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The diverse roles of T cell subsets in asthma

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Summary

T cells are coordinators of the immune response and have been shown to play a central role in the pathophysiology of asthma. A good understanding of the T cells functions in asthma is important for therapeutic reasons, in particular for the choice of biological treatments in severe asthma. Although classically considered a Th2 disease, it is now clear that other types of T cells contribute for the pathophysiology and the heterogeneity of asthma. We here review how the different subsets of T cells are involved in the different phenotypes/endotypes of asthma and how this may influence the treatment of the disease.

Impact statement

This review highlights the heterogenity of ashtma and the significant role of other types of T-cells aside from Th2 (including unconventional subsets) in its development and symptoms.

Abbreviations
IL: *Interleukin*
TNF: *Tumour Necrosis Factor*
IFN: *Interferon*
Th: *T-helper*
TSLP: *Thymic Stromal Lymphopoietin*
FDA: *Food and Drug Administration of the United States of America*
EMA: *European Medicines Agency of the European Union*
FeNO: *Fractional exhaled Nitric Oxide*

Introduction

Asthma is a heterogeneous disease. Several distinct clinical phenotypes have been described and it is now debated whether asthma is a "single disease" or, instead, a group of inflammatory lung diseases with similar manifestations but diverse pathophysiology. More recently, these underlying inflammation mechanisms have been used to classify asthma into "endotypes" (1). Although several markers have been proposed to distinguish specific asthma endotypes (*e.g.*, IgE sensitization in allergic asthma, peripheral eosinophil counts in eosinophilic asthma, periostin in Th2-high asthma, *etc.*), the most consensual distinction is based on the $CD4^+$ T-helper (Th) cytokine profiles involved (*i.e.*, Th2-high *vs* Th2-low), therefore illustrating the central role of these cells in asthma.

T cells are central coordinators of the immune response. T-cell receptor (TCR) recognition is crucial for the initiation and specificity of the adaptive immune response, while T cell dervied cytokines tailor the type of immune response. Asthma is an inflammatory lung disease and T cells have been shown to play a central

role in the pathophysiology of the disease. A good understanding of T cells in asthma is also important for therapeutic reasons, in particular for the choice of biological treatments in severe asthma. T cells are generally divided into two major types: $CD4^+$ T cells and $CD8^+$ T cells. $CD4^+$ T cell are activated by antigen presented by major histocompatibility complex (MHC) class II on the surface of professional antigen-presenting cells (APC), whereas $CD8^+$ T cells recognize antigens presented by MHC class I on the surface of all nucleated cells. As discussed below, each of these types can be subdivided into several functionally distinct subtypes.

Classically, asthma has been considered a Th2-mediated inflammatory disease. However, more recent studies have identified contributing roles for other types of T cells in the pathophysiology and the heterogeneity of the disease. In this chapter, we review how the different types and subtypes of T cells are involved in asthma and its phenotypes/endotypes.

Types of T cells and their roles in asthma

αβ $CD4^+$ T cells

Also termed "helper T cells" (or Th cells), αβ $CD4^+$ T cells have the capacity to produce high quantities of cytokines and influence a variety of immune cells to determine effector immune responses. Naïve Th cells (Th0) are produced in the thymus without a defined cytokine profile. Matured dendritic cells (DCs) present antigens in MHC class II to naïve Th0 cells that bear TCRs specific for the antigen and, together with co-stimulation (*e.g.*, CD80, CD86, OX40L) and cytokines, stimulate T cell proliferation and differentiation into effector Th polarizations (defined by the major profile of cytokine production). From then on, Th cells may behave to promote inflammation (*e.g.*, Th1, Th2, Th17, *etc.*) or regulation (*e.g.*, Tr1, $Foxp3^+$ induced-Tregs). It is now over 30 years since the original Th1 *vs*. Th2 functional distinction of $CD4^+$ T cell subsets was published by Mosmann (2, 3). Th1 cells are polarized by IL-12, express the transcription factors t-bet and STAT4, and produce high amounts of IFN-γ and IL-2 to stimulate macrophages, $CD8^+$ T cells, IgG B cells, and IFN-γ $CD4^+$ T cells to combat intracellular bacteria and protozoa. On the other hand, Th2 cells are induced, upon activation, by the presence of IL-4 and IL-2, and the key transcription factors for the Th2 program are STAT6 and GATA3. Th2 cells produce high amounts of IL-3, IL-4, IL-5, IL-9, IL-13 and GM-CSF (encoded in the IL-4 gene cluster on chromosome 5q31) and stimulate eosinophils, basophils, and mast cells as well as B cells, and the humoral adaptive response (antibody production), typically against extracellular pathogens.

