Endotypes and Immune Cells in Severe Asthma

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Matija Rijavec[1,2] and Peter Korošec[1,3]

Abstract

Severe asthma accounts for a small proportion of asthma prevalence, however due to high requirements for treatment the majority of medical resources are directed toward those patients. Asthma is a highly heterogeneous disease, an umbrella diagnosis for several diseases with variable clinical presentations (phenotypes) and distinct mechanistic pathways (endotypes), and its pathophysiology is not yet completely understood. Thus despite similar clinical symptoms, asthma patients may respond very differently to the same therapeutic interventions. Asthma endotypes are currently regarded as type 2 high (T2-high) or non-T2. Th2 cells, innate lymphoid cells, eosinophils and mast cells are the most important cell types associated with T2-high asthma, on the other hand neutrophils, Th1 and Th17 cells are involved in non-T2 asthma. As more and more innate and adaptive immune cell types and mediators are identified as important drivers of asthma, asthma endotype definitions are still fluid and continue to evolve. The identification and understanding of the molecular mechanisms of different asthma endotypes, that reflect a highly variable response to different treatments, will lead to more precise asthma management and better outcomes in patients.

Keywords: severe asthma, endotype, phenotype, immune cells, eosinophils, innate lymphoid cells, mast cells, basophils

1 University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia

2 Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

3 Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Introduction

Severe asthma remains a worldwide problem. Even though accounts for a small proportion of asthma prevalence affecting a minority of patients, it is characterized by high requirements for treatment to partly or completely control severe and frequent symptoms, and as a result, the majority of medical resources are directed toward those patients[9,12,14-19]. Asthma is a highly heterogeneous disease and its pathophysiology is not yet completely understood. Large asthma clinical heterogeneity and high variability in treatment response extend beyond clinical phenotypes, and over the last two decades several genetic, immunologic, and environmental factors that contribute to asthma risk, pathogenesis and underlying asthma endotypes have been determined[6,17,18]. The identification and understanding of the molecular mechanisms of different asthma endotypes, that reflect a highly variable response to different treatments (related to certain clinical phenotypes), will lead to more precise asthma management and better outcomes in patients[6,13,17,18].

Phenotypes and Endotypes in Severe Asthma

Asthma heterogeneity reflects different underlying mechanisms. Asthma is nowadays

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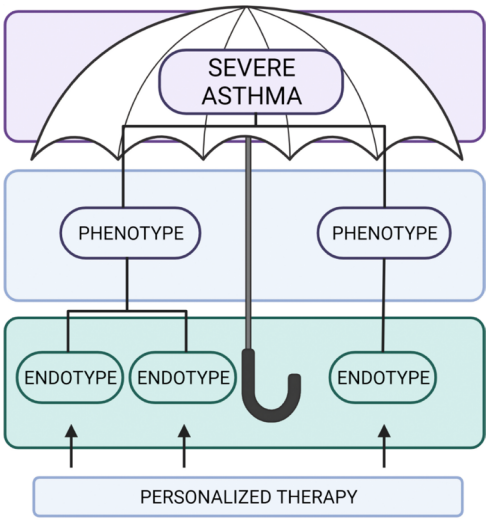

Figure 1. Association between phenotypes and endotypes in severe asthma. Severe asthma is considered an umbrella diagnosis linked to specific pathogenesis. There are many possible phenotypes (clinical presentations) and each phenotype is associated with distinct endotypes (distinct mechanistic pathways) that can be targeted with personalized therapy. Adapted from Fitzpatrick & Bacharier, 2019.

considered an umbrella diagnosis for several diseases with variable clinical presentations (phenotypes) and distinct mechanistic pathways (endotypes) (Figure 1)[13]. Hence, phenotypes are defined as „observable characteristics that result from a combination of hereditary and environmental influences" such as clinical presentation, symptoms, triggers and allergic features. However, the strategy was recently evolving to associate molecular mechanisms to phenotype and asthma endotypes describe these distinct pathobiological pathways at a cellular and molecular level. Thus despite similar clinical symptoms, asthma patients may respond very differently to the same therapeutic interventions. Why? Because of distinct endotypes (mechanistic pathways). Furthermore, the precise definition of these endotypes is central to asthma management due to inherent biological therapeutic implications[8,11,13].

