



Asthma heterogeneity and therapeutic options from the clinic to the bench

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Purpose of review

Asthma is a chronic pulmonary inflammatory disease affecting all races, children and adults. The causative or intrinsic development of asthma is diverse, ranging from episodic exposure to environmental insults to physical induction in exercise.

Recent findings

Asthma uniquely manifests in affected individuals as episodes of airway hyper-reactivity, chronic airway obstruction, and inflammation. The diversity in asthmatic phenotypes and the mechanisms that lead to initiation and perpetuation of the lung disease continue to make asthma a challenge to treat, classify, and manage therapeutically.

Summary

This review will focus on the newest of mechanistic insights into asthma development, therapeutic innovations, and clinical applications.

Keywords

asthma genetics, asthma therapeutics, cellular mechanisms

INTRODUCTION

Asthma is a heterogeneous condition of unclear, but likely multifactorial origins. It is characterized by varying degrees of airflow obstruction, airway inflammation, and episodes of cough, wheezing, and shortness of breath. Further, airway structural changes and remodeling develop in some patients with asthma. The onset of asthma often occurs during childhood, but symptoms can also begin in adulthood. Atopy is a risk factor for the development of asthma, particularly when the onset is during childhood, but not all individuals with asthma are also atopic. The variability in the presentation, natural history, and response to therapies of children and adults with asthma presents challenges to both clinicians and researchers interested in understanding the origins and best treatments for the disease. Despite these challenges, there have been numerous recent publications that have advanced our understanding of asthma, many of which will be reviewed here.

GENETICS

It has long been recognized that genetic predisposition plays an important role in the development of allergy and asthma, with heritability estimates up to

60% [1]. Although lagging behind other complex diseases, recent large-scale genome-wide association studies (GWAS) of asthma have shed some insight into the origins of the disease and potential therapeutic targets. GWAS studies have limitations [2] and require careful planning, analysis, and interpretation [3]. Findings must be replicated, and because the single-nucleotide polymorphisms (SNPs) identified may be in linkage disequilibrium with the actual SNP of interest, follow-up investigations that establish the functional variants responsible for the association are necessary. The GABRIEL (A Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community) Consortium published results in 2010 of the largest GWAS study in asthma to date [4^{••}]. The study included 10 365 patients

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Curr Opin Allergy Clin Immunol 2012, 12:60–67

DOI:10.1097/ACI.0b013e32834edb5b

KEY POINTS

- Asthma is a heterogeneous condition, with complex underlying pathophysiology, genetics, and clinical phenotypes.
- Identifying new and innovative therapeutics is complicated by the availability of appropriate in-vivo modeling of the different phenotypes and translating observations from the bench to the bedside.
- Studies have focused on different cell types, cytokines, and other inflammatory products, but in the end it is ultimately the combined milieu of environment and genome that will best define the mechanism of the asthmatic disease and the appropriate intervention.

with asthma and 16 110 healthy control individuals (who were European or of European descent) and were recruited from 23 different studies. Associations were observed between asthma and six SNPs, implicating *IL1RL1/IL18R1*, *HLA-DQ*, *IL33*, *SMAD3*, *IL2RB* in the entire population and *ORMDL3/GSDMB* only in those with childhood onset of asthma. A smaller study [5[•]] conducted in a more homogenous population of severe or difficult-to-treat asthmatics, The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) population, found associations between multiple SNPs in the RAD50-IL13 region and the HLA-DR/DQ region.

In a recently published study [6^{••}], investigators used a family based screening algorithm that addresses some of the criticisms of GWAS by reducing the likelihood of false-positive associations and increasing the likelihood that the correlations defined by the GWAS will and can be replicated. Investigators screened triads of children randomly assigned to inhaled corticosteroids in the context of the Childhood Asthma Management Program [7] and their parents for SNPs associated with an improvement in FEV₁ with treatment. After identifying an association at SNP rs37972 and replicating the finding in three of four other populations assembled during asthma clinical trials (totaling 935 patients, children and adults), they characterized the functional consequences of the identified SNP and a second SNP in complete linkage disequilibrium (rs37973) as resulting in downregulated expression of the glucocorticoid-induced transcript gene 1 (GLCCI1). Among all participants in the replication populations, those who were homozygous for the mutant allele were 2.36 times more likely to have a poor response to inhaled corticosteroids than those homozygous for the wild-type allele (95% confidence interval, CI 1.27–4.41).

