

Asthma from immune pathogenesis to precision medicine

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ABSTRACT

Asthma is characterized by multiple immunological mechanisms (endotypes) determining variable clinical presentations (phenotypes). The identification of endotypic mechanisms is crucial to better characterize patients and to identify tailored therapeutic approaches with novel biological agents targeting specific immunological pathways. This review focused on summarizing the major immunological mechanisms involved in the pathogenesis of asthma, as well as on discussing the emergence of phenotypic features of the disease. Novel biological agents and other drugs targeting specific endotypes are discussed, as their use represent a precision medicine approach to the disease that is nowadays mandatory particularly for treating more severe patients.

1. Introduction

Asthma is a chronic inflammatory disease of the conducting portion of the airways and it is estimated to affect about 340 million people [1]. These individuals face unbarring burdens, from reduced quality of life to premature death. Ranked as ‘the 16th leading cause of years lived with disability, and 28th leading cause of burden of disease, as measured by disability adjusted life years’, asthma poses as a health, economical and humanitarian burden [2]. In amenable patients, the disease takes its toll by presenting as shortness of breath, wheezing, chest tightness and cough [1].

In the past, asthma has been viewed as a single disease-entity, a chronic inflammatory disorder characterized and defined as two distinct forms: an allergic and a non-allergic phenotype. Today there is direct evidence that connects asthma to various inflammatory pathways [3]: it is universally accepted that asthma is not solely a simple singularly classified disease with a regularly variable clinical presentation, but a multifaceted heterogeneous syndrome that is a result of various mechanistic inflammatory pathways [4].

Therefore, it requires a ‘precision-medicine’ approach for its diagnosis and management [5]. With this term we refer to a novel clinical approach targeting the endotype, which is the mechanism(s) of the disease of the individual patient [5]. The endotypic target might be revealed by ‘omic’ sciences (i.e.: proteomics, tran-scriptomics, metabolomics, genomics...) detecting biomarkers related to the molecular mechanisms of the patient’s disease and therefore leading the clinician to choose the correct therapeutic approach for each single individual

[6].

This review article focuses on summarizing the major components of immunological mechanisms underlying the pathogenesis of asthma, putting them in relation with phenotypic presentation of the disease. It also reviews the novel therapeutic approaches for asthma based on targeting specific immunological mechanisms.

2. Immunopathology of asthma

Both innate and adaptive immune systems, together with epithelial cells, are involved in the pathogenesis of asthma inducing its main clinical features: bronchial obstruction and hyper-reactivity, mucus overproduction, and airway remodeling [7]. The complex interaction between different immunological pathways accounts for the heterogeneous presentation of asthma in the so-called “phenotypes” [3].

Traditionally, asthma was considered an eosinophilic mediated disease characterized by the overexpression and activation of T helper type 2 (Th-2) cells [4]. Furthermore, studies revealed that a form of neutrophilic asthma, with concomitant T helper type 17 (Th-17) cells involvement, also exists [8,9]. Another astonishing discovery was that type 2 innate lymphoid cells (ILC-2) and basophils could also elicit eosinophilic inflammation associated in subgroups of asthmatic patients [10]. This discovery was crucial, as it unraveled that asthma is truly an irregularly heterogeneous disease. This theory was solidified when different cytokine-targeted therapies clinical trials were conducted, and displayed improved symptoms in patients with different forms of the disease [11–14]. Thus, our motive shifted entirely to better

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understand the different classifications of the types of the disease, in order to offer a safer, more precise and more effective treatment.

2.1. Innate immunity in asthma

Historically airway inflammation in asthma was considered exclusively part of adaptive immunity. However, the discovery of ILC-2 and of their potential role in atopic disorders [10], contributed to better understand that innate immunity response significantly contributes to determine the inflammatory response that characterize the different asthma phenotypes.

Innate lymphoid cells (ILCs) are lineage and antigen negative lymphocytes: this means that they does not express any markers or receptors for myeloid, dendritic, antigen specific B or T cells [15]. Three distinctive forms of ILCs have been identified so far: group 1 ILCs (ILC-1) producing interferon- γ (IFN- γ), ILC-2 producing cytokines classically associated with Th-2 cells (IL-4, IL-5, IL-13...), ILC-3 producing IL-17 and/or IL-22 [15].

