especially important in patients with neutrophilic asthma. However, the effects of IL-17A in the airways might be much broader than the simple induction of IL-8 by epithelial cells. Indeed, experiments on primary human bronchial epithelial cells showed the induction of CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8/IL-8, IL-6 and IL-19 proteins after IL-17A signalling (reviewed in [38]). IL-6, IL-8 and IL-11 as well as IL-6, IL-1 β , IL-8, IL-11 and eotaxin production by primary bronchial fibroblasts and human smooth muscle cells, respectively, has also been reported in response to IL-17A (reviewed in [38]). Among those, in addition to IL-8, CXCL-1 and IL-1 β might also be of particular importance to recruit neutrophils to the airways [31] (Fig. 1). Activation of the NLRP3-inflammasome cascade with subsequent cleavage of pro-IL1 β to its active form has been demonstrated in patients with neutrophilic asthma [39,40]. In addition, several other manuscripts have recently shown

increased expression of IL-1 β in sputum of a subgroup patients with asthma compared with controls [41,42,43]. On the other hand, not all data pointed towards a link between IL-17A and neutrophilia in human asthma. Indeed, other authors did confirm IL-17A expression in bronchial biopsies from severe asthmatic patients, but could not find a significant correlation with airway neutrophilia [44]. Furthermore, to highlight the complexity of human asthma, sputum IL-17A mRNA expression was also associated with sputum IL-5 mRNA expression in a subgroup of severe asthma patients [45], which probably also explains why in some severe patients, neutrophilia and eosinophilia are both similarly increased [38].

IL-17A is typically reported to be produced by a separate T-cell lineage, called Th17 cells [33,46], although recent data suggest that T cells with dual production of Th2 as well as Th17 cytokines might also exist and could play a role in human asthma [47,48]. Furthermore, IL-17A-producing innate lymphoid cells (ILC), called ILC3, have been shown to be involved in severe obese asthma [49]. These newly described cells do not express T-cell receptors and can be stimulated directly by specific cytokines [50]. Surprisingly, no data on the absence or presence of correlated airway neutrophilia in these obese individuals were reported [49]. The observed correlation of sputum IL-17A mRNA levels and associated neutrophilia in obese asthmatic individuals in a recent study, suggests that neutrophils could also be associated with IL-17A production, most likely by ILC3 [51]. IL-17A, however, could also be produced by neutrophils themselves, by B cells, natural killer cells and $\gamma\delta$ T cells [38]. The impact of those IL-17A-producing cells in the asthmatic airways is less clear, although it is tempting to speculate that the auto-crine production by neutrophils [52] could be important for the chronicity of the neutrophil asthmatic subtype.

Once they migrate to the airways, neutrophils can undergo programmed cell death (apoptosis) or alternately they can undergo necrosis or NETosis, depending on the level of activation [53]. NETosis involves the formation of neutrophil extracellular traps (NET), where a blend of granular and nuclear content is expelled from the neutrophil to trap and kill the pathogen in its web like structure [54]. Although important for host defence against infection, emerging data from recent studies indicate that exaggerated NET formation and/or delayed clearance may inflict damage to the lung epithelial and endothelial cells [55] and can further contribute to airway obstruction [56]. In severe asthma, inflammatory cytokines such as the chemoattractant CXCL-8 too can trigger NETosis [57]. Recent work has shown an increased presence of NETs in the