These findings are a promising step towards understanding the heterogeneity in response to inhaled corticosteroids seen in clinical trials and clinical care, but will require additional studies using stratified clinical trials before widespread acceptance.

PHENOTYPING

The heterogeneity of clinical phenotypes of asthma contributes to the difficulties in performing asthma GWAS studies and in replicating results. Several studies [8[•],9,10] published recently have focused on better understanding and defining the many phenotypes of asthma. The Severe Asthma Research Program (SARP) applied unsupervised modeling methods to classify participants in its cohort of 726 participants into one of five clusters [11^{••}] outlined in Table 1.

This analysis was performed using cross-sectional data, and biomarkers such as bronchial hyper-reactivity, percentage eosinophils, total IgE, and the fractional concentration of exhaled nitric oxide (F_ENO) were available only on a subset of participants and could therefore not be included in the analysis. Longitudinal studies will be necessary to determine the value of the phenotypes identified in the cluster analysis in predicting response to therapies and the disease course.

The clusters identified in the SARP analysis were identified primarily using lung function, age of disease onset, and other clinical markers. Other attempts at defining asthma phenotypes have used molecular approaches. Asthma has been classically described as a disease of excessive T_{H2}-type inflammation, but the heterogeneity in response to therapies that target these pathways suggests that the underlying inflammation may be heterogeneous as well. Using gene-expression analyses of airway epithelial brushings, biopsies, and bronchoalveolar lavage fluid obtained from 42 patients with asthma [untreated with inhaled corticosteroids (ICS)] and 28 healthy volunteers, investigators found that asthmatics were divided into two distinct subgroups based on degree of T_{H2} inflammation, described as T_{H2}-high and T_{H2}-low [12]. Patients with T_{H2}-high gene expression, but not T_{H2}-low, had improvements in FEV₁ after an 8-week, randomized, placebo-controlled trial of ICS, suggesting phenotypes translate into clinically meaningful differences in response to therapy.

KEY CLINICAL TRIALS

Ongoing concerns about the association of long-acting beta agonist (LABA) drugs with asthma-related deaths has prompted recent analyses of large

Table 1. Severe asthma research program phenotypic clusters

Cluster type	Percent of cohort	Childhood onset	Women/men	History of atopy	Control medication use	Lung function	Healthcare use
Cluster 1	15	Yes	Predominantly women	Yes	Minimal	Normal range	Mild
Cluster 2	44	Yes	Predominantly women	Yes	Slightly higher	Border-line normal range	Mild
Cluster 3	8	No	Predominantly women	Yes and no	High usage	Border-line normal range	Moderate
Cluster 4 (ATS: Severe)	33	Yes	Women and men	Yes	High usage	Significant dysfunction	High
Cluster 5 (ATS: Severe)		Yes and no	Predominantly women	Yes and no	High usage	Significant dysfunction	High

pharmaceutical company databases of patients in over 200 asthma clinical trials [13[¶],14[¶]]. Glaxo-SmithKline conducted a meta-analysis of asthma deaths in 215 studies that compared salmeterol with other non-LABA treatments for asthma in 106 575 participants [15]. Analyses were conducted in participants who were treated with LABA monotherapy as well as those treated with LABA in combination with inhaled corticosteroids. Among those using LABA without inhaled corticosteroids, there was a 7.3-fold increased risk of asthma-related death (95% CI 1.8–29.4), whereas there was a nonstatistically significant increased risk of asthma-related death among those prescribed LABA in combination with inhaled corticosteroids (odds ratio 2.1, 95% CI 0.6–7.9). AstraZeneca conducted an analysis using their database of asthma studies containing 13 542 participants treated with the LABA formoterol and 9968 non-LABA participants and did not find an increase risk of all-cause mortality among those taking LABA [14[¶]]. Concerns about the inadequate power of previous studies to detect asthma-related or all-cause mortality has prompted the Federal Drug Administration to mandate that all manufacturers of LABAs in the USA conduct large-scale clinical trials designed and powered to address the safety of LABAs plus inhaled corticosteroids compared to inhaled corticosteroids alone [15]. These studies will include children, adolescents, and adults, will share oversight by a common independent steering committee and data-monitoring committee, and are expected to include a total of 46 800 adolescents and adults and 6200 children.

