

Immunobiology of Critical Pediatric Asthma

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Abstract Asthma is a heterogeneous disease with numerous clinical phenotypes. Severe asthma constitutes about 10 % of all cases of asthma. There is significant geographic and regional variation in the incidence and severity of asthma. Other important factors include gender, ethnicity, living environment, lifestyle, socioeconomic class, and pathophysiology. These factors can often be identified as either genetic or environmental influences on asthma severity. The immune system derangements in severe asthma are poorly understood. Many molecules and cell types have been implicated in severe asthma, including neutrophils, airway epithelial cells, thymic stromal lymphopoietin, and even filaggrin. Recently, vitamin D has been thought to have a role in the severity of asthma. Aspirin exacerbated respiratory disease is an example of a phenotype that includes severe asthma as a feature. This suggests a role of leukotrienes or prostaglandins in the pathogenesis of severe asthma. Both the innate and adaptive immune system may play a role in the development of severe asthma. Besides filaggrin, other factors of the innate immune system, including TLR4 and TLR9 have been implicated in asthma. Airway epithelial cells possess pattern recognition receptors that recognize danger or pathogen-associated molecular patterns, and the result of binding of the ligand is the triggering of a signaling pathway that ultimately can lead to an activation of inflammatory mediators through the action of calcineurin and NF- κ B. Components of the adaptive immune system, including T_H2 and Th17 cells, have been implicated in the pathogenesis of asthma. The fact that so many

molecules and cells may be variably involved in asthma patients, coupled with the presence of redundant pathways that lead to secretion of inflammatory mediators, make the development of effective drugs for the treatment of asthma extremely difficult. A better understanding of the heterogeneity and what drives this diversity on a genetic and epigenetic level will help to develop strategies for novel therapeutic agents or methods.

Keywords Severe asthma · Asthma phenotypes · Thymic stromal lymphopoietin · T-helper cells · Dendritic cells · T_H2 cells · Eosinophils · Eosinophilic asthma · Leukotrienes · Innate immunity · Toll-like receptors · Airway epithelial cells · Prostaglandins · Biological response modifier therapy · Immunotherapy · Vitamin D

Introduction

Children with severe asthma account for 5 % to 10 % of all pediatric patients diagnosed with asthma [1]. Most clinicians utilize the National Heart Lung and Blood Institute (NHLBI) Expert Panel Report-3 (EPR-3) or the Global Initiative for Asthma (GINA) to understand the clinical definition of asthma of varying severity. But the World Health Organization characterizes severe asthma by that which is characterized by persistent chronic symptoms or severe exacerbations or death and/or adverse reactions to medications and/or chronic mobility (including impaired lung function or reduced lung growth in children). This definition also categorizes severe asthma into three categories—untreated severe asthma, difficult-to-treat asthma, or treatment-resistant severe asthma. Treatment-resistant severe asthma can further be divided into two categories—(1) those patients who are not controlled despite the highest level of recommended therapy, refractory asthma, corticosteroid-resistant asthma or (2) asthma for which control

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can be maintained only with the highest level of recommended treatment [2, 3]. The treatment of asthma has been extensively reviewed [4–13]. This article focuses on the pathophysiology of severe pediatric asthma.

Phenotypes and Endotypes of Pediatric Asthma

Asthma is a heterogeneous disease comprised of syndromes with similar, yet distinctly different, causes, natural histories, and outcomes. An asthma phenotype includes clinical, physiological, morphologic, and biochemical characteristics, as well as the response to different treatments [1]. Since there are so many factors taken into account when distinguishing distinct phenotypes, there remains much controversy in the literature on how to classify the characteristics of each group [3, 14]. The Tucson Study of Childhood Asthma classified pediatric asthma patients into four groups, namely, non-wheezers, early transient wheezers, late-onset wheezers, and persistent wheezers [3] (Fig. 1). In the early 2000s, the Avon Longitudinal Study further reclassified these into six groups along the same lines. The severe asthma phenotype differs in children and adults. It is not known whether there is a linkage, i.e., whether one particular childhood asthma phenotype leads to a particular adult asthma phenotype.

Phenotypes, while helpful clinically, provide little to no insight into the underlying disease process. Without an understanding of the disease process or knowledge of specific biomarkers, phenotypic identification and characterization provide little guidance towards targeted drug therapy. A new term, “endotype”, has gained popularity to better define the distinct subtypes of asthma. First introduced in 2008 by Anderson [15], an endotype refers to a subtype of a condition which is defined by a distinct functional or pathophysiological mechanism or treatment response. Multiple endotypes have been proposed for asthma [16, 17], but most define subclasses of adult patients (Table 1).