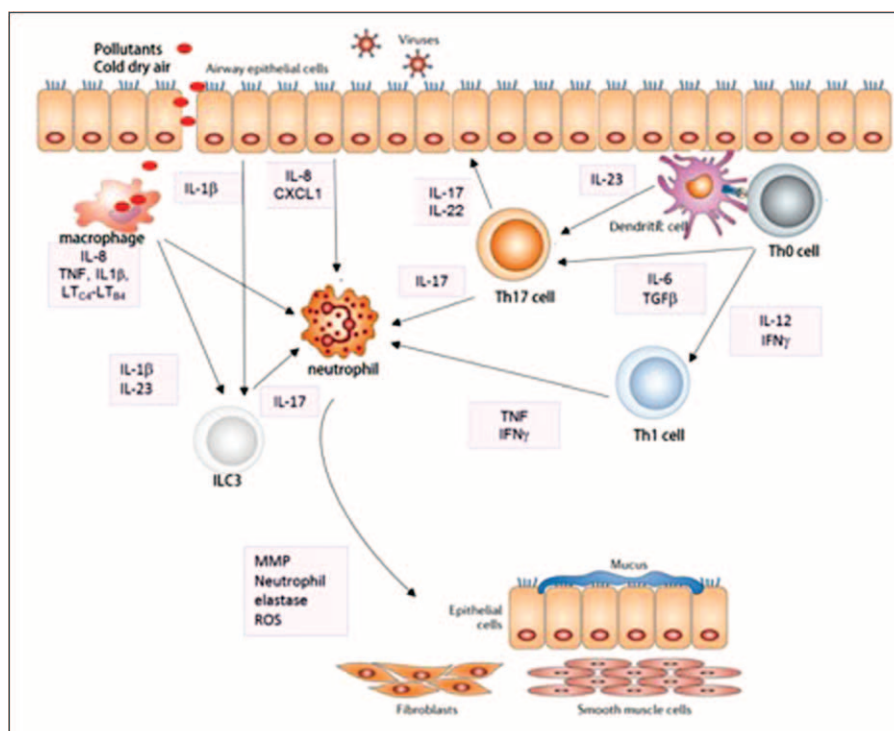


FIGURE 1. Potential interactions between neutrophils and airway cells in asthma; adapted from [88]. IFN, interferon; IL, interleukin; ILC, innate lymphoid cell; LT, leukotriene; MMP, matrix metalloproteinase; ROS, reactive oxygen species; TGF, transforming growth factor; Th, T helper; TNF, tumor necrosis factor.

sputum from adults with neutrophilic asthma and COPD. The increased presence of NETs was negatively associated with the percentage predicted forced expiratory volume in one seconde and asthma control questionnaire (ACQ) scores of asthma participants [58^{***}]. NETs have been shown to amplify the response to an exacerbation via elevated airway inflammatory cell infiltration, mucus production and airway hyperreactivity in a mouse model of type 2 allergic asthma, following a rhinovirus challenge. Removal of NETs by DNase resulted in improved immunopathology in mice, suggesting role of NETs in pathogenesis of rhinovirus-induced exacerbation in type 2 asthma [59].

ENVIRONMENTAL TRIGGERS LINKED WITH THE IL-17A–NEUTROPHIL AXIS IN ASTHMA

If IL-17A would be the most important cytokine for neutrophil recruitment to the airways, it could be of utmost importance to determine, which stimuli could especially induce IL-17A release in the airways. Among 235 children with allergic asthma, high diesel exhaust particle (DEP; $>0.464 \mu\text{g}/\text{m}^3$)-exposed children with allergic asthma ($n = 59$) had nearly six times higher serum IL-17A levels compared with low DEP-exposed children. In parallel,

high DEP-exposed children had a higher chance to have severe asthma. This points to higher IL-17A-induced neutrophilia in the DEP-exposed children. Unfortunately, sputum neutrophilia in these children was not assessed [60].

Similarly, 2-h exposures to 0.4 ppm ozone and filtered air induced neutrophilia in BAL and induced sputum from normal healthy individuals. Most likely, neutrophils are attracted by the induction of IL-8 in these models, but, however, no data on IL-17A at that time could be reported [61,62].

Likewise, cigarette smoke in asthmatic individuals also increased their sputum IL-8 levels in parallel to increased neutrophil percentages [63]. In a more recent and extensive study, smokers with asthma had higher baseline sputum IL-17A as well as IL-8 (but also and even more clear sputum IL-6, IL-7 and IL-12) levels than nonsmokers with asthma, but their sputum neutrophilia was only slightly higher than in nonsmokers. Surprisingly, steroid treatment increased their IL-17A levels whereas decreasing their neutrophilia. However, both purely neutrophilic asthmatics and nonneutrophilic asthmatics have been studied together, which makes it difficult to disentangle the effect of steroids on pure neutrophilic asthmatic airway inflammation in that study [64].

On the other hand, airway neutrophil recruitment induced by high-intensity physical exercise in

elite athletes in the complete absence of increased IL-17A levels has also been observed [65]. Swimming in chlorinated pools induced IL-1 β , tumor necrosis factor (TNF) and IL-6 mRNA levels in elite swimmers and IL-6 mRNA levels in all individuals, even in controls, which could explain the increase in neutrophilia in those conditions. Cold air exposure of asthmatic patients either in a ski resort or at high altitude, results in sputum IL-17A upregulation as well as in associated airway neutrophilia [66].