ILC-2 cells play an integral role in the rapid inflammatory response of helminthic and viral infections [15]. These cells showed abundance in several tissues, including the lung, and seem to be crucial in orchestrating the airway inflammation in eosinophilic phenotypes of asthma, regardless of whether these patients are atopic or not [10]; this is due to the lack of antigen-specific receptors on these cells. However, they respond to epithelium-derived signals mediated by the so-called "alarmins" (IL-25, IL-33 and thymic stromal lymphopoietin - TSLP) produced by epithelial cells themselves [16].

Alarmins are produced in response to epithelial injury and through pattern-recognition receptors, therefore activating ILC-2 and cells of the adaptive immunity [16]. When they are activated, they cause the release of cytokines that will synchronize proinflammatory mechanistic pathways in asthma [16]. For example, the proteolytic epithelium injury due to the exposure to allergens containing proteases (such as *Alternaria alternata* or house dust mites) may induce the epithelial release of IL-33 which activates ILC-2 cells [17,18]. Proteolytic epithelial damage might also induce the production of Leukotriene D4 (LTD4) that, binding with the cysteine leukotriene receptor (CysLT1R) and therefore regulating the activation and proliferation of ILC-2 cells [19]. Moreover, ILC-2 cells might be activated also in response to viral infections affecting airway epithelial cells [20,21], contributing to explain why asthma exacerbations often occur during viral infective episodes [22].

Interleukin 33 (IL-33) is a proinflammatory cytokine belonging to the IL-1 family and expresses an IL-1-like domain on one of its end chains [23]. This is the predominant reason for the binding capability of IL-33 to the ST2 receptor, also referred to as the Interleukin 1 receptor like 1, a protein belonging to the family of Toll-like receptors and expressed on many immune cells, including Th-2 and ILC-2. The ST2/IL1RL1 receptor functions intracellularly to induce its proinflammatory response by the nuclear regulation of the NF- κ B and MAP kinase pathways. This brings about the main downstream function of IL-33, which is to induce the expression and production of IL-13, an imperative mediator in asthmatic eosinophilic inflammation, by ILC-2 [24]. Cellular injury dictates the fate of the response, where repairable damages constitute a passive release of IL-33. Necrotic cellular damage depicts a more active pumping of IL-33, while the release of the cytokine IL-33 will inevitably induce a pathological type 2 cytokine release.

Thymic stromal lymphopoietin (TSLP) is a IL-2 family cytokine, and it is expressed by multiple cells including epithelial cells of the lung [25]. TSLP plays a crucial role in mediating corticosteroid resistance associated with ILC-2 airway inflammation and driving the upregulation of the adaptive immune response in asthma [26]. TSLP binds to a heterodimer surface receptor called thymic stromal lymphopoietin receptor (TSLPR) expressed on multiple cells including T, B, NK cells, monocytes, basophils, eosinophils, ILC-2 and dendritic cells [27,28]. TSLPR is also expressed on the surface of epithelial cells [29]. The

cytokine amplifies a genomic response in innate and adaptive immune cells to increase the production of IL-4, IL-5 and IL-13. This directive constitutes the development of the pathogenic endotype, for a significant production of proinflammatory cytokines, that will facilitate the pathogenesis of asthma [3,4].

Interleukin 25 (IL-25) is the final cytokine of interest in the Alarmin triad. This cytokine upregulates downstream pro-inflammatory cascade: IL-25 release is determined by epithelial cellular injury, which is correspondingly determined by the protease exposure from foreign antigens [30–32]. Moreover, IL-25 uses signaling activation to induce the NF- κ B signalling pathway. In return, the Type 2 inflammatory response is activated by the release of Th2 cytokines IL-4, IL-5 and IL-13 [30,31]. The IL-25 receptor is countlessly expressed on many cells in the airway such as smooth muscle, epithelial, eosinophils, NKT, ILC-2 and antigen presenting cells. The variable expression allows the release of IL-25 to adequately upregulate the release of proinflammatory cytokines [32]. Furthermore, IL-25 has other critical roles in neovascularization of the lung, by stimulating directly angiogenesis [33]. Previous literature also suggests IL-25 to be more closely related to an allergic phenotype of the disease, as mRNA levels of the cytokine are vastly increased in subjects with serum IgE, as well as eosinophilic activated asthma, when compared to normal healthy subjects [34]. Additionally, IL-25 levels are also positively associated with increased airway smooth muscle contractility [35].

