



T2-low asthma: current approach to diagnosis and therapy

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Purpose of review

Asthma is a heterogeneous disease not only on a clinical but also on a mechanistic level. For a long time, the molecular mechanisms of asthma were considered to be driven by type 2 helper T cells (Th2) and eosinophilic airway inflammation; however, extensive research has revealed that T2-low subtypes that differ from the dominant T2 paradigm are also common.

Recent findings

Research into asthma pathways has led to the recognition that some asthma phenotypes show absence of T2 inflammation or alternate between T2 and non-T2 responses. Moreover, numerous immune response modifiers that block key-molecules such as interleukin (IL)-5, IL-13, and immunoglobulin E (IgE) have been identified. Along the way, these studies pointed that T2-low inflammation may also be responsible for lack of responsiveness to current treatment regimes.

Summary

Asthma pathogenesis is characterized by two major endotypes, a T2-high featuring increased eosinophilic airway inflammation, and a T2-low endotype presenting with either neutrophilic or paucigranulocytic airway inflammation and showing greater resistance to steroids. This clearly presents an unmet therapeutic challenge. A precise definition and characterization of the mechanisms that drive this T2-low inflammatory response in each patient phenotype is necessary to help identify novel drug targets and design more effective and targeted treatments.

Keywords

asthma endotypes, noneosinophilic asthma, T2 inflammation

INTRODUCTION

Airway eosinophilia has been a distinctive feature of asthma since Paul Ehrlich used eosin in the late 1800s and demonstrated eosinophils in Charcot-Leyden crystals and in Curschmann's spirals [1]. But it is over the last 30 years that the role of the eosinophil and the relation with helper T-cell (Th) function in asthma has been established. This has been driven by the major advances in molecular biology and imaging techniques, which enabled the characterization of Th cells, and the discovery and characterization of cytokines and chemokines, and it has also been driven by the work of many dedicated researchers worldwide.

Because the majority of studies in asthma involved allergic asthma, which lends itself to experimental challenges and, therefore, makes the study of the asthmatic reaction and its time-course possible, it has been accepted that asthma is a Th2 immunologic disease. However, over the past 10–15 years, the study of severe asthma, which as often as not is nonatopic, showed that in many

cases, inflammation in asthma is not characterized by eosinophilia and T2 type cytokines but may in fact be T2-low or negative. Wenzel *et al.* [2] showed that biopsies from asthmatics may show eosinophilia, but there are also patients who exhibit neutrophilia, a mixed cellularity or that are paucigranulocytic. Severe asthma cohort studies such as the ENFUMOSA/BIOAIR studies [3], the TENOR/SARP studies [4–6], the Belgium Severe Asthma study [7] and, more recently, the U-BIOPRED study [8] also show that some patients have stable eosinophilic phenotypes over time whereas others are

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KEY POINTS

- Asthma pathogenesis is characterized by two major pathophysiological pathways, a T2-high featuring increased eosinophilic airway inflammation, and a T2-low with either neutrophilic or paucigranulocytic airway inflammation.
- The mechanism of T2-low asthma is not well understood; however, Th1/Th17 pathway activation, innate immune defects, tissue remodeling and neurogenic inflammation are possibly implicated.
- Specific biomarkers for phenotyping T2-low asthma are currently lacking and present an unmet need.
- Current treatment approaches for patients with neutrophilic, T2-low inflammation have limited efficacy, and these patients have poor disease control and impaired quality of life and present a future therapeutic challenge.

steadily noneosinophilic or alternate between phenotypes.

T2-low asthma is a problem urgently needing solution: These patients have poor response to steroids, which combined with bronchodilators, are the cornerstone of severe asthma treatment [9]. Moreover, these patients are not candidates for treatment with the newer targeted medications such as anti-IgE or anti-IL-5 [10]. Therefore, there is an imperative need to decipher the mechanisms leading to T2-low asthma and to design treatment aiming at them. Moreover, there is an unmet need to identify biomarkers that can help diagnosis and endotyping. This review aims to discuss the diagnostic approach to T2-low asthma characterized by noneosinophilic airway inflammation and consider older as well as more recent therapeutic approaches, such as novel small molecules and biologics agents, in the treatment of noneosinophilic asthma.