Until the results of the LABA safety studies are available (estimated publication 2017), clinicians who care for patients with asthma who have not responded to inhaled corticosteroids face difficult decisions about the best step-up therapy to offer. Using a three-arm, placebo-controlled, triple-dummy, crossover study design, the Asthma Clinical Research Network evaluated the addition of tiotropium bromide, a long-acting anticholinergic agent, to doubling the dose of ICS or the addition of LABA [16[¶]]. The addition of tiotropium was superior to doubling the dose of ICS with respect to the primary outcome of morning peak expiratory flow, and noninferior to the addition of salmeterol. Similar results were seen with secondary outcomes, including asthma control days and FEV₁. Adverse effects were similar among treatment groups, although the 14-week treatment period limits the ability to comment on long-term safety of any of the therapies tested. This study does give clinicians an additional option to consider for adults who have asthma that is not well controlled on low-dose ICS.

Biologic treatments that target specific inflammatory pathways implicated in asthma are intriguing. The Th₂ cytokines IL-4 and IL-13 have been proposed as potential candidates for novel therapeutics for asthma. A phase 2 study [17] of AMG 317, a human monoclonal IgG₂ antibody with the IL-4 receptor α chain, important in both IL-4 and IL-13 signaling, failed to show efficacy among the 294 moderate to severe participants with asthma randomized to placebo or one of three doses. Subgroup analysis suggested those with more severe disease were more likely to respond to AMG 317. In a separate study [18^{*}], researchers hypothesized that Lebrikizumab, an IgG₄ monoclonal antibody to IL-13, might be more effective in participants with evidence of Th₂-type inflammation. In this randomized, placebo-controlled, parallel group study, participants with asthma inadequately controlled on ICS were stratified by periostin levels, a surrogate for IL-13 activity. There was a significant increase in FEV₁ among all participants randomized to Lebrikizumab (5.5 percentage points, $P=0.02$), but this result was driven by higher responses among those with high periostin levels (8.2 percentage points, $P=0.03$ for periostin high, and 1.6 percentage points, $P=0.61$, for periostin low subgroups). These studies highlight the importance of addressing the heterogeneity of asthma when designing studies for novel therapies to improve our ability to personalize approaches to patients for whom standard asthma therapy is not sufficient.

THERAPEUTIC APPLICATIONS BASED ON BASIC SCIENCE

Therapeutic development in asthma has been challenging because of the heterogeneity in the disease [19]. One of the major hurdles with therapeutic

development has been the difficulty in establishing an in-vivo model which mimics the pathophysiology of the specific phenotypes of asthma [20,21]. Although the animal models (in particularly murine models discussed below) have enabled some investigation into the mechanisms of asthma development, there has been considerable difficulty in translation into clinical trials. The studies in animal models have not always translated into effective human treatments, which has impacted the versatility, clinical relevance, and perceived validity of the in-vivo murine model for testing new therapeutics [22]. The current widely used pharmaceuticals are highlighted in Table 2. These drugs are used to treat acute exacerbations and to control chronic inflammation and remodeling [9,23,24].

ANIMAL MODELS

Observations from in-vivo models have contributed to our understanding of the pathophysiology underlying asthma phenotypes [25]. The only animals that appear to develop asthma intrinsically are horses and cats; however, because of their size, costs, and lack of congenic resources, they are not reasonable models for basic science [26–28]. As most other animals do not develop spontaneous asthma, the atopic phenotype of asthma is the most frequently developed and utilized in-vivo model. The most common model used is the antigen-challenged mouse, with investigator-specific antigen, sensitization, and challenge protocols, allowing versatility but also diversity in observations. In the end, the murine model does not develop spontaneous bronchoconstriction but does develop airway hyper-reactivity to stimulation which has dictated the primary outcomes of the in-vivo studies. This approach probably explains some of the frustrations

Table 2. Classification of current asthma treatment therapeutics

Drug class	Example	Point of care	Mechanism
Corticosteroids	Fluticasone, budesonide, ciclesonide	Controller therapy	Anti-inflammatory
B-Agonists	Albuterol/levalbuterol	Short acting: rescue therapy;	Smooth muscle relaxation
	Epinephrine/terbutaline	Long acting: controller therapy in combination with other therapies	
Anticholinergics	Tiotropium, ipratropium	Rescue therapy	
Methylxanthines	Theophylline, aminophylline	Rescue or controller therapy depending on route of administration	
Cysteinyl leukotriene inhibitors	Montelukast	Controller therapy	Anti-inflammatory
Anti-IgE	Omaluzimab	Controller therapy	Monoclonal antibody to IgE, blocks mast cell degranulation and histamine release

in translating observations in the mouse model to clinical disease in humans [22].