As more and more innate and adaptive immune cell types and mediators are identified as important drivers of asthma, it is evident that asthma endotype definitions are still fluid and continue to evolve[13]. Asthma endotypes are currently regarded as type 2 high (T2-high) or non-T2. Why not adaptive Th2 high or non Th2 endotypes? Recently, a substantial body of evidence has proven that group 2 innate lymphoid cells (ILC2s) also play an equally critical role in type 2 immune responses. ILC2s are particularly high in airway tissues and produce large quantities of IL-5 and IL-13 in response to alarmins, mediators released from epithelial cells in response to stressors, such as infection or inflammation. In asthma, ILC2s appear to play an early and key role in augmenting the type 2 responses in the airway. Together, Th2 cells (adaptive) and ILC2s (innate) are the primary regulators of T2 immunity and express the master transcription factor GATA3. Therefore, Th2-high inflammation is labelled as type 2 (or T2) inflammation, to account for the role of both adaptive Th2 and innate ILC2 immune cells[5,7,11,13].

In T2-high asthma, there is an interplay of several individual pathways and cells (Figure 2). Alarmins, like TSLP, IL-25 and IL-33 are airway epithelial-derived mediators that respond to infection and inflammation. IL-33 and IL-25 mainly activate ILC2s, while TSLP also primes dendritic cells (DCs), and consequently B- and T-cells. Recent data suggest that IL-33 appears to be the most potential amplifier of T2-high asthma[1]. Alarmins serve to activate ILC2s. ILC2s are lineage-negative cells that lack lymphocyte surface markers and antigen-specific receptors and produce 10-fold more IL-5 and IL-13 compared with activated Th2 cells. On the other hand, adaptive pathways involve allergens-activated DCs that induce the expression of a Th2 pathogenic signature in the presence of the master

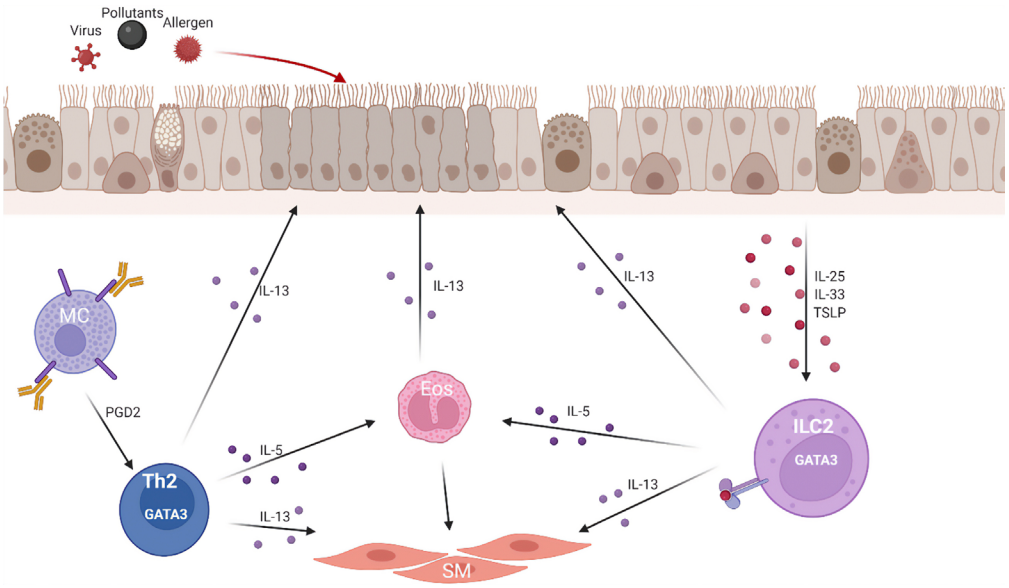

Figure 2. Mechanisms of T2-high asthma. Eos, Eosinophils; GATA3, GATA3 transcription factor; MC, mast cells/basophils; SM, smooth muscle cells; Th, T helper cells. Adapted from Corren, 2019.

transcription factor GATA-3. Th2 cells then stimulate type 2 immunity through the secretion of the cytokines IL-4, IL-5, and IL-13. Importantly, both IL-4, as well as IL-13, utilize a common IL-4Rα chain. IL-5 plays a pivotal role in promoting the differentiation and maturation of eosinophils, as well as their subsequent mobilization and survival. Furthermore, T2 cytokines have effects on goblet cells (mucus), fibrogenic functions (remodelling), and hyperresponsiveness[5,7,11].