Concerning the functions of Th-2 and ILC-2 cells in the pathophysiology of asthma, the two immune cells share mechanistic features, the most common being the upregulation of the transcription factor GATA-3, which drives the production type 2 cytokines and chemokine receptors [36,37]. Among these cytokines, IL-5 is primarily important in the controlled maintenance of the development of eosinophils in the bone marrow [38]. More importantly, IL-5 signals for chemotaxis of eosinophils to survive and release subsequent cytokines, in order to shape the underlying pathogenesis of airway eosinophilia. Another collective effort for IL-5 and IL-13 is to prime bronchial hyper-reactivity as well as inducing other molecules such as eosinophil cationic protein, major basic protein, tumor necrosis factor and eicosanoid pathway metabolites [38,39]. IL-13 overexpression in the lung directs the signalling of ICAM-1 and VCAM-1 alongside IL-4 [39]. This is released by Th-2 cells, and causes the formation of perivascular and peribronchial infiltrates to enter the lung interstitium and induce bronchial smooth muscle hyperreactivity, chemokine induction and epithelial damage. IL-13 and IL-4 also induce goblet cell metaplasia and increase mucus production in the lung, another typical feature of asthma [40].

2.2. Adaptive immunity in asthma

Traditionally, the immunopathology of asthma was described as a linear progression separating the innate and adaptive immunity from each other. This idea caused researchers to diverge from the common cascade, although it did provide the ability to distinguish between the innate and adaptive immunity mediated inflammatory response in asthma. Nonetheless, the pathophysiology of asthma is that of a common cascade, with signalling molecules shared by both types of immunity.

Therefore a comprehensive outlook of the system is needed, also to ensure a more directive approach of the overlapping syndromes of eosinophilic and neutrophilic asthma. Traditionally, asthma was classified as an allergic inflammatory disease, characterized mainly by eosinophilia and immunoglobulin E (IgE), associated with airway hyperresponsiveness, smooth muscle cells (SMC) hypertrophy, airway remodeling and increased mucus production.

The immune recognition of environmental allergens is initiated by antigen presenting cells (APCs), such as dendritic cells (DCs) (but also B lymphocytes, and many other cell types); these will induce the maturation of T-naïve cells into Th-2 cells, able to produce cytokines such as IL-4, IL-5 and IL-13 [7,24]. IL-4 produced by Th-2 cells activates B

cells to differentiate into plasma cells and switching antibody production to the IgE isotype [41]. The cross-linked binding of allergen-specific IgE molecules, the allergen itself and effector cells like mast cells and basophils, determines the degranulation of preformed (i.e.: histamine, tryptase) and other mediators (i.e.: leukotrienes, prostaglandins) contributing to the development common symptoms of asthma [7,24]. Moreover, IL-4 contributes to polarize T-naïve cells to the Th2 cells, therefore further enhancing the entire Th-2-mediated inflammatory response [42]. Other relevant activities of IL-4 in the asthma pathogenesis are the induction of VCAM-1 expression, which contributes to direct the migration of eosinophils, basophils, monocytes and T cells to the site of allergic inflammation [43], and the induction of mucin gene expression with subsequent increase in airway mucus production [44].

Broncho-alveolar lavage (BAL) studies on asthmatics revealed that IL-4 is central in the immunopathogenesis of allergic phenotypes of asthma [45,46], while its mRNA expression was not found in non-allergic asthmatics [46]; interestingly, nonallergic but eosinophilic asthmatics had elevated levels IL-5 (as it happens also in allergic patients) suggesting its crucial role in determining eosinophilic airway inflammation even in absence of allergic stimulation [46].

More recently, another mechanism of upregulation of mast cells was described and it passes through the production of IL-9 by both Th-2 and ILC-2 cells [47].

Another adaptive immune system mechanism that seems to be implicated in the pathogenesis of asthma, mainly neutrophilic phenotypes [8,9], has IL-17 (IL-17A and IL-17F) as the pivotal cytokine. Th-17 cells, other T cells, NK cells, ILC-3 and mast cells express this cytokine. With an abundance of IL-17A and IL-17F, neutrophil influx is regulated indirectly through the induction of airway epithelial cells and stromal cell production of cytokines, which influence the chemotaxis of neutrophils to the scene. Relating back to neutrophilic asthma pathogenesis, IL-17 is the dominant signalling molecule that elicits the activation of its receptor and it is expressed on smooth muscle cells, inducing smooth muscle cells hypertrophy [48]. IL-17 receptor is also expressed on epithelial fibroblast, macrophages and endothelial cells, contributing to explaining the potential role of IL-17 in airway remodeling [49]. Although the mechanism is still unclear, researchers believe that cytokines released by neutrophils such as IL-6, transforming growth factor beta, IL-1beta, tumor necrosis factor alpha, IL-21 and IL-23 in variable combination, can activate key transcription factors such as STAT3 and RORC2 to induce the differentiation of naïve CD4⁺ T cells to Th-17 cells [50].