The potentially evolving endotype most applicable to pediatric asthma is the “early onset allergic” endotype, encompassing about 40 % of all severe asthmatics [17]. These patients have a childhood onset of severe asthmatic disease that is persistent and likely progressive to adulthood. They have a history of allergic reactions, and a family history positive for asthma. Studies have suggested various biomarkers to assist with diagnosis and treatment, but none, to date, have been completely specific for early-onset allergic asthma. Because endotypes attempt to address the pathophysiology of the disease, and because asthma is essentially a disease with significant immunologic characteristics or dysfunction, many of the endotypes that are or will be used to characterized subsets of asthma will most likely be based on different immunobiology that is predominant in that subset. At

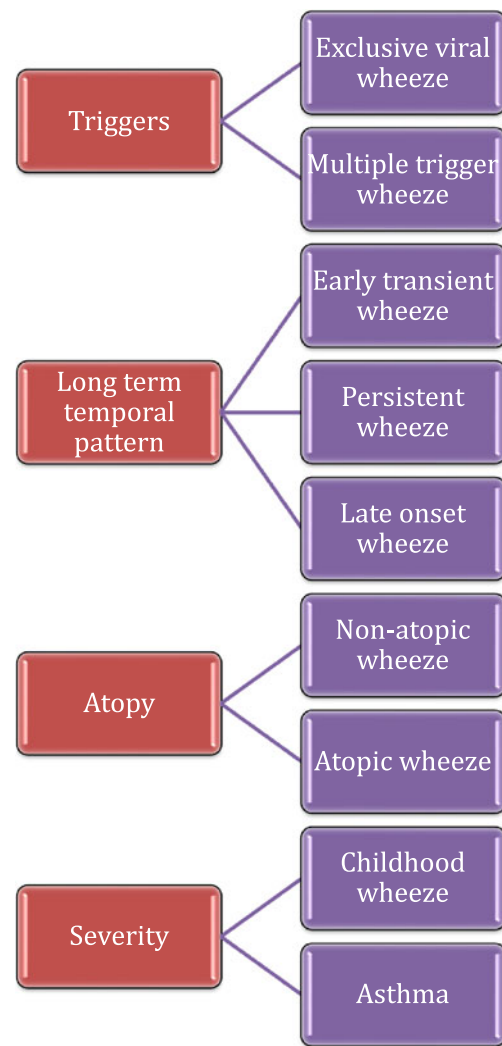


Fig. 1 Phenotypic distribution strategies in childhood asthma

the present time, our understanding of the exact mechanism of any of the endotypes of asthma is for the most part incomplete.

Immunological Characteristics of Pediatric Asthma

Innate Immunity and Critical Pediatric Asthma

The concept that adaptive immunity and T_H2 cells play a significant role in pediatric asthma is well established. However, several innate molecules, cytokines, and target cells have been shown to induce airway hyper-reactivity (AHR) in mice, independently of T_H2 cells or adaptive immunity, supporting the idea that there are many pathways to the development of asthma. Each of these pathways may have a distinct endotype associated with it, though it is likely there is some overlap in patients, which would explain why targeting a solitary pathway may be therapeutically ineffective in patients [18].

Table 1 Endotypes of pediatric asthma

Endotype	Natural history	Clinical	Genetics	Biomarkers	Response to Rx
Aspirin-sensitive	Adult onset, after NSAID ingestion, severe disease, poor prognosis	Airway obstruction+chronic rhinosinusitis+nasal polyps	LT-related gene polymorphisms	Eosinophilia, ↑ urinary LTs	Responds well to anti-LT, especially 5-LO inhibitors
ABPM	Typically adult onset 2° to airway colonization by mold, esp <i>A. fumigatus</i>	Severe cough, mucoid impaction, bronchiectasis, fixed airflow obstruction	HLA polymorphisms and CFTR variants (non-CF pts)	Eosinophilia, ↑ total IgE, ↑ mold-specific IgE/IgG	Systemic glucocorticoids, antifungals, omalizumab (?)
Hypereosinophilic	Typically adult onset; minimal atopy	Severe exacerbations (20 % of severe asthmatics)	No evidence	Eosinophilia	Glucocorticoid-sensitive, anti-IL-5
Neutrophilic	Unknown	Fixed airway obstruction	Unknown	Unknown	Macrolides (?)
Early onset allergic	Childhood onset, severe, persistent disease	Aeroallergen sensitivity, allergic rhinitis	17q12–21 T _H 2 pathway	FeNO, increased specific IgE periotin	Mild response to glucocorticoids and T _H 2 blockers
Cross-country skiers with asthma	Skiing-related; minimal atopy; URI common.	Mild–moderate severity related to strenuous skiing-related exercise	Unknown	Normal FeNO and blood eosinophil count; ↑ LTE ₄ in urine	Poor response to inhaled glucocorticoids; improvement when not skiing

ABPM allergic bronchopulmonary mycoses, CF cystic fibrosis, FeNO fractional exhaled nitric oxide, LT leukotriene, LTE₄ leukotriene E₄, 5-LO 5-lipoxygenase, URI upper respiratory tract infection

The Airway Epithelium and Severe Asthma

The airway epithelial cells (ECs) are pivotal in protecting the airway from invasion by allergens, viruses, and environmental pollutants. ECs express pattern recognition receptors (PRRs), which identify and respond to pathogen-associated molecular patterns (PAMPs) found in microbes or to damage-associated molecular patterns (DAMPs) released upon tissue damage, cell death, or cellular stress [19]. DAMPs have also been referred to as “danger-associated molecular patterns to include all non-pathogenic entities that may be dangerous to the host. Activation of epithelial PRRs result in the release of cytokines, chemokines, and antimicrobial peptides which stimulate cells of both the innate and adaptive immune system [20].