DEFINITION OF TH2-LOW/HIGH ENDOTYPES

Although T2-high asthma with eosinophilia is easy to distinguish, there is no clear distinction or a widely accepted definition of T2-low disease. The T2-high asthmatic endotypes are associated with increased epithelial expression of T2 cytokines, such as interleukin (IL)-4, IL-5, and IL-13. Although heterogeneous in nature, the designation of the T2-high endotype has primarily been based on the presence of eosinophilic airway inflammation, usually identified on the basis of sputum or blood eosinophilia, yet with no universally accepted thresholds [11–13,14[¶],15]. The definition of the

T2-low endotype is even more elusive. In most cases, the T2-low endotype is defined by the absence of markers of T2 inflammation and is usually characterized by neutrophilic or, less commonly, paucigranulocytic infiltration in the airways [16].

BIOMARKERS FOR T2-LOW ASTHMA

In a landmark study of Wenzel *et al.* [2], a T2-low group has been described, especially among severe asthmatics, based mainly on the absence of eosinophilic inflammation in lung biopsy samples. Patients with T2-low asthma can be further divided in two different phenotypes according to the level of neutrophilic inflammation, paucigranulocytic and neutrophilic, especially on bronchial biopsies [2,16]. The problem is that there is no consensus regarding the percentage of sputum neutrophils required to define the neutrophilic asthma phenotype and different cut-off values have been used in the literature with a wide range between 40 and 76% [17–19]. Furthermore, sputum neutrophils do not seem to correlate with other measures of airway inflammation in asthma, raising concerns about their value for identifying specific neutrophil-induced airway pathology [20].

T2-low asthma is not so rare as previously thought, especially among severe asthmatics. In the Belgian severe asthma registry, eosinophilic asthma (sputum eosinophils $\geq 3\%$) was the predominant phenotype (55%) but neutrophilic (sputum neutrophils $\geq 76\%$) and paucigranulocytic asthma accounted for 22 and 17%, respectively [7]. Moreover, T2-low asthma – especially neutrophilic asthma – was associated with more severe asthma, reduced pulmonary functions and poor asthma control [3,19,21]. Paucigranulocytic asthma is even less well described and studied. In a recent study from Belgium, paucigranulocytic inflammatory phenotype was presented in 38% of patients (defined as a sputum eosinophil count $< 3\%$) and in 29% when the cut-off point for eosinophils was reduced to 1.01% [22[¶]].

Specific biomarkers, other than sputum differential cell counts, to discriminate T2-low asthma from T2-high endotype do not exist yet, at least in an everyday clinical setting [23]. However, several biomarkers have been studied in the airways and blood of T2-low asthmatics, giving promising for their future use in clinical practice. Interleukin-8 (IL-8) activates neutrophils inducing chemotaxis, exocytosis and the respiratory burst [24]. In a groundbreaking study from Gibson *et al.* [25], sputum IL-8 levels were highest in patients with noneosinophilic asthma and correlated with sputum neutrophilic count. Furthermore, in treatment-resistant severe

asthmatics sputum IL-8 concentration significantly and positively correlated with airway colonization with potentially pathogenic micro-organisms and neutrophilic airway inflammation [26]. IL-8 (also named CXCL8), as well as other neutrophil chemoattractants such as CXCL1 and CXCL5, modulate their action through the CXC chemokine receptors CXCR1 and CXCR2 [27]. Expression of both receptors was significantly increased in the sputum of neutrophilic asthma [28,29]. Myeloperoxidase (MPO) and neutrophil elastase are two other important biomarkers of neutrophil activation in asthma. Increased levels of both sputum MPO and neutrophil elastase have been reported in severe asthma associated with neutrophilic phenotype [29,30], although their serum levels seem to have limited value [31]. Tumor necrosis factor- α (TNF- α), a proinflammatory cytokine implicated in many aspects of severe refractory asthma, have also been studied in low-Th2 asthma. High serum and sputum levels of TNF- α have been reported in severe asthma, associated with neutrophilic accumulation and activation [28,32[■],33]. But the enthusiasm about the role of TNF- α as a biomarker in severe asthma has gradually faded, after the disappointing results of clinical studies with TNF- α inhibitors [34]. Finally, IL-17, a biomarker of Th17 pathway activation leading to nontype 2 inflammation, have been investigated in neutrophilic asthma. A strong correlation between IL-17 and both IL-8 and neutrophils was proven in induced sputum and blood of severe asthmatics [35–37].