Th2 cells

Ever since Mosmann (2), the functional Th1/Th2 divide has been used to classify inflammatory diseases according to the predominant type of cells and/or cytokines. The majority of asthma patients are atopic and have an allergic pattern of inflammation in their airways. Th2 cells were identified as the primary cells involved in allergic asthma more than 25 years ago (4). Mice deficient in t-bet (the Th1 master-regulator transcription factor) develop spontaneous allergic/Th2/eosinophilic airway inflammation and hyperreactivity (5), while knocking-down GATA-3 (the Th2 transcription factor) prevented airway hyperreactivity and inflammation (6). Many other compelling data in humans and mice have demonstrated the central role of Th2 cells in allergic asthma (7, 8). Th2 cytokines are main drivers of allergic inflammation in atopic asthma (8) and have been associated with the activity of disease, symptom scores, airway eosinophilia, and bronchial hyperresponsiveness (7). IL-4 upregulates the Th2 transcription factor GATA-3 in naïve T helper cells and is therefore essential for Th0 to Th2 differentiation and the production of IL-5, IL-9, IL-13, CCL17 and eotaxin (a pivotal chemokine in eosinophil recruitment). IL-4 also increases B cell proliferation and differentiation to plasma cells, and it increases antibody production and isotype class-switch to IgE. IL-5 also helps B cells differentiation and isotype class switch to IgE acts, promotes basophil differentiation and histamine release, and it is a crucial cytokine in eosinophils function (promoting the production, differentiation, recruitment, activation and survival of these cells). IL-13 shares some functions with IL-4, but it also stimulates goblet cell hyperplasia and mucus production, and increases smooth muscle contractility and bronchial hyperreactivity. It is noteworthy that Th2 cells are involved also in some non-atopic asthma patients (9). Many patients with high eosinophil count in sputum and blood do not show increased total IgE or allergen-specific IgE. This subset of asthma has been termed "eosinophilic" asthma and is also believed to be Th2-driven (10). Several monoclonal antibodies targeting Th2 cytokines have shown great benefit in severe asthma, both allergic and/or "eosinophilic", once more demonstrating the importance of Th2 cells and their cytokines in the physiopathology of these asthma endotypes. Currently, there are 5 monoclonal antibodies approved by the European Medicines Agency of the European Union, (EMA) and the Food and Drug Administration of the United States of America (FDA) for the human treatment of Th2-mediated asthma:

1. Omalizumab targets the Cε3 domain of free IgE and prevents IgE interaction with the high-affinity receptor (FcεRI) on mast cells, basophils, eosinophils, Langerhans cells and dendritic cells. Although not directly targeting Th2 cells or cytokines, it is well recognized that IgE production is a result of Th2 activation and IgE-mediated degranulation and activation of mast cells and basophils is responsible for late phase Th2-type inflammation. Omalizumab was approved for the treatment of moderate-to-severe allergic asthma by the FDA in 2003 and the EMA in 2005;

2. Mepolizumab and Reslizumab directly bind to and neutralize the cytokine IL-5, limiting its availability for eosinophils and other cells. Mepolizumab received approvals for severe refractory eosinophilic asthma by the FDA and EMA in 2015, while reslizumab was approved for similar indications by the FDA and EMA in 2016;
3. Benralizumab binds to the α-subunit of the IL-5 receptor (IL-5Rα) targeting the cells that express it for destruction by antibody dependent cell cytotoxicity. Benralizumab received approvals by FDA in 2017 and EMA in 2018 for severe eosinophilic asthma;
4. Dupilumab targets IL-4Rα, therefore inhibiting both IL-4 and IL-13 signaling. It was FDA and EMA approved in 2019 for asthma with type 2 inflammation (characterized by raised blood eosinophils and/or raised FeNO).

It should be borne in mind Th2 cytokines are produced by conventional αβ $CD4^+$ T cells but also other T cells, such as "unconventional" T cells (*i.e.*, iNKT or γδ T cells, as discussed below), and even from other non-T immune cells.