As dendritic cells (DCs) express PRRs, it was initially believed that they also directly recognize inhaled foreign substances. However, studies using mice lacking Toll-like receptor 4 (TLR4) showed that recruitment, activation, and intraepithelial migration of DCs in response to inhaled endotoxins require only epithelial triggering of TLR4 [21]. Many allergens, such as Der p 2 and Der p 7 from house dust mite (HDM) and Fel d 1 from cats, activate TLR4 signaling [22]. TLR4 expression is also upregulated by viral infection of the airway epithelia with respiratory syncytial virus (RSV) and exposure to cigarette smoke [23, 24], potentially contributing to the allergic sensitization and inflammation experienced early in life from these exposures.

HDM triggering of TLR4 on ECs also induces production of thymic stromal lymphopoietin (TSLP), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-25, and IL-33. These factors activate DCs that prime T_H2 responses by inhibiting production of IL-12 and tipping the balance toward a T_H2 paradigm. The result is the release of chemokines that attract T_H2 cells, or upregulate surface molecules, such as OX40L, that trigger T_H2 cell development [19].

Cytokines derived from the lung epithelium also activate innate immune cells such as basophils, eosinophils, mast cells, and non-T and non-B lymphoid cells [25–28], which act to further potentiate T_H2 development. The epithelial response to allergens and pollutants are maintained through the action of T_H2 effector cytokines, creating a feedback loop that can perpetuate asthmatic disease [19] (Table 2).

Toll-Like Receptors and Asthma

TLR4 Toll-like receptors recognize molecular patterns from bacterial and fungal pathogens, resulting in production of proinflammatory antimicrobial mediators [29]. TLR4, the first mammalian TLR identified, is activated by lipopolysaccharide from Gram-negative bacteria and CD14, leading to increased expression of TNF-α, IL-1, and IL-12 via signaling through a common adaptor protein MyD88 [30]. TLR4 is

Table 2 Immunological determinants of severe asthma

Cellular or molecular biomarker(s)	Different in severe asthma	Details
Eosinophils	Yes	Increased eosinophils in BAL and peripheral blood
Neutrophils	Yes	Increased in airway secretions in severe asthma
T-helper cells	Yes	T _H 2 skewing may predispose to more severe asthma
Dendritic cells	Unknown	
Airway epithelial cells		Physical and functional barrier may be critical in preventing severe asthma (Holgate et al. [136], epithelium)
T _H 2 cytokines	Yes	Polymorphisms in IL4R α and TNF α have been associated with severe asthma
Th17 cytokines	Yes	IL-17 in sputum correlates with the severity of AHR
IL-33	Unknown	
Leukotrienes	Yes	In AERD
IFN- γ	Yes	Higher numbers of peripheral blood CD8+ T cells expressing IFN- γ in asthmatic airways correlates with severity of asthma (Cruz et al. [137], pathogenesis)
Filaggrin	Yes	Filaggrin null mutations associated with severe asthma in children (Palmer et al. [138], filaggrin)
TSLP	Yes	TSLP mRNA expression correlates with symptom severity
TLR4	Yes	Certain TLR4 polymorphisms have been associated with milder asthma and other have been associated with lower FEV1
CD14	Yes	Variants in the CD14 promoter have been associated with lower IgE levels (Leung et al. [139], polymorphism)

highly expressed by cells of the innate immune system, particularly dendritic cells and mononuclear phagocytes [31].

Research in mice with defective TLR4 signaling showed a decrease in eosinophils and plasma IgE levels, as well as IL-4 and IL-5, suggesting a decreased T_H2 response in the absence of TLR4. These results suggest that TLR4-mediated signals are necessary for development of T_H2 immune responses to non-microbial protein allergens [32]. RSV infections during childhood have been identified as a major risk factor for the development of asthma [33]. One study demonstrated that the innate immune response to RSV is mediated by CD14 and

TLR4 [34]. This finding suggests one mechanism by which certain viral respiratory infections might act as cofactors for allergen-induced asthma [32].

The discovery of the ability of TLR-4 to recognize the house dust mite allergen in epithelial cells and its eventual activation of DC has led to investigation of an intrapulmonary TLR-4 antagonist. In a murine model of HDM-induced experimental asthma, TLR-4 blockade led to decreased airway eosinophilia and suppression of bronchial hyperresponsiveness [21]. TLR agonists that can specifically activate and assist plasma DCs in patients with allergic asthma can also be of therapeutic benefit [35].

In a study by Zhang et al., no association was found between asthma susceptibility and single nucleotide polymorphisms in TLR4. However, interestingly, there appeared to be an association between the TT homozygote of rs1927914 and lower FEV1 in the TLR4 gene. This finding was also discovered with respect to the GG genotype of both rs10983755 and rs1927907. All of these associations were statistically significant. The authors thus concluded that the A allele of rs1927907 and rs10983755, as well as the C allele of rs1927914 may in fact confer a protective effect with regard to asthma severity [36]. The protective effect of certain TLR polymorphisms on asthma severity has also been shown in children, such as in a study by Sackesen, who demonstrated that TT homozygosity at the CD14-159 promoter was independently and statistically significantly associated with lower IgE levels ($P=0.021$), and also that 2 polymorphisms of the TLR4 gene (TLR4-A896G and TLR4-C1196T) were associated with milder asthma with atopy [37].