2.3. Eicosanoid pathway

The metabolism of arachidonic acid plays a crucial role in the pathogenesis of inflammation in asthma. The phospholipid bilayer is metabolized by Phospholipases A2 (PLA2) to synthesize arachidonic acid, which has two subsequent pathways: the cyclooxygenase and 5-lipoxygenase produce Prostaglandin H2 (PGH2) and Leukotriene A4 (LTA4) respectively. Metabolites of PGH2 synthesize multiple prostaglandins, including Prostaglandin D2 (PGD2). Both the cyclooxygenase and 5-lipoxygenase pathways play relevant roles in asthma [24].

2.3.1. Prostaglandin D2

Prostaglandin D2 is largely released by mast cells, although other cells play a vital role, including Th-2 cells; the latter induce the B cells to stimulate the release of IgE, which binds to mast cells and allow a massive release of cytokine mediators and arachidonic acid metabolites. More so, eosinophils play a small role in the synthesis of PGD2 [51]. The prostanoid PGD2 complexes with two major receptors, each with variable affinities to carry out its function: D-prostanoid 1 (DP1) and DP2 (also called chemoattractant receptor-homologous molecule expressed on Th2 cells: CRTH2). DP1 is a Gs-protein coupled receptor that is expressed on goblet cells, serous glands, endothelium, T_H2 cells,

dendritic cells, basophils and eosinophils [52]. The activation of DP1 causes a surge of increased concentrations of intracellular cyclic AMP, activating a cellular response; DP1 mediates its function by both preventing apoptosis, and immune cell recruitment to the site of inflammation and its subsequent degranulation [52]. DP2 (CRTH2) is expressed on eosinophils, basophils, Th-2 and ILC-2 cells [53]. The activation of the PGD2-CRTH2 complex induces multiple pro-inflammatory actions [53]. Primarily, the PGD2-CRTH2 induces the migration and degranulation of immune cells. Furthermore, on IL-13/IL-4 producing Th-2 cells, CRTH2 signal is augmented when complexed with PGD2. This increases the release of cytokine mediators of immune cell chemotaxis and degranulation in the lung. CRTH2 also stimulates ILC-2 to mediate type 2 inflammation and ultimately leading to airway inflammation, obstruction and hyperresponsiveness [53].

2.3.2. Cysteinyl leukotrienes

Cysteinyl leukotrienes (CysLTs) are 5-lipo-oxygenase metabolites that have a cysteine molecule forming a portion of their end structure. They are released by eosinophils, basophils, mast cells and macrophages, through the expression of IL-4 [54,55]. This process regulates the STAT6 transcriptome pathway, as well as the upregulation and the consequent production in asthmatic inflammation [55]. CysLTs carry out variable outcomes depending on the receptor complex expression that they induce. The first receptor CysLT1 is vital in inducing bronchoconstriction, as it is expressed on smooth muscle cells [56]. Mucous secretion and alveolar edema are also consequences of its expression on mast cells, eosinophils, monocytes, macrophages and B cells. The upregulation of CysLT1 receptor is produced at the genomic level, due to the expression induced activity of pro-inflammatory type 2 cytokines, IL-13 and IL-4 [56]. On the other hand, studies of the CysLT2 receptor showed that when complexed, it activated vascular permeability, inflammation and tissue fibrosis [57]. The importance of CysLT2 is a crucial discovery, as its expression on mast cells and eosinophils constituted an asthmatic eosinophilic phenotype [58].