Eosinophils are the hallmark cell type associated with T2-high asthma and have pleiotropic effects on various inflammatory cells. Upon stimulation, they release a myriad of inflammatory mediators chemokines, and cytokines including IL-5, IL-13, eotaxin, cysteinyl leukotriene (CysLT), major basic protein (MBP), eosinophil peroxidase (EPX), and eosinophil cationic protein (ECP). CysTL is a potent bronchoconstrictor that acts in synergy with IL-33 and further drives the self-amplifying loop that characterizes T2 inflammation. Eosinophils also activate bronchial fibroblasts. Very important from the therapeutic target, IL-5 plays a pivotal role for eosinophils (differentiation and survival)[5,7,11,13].

While the role of mast cells degranulation in acute asthma exacerbation is well established especially in allergen driven exacerbations, the functional significance of basophils in asthma has recently gained attention. Notably, it was shown that basophils have been recruited in the bronchial walls of T2-high asthma. Moreover, it is well known that in humans basophils are one of the major producers of IL-4 and can thus directly modulate T2 inflammation. IgEs, which on mast cells and basophils through FcεRI mediate immediate hypersensitivity response to allergens, can also facilitate and modulate antigen presentation by dendritic cells and response to viruses. Additionally, recent data strongly suggest that an altered functional subtype of mast cells may have greater potential to generate PGD2. These PGD2-high mast cells strongly predict poorly controlled T2-high

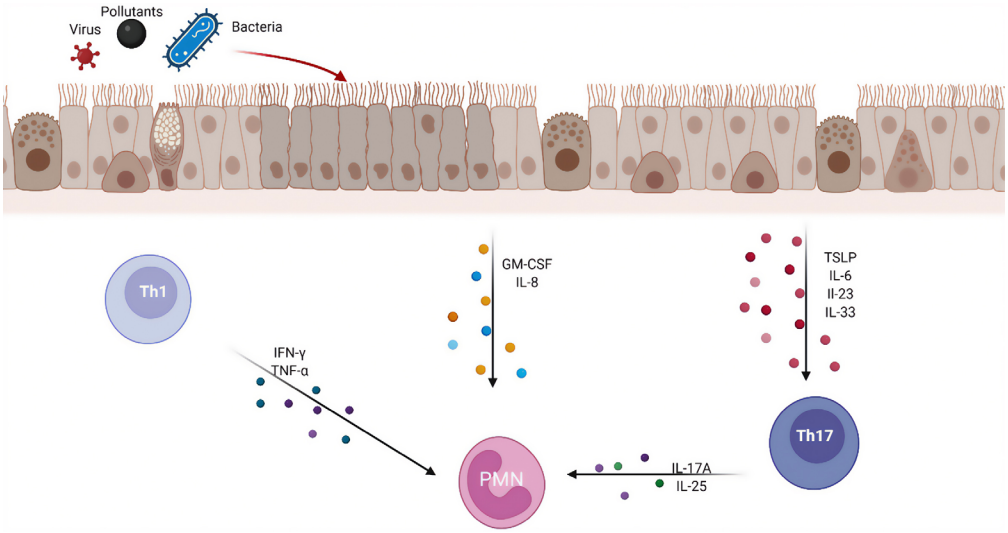

Figure 3. Mechanisms of non-T2 (T2-low) asthma. PMN, polymorphonuclear cell; Th, T helper cells. Adapted from Corren, 2019.

asthma and are associated with more severe disease (targeting CRTH2)[5,7,11,13].

Non-T2 (T2-low) asthma is typified by the absence of markers of T2-high disease. It is generally characterized by neutrophilic (sputum neutrophils > 40–60%) or paucigranulocytic (i.e., normal sputum levels of both eosinophils and neutrophils) inflammation and a lack of response to corticosteroid therapy. Mechanisms underlying recruitment and maintenance of neutrophilic airway inflammation are yet unknown: the role of the neutrophil itself is in debate. It has been linked with the Th1 and/or Th17 cells, cytokines IL-17A, IL-17F, IL-22, IFN-γ, TNF-α, IL-1β, IL-6, IL-8, and NLRP3 inflammasome. It is also possible that some non-T2 endotype are labelled as such only because steroid therapy has masked the T2 signature (Figure 3)[5,7,11,13].