TLR9 One of the strategies to treat asthma has been to develop a method by which to reverse the skewing of the T cell response to a predominantly T_H2 paradigm that is seen in allergic asthma. One method has been to mimic the presence of infections by stimulating pathways that are responses to natural infections. The idea is that one can counterbalance T_H2 cytokines by inducing a T_H1 response. By targeting the innate immune system, researchers have utilized Toll-like receptors that recognize pathogen or danger associated molecular patterns (PAMPs or DAMPs). Specifically, TLR-9 agonists have already been studied in clinical trials of persistent allergic asthma. A study of the TLR-9 agonist QbG10 on 63 adult asthmatic patients has shown improved overall patient reported scores in the treatment group compared to controls treated with placebo in a double-blinded trial. After 12 weeks of treatment with this molecule, the proportion of patients who reported “well control” asthma based on the Asthma Control Questionnaire (ACQ) was 2/3 compared to 1/3 in the placebo group. Moreover, those on placebo had a worsening FEV1 whereas it was stable in the treatment group. This illustrates not only the role of pattern recognition receptors in the evolution of asthma as a disease, but also how new treatment

strategies can potentially target this area of immunology to develop new drugs [38].

Complement and Severe Asthma

Mannose Binding Lectin and Asthma The role of complement in asthma is unclear. Mannose-binding lectin (MBL) is an important initial trigger of the alternate pathway of complement. Complement can play a role in immune activation, and the alternate pathway does not require antibody for it to exert its function. A study of MBL levels in 72 asthmatic children and 30 healthy age-matched controls demonstrated that serum MBL levels were elevated in asthmatic children during an acute exacerbation and also between exacerbations. Interestingly, MBL levels in the asthmatic children correlated with peripheral blood eosinophilia. Currently, the significance of this association is unclear, and it is also unknown as to whether or not MBL plays an active role in pathogenesis, or perhaps only serve as a biomarker for asthma. Specifically, there have been no studies linking MBL levels to the severity of asthma.

Cellular Immunity and Critical Pediatric Asthma

Eosinophils and Neutrophils

Increased numbers of eosinophils in peripheral blood and airway secretions are one of the hallmarks of asthma. Early necropsy data and later biopsy studies have consistently revealed eosinophilic inflammation within the airway mucosa of patients with asthma [39]. These findings are supported by studies showing elevated levels of eosinophil degranulation products in bronchoalveolar lavage (BAL) fluid [40] and bronchial biopsies of patients with asthma of varying severity [41]. Even further, the consistent demonstration that factors that improve asthma control, most notably inhaled corticosteroids, also improve underlying eosinophilic airway inflammation [42], whereas factors associated with a loss of asthma control, such as exposure to environmental allergens, lead to a worsening of eosinophilic airway inflammation [43].

It has been demonstrated that there is a direct association between asthmatic disease severity and the number of eosinophils in peripheral blood and bronchial lavage fluid [41]. In patients with symptomatic asthma, 70–80 % of corticosteroid-naïve patients and 50 % of corticosteroid-treated patients have a raised sputum eosinophil count [44]. The absence of sputum evidence of eosinophilic airway inflammation in the remainder suggests that factors independent of eosinophilic airway inflammation are also contributing to airway dysfunction and symptom [39]. FeNO has been utilized as a biomarker for eosinophilic inflammation in both children and adults with asthma. It has been proposed that FeNO may assist in the differentiation of the various phenotypes of asthma, with

elevated FeNOs observed in eosinophilic inflammation but not in noneosinophilic inflammation in children who are non-responsive to corticosteroids [45, 46]. In 2011, the American Thoracic Society published their official guidelines on the clinical practice of using FeNO as a reflection of eosinophilic inflammation, vis a vis, control of asthma. Evidence has confirmed that a low FeNO normally reflects either noneosinophilic inflammation or no inflammation or the airways [47].

Eosinophilia of the airways is thought to be mediated through the action of T_H2 cytokines, particular IL-5. Certain characteristics have been identified in patients with both severe asthma and serum or airway eosinophilia. Tissue lymphocytes (CD3+, CD4+, and CD8+), mast cells, and macrophages were increased, the subepithelial basement membrane zone was thicker, and they have a better short-term response to inhaled corticosteroid treatment [48].

In contrast, neutrophilic inflammation is due to innate and cell-mediated immune responses. There are many factors thought to evoke responses via these pathways, such as endotoxins, viral and bacterial infections, cigarette smoke, environmental pollutants, and occupational agents [39].

Neutrophils, despite being the most abundant cell type in sputum (of both healthy and asthmatic patients) [49], are not increased in the airway secretions of patients with mild or moderate asthma. However, in some patients with severe asthma, neutrophils are found in increased numbers in the airways [50]. Airway neutrophilia persists at the time or asthma recovery, which leads to the question whether these neutrophils play a role in the initiation of attacks, or its resolution, or both [48].