3. From endotypes to phenotypes

As already stated, asthma presents a variable mechanistic pathology; the heterogeneous disease further encompasses a vast array of inflammatory entities that make up the clinical presentation. Above, we outlined the immunological engine that drives the pathology to alter the normal function in the lung, and how mechanistic pathways (the so-called “endotypes”) lead to a common outcome. However, the heterogeneity of asthma does not only regard the endotypes, but also the clinical and inflammatory features that might be variable: those patients having similar clinical and inflammatory features can be classified as part of specific “phenotype” of asthma [3,4,59]. The heterogeneity of asthma clinical presentation is further complicated and increased by the presence of comorbidities directly impacting on asthma severity [60,61] or by nutritional factors (i.e.: iron or vitamin D deficiency) [62,63].

Endotypic classification gave insight to variable immune-pathogenesis of asthma. As we outlined above, the disease can take different forms, from the release of type 2 cytokines, where a more eosinophilic outcome presents itself, to the other end of the spectrum where T helper 17 cells regulate neutrophilic inflammation of the airways [7,24]. Regardless of the nomenclature, studies refer to an ‘endotypic range’, classified as complex type 2 high or low, where one end of the spectrum is eosinophilic and the other end is neutrophilic [64]. The existence of a ‘mixed’ endotype has been also described as a possible presentation in patients, which can be an overlap of these syndromes [65].

3.1. Eosinophilic phenotypes

3.1.1. Early onset, allergic asthma

Allergic asthma is the most common asthma phenotype, accounting

ALLERGIC EOSINOPHILIC AIRWAY INFLAMMATION

NON-ALLERGIC EOSINOPHILIC AIRWAY INFLAMMATION

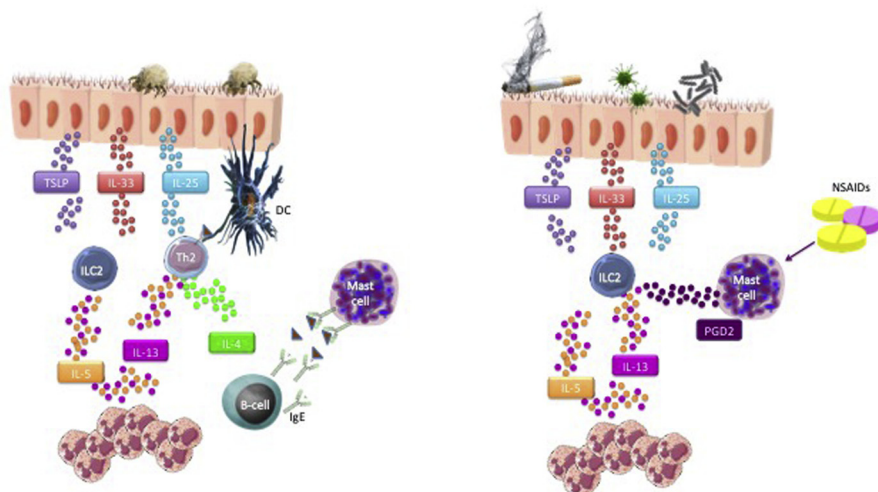


Fig. 1. Eosinophilic endotypes of asthma: an allergic (left) and a non allergic (right) endotype can be distinguished. In both endotypes the interaction between antigens (allergens in case of allergic endotype) and/or other irritants induces airway epithelial cells to produce alarmins (IL-25, IL-33, TSLP) that interact with innate and adaptive immune system cells to recruit and activate eosinophilic inflammation.

for at least half of the patients [66]. The underlying immunological mechanisms have been described above when referring about the interaction between allergens, APCs, Th-2 and B-cells, IgE production and subsequent activation of mast-cells and basophils degranulation. Allergic asthma is usually referred to as early onset eosinophilic asthma, frequently associated with family history of atopy, and with a well-defined steroid sensitive prognosis [67]; however, also an allergic severe asthma phenotype, not entirely responding to corticosteroids, has been described and seems to be one of the most common severe asthma phenotype [68–70]. Allergic asthma is often associated with other allergic comorbidities, such as allergic rhinitis and atopic dermatitis [68–70]. Proposed indicative biomarkers of atopic eosinophilic asthma are elevated blood and sputum eosinophil count, serum IgE levels, elevated fractional exhaled nitric oxide (FE_{NO}) and serum and sputum periostin levels [67].

3.1.2. Late onset eosinophilic asthma

A group of patients with asthma does not exhibit any symptom until the adulthood and tends to have a less atopic disease but a more severe prognosis. These patients, commonly referred as late-onset asthmatics, often are characterized by persistent airway and peripheral eosinophilia poorly responder to corticosteroid treatment [67,70,71].

