Review

The Th2/Th17 pathway in asthma and the relevant clinical significance

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Key words:
- Th2/Th17
- Asthma
- Phenotypes
- Biomarkers
- Treatment

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SUMMARY

Asthma is a heterogeneous chronic disease of the airways, characterized by different phenotypes. The principal pathophysiological pathway appears to be Th2 dependent eosinophilic inflammation mainly produced by T helper 2 (Th2) cells. More recently epithelial innate lymphoid cells (ILC2) cell shave been implicated as another source of Th2 cytokines leading to bronchial eosinophilia without previous allergen sensitization. Another pathogenic pathway is the non-Th2 type, mediated by Th1 and especially Th17 lymphocytes, responsible for neutrophilicin flammation. Furthermore, recent studies have associated Th-17 cells with allergic inflammation and eosinophilic asthma. Ongoing clinical trials are expected to further elucidate the role of different cells in the evolution of asthmatic inflammation and also the role of established or novel potential biomarkers in routine clinical practice aiming to maximize drug efficacy in asthmatics. In the present review, we summarize the above mentioned mechanisms focusing on T-helper cell subset plasticity which led to the identification of dual positive Th2/Th17 inflammation. ***Pneumon 2018, 31(3):174-182.***

INTRODUCTION

Asthma is a heterogeneous disease of the airways characterized by airway inflammation and bronchial hyperresponsiveness (BHR) leading to reversible airway obstruction[1]. This chronic disease affects many people, men and women, young and old, worldwide. It is defined by a history of respiratory symptoms (such as wheeze, shortness of breath, chest tightness and cough), which vary over time and in intensity[2]. The majority of asthma patients are well controlled by conventional therapies such as inhaled corticosteroids. However, about 5-10% of asthma patients have a severe and complex condition, described as "fatal or near fatal asthma", "severe asthma", "steroid-dependent asthma", "steroid-insensitive asthma", "difficult to control asthma", "poorly controlled asthma", "brittle asthma", or "irreversible asthma"[3].

For over 20 years, asthma has been considered a Th2-type dependent allergic disease, characterized by Th2 cells producing high levels of type 2 interleukins (ILs), such as IL-4, IL-5 and IL-13. Besides, other studies suggested that Th-1 cells producing interferon (IFN)-γ display a regulatory function in allergic asthma[4]. Although the Th-1/Th2 mechanism provided the initial framework for asthma management, the discovery of a distinct subpopulation of CD4+ T cells that produce IL-17A, IL-17F, IL-22, TNF-α, and IL-21 led to a major revision of the Th-1/Th2 hypothesis (Figure 1). Th17 cells are differentiated and activated by several cytokines such as transforming growth factor TGFβ, IL-6 together with IL-21 and IL-23[5].

In addition to Th2 and Th17 cells, the heterogeneity of asthmatic patients suggests that also other factors must be involved in regulating asthma inflammation. Indeed, recent studies have implicated innate lymphoid cells (ILCs) of non-T, non-B effector cells that are antigen-nonspecific, have conserved effector cell functions and play crucial roles in tissue homeostasis, repair and remodeling and in innate immunity to pathogenic and nonpathogenic microorganisms[6]. ILCs are classified into three categories (Type 1, Type 2 and Type 3 ILCs) depending on their ability to produce Th1, Th2 and Th17 cell-associated cytokines[6]. In specific, ILC type 2 (ILC2) have been associated with asthma by producing a broad array of cytokines, including IL-5, IL-13 and IL-17[7].

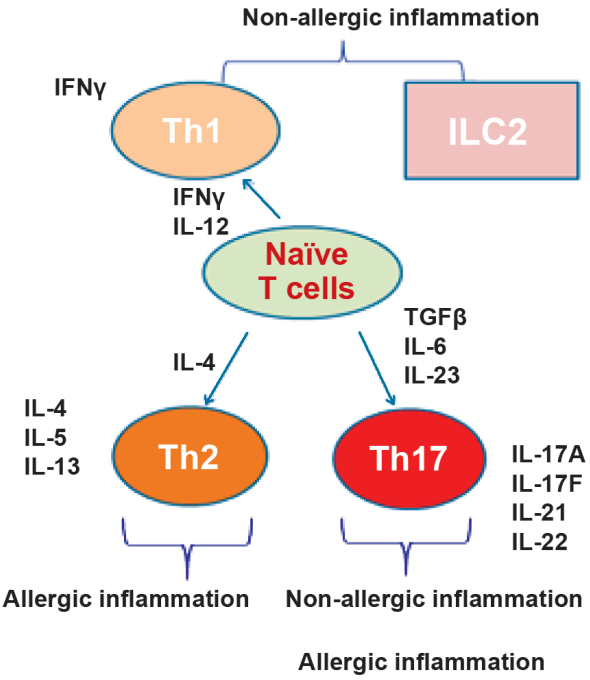

FIGURE 1. Type 2 Innate Lymphoid Cells and Th cell subsets involved in allergic and non-allergic inflammation in asthma.