There has been less consistent information on the presence of markers of neutrophilic airway inflammation. Compared with eosinophilic asthmatics, those with noneosinophilic disease was associated with normal thickness of the subepithelial basement membrane and a lower number of CD3+ cells and macrophages [51]. Sputum neutrophilia and elevated sputum supernatant IL-8 have been found in this phenotype [52] but not consistently, and excess neutrophils in bronchial biopsy have not been reported consistently [51, 53]. Likely, there are different subgroups of patients with noneosinophilic asthma.

There appear to be differences in the epidemiology, natural history, and response to treatment in those with noneosinophilic asthma compared to those with eosinophilic disease. Noneosinophilic, neutrophilic asthmatics were more likely to be female, nonatopic, and to have an onset of symptoms in middle age [39]. Exacerbation frequencies were substantially lower in patients with noneosinophilic asthma when treated with standard management [54]. This subgroup of patients have also been noted to have less severe exacerbations of their asthma [51] with significantly smaller reductions in FEV_1 than those with eosinophilic inflammation [55].

Since the early days of corticosteroid treatment for asthma, the absence of eosinophils in sputum smears were associated with a poor clinical response [56]. Short-term improvement in airway responsiveness, symptoms, peak expiratory flow variability, and FEV₁ in response to inhaled budesonide was significantly less in those with asthma and a sputum eosinophil count <3 % [57]. Though it is too early to state that corticosteroids serve no role in noneosinophilic asthma, there is a possibility that they can safely be withheld or be substituted with better alternative therapies.

Dendritic Cells

Lung DCs are the main antigen-presenting cells in pediatric asthma, and are necessary for T_H2 cell stimulation during airway inflammation. In the absence of CD11c positive DCs, T_H2 cells will not produce IL-4, IL-5, or IL-13. Lung DCs upregulate expression of several costimulatory molecules (such as CD80 and CD86) and chemokines (such as CCL17 and CCL22) that attract T cells, eosinophils, and basophils into the lungs [18]. The role of DCs in asthma has mostly been elicited from mouse models. Studies have shown substantial increases in the amount of DCs in the lungs of murine and rat models subsequent to exposure to allergens [21, 58, 59]. Additionally, ovalbumin-pulsed DCs placed directly into the airways of naïve animals resulted in both ovalbumin sensitization and a T_H2 response, complete with eosinophilic airway inflammation, goblet cell hyperplasia, and bronchial hyperreactivity following re-administration of ovalbumin aerosol [60, 61].

Plasma DCs (pDC) also represent the major source of IFN- α upon viral stimulation [62]. In studies [63–65], it appears that patients with asthma may indeed have a decreased viral-induced IFN- α response from pDCs. This finding, coupled with the data that IFN- α/β can block human T_H2 development and inhibit IL-4, IL-5, and IL-13 secretion via GATA-3 suppression [66], suggests a role of plasma dendritic cells in suppressing the immunologic changes seen in patients with asthma. Type 1 interferons also are known to negatively regulate T_H17 development in mice and humans [67, 68], suggests that the failure of pDCs to secrete IFN- α/β may be partially responsible for viral-induced asthma exacerbations. Again, data regarding the relationship between dendritic cell function and the clinical severe phenotype of asthma in children are non-existent. Mechanisms that specifically target DCs have been suggested for future research. One of these targets include specific blockade of TSLP or IL-25 within the respiratory tract to decrease the DC-driven T_H2 inflammatory airway response to allergens [35]. Other ways to specifically decrease the DC's ability to stimulate T_H2 inflammation in the lung would be to selectively inhibit the DC secretion of CCL22 and CCL17, major attractants of T_H2 effector cells to the lungs [35].

T Cells and Their Cytokines in Severe Asthma

T cells are the driving force behind inflammatory diseases and asthma is no exception. Most of the subsets of T cells, including T_H1, T_H2, T_H17, T_H9, T regulatory cells, and cytotoxic T cells play some role in asthma. It is impossible to separate the discussion of T cells and cytokines, many of which either are secreted by T cells or act on them, and therefore the subsequent discussion will address both T cell subsets and their corresponding related cytokines. T cells may also play a role in the development of severe asthma, especially in children, where atopy is considered a phenotypic characteristic of severe asthma.

Key Cytokines in the Pathogenesis of Critical Pediatric Asthma

TSLP

Thymic stromal lymphopoietin is an IL-7-like cytokine, released by primary human epithelial cells in response to particular microbial products, physical injury, or inflammatory cytokines [18]. TSLP is expressed primarily by epithelial cells (of the lung, skin, and gut) [69] and keratinocytes and is also produced by DCs, mast cells, smooth muscle cells, and lung fibroblasts [70].

DCs activated by TSLP in vitro promote the development of T_H2 memory/effector cells [70]. TSLP expression has been observed in the airways of asthmatic patients and TSLP mRNA expression is noted to be directly correlated with symptom severity [71]. TSLP receptor-deficient mice have noticeably less allergen-induced AHR [72], and mice with over-expressed TSLP in the lungs had an amplified T_H2 inflammatory response and remodeling when challenged with ovalbumin [72].