THE CYTOKINE STEW

The historical focus of the asthma research community on T_{H2} inflammation has recently broadened to include other cell types increasingly recognized as important in the cause of asthma development. Recent data support the involvement of T_{H1} , T_{H17} , T-regs, $CD8^+$ T cells, natural killer T cells (NKT), and $\gamma\delta$ T cells [29]. Further, in addition to the traditional cytokine mix defined by IL-4, IL-5, and IL-13, new cell-specific mediators such as IL-33 and IL-25 have been identified [30]. Cytokines implicated in the response to antigen challenge can be produced by a variety of cells even beyond the T-cell network. Figure 1 summarizes these cytokines and their potential roles in the development of airway disease [31]. It is thought that the resulting inflammatory milieu supports ongoing airway reactivity,

inflammation, and finally remodeling in chronic disease.

CURRENT VIEW OF THE CELLULAR COMPONE OF ASTHMA

T cells, the key players in the adaptive immune response against infections, are thought to be recruited into the asthmatic airway following antigenic challenge [32]. The accumulation of activated T cells in and around the airways of asthmatics is thought to orchestrate the inflammatory response through the interactions with neutrophils, eosinophils, and macrophages. T cells have been a focal point of asthma research, making them a target for pharmacological intervention. Although traditionally, the focus has been on $CD4^+$ T_{H2} cells, research has begun to identify many other potential T cell contributors to the pathophysiology of asthma.

T_{H1} cells are present in the bronchoalveolar lavage fluid of asthmatic patients along with an