Asthma was initially categorized in terms of 'allergic' or "nonallergic" asthma. A distinction was then made when sputum became available between eosinophilic and non-eosinophilic asthma[2]. The last decade, a global approach for the understanding of asthma pathogenesis has introduced the concept of phenotypes as a grouping of clinical/physiologic characteristics, triggering factors and inflammatory components[2]. A new approach includes the addition of genetic or blood biomarker for the classification of disease entities within the asthma syndrome which led to the introduction of the term endotype[3]. Indeed, asthma endotyping has shed light into key pathogenic mechanisms for this complex disorder[8].

Recent discoveries revealed possible subgroups of Th2 high asthma that differ in terms of both the presence of underlying allergy and the potential source of type 2 cytokines. The current concept involves Th2-high asthma, eosinophilic, characterized by high levels of type 2 interleukins (ILs), and involves type 2 helper T cells (Th2 cells), mast cells, basophils, B cells and ILC2s[4,8,9].The fact that ILC2 produce Th2 cytokines could explain severe eosinophilic inflammation, when classical Th2 mediated allergy is absent, which is further supported in other studies[10].

On the other hand lays Th2-low/Non-Type 2 as non eosinophilic asthma, where Th17 cells are involved (IL-17A, IL-23, IL-22, IL-6), mostly characterized by neutrophilic inflammation[11]. An interesting issue for Th2 and Th17 cells is the qualitative difference concerning their response to glucocorticoid treatment, as IL-17 production was shown to be less susceptible to inhibition by glucocorticoids when compared to IL-4 and IL-5 production[12].

According to recent literature possible endotypes associated with eosinophilic phenotypes include, early onset allergic asthma with or without obesity, aspirin sensitive asthma and late onset eosinophilic asthma, exacerbation prone asthma, and exercise induced asthma,whereas those associated with non-eosinophilic asthma and for which the pathobiologic pathways are not yet defined, include those patients with obesity-related late onset asthma, asthma with fixed airflow obstruction and very little inflammation (paucigranulocytic), and asthma associated with neutrophilia[11].

BIOMARKERS IN ASTHMA

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of

normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention"[9]. Cytokines play a significant role in the pathogenesis and chronic inflammation of the airways in asthma and therefore, interleukins as biomarkers could identify endotypes and subtypes of asthma, reflecting the predominant pathophysiological mechanism[13]. The use of biomarkers can potentially help avoid unnecessary morbidity from high-dose corticosteroid therapy, and allow the most appropriate and cost-effective use of targeted therapies[14]. As cytokines include markers of inflammation, several studies have been trying to identify molecular endotypes based on serum cytokine profile[15]. However, the persistent airway inflammation in asthma is caused by a large variety of cytokines that recruit, activate and perpetuate the inflammatory cells in the asthmatic airways. As a result, several attempts to inhibit inflammatory cytokines in asthma with blocking antibodies have shown poor results so far and besides studies of targeted therapy in non-phenotyped asthma did not show profound or even any efficacy[16]. Furthermore, it is a fact that there is no consensus how to best identify asthma endotypes and what therapy to use for a given endotype. Apart from cytokine profile, the use of blood eosinophils can facilitate individualized treatment and management of asthma.

Th2-specific biomarkers identified so far include sputum/blood eosinophils, total serum IgE, the fraction of exhaled nitric oxide (FeNO) and bronchial epithelium-derived proteins such as periostin and DPP4[11]. The first biomarker proposed to predict corticosteroid response was the eosinophil count in sputum and blood[17]. Furthermore, total serum IgE is used specifically to identify allergic asthma phenotype[2]. However, IgE has a low sensitivity and correlates poorly with eosinophilic inflammation[18]. FeNO is also a biomarker of Th2 inflammation[19] and is suggested to be used as a predictor of steroid responsiveness more consistently than other parameters[20]. According to recent literature, in patients with mild to severe asthma, blood eosinophils have the highest accuracy in the identification of sputum eosinophilia when compared to serum periostin and exhaled nitric oxide (FeNO)[21]. Indeed, serum periostin, exhaled nitric oxide and blood eosinophil counts are the most promising biomarkers until now that could identify patients patients most likely to derive benefit from biologic agents targeting IgE, IL-5 and IL-4/13[22].