In real life clinical settings, the most useful tool to identify T2-low asthma phenotype is not the presence of one of the above-mentioned biomarker but the absence of biomarkers of T2-high asthma. Exhaled NO, blood eosinophils, total IgE and serum periostin are well-studied and established biomarkers for T2-high asthma [38]. In a recent study by Busse *et al.* [39[■]], the cut-off points used to define a high level of T2 immune activation were: IgE ≥ 100 IU/ml, eosinophil count ≥ 300 /ml and $F_{ENO} \geq 30$ parts per billion. Patients were then classified as having either a high (elevation in two or more T2 biomarkers) or a low (no elevation or up to one elevated T2 biomarker level) T2 immune profile [39[■]]. This approach seems reasonable and could be used in clinical practice until a relevant T2-low biomarker is identified and validated in well-designed studies.

MECHANISMS OF T2-LOW/HIGH AIRWAY INFLAMMATION

T2-mediated airway inflammation is a central mechanism of disease in many, if not most, patients with severe allergic asthma (Fig. 1). It is generally

accepted that events occurring at the level of the bronchial epithelium (i.e., exposure to allergens) result in epithelial damage and activate the epithelial-mesenchymal unit (EMU) through major regulators such as the thymic stromal lymphopoietin (TSLP), IL-25 and IL-33 [40[■]]. This leads to increased mucosal expression of IL-4, IL-5 and IL-13 [41] and other type 2 cytokines such as osteopontin, periostin, and activin-A [42–44] that are essential for the initiation and perpetuation of the inflammatory process. Downstream events in the airways of asthmatics are orchestrated by Th2 lymphocytes and the group 2 innate lymphoid cells (ILC2s) recently acknowledged to participate in nonatopic eosinophilic inflammation [40[■],45,46], while other cells, such as eosinophils, basophils and B cells, are involved and result in eosinophilic airway inflammation and airway remodeling changes, both hallmarks of the T2-high endotype.

The mechanism that drives T2-low asthmatic inflammation, however, is currently less understood, although it has been evident from the time of Sally Wenzel's landmark study [2] that some patients with late-onset, more severe asthma have a T2-low endotype with neutrophilic, rather than eosinophilic, airway inflammation and less reversible airway obstruction. No proven pathways for this T2-low endotype exist currently, but inherent airway smooth muscle abnormalities, IL-8 and/or IL-17-mediated inflammatory processes have been proposed as possible mechanisms. Moreover, T2-low asthma is often associated with different harmful stimuli such as certain occupational exposures, smoking, and viral infections [47–49]. Severe asthma patients with predominantly neutrophilic airway inflammation exhibit a mixed Th1 and Th17 inflammatory profile with IL-8 and IL-17, respectively, having pivotal roles in driving airway inflammation in this Th2-low milieu [50–52,53[■],54]. Important sources of IL-17 in this context are not only Th17 cells but also the newly identified type 3 population of innate lymphoid cells (ILC3s) [55[■]], which have been shown in abundance in the bronchoalveolar lavage fluid (BALF) of obese individuals with severe asthma [27].