The interplay between the bronchial tissue and the adaptive immune system in asthma has been the focus of recent interest. Several cytokines produced by the tissue may play a role in the initiation and amplification of Th2 inflammation in asthma, among them thymic stromal lymphopoietin (TSLP), IL-25, IL-33, and osteopontin (11, 12). TSLP is produced by epithelial cells in response to proinflammatory stimuli (such as allergens, pollutants, infections, *etc.*) and acts on dendritic cells to upregulate surface OX40 ligand to promote Th2 differentiation (13). TSLP is increased in the airways of patients with asthma and correlates with the severity of disease. IL-25 is produced by tissue eosinophils and basophils and synergizes with TSLP to increase GATA-3 expression in memory Th2 cells stimulating cell proliferation and cytokine production (14). IL-25 may also act on innate cells to stimulate the production of Th2 cytokines independently of T cells (*e.g.*, nuocytes (15)). Also, IL-33 is produced by airway epithelial cells, fibroblasts and smooth muscle and induces dendritic cells production of IL-5 and IL-13, therefore stimulating Th2 polarization. IL-33 expression is also increased in the airways of patients with asthma (16).

The clinical importance of these tissue-derived Th2-stimulating cytokines in asthma has also been explored using blocking monoclonal antibodies *in vivo*. Two phase II trials with the monoclonal antibody tezepelumab/AMG 157, a human IgG2λ mAB targeting TSLP, showed promising results in severe asthma: reducing the levels of blood and sputum eosinophils, FeNO and allergen-induced bronchoconstriction (17), and reducing the annualized asthma exacerbation rates by 61-71%, regardless of blood eosinophil levels at enrolment (18). Two phase III trials with tezepelumab are currently ongoing.

Th1 and Th17 cells

Importantly, not all patients with asthma present high levels of Th2 cytokines, local or systemic eosinophilia, or allergic sensitization. These patients usually present a predominance of neutrophils over eosinophils in the airway; therefore, this subset of asthma has been designated "neutrophilic" or "intrinsic", and more recently "non-Th2" or Th2-low". In "non-Th2" asthmatics, disease symptoms tend to initiate later in life, present a more severe clinical evolution, worse response to corticosteroids, and less reversible airway obstruction. It is believed to be mediated by Th1 and Th17 cells (19).

IFN-γ-producing Th1 cells have been found among the bronchoalveolar lavage (BAL) T cells in asthma patients (20). Also, Th2-independent triggers, such as viruses, air pollution, and exercise, can induce and/or exacerbate asthma symptoms in some patients, and the serum levels of IFN-γ increase during acute asthma exacerbations. Th1 may be involved also in children with severe asthma, in which memory $CCR5^+$ Th1 cells were shown to be the most significant type of $CD4^+$ T cell in the bronchoalveolar lavage (BAL), together with Th17 cells and IFN-γ^+ IL-17^+ double positive cells, independently of the allergic status (21). The therapeutic effect of IFN-γ blockade in asthma has not been assessed in humans so far.

Th17 cells are induced by a combination of TGF-β and IL-6, leading to the expression of the transcription factor RORγT, and are characterized by the production of IL-17A, IL-17F and IL-22. These cytokines can induce epithelial cells, endothelial cells, fibroblasts, neutrophils and eosinophils to produce IL-6, GM-CSF, CXCL10 and CXCL8, therefore stimulating "neutrophilic inflammation".

Th17 cells are known to regulate neutrophilic and macrophage inflammation in autoimmune diseases and, more recently, have been implicated in neutrophilic airway inflammation (22), in asthma, and in corticosteroid insensitivity (23). Others have found that IL-17A and IL-17F in asthma did not correlate with neutrophilic inflammation (24). Circulating Th17 cell numbers and IL-17 plasma concentrations are increased in asthmatic patients and correlate with disease severity. In mouse models of severe asthma, specific roles for Th1 cytokines (IFN-γ gene knockout) were demonstrated in airway hyperresponsiveness (AHR) but not in airway inflammation while IL-17RA knock-out mice developed AHR but airway inflammation was lower (25).

In allergic asthma, the role of Th17 cells has not been fully elucidated and contradictory results were found in mouse models. IL-17 may play a dual role: it seems to be essential during allergen sensitization (26), but later, once sensitization is established, IL-17 attenuates the allergic response and may reduce asthma chronic manifestations (26). More recently, it was described in humans a novel subset of $CD4^+$ T cells with memory and effector phenotypes that, simultaneously, expresses both GATA3 and RORγT, and produces both Th2 and Th17 typical cytokines (27). These cells showed increased numbers in the circulation of patients with bronchial asthma and may play some role in the

pathogenesis of this disease. These dual-positive Th2/Th17 cells were found also in the BAL and may characterize a population of patients with severe asthma (28).