The combined evaluation of FeNO and peripheral blood eosinophil counts represent two significant biomarkers of asthma, comparable to sputum eosinophil count and are often used to distinguish asthma phenotypes and even identify responders to inhaled corticosteroid treatment[2,23]. An count of >400 eosinophils/μL is associated with more severe asthma[24].Concerning the non Th2 pathway biomarkers, data are limited compared to Th2 type. Sputum neutrophils, mixed granulocytic and paucigranulocytic patterns include the most commonly used biomarkers[25].

Th2 cytokines

Th2 cytokines (IL-4, IL-5, IL-9, IL-10, and IL-13) have a substantial effect on the pathogenesis of atopic diseases[26]. It is currently suggested that apart from Th2 cells, ILC2s are also responsible for the production of the majority of Th2 cytokines in the airway[27].

IL-4/IL-13

Th2 cytokines IL-4 and IL-13 share significant pathways and many biological activities concerning asthma. In specific, they play an important role in the identification of the presence of eosinophilic inflammation and also are key factors in IgE synthesis by B cells, mucus production, bronchial fibrosis and airway hyperresponsiveness in asthma[28,29]. There is a plethora of studies associating IL-4, IL-13 and asthma. When comparison was made between asthmatics and healthy controls, levels of IL-mRNA, protein levels in serum, bronchoalveolar lavage fluid (BAL), bronchial biopsies and exhaled breath condensate were found higher in asthmatics[30-34]. Similarly in other studies, increased IL-13 mRNA and protein levels are also found in sputum, BAL and bronchial biopsies of patients with asthma when compared to controls[35-37].

Furthermore, it is well-known that single-nucleotide polymorphisms (SNP) can be used to assess genetic disorders. A recent meta-analysis showed that IL-4 C-589T and C-33T were associated with asthma in Europeans[38]. As for IL-13, two SNPs, positioned at regions +2044G/A and +1923C/T have been suggested to play critical role in the development of asthma[39-41].

Anti-IL-13 and anti-IL-13/anti-IL-4 receptors targeted therapies with humanized monoclonal antibodies are currenlty used as add-on therapy in patients with Th2-high inflammation with uncontrolled asthma despite maximum therapy. Specific studies have shown that Th2-high and especially periostin-high groups of asthmatics with moderate-to-severe uncontrolled asthma are suggested to compose the group that could benefit from anti-IL-13 therapy[42]. Anti-IL-13 biologic agents (anrukinzumab, lebrikizunab and tralokinumab), are currently under clinical

evaluation to further elucidate the use of predictive Th-2 biomarkers[43].

Besides, IL-4 and IL-13 share some structural similarities and they bind the IL-4Ra/IL-13Ra1 receptor complex by which the transcription factor STAT-6 is activated[44]. Based on this concept, biologic agent such as dupilumab that target the dual cytokines IL-4/13 may be more encouraging approach for those patients suffering from refractive difficult-to-control eosinophilic asthma. Indeed, recently Wenzel et al reported that dupilumab increased lung function and reduced severe exacerbations in patients with uncontrolled persistent asthma irrespective of baseline eosinophil count and had a favourable safety profile[45]. More recently, another study reported that in patients with glucocorticoid-dependent severe asthma, dupilumab treatment reduced the rate of severe exacerbations and the use of oral steroids along with increasing the FEV_1[46]. Significantly lower rates of severe asthma exacerbations, as well as better lung function and asthma control were also reported in another study in patients treated with dupilumab[47].

IL-5

IL-5 is produced by CD4 + Th2 lymphocytes and ILC2 cells and, to a lesser extent, also by natural killer T (NKT) cells, mast cells, and eosinophils themselves[48]. Multiple studies have demonstrated the link between IL-5 and asthma as higher IL-5 mRNA levels[49] and IL-5 levels in sputum[50] have been associated with increasing eosinophil production or acute asthmatic exacerbations[51]. As for genetics, IL-5 C-746T was found to influence atopic outcomes[52]. Based on the above, IL-5 has been considered a suitable target for add-on biological therapies of severe eosinophilic asthma[53,54]. In particular, the anti-IL-5 antibodies developed, include mepolizumab and reslizumab, and the IL-5 receptor antagonist benralizumab which are evaluated for the treatment of refractory eosinophilic asthma[55-57]. Apart from mepolizumab and reslizumab, already FDA approved[55], benralizumab has also recently obtained the approval of FDA on the basis of several successful randomized controlled trials[58].