Several in-vivo and ex-vivo studies have associated low Th2-asthma with persistent bacterial inflammation and potent Th1 and Th17 responses [26,56–58,59[■]]. Moreover, IL-8 can act as a potent inducer of neutrophilic inflammation and is mainly produced by the bronchial epithelium in response to harmful stimuli under the influence of cytokines IL-17 and IL-22 by Th17 and ILC3 cells [60]. Evidence regarding the contribution of IL-8 in the molecular pathways of the T2-low endotype emerged 15 years ago via

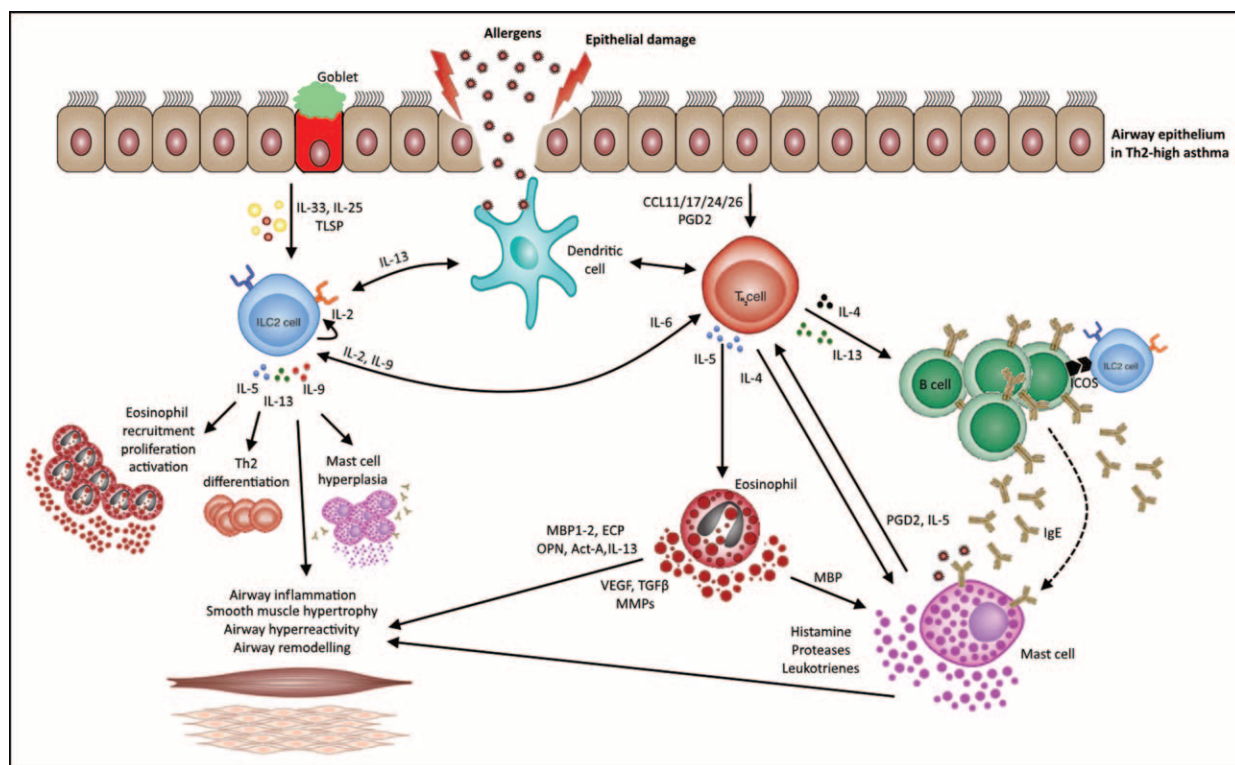


FIGURE 1. Schematic diagram of potential pathways leading to eosinophilic inflammation and airway remodeling in T2-high asthma. Recognition of allergic epitopes by dendritic cells leads to antigen presentation and differentiation of naïve T cells to Th2 cells. The production of the T2 cytokines such as IL-4 and IL-13 lead to increased IgE production by B cells while IL-5 promoted the recruitment and proliferation of eosinophils. ILC2 produce the effector cytokines IL-5 and IL-13 directly under the effects of epithelium-derived TSLP, IL-25 and IL-33 (alarmins). Both pathways ultimately drive eosinophilic airway inflammation and establish airway remodeling changes. Act-A, activin-A; CCL, C-C motif chemokine; ECP, eosinophilic cationic protein; IL, interleukin; ILC, innate lymphoid cell; MBP, major basic protein; MMP, matrix metalloproteinase; OPN, osteopontin; PGD2, prostaglandin D2; PRR, pattern recognition receptor; TGF, transforming growth factor; Th, helper T cell; TSLP, thymic stromal lymphopoietin; VEGF, vascular endothelial growth factor.