Although the mechanism of disease progression in adult onset eosinophilic asthma is not fully determined, typically this phenotype shows an involvement of ILC-2 driven inflammation mediated by cytokines such as IL-5 and IL-13 [72]; the final result is an intense eosinophilic airway and systemic inflammation that is frequently associated with the comorbidity chronic rhinosinusitis with nasal polyps (CRSwNP), which often precedes the onset of asthma [70,73] and determine low probability of asthma remission [74]. A subgroup of patient with late-onset eosinophilic asthma and CRSwNP highly expresses *Staphylococcus aureus* enterotoxin specific IgE, high levels IgE and of IL-5 [75]; in some cases, sputum eosinophilia is accompanied by neutrophilia, implicating two concomitant immunological mechanisms involved: Th-2 and Th-17 expression [76].

Indicative biomarkers for eosinophilic late onset asthma are elevated FE_{NO}, sputum and peripheral blood eosinophilia, and at least in a subgroup of patients also elevated serum IgE levels [67].

3.1.3. Non-steroidal anti-inflammatory drugs exacerbated respiratory disease (N-ERD)

Non-Steroidal Anti-Inflammatory Drugs Exacerbated Respiratory Disease (N-ERD) (also known as Aspirin-exacerbated respiratory disease, AERD) is a prominent severe adult onset phenotype of asthma [77]. N-ERD is commonly associated with gene polymorphisms in

prostaglandin E₂ receptor 2 and cysteine leukotriene receptor 1 [77]. Pathological pathways show increased Cys-LTs production, indicating a dysregulated arachidonic acid metabolism that presumably stimulates the progression of the disease [77].

Classified as a Type-2 airway inflammatory disease, it is generally characterized by increased peripheral and sputum eosinophilia [77], high prevalence of CRSwNP as comorbidity, and non-steroidal anti-inflammatory drug hypersensitivity. Indicative biomarkers for N-ERD are, elevated blood and sputum eosinophil count, possibly associated with increased serum IgE levels, and most importantly elevated 24-h urinary Leukotrien E₄ (LTE₄) [67,77].

3.1.4. Exercise-Induced bronchoconstriction (EIB)

Exercise-Induced bronchoconstriction (EIB) is a classical feature surrounding the common conception of asthma. Excessive exercise causes an evaporative force on the epithelial cells, forcing the loss of water and inducing the release of pro-inflammatory mediators into the interstitium, which prime mast cell degranulation [78]. The release of cytokines and signalling molecules from mast cells, induces airway hyperresponsiveness and airway remodeling. At the same time, pro-inflammatory mediators released from epithelial cells cause bronchoconstriction [78]. Symptoms for EIB are intermittently heightened throughout the exercise process. This phenotype has a genetic predisposition, through a polymorphic loss of function of aquaporin channels. This results in the dehydration of the cells. EIB is a component of Th-2 cytokine induced pathway, alongside a partial dysregulation in the arachidonic acid metabolism pathway [67,78].

The main endotypic mechanisms involved in eosinophilic (allergic or non allergic) airway inflammation in asthma are summarized in Fig. 1.

3.2. Non-eosinophilic asthma phenotypes

Clinicians identified two forms of the phenotype that share a common genetic and molecular mechanism of pathogenesis. The first type of non-atopic neutrophilic asthma is the so-called “pauci-granulocytic asthma”: generally a mild form of asthma that lacks the presence of airway inflammation [79]. Patients with elevated neutrophil count represent the second type: the properly called “non-atopic neutrophilic asthma” phenotype that is generally an adult onset phenotype of the disease, with variable severity [8,80]. The phenotype is thought to be mediated by the immune cell Th-17, polymorphic changes in altered mRNA expression of NLRP3 gene that produces cryopyrin, an inflammasome, and increase in IL-1β expression [80]. Indicative biomarkers for non-atopic neutrophilic asthma are induced

sputum neutrophil count, and elevation of bronchoalveolar lavage Matrix Metalloproteinase-9 (MMP-9), which is a regulatory protein of neutrophil migration across the endothelial membrane [67].