High Th1 is marked by the production of IFN-γ. Elevated IFN-γ was associated with high airway resistance, increased inflammatory infiltrates, and corticosteroid refractoriness. Corticosteroids may not only be inefficacious in these patients but may exacerbate the underlying inflammatory state through increased Th1 recruitment. High Th17 is marked by increased levels of IL-17A, IL-17F, and IL-22 and IL-17F frequent exacerbator endotype has been recently described. Additionally, IL-6 has been recently shown to cause systemic inflammation in a subgroup of asthma patients with obesity and severe disease[5,7,11,13].

Currently, due to the availability of therapies targeted toward T2 cytokines, the approach is to divide patients into those with T2-high and non-T2 (T2-low) asthma. There continue to be critical unanswered questions in severe asthma, mainly since our understanding of the inflammatory microenvironment in the lower airway and the contributions to the clinical expression of the disease remains incomplete. Recent advances have provided further insight into molecular mechanisms underlying steroid resistance, tissue remodelling, and disease exacerbations. The accurate translation of discoveries from these studies will require careful clinical

characterization for the design of clinical trials and the development of new biologic therapies[13].

Immune Cells Drivers of Severe Asthma

Eosinophils

Eosinophils have been widely considered to play a prominent role in the pathogenesis of T2-high asthma and have pleiotropic effects on various inflammatory cells. Eosinophils are implicated in several pathologic processes including epithelial damage, smooth muscle hypertrophy, neural plasticity, and impaired tissue repair processes, promoting chronic airway remodelling and airflow obstruction. Additionally, blood eosinophilia or an increased number of eosinophils in blood positively correlated with increased disease severity, worse disease control, and increased risk of severe exacerbations. Eosinophilia results from the stimulation of eosinophil production from hematopoietic stem and progenitor cells. The differentiation and survival of eosinophils involve signalling by IL-3, IL-5, and GM-CSF. Of these three cytokines, IL-5 plays the most critical role, which is very important from a therapeutic view as a target. Tissue recruitment involves the activation of their surface integrins in response to chemotactic factors including eotaxins and lipid mediators. Once infiltrated, eosinophils may undergo activation. Upon stimulation and activation, eosinophils release a myriad of signature mediators, chemokines, cytokines and growth factors including ECP, eosinophil-derived neurotoxin (EDN), EPX, MBP, IL-5, IL-13, eotaxin, CysLT, and transforming growth factor (TGF)-β1. Among these factors are several key actors of T2 immunity and tissue remodelling, most notably IL-4, IL-13, and TGF-β1. Furthermore, CysTL is a potent bronchoconstrictor that acts in synergy with IL-33 and further drives the self-amplifying loop that characterizes T2 inflammation[13,20].

Innate Lymphoid Cells

It is 12 years since the discovery of innate lymphoid cells (ILCs)[3]. ILCs reside at barrier surfaces and regulate tissue homeostasis, immunity, and disease pathology. Cytokine-producing ILCs are divided into 3 groups, group 1 ILCs (ILC1s), group 2 ILCs (ILC2s), and group 3 ILC3 (ILC3s), based on their functional similarities to the main groups of adaptive T helper (Th) cells. ILC1s are the innate equivalents of Th1 cells and produce IFN-γ and TNF-α. ILC2s are the innate equivalents of adaptive Th2 cells. On activation, they secrete type 2 cytokines including Il-4, IL-5 IL-9, and IL-13. They also produce amphiregulin and IL-10. ILC3s are the innate equivalents of Th17 cells. ILC3s produce IL-17A/F and IL-22.

Recent data suggest that ILC2s might be highly important in the pathogenesis of asthma[3]. They respond rapidly to allergen exposure and environmental insults in mucosal organs, producing type 2 cytokines. It was shown that epithelium-derived cytokines IL-25, IL-33, and TSLP activate ILC2s resulting in eosinophilia, mucus hypersecretion, and remodelling of mucosal tissues. Increased ILC2s have been reported in blood and BAL from patients with asthma as compared with healthy controls, and in blood and induced sputum of patients with severe asthma in comparison with mild asthma. The number of circulating ILC2s correlated with eosinophil counts in induced sputum and blood and ILC2s frequencies were also increased in induced sputum of pediatric patients with severe asthma. Regarding the activation status of circulating ILC2s subjects with severe asthma or uncontrolled asthma had increased numbers of IL-5+ and IL-13+ ILC2s cells. Besides IL-25, IL-33, and TSLP cytokines eicosanoids likely play a critical role in promoting migration and activation of ILC2s in asthma. Involvement of ILC2s was also observed with viral triggers of asthma besides allergens, both in respiratory syncytial virus infection in mice and experimental rhinovirus