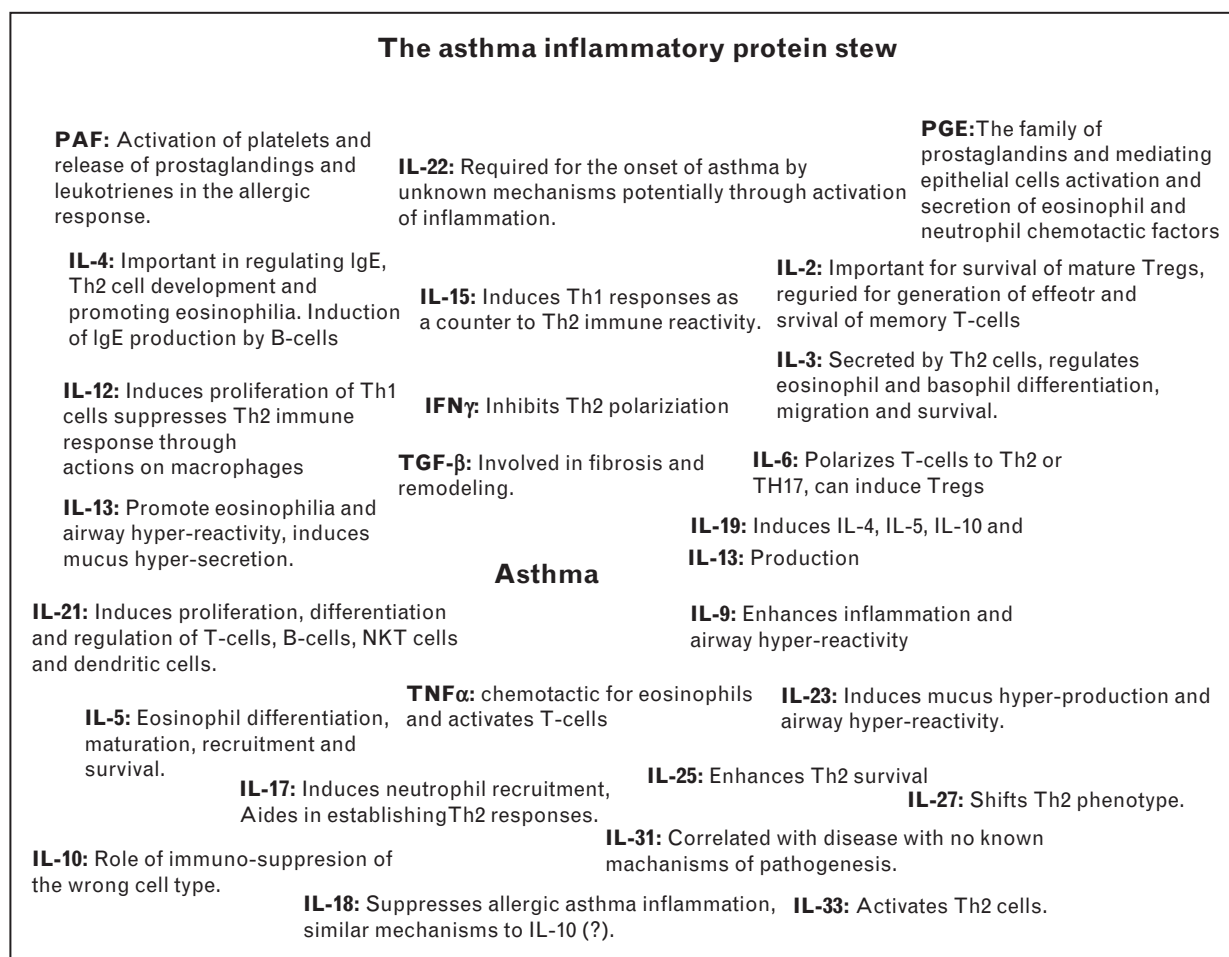


FIGURE 1. Cytokine stew of asthma. The interplay of inflammatory proteins and cytokines in the onset and maintenance of inflammation and induction of remodeling in asthma.

increase in the levels of the T_{H1} cytokine IFN γ [20,33]. Deletion of the T_{H1} -dependent transcription factor T-bet in mice subsequently sensitized and challenged show spontaneous development of airway hyper-reactivity and eosinophilia, suggesting that T_{H1} cells are essential in asthma pathogenesis even in the absence of any antigenic challenge. It is interesting that these studies suggest that the intrinsic defect was in the absence of definable activation of T_{H2} cells. To further support this finding, mice deficient in the IFN γ receptor, the receptor for a T_{H1} cytokine, also show an enhanced response to allergen postchallenge [33].

Following sensitization and challenge, T cells of the T_{H2} phenotype release a group of cytokines including IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF [29]. These cytokines promote the activation, proliferation, and recruitment of neutrophils, macrophages, and eosinophil cells into the airways typical of the clinical signs associated with asthma. As with any network biological process, it is almost impossible to define the specificity of each cytokine's role in the asthma pathophysiology, especially with regards to the multiple cellular sources and combinations.

T_{H17} cells are a distinct subset of T cells that are differentiated by the cytokines IL-6, IL-1 β , TGF- β , and IL-23 [34,35]. Increased numbers of T_{H17} cells are found in the airways of asthmatics. Intra-tracheal instillation of IL-17 prompts a neutrophilic influx into airways and activates a variety of downstream pathways of NF κ B and mitogen-activated protein kinase, stimulating transcription of inflammatory. These mediators are also chemotactic for neutrophils and eosinophils, documented to participate in asthma pathophysiology [36].

T-regs, formally called suppressor T cells, are important in immunological tolerance and limiting T-cell-mediated immune response [37–39]. T-regs control the immune response through the production of anti-inflammatory cytokines IL-10 and TGF- β and inhibition of co-stimulation with antigen-presenting cells such as macrophages, dendritic cells, and B lymphocytes. T-regs are naturally occurring cells characterized by the cell surface expression of CD4⁺CD25⁺ T cells and expression of the transcription factor forkhead box protein 3 (FoxP3) [40]. Clinically, some studies have shown that there is a correlation between the suppression of the T_{H2} responses to allergens because of the absence of CD25⁺FoxP3⁺ and IL-10 (both FoxP3⁺ and FoxP3⁻). Furthermore, some studies have correlated the absence of FoxP3⁺ T-regs in scenarios of pediatric and adult asthma [40].

To add to the complexity of the asthmatic T-cell phenotype, beyond the traditional concept of T_{H1}

versus T_{H2} , T_{H17} , and T-regs, is the potential participation of CD4⁺ NKT cells, $\gamma\delta$ T cells, and CD8⁺ lymphocytes [29,41]. NKT cells produce similar cytokines to the conventional CD4⁺ T cells; however, their unique participation appears to be in the response to glycolipids, including surfactant phospholipids [42]. The role of NKT cells in acute exacerbations and chronic airway remodeling is still unclear. The $\gamma\delta$ T cells are unique T cells capable of recognizing small organic molecules such as alkylamines and lipids presented by CD1⁺ on dendritic cells, similar to NKT cells [29,43]. In response to stimulation, $\gamma\delta$ T cells secrete T_{H2} -type cytokines which are required for eosinophilic inflammation and airway hyper-reactivity to allergen challenge. Recently, it has been suggested that there is an association between severe asthma and the activation of circulating CD8⁺ T cells [44]. In these studies, the CD8⁺ cells produced higher amounts of IL-4 contributing to the asthma phenotype [44]. NKT cells, CD8⁺ cells, and $\gamma\delta$ T cells might be involved in the pathogenesis of allergic asthma through the release of T_{H1} , T_{H2} , and T_{H17} cytokines. The contribution of these different types of T cells and their cytokines may be related to the asthma phenotypes and why it is difficult to correlate T cells with a specific phenotype.