IL-17A/IL-23

A distinct subpopulation of CD4+ T cells produce Th-17 cells that by secreting IL-17, orchestrate the recruitment of neutrophil granulocytes in the lungs[59]. Furthermore, IL-17 contribute to the development of airway fibrosis during asthma by enhancing the production of profibrotic cytokines, proangiogenic factors, and collagen[60]. Besides, in vitro studies investigating the role of epithelial-mesenchymal transition (EMT) in asthma have shown IL-17 synergization with IL-4 and TGF-β promotes EMT with the expression of mesenchymal markers[61,62].

Th17 cells produce IL-17A, IL-17F, IL-21 and IL-22 cytokines[59]. IL-17A has been involved in severe asthma characterized by airway intense neutrophil infiltration and less responsive to corticosteroids[59,63]. Furthermore, these steroid-insensitive patients could be classified as a Th2 low phenotype of asthma[2]. Clinical studies have shown that levels of IL-17A is sputum, bronchoalveolar lavage fluid (BALF) and serum of asthmatic patients are significant higher in asthmatics than in healthy subjects and that these are correlated with the severity of disease[64]. Additionally, in a study on pre-school children with history and physical exam in favor of asthma which cannot be tested by spirometry, they concluded that IL-23 serum levels might be an auxiliary biomarker for the diagnosis of asthma[65].

Recently, Fattahi et al showed that atopy is associated with lower numbers of IL-17 cells in asthmatic airways[66]. Current data suggest that IL-17 has also been implicated in Th2 cell-mediated eosinophilic airway inflammation in mouse models of asthma[67] along with increased levels of IL-23[68] or in asthmatic patients with allergy after a challenge with house dust mite[69]. Furthermore, recently Camargo et al reported that inhibition of IL-17 even in exacerbated asthmatic patients significantly contributed to the control of Th1/Th2/Th17 inflammation, chemokine expression, extracellular matrix remodeling, and oxidative stress in a murine experimental asthma model exacerbated by Lipopolysaccharide (LPS)[70]. As for genetics, a recent meta-analysis concerning the association between IL-17A polymorphisms and asthma risk suggested that the IL-17A -737C/T polymorphism provides protection against the disease, whereas the IL-17A -197G/A polymorphism does not contribute to asthma risk[71].

However, so far, results from clinical trials targeting IL-17 Receptor (Brodalumab) including moderate to severe asthmatics, showed no improvement in asthma outcomes. This fact could be apparently attributed to inadequate selection of patients with asthma[72]. More specifically, although there was no effect of brodalumab on the primary outcome (the Asthma Control Questionnaire score), researchers,based on a subgroup analysis, suggested a new phenotype including patients with high reversibility of FEV1 in response to albuterol and a new

endotype which is IL-17R-dependent[60]. In future trials targeting IL-17 pathway, the selection of patients based on sputum neutrophilia could exclude Th2 high asthmatics that are less likely to respond to an IL-17-targeted therapy.

Dual positive Th2/Th17 cells

Athough T-helper cells were thought to be fully differentiated, expressing a master regulatory transcription factor and their development from naive CD4 cells was considered to be lineage specific[73], Cosmi et al demonstrated that there is great plasticity in human Th17 cells even toward the Th2 phenotype, suggesting the existence of CD4+ T cells able to produce both Th17 related (IL-17A) and Th2 (IL-4) related cytokines[74]. Besides, several in vitro studies and animal studies suggest that T-helper cell subsets display plasticity by changing their transcription factor or by expressing multiple transcription factors[75-79].

Asthmatic patients can suffer from a predominant eosinophilic inflammation usually seen in mild-to-moderate disease, from neutrophilic inflammation in more severe disease or even mixed eosinophilic/neutrophilic inflammatory response[80]. The underlying T cell response is predominated by Th2, Th17, or a mixed Th2/Th17 cell immune response.

A recent study showed that asthma is associated with a higher frequency of dual-positive Th2/Th17 cells in BAL fluid[12]. They concluded that Th2/Th17 (predominant) subgroup of asthmatic patients manifested glucocorticoid resistance in vitro and also had the greatest airway obstruction and hyperreactivity compared with the Th2 (predominant) and Th2/Th17 (low) subgroups. Moreover, in experimental animal models, IL-17 has not only been involved to produce airway intense neutrophil infiltration but to exacerbate Th2 cell mediated eosinophilic airway inflammation and hyperresponsiveness[67,68].