Gibson *et al.* [25]. The same group has also shown that both IL-8 type A (IL-8RA) and B (IL-8RB) receptors are increased in asthmatics with neutrophilic asthma, who also have increased systemic inflammation [29]. More importantly, they have demonstrated that asthmatic patients with evidence of increased systemic inflammation differentially express genes in the lung, including the genes for IL-8RA, and are more likely to have neutrophilic airway inflammation, suggesting that targeting systemic inflammation might be beneficial for controlling their asthma. Lastly, experimental data coming from animal models show that IL-8 exerts its aforementioned functions through an NF- κ B-dependent pathway, leading to the development of steroid-resistant neutrophilic airway inflammation [61]. Potential pathways leading to noneosinophilic inflammation in Th2-low severe asthma are depicted in Fig. 2.

MANAGEMENT OF T2-LOW ASTHMA

The lack of an effective controller medication for patients with T2-low asthma is a considerable clinical problem that currently has no obvious solution. Any treatment approach should take under consideration the prevailing features in each patient, such as the profile of airway inflammation (neutrophilic or paucigranulocytic, Th1 or Th17-high), the degree of corticosteroid insensitivity and the possible involvement of noninflammatory pathways such as bronchial hyperreactivity and airway remodeling [62²²].

An essential, but often overlooked, intervention that has a low cost and may dramatically improve asthma control in such patients is smoking cessation and avoidance of exposure to environmental/occupational pollutant agents. Smoking is a well-known factor that not only aggravates asthma symptoms and worsens asthma control