The expression of TSLP in asthma is allergen driven. The allergen activates mast cells via IgE and Fc ϵ RI, which leads to mast cell release of TSLP and various inflammatory cytokines and other mediators. These cytokines likely signal lung epithelial cells to further produce TSLP, priming the DCs to secrete T_H2-attracting chemokines as well as the OX40 ligand. OX40 ligand is one of the signals required from DCs to prime naïve T-cells to differentiate into CD4⁺ T_H2-type T cells and further proliferate. The T_H2 cytokines act in a positive feedback loop to the lung epithelial cells to further stimulate TSLP production [73].

Viral infection, together with cytokine production, is a primary driver for TSLP production. It has been shown that rhinovirus and RSV can both upregulate the expression of TSLP in human bronchial epithelial cells [74].

T_H2 cytokines (IL-4, IL-4R α , IL-5, IL-9, and IL-13)

The predominant cell type involved in the regulation of pathophysiology in asthma is the CD4 T-cell of the T_H2 subtype characterized by the expression of cytokines such as IL4, IL5, IL9, IL10, and IL13 [75]. The exact role of each of these cytokines, in regards to the key pathophysiologic changes seen in the lungs of asthmatics, and the severity of their disease, is still not entirely known.

Interleukin-4 (IL-4) mediates important pro-inflammatory functions in asthma including induction of the IgE isotype switch, expression of vascular cell adhesion molecule-1 (VCAM-1), promotion of eosinophil transmigration across endothelium, mucus secretion, and differentiation of T helper type 2 lymphocytes leading to cytokine release [76].

Interleukin-5 (IL-5) is produced by a number of cell types, and is responsible for the maturation and release of eosinophils in the bone marrow. In humans, IL-5 is a very selective cytokine due to the restricted expression of the IL-5 receptor on eosinophils and basophils [77].

Interleukin-9 (IL-9) is also produced by a variety of cell types and plays a central role in atopic asthma as suggested by several studies. In addition to its activity as a growth factor for T lymphocytes, IL-9 has been shown to have direct activity on additional cell types involved in the asthmatic inflammatory response, such as on mast cells, B cells, and eosinophils [78]. IL-9 plays a role as a mast cell growth factor, upregulating expression of mast cell proteases [79, 80]. Additionally, IL-9 upregulates the expression of the α chain of the high affinity IgE receptor and induces IL-6 secretion in mast cell lines [80, 81]. IL-9 has also been shown to potentiate the release of IgE from primed human cells in vitro and in vivo [82–84]. Lastly, intratracheal instillation of recombinant murine IL-9 led to an increase of eosinophils and IgE levels in BAL [78].

Nearly 15 years ago, two landmark articles demonstrated IL-13 to be a “central mediator of allergic asthma.” [85, 86]. IL-13 production in the airway promotes the survival and migration of eosinophils [87], activation of macrophages [88], increased permeability of and mucus production by airway epithelial cells [89, 90], production of inducible nitric oxide synthase by airway epithelial cells [91], and transformation of airway fibroblasts to myofibroblast, leading to collagen deposition [90]. IL-13 also stimulates airways hyperresponsiveness [92]. The role of T_H2 cytokines in severe asthma is not entirely clear. Cellular infiltration that is regulated by T_H2 cytokines may indicate a role for certain ones, such as the eosinophilic proliferation and differentiation activities attributed to IL-5, or the IgE class switching mediated by IL-4. On the other hand, polymorphisms of T_H2 cytokines and their receptors have been associated with severe asthma, as discussed below under “Genetics and Severe Asthma”. Several drugs targeting these cytokines have already been extensively studied in the treatment of asthma.

IL-25

IL-25, a member of the IL-17 family, is expressed in human lung epithelial cells after exposure to allergens [93]. IL-25 is also secreted by activated eosinophils, by bone-marrow-derived mast cells, by basophils after Fc ϵ RI cross-linking and by c-Kit⁺ cells after stimulation with stem cell factor in the lung [94]. In mouse models of asthma, several reports have shown that IL-25 amplifies T_H2 cytokine production and eosinophilia. Ovalbumin (OVA) sensitization and challenge in wild-type BALB/c mice results in more IL-25 mRNA in the lung. Conversely, treatment with soluble IL-25 receptor fusion protein or antibody to IL-25 before OVA sensitization and challenge results in less AHR, airway inflammation, and OVA-specific serum IgE [95, 96].

IL-33

IL-33 is a member of the IL-1 cytokine family and can be detected in many cell types [97]. IL-33 acts in synergy with stem cell factor and the IgE receptor to activate primary human mast cells and basophils [98]. IL-33 also acts to enhance the survival of eosinophils and eosinophil degranulation in humans [99]. In mice, viral infection or exposure to pathogen-derived products, irritants, or allergens can enhance IL-33 production [21, 100]. Challenge with OVA has also been shown to induce production of IL-33 protein in the lung, which correlates with the maintenance of asthma [100]. Administration of IL-33 with OVA leads to enhancement of airway inflammation in an IL-4-independent mechanism [101]. Conversely, administration of IL-33 antibodies or neutralizing antibodies to ST2 (the receptor for IL-33) decreases eosinophilic inflammation in the lung and AHR [102, 103]. The full mechanism in which IL-33 functions in asthma is not fully understood as much of IL-33’s role in the development of asthma has been elucidated from mouse models.