Typical clinical conditions that are associated with neutrophilic inflammation in asthma are: the smoking habit, obesity and elderly [67]. Asthma in smokers is characterized by a mixed inflammatory form, with a higher inclination to neutrophilia than eosinophilia, in which the Th-17 driven neutrophilic inflammation is typified by oxidative stress, which induces extensive airway remodeling [81]. Obesity related asthma is a gender determined phenotype and is more commonly present in females; this phenotype is characterized by a cascade of oxidative stressor induced pathways as well as elevated levels of leptin [82]. Comparatively, studies also indicate that this phenotype has an increased innate immune activation with particular activation of ILC-2 cells immune pathways [83]. Obesity-related asthma reveals an elevation in IL-6, an endogenous pro-inflammatory cytokine of neutrophils, which is concomitantly compliant with an elevation in neutrophil count [82]. Therefore, a potential peculiar indicative biomarker in obesity related asthma might be the elevation of serum IL-6 [67]. Neutrophilic asthma in elderly is a steroid resistant form of the disease, classified as an immunosenescent inflammatory disease mediated by Th-17 cells [84]. Whilst pathologic mechanisms of the disease remain unknown, studies presume that the respiratory system has a decreased function due to inflammatory induced airway remodeling [67,84].

4. Immunological therapeutic targets

The use of standard anti-inflammatory (mainly inhaled corticosteroids, ICS) and bronchodilator medications, that are still the main treatment options of asthma, do not take in account the heterogeneity of asthma phenotypes and endotypes [3,4]. This “one size fits all” strategy may be effective for the majority of patients, but about 5–10% of asthmatics do not reach clinical and functional control even using high dose of ICS plus other controllers and/or with chronic use of oral corticosteroids (OCS); these patients are defined as “severe asthmatics” [85] and they deserve a more personalized and precision therapeutic approached based on the identification of phenotypes and endotypes, and targeting specific endotypic mechanisms [5]. Here below we summarize the most relevant therapeutic approached based on asthma endotypes.

4.1. Anti-IgE therapeutic strategies

The first biologically targeted treatment in asthma was Omalizumab, an anti-IgE monoclonal antibody used for the treatment of severe allergic asthma [86]. Mechanistically, the drug binds to IgE and blocks the interaction of IgE using downstream receptors and ultimately limits its action. Primarily, an overall decrease in airway inflammation is achieved due to the inhibition of IgE, an increase in eosinophil apoptosis, a decrease in the IgE receptors on basophils and mast cells subsequently decreasing the release of mediators. On the other hand, inhibition of IgE halts airway remodeling, due to a decrease of growth factor release upon epithelial and smooth muscle cells in the airway [87]. Lastly, anti-IgE inhibits T cell maturation inducing a reduced production of IgE by plasma cells [88]. Omalizumab has been the only marketed biological drug for asthma for more than a decade, demonstrating its efficacy mainly in the severe allergic phenotype of asthma, reducing the exacerbation rate and improving the overall quality of life of treated patients [86]. The current clinical indication for Omalizumab is for severe asthmatics with high serum IgE levels and proven sensitization to a perennial allergen [86].

4.2. Anti-IL5 therapeutic strategies

Given the pivotal role of IL-5 in determining and maintaining the eosinophilic inflammation in all the Type 2 phenotypes of asthma,

novel biological drugs targeting this cytokine recently emerged as therapeutic option for severe eosinophilic asthmatics [38]. Mepolizumab is a monoclonal antibody targeting circulating IL-5, inducing a decrease proliferation, maturation and survival of eosinophils [89]. It has been shown to be effective in preventing asthma exacerbation [11], improving asthma-related quality of life [90] and reducing the need of use OCS treatment [91] with potential subsequent reduction of risk for OCS-related adverse events [92,93]. Mepolizumab is administered subcutaneously, every four weeks and at a fixed dose of 300 mg. Currently its use is indicated for severe eosinophilic asthmatics with evidence of blood eosinophils greater than 300/mcl [89].

Reslizumab is another monoclonal antibody directed towards IL-5, but unlike Mepolizumab it is administered intravenously and with a variable dose (from 100 to 575 mg) depending on the weight of the patient [94]. Studies have shown a reduction in exacerbations risk of asthmatic patients, a positive effect on their overall quality of life [13].

A different mechanism of action characterizes the third biological targeting the IL-5 cascade: Benralizumab is a monoclonal antibody targeting IL5-receptor alpha [95]. The inhibition of IL5-R alpha induces an antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism mediated by Natural Killer (NK) cells against eosinophils and basophils [38,95,96]. Also in the case of Benralizumab, the main outcome achieved in treated patients were the reduction of asthma exacerbations [12] and of OCS use [97,98], and the improvement on quality of life [99].