Lastly, obesity has been linked to increased IL-17A expression and neutrophils in sputum of asthmatics [51]. Activation of the NLRP3-inflammasome pathway might be the upstream event driving the neutrophilic inflammatory response in patients with obese asthma [67]. Recently, it has been shown that a meal with saturated fatty acids led to increased NLRP3 expression and neutrophils in non-obese asthmatics [67].

Recent advances on how neutrophilic inflammation is induced in the airways of patients with asthma provide us with a better view on the asthma phenotypes that may potentially benefit from neutrophil-targeted therapies.

TARGETING NEUTROPHILIC ASTHMA

Variable success was achieved with clinical trials evaluating compounds targeting the IL-17A–neutrophil axis (Table 1). Also other biologicals targeting nontype 2 inflammatory pathways, such as anti-TNF have not been able to fully fulfil their promise. Several factors may account for this variable response: type of stimulus evoking asthma symptoms, heterogeneity in phenotype and/or endotype, concomitant activation of other inflammatory pathways and outcome parameters.

Anti-IL17RA

IL-17A, IL-17F and IL-17E (or IL-25) exert their effect via the IL-17R (receptor). The functional IL-17R is a heterodimeric complex constituting at least the IL-17RA or IL-17RC subunit. Given the role of IL-17A in neutrophil recruitment and airway smooth muscle contractility and migration, blocking this molecule or downstream signalling pathway bears the potential of cutting down key asthma features in patients with neutrophil predominant asthma. A phase II study with brodalumab (anti-IL17RA mAb) by Busse *et al.* [68] could, however, not demonstrate a treatment effect. Patients included in the study were inadequately controlled moderate-to-severe asthmatic patients despite inhaled corticosteroid treatment. A post hoc analysis of patients with high reversibility did show improved asthma control

with the intermediate dose of brodalumab but not with the highest dose.

Antitumor necrosis factor

Inhalation of recombinant TNF has been shown to increase sputum neutrophils and induce airway hyperreactivity in normal human individuals [69]. Patients with severe asthma show increase levels of TNF in BAL and bronchial biopsies compared with patients with mild–moderate asthma and controls [70]. Early studies with etanercept, a soluble TNF receptor fusion protein, demonstrated beneficial effect on asthma control, lung function parameters and quality of life [71,72]. However, these studies were performed in small numbers of patients. Follow-up studies with not only etanercept but also golimumab (anti-TNF mAb) could not confirm these initial promising results [73,74]. In addition, safety concerns were raised in the golimumab trial, where serious infections were detected more frequently in the active group compared with the placebo group.

CXCR2 antagonists

Neutrophils may also be directly targeted by antagonists of the CXCR2 receptor, the high-affinity receptor of IL-8. Two-placebo controlled trials with CXCR2 antagonists showed dose-dependent reductions in blood neutrophil counts [75,76]. Nevertheless, these studies did not show better asthma control, lung function or reduced exacerbation rates. Furthermore, the use of some of these compounds was associated with significant neutropenia.

Macrolides

Macrolides, which are generally used as antimicrobial agents against airway infections, are also emerging as anti-inflammatory agents [77] with the analogues like clarithromycin have been found improving quality of life by reducing CXCL-8 levels and neutrophil numbers in airways of refractory asthma participants, during a short-term treatment (8 weeks) [78]. Recent work suggests that macrolides exert their anti-inflammatory action by inhibition of NF- κ B receptor of airway epithelial cells resulting in suppression of CXCL-8 release [79]. In addition to clarithromycin, azithromycin has been reported to be effective in reducing severe exacerbations (hospitalization, emergency room visits and/or need for systemic corticosteroid for at least 3 days) in non-eosinophilic asthma participants [80]. The efficacy of azithromycin in asthma was further confirmed in a recent study by Gibson *et al.* [81[■]], which find it effective in reducing the rate of exacerbations