infection in humans. Recently, studies have been initiated to elucidate the effects of asthma treatment on ILC2s and to modulate ILC2s as a potential treatment option for asthma as ILC2s may contribute to steroid resistance and persistent airway pathology. Further studies using anti-IL5 and IL4/13 biologics likely will provide useful information to dissect the roles of ILC2s and innate type 2 responses in the pathophysiology of asthma and, at the same time, will help to develop novel treatment strategies for asthma by targeting ILC2s[3]. Further studies are also needed to elucidate the possible role of ILC1s and ILC3 responses in asthma.

Mast Cells and Basophils

The evidence that mast cells degranulation followed by the release of various mediators represent important contributors to the pathogenesis of asthma is strong. Mast cells normally reside in the lungs, and on activation by IgE-dependent or other mechanisms, they can release a diverse spectrum of mediators that in turn can rapidly induce local effects on blood vessels, nerves, and mucous glands, as well as on epithelial cells, airway smooth muscle cells, and immune cells[4]. Among the mast cells secreted mediators histamine, prostaglandin (PG) D2, and leukotriene (LT) C4 are capable of inducing bronchoconstriction, mucus secretion, and mucosal oedema, all asthma characteristics. Additionally, recent data strongly suggest that an altered functional subtype of mast cells may have greater potential to generate PGD2. These PGD2-high mast cells strongly predict poorly controlled T2-high asthma and are associated with more severe disease (targeting CRTH2)[5,7,11,13]. Besides, mast cells also synthesize and secrete a large number of proinflammatory cytokines (including IL-4, IL-5, and IL-13), which regulate both IgE synthesis and the development of eosinophilic inflammation, and several profibrogenic cytokines, including TGF-β. Furthermore, major mast cells' secretory products, serine proteases tryptase, chymase, and carboxy-peptidase, can interact with various cell types and direct their activity via protease-activated receptors and other processes[4]. On the other hand, the functional significance of basophils in the pathogenesis of asthma has gained attention only recently. Similarly, as mast cells, IgEs on basophils through FcεRI mediate immediate hypersensitivity in response to allergens, and can also facilitate and modulate antigen presentation by dendritic cells and response to viruses. Basophils are recruited in the bronchial walls of T2-high asthma. Basophils and eosinophils appear to be closely linked by directly or indirectly influencing each other since they are responsive to similar cytokines and chemokines[10]. Basophils activation leads to the release of immunoregulatory and effector mediators, including IL-4 and IL-13, histamine, and LTC4. Moreover, it is well known that human basophils are one of the major producers of IL-4 and can thus directly modulate T2 inflammation[4,5,7,11,13].

The pathophysiologic mechanisms driving corticosteroid insensitivity and severe asthma are still unclear and evidence suggests that mast cells and basophils might have a role in it. *In vitro* studies using various cell types showed that different mediators produced by activated mast cells and/or basophils, including cytokines, can interfere with the therapeutic action of corticosteroids. Mediators released by activated mast cells have been shown to decrease the anti-inflammatory action of glucocorticoids in airway smooth muscle cells by reducing the expression of anti-inflammatory genes. Mast cells infiltration and interactions have been described in different compartments of the airways, including epithelium, submucosa and airway smooth muscle. Therefore, mast cells' airway infiltration, release of mediators and interaction with lung structural cells may contribute to the corticosteroid insensitivity in severe asthma[2,4].

Conclusion

For many years, severe asthma has been recognized as a subset of asthma that is poorly managed by standard therapy for asthma. Nowadays asthma is considered an umbrella diagnosis for several diseases with variable clinical presentations (phenotypes) and distinct mechanistic pathways (endotype). The precise definition of these endotypes and deciphering the complex interplay of several individual pathways and immune cells is central to asthma management due to inherent biological therapeutic implications. The identification and understanding of the molecular mechanisms of different asthma endotypes, that reflect a highly variable response to different treatments, will lead to more precise asthma management and better outcomes in patients.

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