AIRWAY EPITHELIUM

Airway epithelial cells are the initial site of the immune response to antigenic challenge [45], providing costimulation to dendritic cells that lie just beneath the epithelium [46]. Upon inhalation of particulates or antigens, airway epithelium produces a variety of cytokines which recruit inflammatory cells (chemokines), activate immune cells (TNF α and IL-6), or produce reactive oxygen species. These products change the milieu at the epithelial cells airway lumen interface as well as the interstitial space. This change in milieu ultimately provides the conditions for which dendritic cells and macrophages respond to stress as in exercise-induced asthma or antigen exposure in atopic asthma.

DENDRITIC CELLS

The development of an allergic response is divided into two steps: sensitization to a particular allergen and subsequent response to challenge with the same allergen. Dendritic cells, like airway epithelial cells and alveolar macrophages, have surface pattern receptors that recognize pathogens and antigens [47]. Dendritic cells are the key to pulmonary immune surveillance. Dendritic cells' unique juxtaposition to the airway lumen provides these cells

with the opportunity to present antigen to T_{h2} cells and initiate activation of the specific T-cell response [41].

MACROPHAGES

In addition to the epithelial cell–dendritic cell partnership's role in antigen presentation described above, alveolar and interstitial macrophages also present antigen. Macrophages present antigen to T-cell populations that have preexposure memory. Macrophages are chronic inflammatory cells and are essential in directing the resolution or continuation of inflammatory responses [48]. Studies [49,50] have suggested the skewing of macrophage function from classically activated macrophages to alternatively activated macrophages defines the milieu and development of chronic asthma and remodeling.

Accessory cells defining airway hyper-reactivity

Cross-linking soluble IgE to FcεRI receptors on mast cells, basophils, and eosinophils are essential components of immediate hypersensitivity reactions in allergic inflammation [51].

Eosinophils

Eosinophils are derived from myeloid lineage and participate in antigen presentation. The unique production of cytokines and inflammatory mediators produced by eosinophils contributes to bronchomotor tone involved with airway hyper-reactivity [52]. New research suggests that eosinophils and their distinct products may be important in the switch between the T_{h1} and T_{h2} response in asthma and therefore are important in the regulatory pathways of asthma pathogenesis [20].

Mast cells

Mast cell hyperplasia is a consistent finding in the mucosal epithelium and adjacent smooth muscle [53]. Mast cells populate all vascularized organs and tissues, including the upper and lower respiratory track. Mast cells are exquisitely sensitive to the surrounding milieu and are activated by T_{h2} cytokines, including IL-13 and IL-5. Mast cell degranulation through FcεRI cross-linking triggers results in the rapid release of inflammatory mediators, such as histamine, proteoglycans, and cytokines that contribute to airway hyper-reactivity, airway inflammation, and mucus production [51]. Anaphylatoxins, such as C5a and C2a, generated during the

inflammatory response, also contribute to mast cell activation, perpetuating chronic airway inflammation [20,53,54].

Basophils

Cross-linking basophilic FcεRI receptors result in the production of mediators including histamine, leukotrienes and heparin [51,55]. These products contribute to bronchoconstriction, bronchomotor changes, airway hyper-reactivity and inflammation [51].

CONCLUSION

Asthma is a heterogeneous condition, with complex underlying pathophysiology, genetics, and clinical phenotypes. Identifying new and innovative therapeutics is complicated by the availability of appropriate in-vivo modeling of the different phenotypes and translating observations from the bench to the bedside. Studies have focused on different cell types, cytokines and other inflammatory products, but, in the end, it is ultimately the combined milieu of environment and genome that will best define the mechanism of the asthmatic disease and the appropriate intervention.

Acknowledgements

None.

Conflicts of interest

This work was partially supported by the American Lung Association, The Case Western Reserve Vision Fund. The authors have no conflict of interests or sponsorship relationships in regards to the summary of the topics discussed in this article.

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- of special interest
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