Table 1. Targeted treatment for neutrophilic asthma

Target	Therapy	Population	Treatment period and route of administration	Outcomes	Reference
IL-17RA	Brodalumab	Inadequately controlled moderate-severe asthma (n = 302)	12 weeks, s.c.	No effect on asthma control, lung function, symptom scores	Busse et al. [68]
OTNF	Etanercept	Mild-moderate asthma (n = 10) and refractory asthma (n = 10)	10 weeks, s.c.	Improved PC20, AQLQ and postbronchodilator FEV ₁	Berry et al. [71]
TNF	Etanercept	Severe corticosteroid dependent asthma (n = 39)	12 weeks, s.c.	Improved asthma control and reduction in systemic inflammation	Morjaria et al. [72]
TNF	Golimumab	Severe uncontrolled asthma (n = 309)	52 weeks, s.c.	No effect on lung function, severe asthma exacerbations	Wenzel et al. [74]
TNF	Etanercept	Moderate-severe persistent asthma (n = 132)	12 weeks, s.c.	No effect on lung function, asthma control, asthma exacerbation, AQLQ	Holgate et al. [73]
CXCR2 receptor	SCH527123	Severe asthma with greater than 40% sputum neutrophils (n = 34)	4 weeks, p.o.	Reduced sputum and blood neutrophils and reduction in rate of mild exacerbations	Nair et al. [87]
CXCR2 receptor	AZD5069	Uncontrolled asthma (n = 478)	6 months, p.o.	No effect on severe exacerbations	O'Byrne et al. [75]
Unknown	Clarithromycin	Severe refractory asthma (n = 45)	8 weeks, p.o.	Downregulated IL-8 and neutrophil numbers and improved quality of life	Simpson et al. [78]
Unknown	Azithromycin	Severe asthma (n = 109)	6 months, p.o.	Reduction in severe exacerbation and/or lower respiratory tract infection requiring antibiotics, in noneosinophilic asthma patients	Brusselle et al. [80]
Unknown	Azithromycin	Asthma (n = 420)	48 weeks, p.o.	Reduction in rate of exacerbation and improved asthma related quality of life	Gibson et al. [81 [■]]

AQLQ, asthma quality of life questionnaire; FEV₁, forced expiratory volume in one seconde.

experienced by those with persistent uncontrolled asthma, suggesting its role as a potential candidate for add-on therapy in asthma. In this study, the drug was effective in asthma patients with either eosinophilic or noneosinophilic phenotype.

Other potential candidates

Targeting neutrophil weaponry, such as its proteolytic enzymes like MMP-9 may provide a novel approach in dealing with neutrophil-dominated inflammation. The inhibition of MMPs has been largely exploited outside the respiratory disease [82], such as in cancer [83] and have reported to have limited success as trials of many analogues were terminated in either phase 2 or in phase 3 because of poor bioavailability and adverse reactions like musculoskeletal pain, neutropenia, gastric upset, and so on [84]. Although use of dual MMP-

9 and MMP-2 inhibitor (AZD1236) failed to provide any clinical benefits in a small exploratory study on COPD participants [85], the effect of MMP inhibitor on asthma still needs to be investigated [82]. As we have learnt from the anti-IL-5 studies, targeted therapy to the right inflammatory subtype can result in positive outcomes and perhaps MMP inhibitors may be worthwhile only in those participants with elevated neutrophilic inflammation. MMP-9 can also be downregulated through other pathways. A recent trial of CXCR-4 inhibitor (AMD 3100) in a murine asthma model, suppressed the release of MMP-9 by bronchial epithelial cells after ovalbumin-induced bronchial challenge [86].

Future consideration

The current efforts in designing a neutrophil-targeted therapy for asthma has not yielded any fruitful

outcomes, as many specific therapies such as CXCR-2 inhibitor failed in showing any promising effect on clinical parameters of asthma participants [87]. The emerging knowledge about presence of more than one type of neutrophils in inflammatory airway diseases can provide a more specific approach in dealing with neutrophilic airway inflammation. A detailed assessment of presence and roles of these subsets in airways can provide clinicians much needed help in modulating the use of available conventional therapies accordingly.

CONCLUSION

Neutrophilic asthma occurs in 15–25% of the asthma cases and is more prevalent amongst steroid-dependent severe asthmatic patients. Neutrophilia and airway IL-17A secretion seem to be directly correlated, though the effect of IL-17A on neutrophil attraction most likely occurs indirectly through the release of certain chemokines, such as IL-8, by epithelial cells. However, upon direct triggering, epithelial cells can also produce specific mediators, responsible for direct neutrophil attraction, even in the absence of IL-17A. The specific role of neutrophils in the asthmatic airways remains unexplained.

Several therapeutic strategies interfering with airway neutrophilia, both directly or indirectly, have been evaluated for patients with varying severity of asthma. So far, macrolides have been the only effective treatment validated in a placebo-controlled trial with sufficient number of patients included.

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Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

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