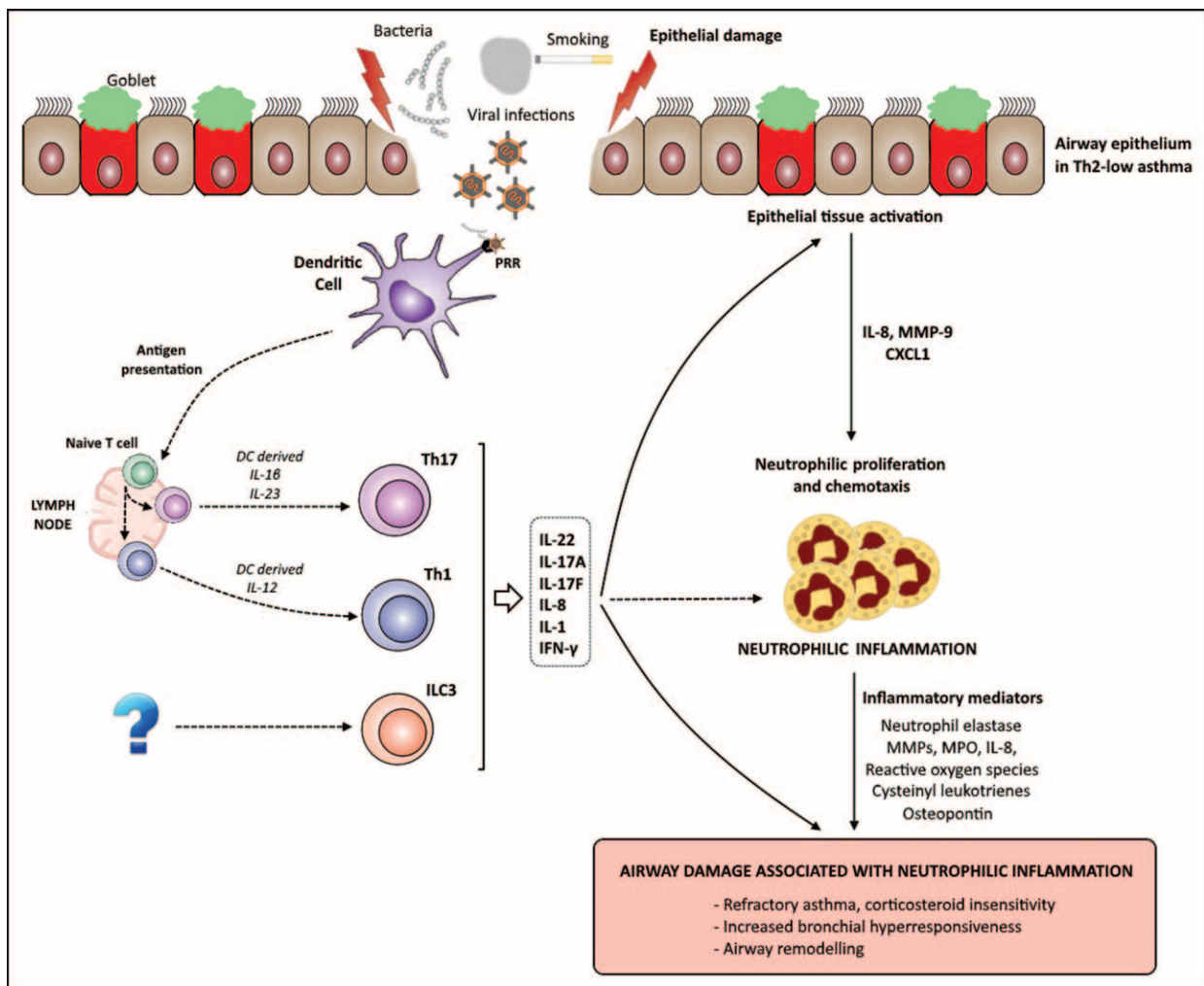


FIGURE 2. Schematic diagram of potential pathways leading to noneosinophilic inflammation in T2-low asthma. There are several inflammatory pathways as possible candidates for driving noneosinophilic airway inflammation in T2-low asthma; however, information on the exact mechanisms is still lacking. It is possible that epithelial damage inflicted by nonallergic stimuli such as bacterial and viral infections, smoking and exposure to environmental/occupational air pollutants may lead to the establishment of a delicately balanced Th1/Th17 milieu in the airways. The release of IL-17 and IL-22 from activated Th17 cells and also ILC3, whose origin is still rather vague, may stimulate the synthesis and release of pro-neutrophilic cytokines from the bronchial epithelium, such as IL-8 and CXCL1. Damaged bronchial epithelium may also be directly associated with the establishment of neutrophilic inflammation. Inflammatory mediators released by neutrophils are implicated in the induction of airway damage, airway hyperreactivity and the development of airway remodeling changes. CXCL, chemokine (C-X-C motif) ligand; IFN, interferon; IL, interleukin; ILC, innate lymphoid cell; MMP, matrix metalloproteinase; MPO, myeloperoxidase; Th, helper T cell.

[63] but is also associated with neutrophilic, difficult-to-treat asthma that is more refractory to corticosteroids and other medications [47,64]. Moreover, smoking cessation that lasts for at least 6 weeks has been shown to ameliorate neutrophilic inflammation and improve corticosteroid sensitivity and lung function [65]. On the other hand, pollutants, such as ozone and nitrogen oxide, induce airway hyperresponsiveness and neutrophilic airway inflammation and cause oxidative injury and remodeling of the airways [66,67].