Despite these relevant functions of IL-17 and Th17 cells in the pathophysiology of asthma (it should be noted that neutrophil-stimulating factors may also be produced by the epithelium), the results of clinical trials with monoclonal antibodies targeting the IL-17 pathway produced disappointing results, and the development of these drugs for asthma has largely been abandoned (although approved and commercialized for psoriasis, for example): brodalumab, a human anti–IL-17RA immunoglobulin G2 (IgG2) monoclonal antibody reached phase II trials, but did not produce significant effects in asthma, except for a group of patients with high-reversibility (29); also secukinumab, an anti-IL17A monoclonal antibody, was discontinued for asthma. Considering the heterogenous pathophysiology (endotypes) of asthma patients, further studies may try and identify particular groups of asthma patients better responding to IL-17 blockade (30). Currently, some phase II trials with monoclonal antibodies targeting Th17 cytokines are under development (*e.g.*, anti-Interleukin-23 subunit p19 inhibitor, anti-IL-17A).

Th9 cells

Th9 cells are induced by the concomitant presence of IL-4 and TGF-β during $CD4^+$ T cell activation and are characterized by the production of high amounts of IL-9 (31). IL-9 enhances Th2 cytokine production, airway mucus production, and eosinophil, basophil and mast cell differentiation, among other functions. Although STAT6, IRF4 and BATF are required for IL-9 production, no lineage specific transcription factor has been identified as a "master regulator" of the Th9 phenotype.

The expression of IL-9 and IL-9 receptor is increased in bronchial biopsies of patients with atopic asthma (32). Th9-related genes (for example, *IL4RA*, *STAT6*, *IL9*, *IL9R*, *SMAD3*, *IL33*, *IL1RL1*) have been linked to the development of asthma in humans, and allergic patients have elevated IL-9 producing T cell numbers (31). Several experiments in mouse models indicate that Th9 cells are critical to generate allergic lung inflammation (32). Taking these data into consideration, enokizumab (also known as MEDI-528), a humanized IL-9 neutralizing antibody, was tested in asthmatic patients. Preliminary studies with Enokizumab showed acceptable safety profile and findings suggestive of clinical efficacy in adult patients with mild to moderate asthma (33). However, another study with Enokizumab in adult patients with uncontrolled moderate-to-severe asthma failed to show any benefits in quality of life, asthma exacerbation rates, or FEV1 values (34), and the drug development for asthma was discontinued.

Follicular helper T cells

Follicular helper T cells (Tfh) are an independent subset of $CD4^+$ T cells that is specialized in helping B cells. Tfh are characterized by the expression of CXCR5, PD-1, BCL6, BTLA4, ICOS and SAP. Bcl6 is a transcription factor required for Tfh cell differentiation and the absence of Bcl6 expression on T cells impedes germinal center formation and B cell responses to protein antigens (35, 36). Tfh may be a particular differentiation of $CD4^+$ T cells that is plastically acquired when these cells enter the germinal centre (35). Tfh cells were shown to be essential for IgE production (37) and also to be required for the development of allergen specific sensitization and allergic asthma (38). These murine studies proposed that IL-4-committed Tfh cells were the precursors to pathogenic Th2 cells in allergic airway disease(38). Also in humans, Tfh cell numbers correlated with the total IgE blood level, and Tfh cells from asthma patients could stimulate IgE production *ex-vivo* in an IL-4-dependent manner (39).

Regulatory $CD4^+$ T cells

Regulatory T cells (Tregs) are a subset of $CD4^+$ T cells that suppress or modulate other immune cells to prevent autoimmunity, uncontrolled inflammation, and also allergy. "Natural" $CD4^+$ $CD25^+$ Tregs (nTregs) are produced in the thymus, express the transcription factor Foxp3, and their TCRs have intermediate affinities for self-antigens, allowing them to prevent autoimmunity. Peripherally "induced" Treg cells (iTreg) can be differentiated from naïve Th0 cells of any specificity and are believed to be important for tolerance induction to non-self-antigens, including allergens. Two major groups of iTregs are considered: $Foxp3^+$ iTregs (induced by TGF-β) and Tr1 cells (induced by IL-10).