4.3. Anti-IL4-receptor alpha therapeutic strategies

Dupilumab is an IgG4 monoclonal antibody that targets the IL-4R alpha chain that is a shared

portion of a common receptor for the pro-inflammatory cytokines IL-4 and IL-13. This dual activity ensure an inhibition of the overall type 2 cytokine drivers of asthmatic inflammation [100]. The drug is currently approved for the use against atopic dermatitis and recently obtained the US Food and Drug Administration (FDA) approval as an add-on maintenance therapy in patients with moderate-to-severe asthma aged 12 years and older with an eosinophilic phenotype or with oral corticosteroid-dependent asthma [101], and for CRSwNP [102]. Clinical trials on Dupilumab for asthma showed a reduction in asthmatic exacerbation rates [14], improvement of lung function and favorable tolerability with glucocorticoid withdrawal underlie the reason for this [103]. Recently published data showing positive effect of Dupilumab on CRSwNP outcomes make this biological promising to treat patients with concomitant asthma and CRSwNP [104].

4.4. Anti-TSLP therapeutic strategies

Tezepelumab is an IgG monoclonal antibody that is specific to the inhibition of the cytokine TSLP. It binds the protein TSLP and blocks its interaction with its receptor complex [25]. Hence, a diminished effect on recruiting antigen presenting cells to mature cells of the adaptive immunity is hindered and an overall suppression of type 2 inflammation ensues [25,105]. In a recent clinical trial, following the administration of Tezepelumab for four weeks, a decrease in blood eosinophil count, IgE and FeNO levels was seen in comparison with levels at the initiation stage [105]. Asthmatic patients who received Tezepelumab, showed a significant clinical decrease in exacerbation episodes and improvement in lung function [105]. This biological is still not on the market and further studies are needed to confirm the positive effect showed in the only clinical trial published so far.

4.5. Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) antagonists

Fevipirant is a reversible, but selective competitive antagonist of CRTH2 commonly expressed on the surface membrane of many

immune cells [106]. The PGD2-CRTH2 complex induces the release of pro-inflammatory cytokines from Th2 cells, as well as the recruitment and degranulation of cytokine mediators from eosinophils and basophils [53]. Moreover, CRTH2 is also expressed on innate cells ILC-2, inducing migration and stimulating type 2 inflammation [53]. These premises make Fevipiprant a novel promising treatment for Type 2 phenotypes of asthma, at least under the biologic point-of-view. Two pivotal multicenter, randomized, double-blind, placebo-controlled phase 3 studies are underway [107,108] to assess the efficacy of Fevipiprant in severe asthmatics in terms of reduction of exacerbation rate and lung function improvement.

5. Conclusions

Asthma is a complex, multi-factorial disease characterized by variable clinical presentations in different subsets of patients, and it is the result of heterogeneous expression of inflammatory pathways, involving both innate and adaptive immune systems. Therefore, the simplistic concept of asthma as a single disease characterized by chronic airway inflammation, bronchial hyperreactivity, airway obstruction and airway remodeling, is nowadays obsolete. In this context, a more personalized approach to asthmatic patients, applying the so-called precision medicine in order to better characterize patients into phenotypes and endotypes, and to choose the right drug for the each single individual (a "tailored-treatment" approach) is now mandatory, particularly for severe asthmatics [5].

The identification of phenotypes passes through a careful evaluation of any single clinical aspect (i.e.: the presence of atopy, comorbidities, clinical presentations...), of lung function patterns (i.e.: the degree of bronchial reversibility, the presence of fixed airway obstruction, the degree of airway hyperreactivity...) and of sputum and systemic inflammatory involvement (i.e.: eosinophilic, neutrophilic, mixed, paucigranular phenotypes); asthma phenotypes are the result of underlying immunological mechanisms (the endotypes): their identification through reliable and possibly non-invasive biomarkers is crucial for establishing a precision medicine based therapeutic approach, for example by using novel biological agents that are showing impressive results in controlling more severe asthmatics [11–14,86–91,94–105].

The precision medicine approach to asthma represents a completely novel paradigm and it will surely give the opportunity not only to treat severe asthmatics in a more appropriate and effective way, but also new insights on those immunological aspects of asthma that still need further investigations.

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Declaration of Competing Interest

The authors report no conflict of interest.

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