IL-17

IL-17-secreting cells are important regulators of both allergic and non-allergic asthma. In humans with asthma, the concentration of IL-17 in sputum correlates with the severity of AHR [104] and with the presence of neutrophils. In the IL-17-dependent model of allergic asthma, allergen sensitization via the airway, which can be enhanced by stimulation of Toll-like receptor 4, can prime strong T_H17 responses which promote airway neutrophilia and acute AHR [105]. IL-17 has also been implicated in the stimulation of eosinophilic inflammation [106]. IL-17A or IL-17 receptor-deficient mice demonstrate impaired T_H2-type allergic airway inflammation [107]. In a non-allergic model of mouse exposed to ozone, IL-17 is required for induction of AHR [108]. Despite the presence of T_H17 cells in the lungs of these mice,

development of AHR could occur in MHC class II-deficient mice, suggesting a response independent of adaptive immunity and T_H2 or T_H17 cells [18].

The Humoral Immune System and Severe Pediatric Asthma

IgE in Pediatric Asthma

Allergen-specific T helper type 2 (T_H2) cells are thought to be present in the lungs of almost all asthmatic patients, especially those with allergic asthma. These T_H2 cells produce cytokines which regulates the allergen-specific synthesis of IgE (IL-4), the recruitment of eosinophils (IL-5), the recruitment and growth of mast cells (IL-9), and airway hyper-reactivity (IL-13) [18].

Recent studies have suggested that mechanisms involving IgE and expression of its high-affinity receptor, FcεRI might be partly responsible for the defect in pDC IFN-α secretion in patients with allergic asthma [35]. Patients with increased serum IgE levels appear to have decreased secretion of IFN-α by pDCs after in vitro viral challenges [63]. In support of this finding, IgE cross-linking was shown to substantially inhibit viral-induced secretion of IFN-α by pDCs, further promoting a T_H2 environment [35]. These findings help explain why treatment of patients with cat allergy with anti-IgE (omalizumab; Genetech, Inc, South San Francisco, California, and Novartis, Basel, Switzerland) led to a decreased ability of allergen-exposed DCs to elicit a T_H2 cytokine response [109].

Omalizumab's ability to decrease the sensitivity of many cell types to allergens by decreasing IgE levels paves the way for other therapeutics that also decrease IgE levels and inhibits the interaction of IgE with cellular receptors on specific cell types. These therapeutics would be especially beneficial for atopic patients with viral-induced asthma in whom the interaction of allergen-specific IgE on DCs with inhaled allergen might interrupt the release of antiviral type I interferons. A reduction in IgE levels could improve their response to viruses, decrease their associated T_H2 inflammation, and make them generally less susceptible to virus-induced wheezing [35].

IgA and Severe Asthma

IgA deficiency has been associated with asthma. In a series of 39 adults and children with sIgA deficiency, the incidence of having a diagnosis of asthma prior to diagnosis of sIgA deficiency was 23.1 % in the sIgA group and only 10.3 % in the control group (OR=2.77; 95 % CI, 1.09–7.06, $P=0.03$). A history of ever having had asthma was also higher in the sIgA deficiency group (30.8 %) compared to that seen in controls (10.3 %), (OR=3.57, 95 % CI=1.50–8.51, $P=0.01$). In addition, IgG subclass deficiency was also found to be frequently present in patients with severe asthma. It was thought initially

that this may be due to an association of respiratory infections and IgG subclass deficiency, and the ability of respiratory infections to be an asthma trigger, but there was no difference in IgG subclass levels between the group with respiratory infections and that without among the patients with severe asthma. The question of why there may be an association between severe asthma and IgG subclass deficiency was thus left unanswered.

Vitamin D and Asthma

Over the past few years, the effects of vitamin D levels on immune function have become a heavily invested area of research [110]. Accordingly, there have been many studies examining the relationship of vitamin D deficiency and asthma. Vitamin D has been shown to have both in-utero and post-natal effects on lung development and immune system development and function. In the in-utero period, vitamin D has a role in fetal lung and immune system development [111]. Sufficient vitamin D levels may improve management of respiratory infections early in life by upregulating the production of antimicrobial proteins, such as cathelicidin and beta defensins [112], and decreasing the inflammation associated with viral respiratory infections.

Potential mechanisms for how vitamin D might decrease disease severity of asthma include its effects on immune cells, improved handling or prevention of infections, decreased inflammatory responses, reversal of steroid resistance, and effects on airway smooth muscle mass [113]. In a large study of 1,024 participants of the Childhood Asthma Management Program (CAMP), a randomized trial of inhaled budesonide vs. nedocromil vs. placebo, it was demonstrated that vitamin D deficiency (<30 ng/ml) is associated with increased risks for severe asthma exacerbations leading to emergency department visits or hospitalizations [114].