Addressing neutrophilic airway inflammation with pharmacotherapy is the next logical, but challenging, step. Current treatment approaches usually fail to provide adequate asthma control. Several novel small molecule medications have been developed and are currently under investigation for the treatment of neutrophilic asthma. Such molecules include C-X-C motif chemokine receptor 2 (CXCR2) antagonists [68], 5-lipoxygenase-activating protein (FLAP) inhibitors [69], prostaglandin E3/E4 and various protein kinase inhibitors [62^{***}]. Although

some preliminary results especially for CXCR2 antagonists are promising [68], there are currently no active phase III clinical trials for the aforementioned molecules on ClinicalTrials.gov. Recent clinical trials have also tried to address other inflammatory pathways involved in T2-low asthma, such as blocking Th17 (anti-IL-17) and Th1 (anti-TNF- α) mediated airway inflammation. Although initial results in experimental models were promising [70,71], results from clinical studies were rather disappointing regarding clinical efficacy and moreover, raised concerns over increased risk of severe infections and malignancies with TNF- α receptor blocker treatment [34,72].

The 'off label' use of several drugs licensed for treating medical conditions other than asthma has also been the subject of investigation regarding their anti-inflammatory effects in patients with noneosinophilic T2-low asthma, such as macrolides, statins, and vitamin-D3 [62²²,73,74]. Data regarding their clinical efficacy are still preliminary; however, regarding the use of azithromycin, a significant reduction in exacerbations in noneosinophilic patients has been reported recently in an interesting study by Brusselle *et al.* [73]. Another clinical trial from Australia assessing the effect of low-dose azithromycin in over 300 severe asthmatics, the AMAZES (Asthma and Macrolides: Azithromycin Efficacy and Safety) study, has recently been completed and the results are underway.

Other treatment approaches currently available for T2-low asthma address noninflammatory pathways possibly involved in its pathogenesis. These medications/interventions may be considered for both T2-high or -low severe asthma. Tiotropium has been recently included as a new add-on treatment for Steps 4 and 5 in patients aged ≥ 18 years with a history of exacerbations. Its clinical efficacy has been demonstrated in patients with fixed airway obstruction, an asthma phenotype usually associated with neutrophilic airway inflammation [75]. Finally, bronchial thermoplasty, a new bronchoscopic technique to reduce airway smooth muscle mass, has also been found to improve symptom and reduce exacerbations in patients with severe uncontrolled asthma and chronic airflow obstruction phenotype [76,77].

CONCLUSION

In the past few years, we have come to the realization that asthma, especially severe asthma, is a vastly heterogeneous disease with many endotypes. We now know that inflammation in asthma is often not characterized by eosinophilia and T2 type cytokines but may in fact be T2 low or negative.

Although information about the pathways of T2-low asthma are not well understood, several data point to Th1/Th17 pathway activation, innate immune defects, tissue remodeling and neurogenic inflammation as possible mechanisms. As a result, a clear definition and specific biomarkers for T2-low asthma that would allow us to identify specific pathways and provide targeted treatment are missing. Current therapeutic approaches addressing this subgroup of patients have limited efficacy, and these patients have poor disease control and impaired quality of life. No single medication will be effective for all patients, but some treatments might be very effective in selected patients who are carefully classified. Further studies in this specific population are needed to elucidate the pathogenetic mechanisms of T2-low asthma and to develop more effective therapies.

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Conflicts of interest

K.S. has received speaker fees from Novartis and conference travel grants from Novartis, GSK and Elpen. E.Z. has received speaker fees and conference travel grants from pharmaceutical companies, including AstraZeneca, GSK, Elpen, Boehringer Ingelheim, Takeda, Novartis, Pfizer, UCB, Chiezi, Menarini, Roche, MSD and Schering-Plough. E.Z. has also received payment for consulting and advisory board involvement from AstraZeneca, GSK, Elpen, Novartis and Menarini. M.G. has received speaker fees and conference travel grants from pharmaceutical companies, including AstraZeneca, GSK, Elpen, Boehringer Ingelheim, Novartis, Pfizer, UCB, Chiezi, Menarini, Roche, MSD and Schering-Plough. M.G. has also received payment for consulting and advisory board involvement from AstraZeneca, GSK, Novartis and Boehringer Ingelheim.

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