Early studies have shown that depletion of $CD4^+CD25^+$ Tregs (here including nTregs and iTregs) results in increased neutrophil and T cell recruitment in the airways of the mice, increased IL-4 and IL-5 production, and airway hyperreactivity (40). Importantly, Foxp3 deficiency in humans (IPEX syndrome) and mice (Scurfy) causes rapidly fatal immune dysregulation that includes autoimmunity but also allergic manifestations (41). Adoptive cell transfer studies in mice showed that also Tr1 cells can inhibit Th2 and IgE responses *in vivo* (42).

Altogether, Tregs seem to have important roles in allergy and asthma development (these roles have been reviewed elsewhere (7, 43-46)). Tregs may be an important target also for therapeutic reasons. As described before, a great effort has been placed in understanding and targeting IgE, Th2 cytokines and eosinophils to control asthma inflammation. Comparatively, therapeutic strategies eliciting Tregs and immune tolerance induction to control asthma inflammation have been relatively scarce and remain far from clinical use (7). This possibility of inducing Tregs and tolerance for asthma treatment was shown in mouse models of allergic asthma using CD4 co-receptor blockade: antigen-specific tolerance was achieved and it could prevent airway hyperreactivity and eosinophilia, while at the same time maintaining immune competence to mount Th2 responses to unrelated antigens (47).

αβ CD8⁺ T cells

While the fundamental role of $CD4^+$ T cells in the physiopathology of asthma is undisputed, there is recent evidence indicating that also $CD8^+$ T cells may participate, either as a direct intervenient or as helpers for $CD4^+$ T cells (48). Clinical studies examining a potential role for $CD8^+$ T cells in asthma hinted to a positive correlation with disease severity (49, 50). The decline of FEV1, even if mild, correlated, both at baseline and follow-up, with the number of $CD8^+$ T cells in the airways (49). Also wheezy infants have elevated numbers of $CD8^+$ T cells in bronchoalveolar lavage (BAL) in comparison to controls, even in the absence of viral infections (51). The specific functions $CD8^+$ T cells in the airways are, however, incompletely understood.

Similar to $CD4^+$ T cells, $CD8^+$ T cells can be polarized into functional subtypes depending on environmental stimuli: Tc1 cells that produce IFN-γ and Tc2 cells producing IL-4, IL-5 and IL-13. A vast array of other subtypes have been proposed, such as, Tc9, Tc17, γδ $CD8^+$ cells and also $CD8^+$ regulatory T cells (52, 53).

Tc1 cells may function both as "friend or foe" in asthma physiopathology. These cells play a major role in defending against viral infections (mainly rhinovirus) with the production of IFN-γ, therefore reducing some viral induced asthma exacerbations. On the other hand, Tc1 cells may suppress or exacerbate pulmonary inflammatory response to allergens, depending on the temporal relationship with the progression of allergic sensitization: in mice models, depletion of Tc1-cells prior to systemic ovalbumin sensitization reduced airway hyperresponsiveness (AHR), lung eosinophilic infiltration and IL-5 production at the lymph nodes of the airway (52). Conversely, when cell depletion occurred subsequent to the initial allergen sensitization, the inflammatory responses were potentiated (54, 55). Both Tc1 and Tc2-cells were linked to suppression of allergic asthma and indirect inhibition of IgE production by stimulation of Th0 differentiation into Th1 cells and subsequent production of IFN-γ (56).

Tc2 cells have been found to exacerbate asthma by secretion of high levels of IL-4, IL-5 and IL-13, in a similar manner to Th2 cells (57). The activity of these cells seems to be more focused in the lung tissue in opposition to lymph nodes (58). In opposition to Tc1, Tc2 and $CD8^+$ Tregs, the subtypes Tc9 and Tc17 cells have low cytotoxic activity (59). When combined with Th2 cells, Tc9 induced key features of asthma, such as increased eosinophil numbers in BAL and elevated lung inflammatory score (48). Tc17 produce IL-17, which was shown to be proinflammatory in pulmonary pathology (60). There is a growing body of evidence to suggest that γδ $CD8^+$ cells may be influential in the regulation of airway inflammation by reducing late-phase AHR and airway eosinophilia via an IFN-γ dependent pathway (61). The role of NK-like $CD8^+$ T cells has been reviewed elsewhere (62).

In conclusion, even though $CD8^+$ T cells appear to play a significant role in asthma, the available evidence is conflicting. There are convincing studies that support their beneficial influence during the initial sensitization to the allergen. On the other hand, it has been suggested that these cells may help propagate the chronic inflammation associated with asthma. The better understanding of the subset diversification of $CD8^+$ T cells will be essential in establishing their functional roles in asthma.