Several other case-control studies, in multiple ethnic groups, demonstrated that the prevalence of vitamin D deficiency is greater among asthmatic children than in controls [115–117]. Another analysis of 6,857 participants ≥6 years old showed that vitamin D levels were inversely associated with current wheeze and asthma [118]. Additionally, in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort of over 300 children, serum vitamin D concentrations at age 4 years were inversely associated with asthma at ages 4 to 8 years [119].

Immunotherapy and the Prevention of Severe Asthma

Specific allergen immunotherapy has for a history of over 100 years, with the first report of its use in allergic disease by Noon and Freeman in 1911. Allergen immunotherapy is

typically used to treat allergic rhinitis, but it has been shown that children with allergic asthma tend to have more severe asthma. While it is generally not recommended that allergen immunotherapy be used in uncontrolled asthma patients, there are significant benefits which impact the progression of the atopic march in patients who are on allergen immunotherapy. The immune effects of allergen immunotherapy are not completely known. Several hypotheses include the generation of IgG4 antibodies, the induction of allergen-specific T regulatory cells [120, 121], the generation of regulatory cytokines such as IL-10 [122, 123], and the reversal of the T_H2 predominant skewing of T-helper cell balance that is seen in allergic diseases [124–126]. IgG4 may have several potential effects, ranging from acting as a blocking antibody to reducing degranulation of mast cells and basophils [127, 128], to mediating the presentation of antigens to T cells. Clinically, allergen immunotherapy has been shown to reduce the severity of allergies, and also to reduce the incidence of asthma in allergic patients [129].

Genetics and Severe Asthma

From a genetic standpoint, multiple genes have been implicated in the pathogenesis of asthma. Some genes have been associated with increased susceptibility to develop asthma, while others have been associated with severity. In many cases, these genes code for proteins that have intimate roles in immune regulation, including many cytokines such as IL4R α , IL-5, IL-12, and TNF- α . Specifically, polymorphisms in LTA4H, TNF α , and IL4R α have been significantly associated with severe asthma in children. In addition, two haplotypes have also been found to be associated with severe asthma in children. These include the ATA haplotype of IL-4R α A1199>C, IL-4R α T1570>C, and IL4R α A1727>G, and the CA haplotype of TNF α C-863>A and TNF α G-308>A [130]. A further illustration of the usefulness of this association is that polymorphisms in IL-4 have been associated with changes in FEV1 in patients with severe asthma [131], and that the C-590>T single nucleotide polymorphisms is associated with fatal or near-fatal asthma in adults [132].

Other genes associated with severe asthma may be found in relation to molecules that play a role in pathogenesis of certain asthma endotypes, such as glucocorticoid-resistant asthma or leukotriene-related asthma. As eluded to above, polymorphisms in the LTA4H have been associated with difficult to treat asthma in children. Meanwhile, polymorphisms in the CRHR1 receptor, a major regulator of synthesis of glucocorticoids, is associated with differences in responses to inhaled steroids in asthma patients, although there was no observed association with severe asthma in particular [133].

Future Directions

Current therapy options for asthma focus on symptom management and reducing inflammation, but have not succeeded at altering the natural course of disease. Targeted drug therapy appears to be crucial to both short-term reduction of symptoms and long-term alteration of asthma.

Selective inhibition of key aspects of the epithelial-immune interaction, such as TSLP, GM-CSF, IL-25, and IL-33, may lead to a decrease in allergic inflammation by decreasing the risk of allergic sensitization and preventing airway remodeling. Given the importance of epithelial PRRs in promoting sensitization, targeting these receptors via chronic PRR agonist exposure may reprogram ECs to be less reactive to environmental triggers [19].

TNF- α has been recognized as a potential target, specifically for those with noneosinophilic, neutrophilic disease. Treatment with the TNF- α antagonist, etanercept, has been shown to be associated with a significant improvement in airway responsiveness and airflow obstruction in patients with severe asthma [134]. These effects are thought to be due to overexpression of membrane TNF- α on PBMCs [39]. Moreover, mast cells have been reported to be increased in airway smooth muscle in patients with neutrophilic asthma [53], representing a potential source for TNF- α [135].

Discussion

Asthma is not one disease, but many. The pathophysiologic mechanisms that predominate may vary from one asthmatic to another. Certain immunological factors may contribute to the “severe” asthma phenotype. In children, these patients appear to have a higher degree of atopy, which suggests a greater involvement of T_H2 cells and T_H2 cytokines. But two similarly atopic children may not have the same extent of severe asthma, suggesting also that multiple additional mechanisms may be involved in the pathogenesis. As we progress from discussing clinical and laboratory features (phenotype) to biochemical or biophysiological mechanisms of the disease (endotypes), we obtain a better understanding of the disease from the standpoint of targeting the appropriate molecule or cell to optimize treatment. The genomics and personalized medicine acts of 2006 and 2010 were attempts to incentivize research into customization of therapy for heterogeneous disease states such as asthma.

Current advances in the fields of proteomics, genomics, and metabolomics have improved our understanding of asthma. This has led to the delineation of certain phenotypes and endotypes of severe asthma and the identification of gene polymorphisms that may contribute to asthma severity. More information is needed, as the heterogeneity of the disease still out-strips our knowledge of pathophysiology and mechanisms

of action of the numerous factors that act alone and in concert to generate the clinical manifestations of asthma.

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