However, in another study, Choy et al investigated the potential of Th2 cytokine suppression in promoting TH17 responses in a preclinical model of allergen-induced asthma and concluded that IL-13 and IL-17A reciprocally regulate the expression of their target pathways in the lung[81]. In specific, IL-13 stimulation repressed the expression of the Th17 genes, with a trend for a similar repressive effect of IL-17A stimulation on Th2 genes.

Furthermore, in an effort to discover the possible mechanism of severe late-onset hypereosinophilic phenotype, ILC2 activation along with dual positive Th2/Th17 inflammation has been proposed[11]. Besides, recent data suggest that combination therapies targeting both pathways may maximize therapeutic efficacy across a patient population comprising both Th2 and Th17 endotypes[12].

CONCLUSIONS AND FUTURE PERSPECTIVES

As new research data concerning the different T helper lymphocyte pathogenic pathways are being developed in the asthmatic inflammation cascade, new effective individualized therapies in severe asthma are urgently needed to block specific inflammatory pathways using monoclonal antibodies. The only approved therapies so far include anti-IL-5 IgG (Mepolizumab, Reslizumab, Benralizumab) for severe eosinophilic asthma and anti-IgE (Omalizumab) for the treatment of severe allergic asthma. There is none approved biomarker for Non-Type 2/Th2 low asthma.One could possibly further suggest that combination therapies targeting both pathways might maximize therapeutic efficacy across a patient population comprising both Th2 and Th17 endotypes. Clinically, the ability to identify a Th2 or Th17 high or a mixed asthma phenotype on the basis of testing blood eosinophils may facilitate the use of effective biologically targeted approaches in asthmatic patients. The fact that analyzing the cytokine pattern in serum samples does not give us information on the source of the assessed cytokine may reflect the significance of local environment like lung tissues or intracellular molecular methods in analyzing the cytokine levels.But meanwhile, the feasibility of serum sampling as a noninvasive method to analyze cytokine levels has directed the interests toward noninvasive methods rather than invasive ones.

ΠΕΡΙΛΗΨΗ

Το μονοπάτι Th2/Th17 στο άσθμα και η κλινική του σημασία

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Το άσθμα είναι μια ετερογενής χρόνια νόσος των αεραγωγών, που χαρακτηρίζεται από διαφορετικούς φαινοτύπους. Η κύρια παθοφυσιολογική οδός είναι εξαρτώμενη από την Th2 ηωσινοφιλική φλεγμονή που παράγεται κυρίως από βοηθητικά Τ2 λεμφοκύτταρα (Th2). Πρόσφατες βιβλιογραφικές αναφορές προτείνουν τα μη ευαισθητοποιημένα λεμφοκύτταρα του βρογχικού επιθηλίου (innate lymphoid cells, ILC$_2$) ως μία άλλη πηγή Th2 κυτταροκινών, με αποτέλεσμα την παραγωγή ηωσινοφίλων χωρίς προηγούμενη αντιγονική ευαισθητοποίηση. Άλλο παθοφυσιολογικό μονοπάτι είναι η μη Th2 φλεγμονή που εξελίσσεται μέσω των Th1 λεμφοκυττάρων και των Th17 που εμπλέκονται στην ουδετεροφιλική φλεγμονή. Όμως, πρόσφατες μελέτες έχουν συσχετίσει και τα Th17 κύτταρα με την αλλεργική φλεγμονή και το ηωσινοφιλικό άσθμα. Οι τρέχουσες κλινικές μελέτες αναμένεται να διευκρινίσουν περαιτέρω τον ρόλο των διαφόρων κυττάρων στην εξέλιξη της ασθματικής φλεγμονής και επίσης τον ρόλο των καθιερωμένων ή νέων πιθανών βιοδεικτών στην καθημερινή κλινική πρακτική με στόχο τη μεγιστοποίηση της αποτελεσματικότητας των αντιασθματικών φαρμάκων. Στην παρούσα επισκόπηση, συνοψίζουμε τους προαναφερθέντες μηχανισμούς, επικεντρώνοντας στην πλαστικότητα κι ευελιξία των υποπληθυσμών των Τ-βοηθητικών κυττάρων και στην αναγνώριση της διπλής Th2/Th17 θετικής φλεγμονής.

Πνεύμων 2018, 31(3):174-182.

Λέξεις - Κλειδιά: *Th2/Th17, Άσθμα, Φαινότυποι, Βιοδείκτες, Θεραπεία*

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