γδ T cells

γδ T cells are a subset of $CD4^-CD8^-$ T cells that express an alternative TCR, with γ and δ chains, as opposed to the classical αβ TCR found on most $CD4^+$ and $CD8^+$ T cells (63). γδ T cells represent a small proportion of peripheral blood T cells (less than 10%) but comprise up to 50% of the T cells within epithelium or mucosa-rich tissues, and these are often composed by oligoclonal subpopulations sharing the same TCR chains. γδ T cells were shown to be involved in several mucosal-related pathologies, namely in the gut. In striking contrast to MHC-restricted αβ T cells, γδ T cells recognize a range of antigens without the presence of MHC molecules. Antigens recognized by these cells are largely unknown, but may include phosphorylated microbial metabolites, markers of cellular stress, and also lipid antigens presented by CD1 molecules, in particular CD1d (63).

γδ T cells expressing Th2-type cytokines were reported in BAL fluid after allergen challenge of asthmatic patients. Also, during exacerbations, asthma patients showed increased proportion of γδ T cells and, *ex-vivo*, these cells had increased expression of intracellular TNF-α, IL-4 and IL-10 after stimulation in asthmatic *vs* controls (64). On the other hand, early studies using murine models have shown that airway reactivity is increased in the absence of γδ T cells (65), suggesting a suppressive role of these cells in airway hyperreactivity. In humans, γδ T cell numbers are greater in asthmatic airways during rhinovirus infection and correlate with clinical illness severity, virus load, and airways inflammation (66).

It is important to note that, similar to αβ T cells, γδ T cells are capable of assuming different cytokine production profiles (63) and therefore may play different and opposing roles in asthma, some of which may have contributed to the inconsistent findings on γδ T cells in asthma studies. Also, the localization and TCR specificity may influence γδ T cell roles (46).

Invariant NKT cells

NKT cells are a subset of T cells that coexpress an αβ T cell receptor but also a variety of molecular markers that are typically associated with NK cells, such as NK1.1. NKT cells possess an invariant Va24Ja18 TCR and therefore present restricted variability (and are, therefore, also called invariant NKT cells). The most important ligands for NKT cells are glycolipids, in particular α-galactosylceramide (α-GalCer), which is exclusively presented by the MHC class I-like molecule CD1d. NKT cells are capable of producing high amounts of several cytokines upon

activation, with similar variety of cytokines to conventional CD4+ T cells, including IFN-γ but also IL-4, IL-5 and IL-13. The role of NKT cells in allergy and asthma is still controversial (reviewed in (67)). Some studies suggested a suppressive role of NKT, *e.g.*, α-GalCer-stimulated NKT cells suppressed allergic inflammation of the airways by promoting IFN-γ production (68). However, others have found a high proportion of NKT cells among the CD4+ T cells in the lung of asthmatic patients (69) (a finding that has subsequently been disputed (70)) and several roles for NKT cells in asthma were described (71). Interestingly, mouse studies have shown that CD1d deficiency results in a selective deletion of NKT cells and prevents allergic airway disease (69), and CD1d antagonists prevented the development of allergen-induced airway hyperreactivity (72).

Conclusions

Classically, asthma has been considered a Th2 disease. The role of Th2 cells and Th2 associated cytokines is undisputed in the pathophysiology of asthma, in particular in allergic asthma. Therapeutic agents targeting Th2 cytokines and cells have shown great benefits in patients with moderate-to-severe Th2-high asthma. However, it is now clear that asthma is a heterogeneous disease. Furthermore, the division of asthma into only two clinical forms (Th2-high *vs* Th2-low) is probably an oversimplification. Other types of T cells were shown to contribute to asthma development and symptoms, including some unconventional T cells. It is important to remember that a certain amount of plasticity occurs between T cell polarizations and multiple cytokines can be produced in response to a diversity of stimuli by the same cells. Therefore, it is perhaps not surprising that some T cells that do not adhere to conventional types can be found in the lungs of asthmatic patients. Finally, no targeted therapies have been found to be efficacious for Th2-low asthma. This may indicate that the absence of Th2 markers does not characterize a "unified" non-Th2 endotype.

Conflict of interests

Frederico S. Regateiro received speaker and consultancy fees from AstraZeneca, Novartis, TEVA, Sanofi and Lusomedicamenta, all of which outside the submitted work.

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