THE PARALLEL MARCH OF ASTHMA AND ALLERGY IN CHILDHOOD: A MULTI-PERSPECTIVE APPROACH

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THE PARALLEL MARCH OF ASTHMA AND ALLERGY IN CHILDHOOD: A MULTI-PERSPECTIVE APPROACH

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Could the rings of the truck of a tree be separated? This is how allergy and asthma seem to be intermingled. Image: Luis Garcia-Marcos.

It has not been yet clarified whether allergy and asthma are part of the same condition or they follow a parallel path. This Research Topic aims to try and put some light in this parallel march going through crucial topics: from prenatal events to later risk factors such as obesity; and from basic immunology to immunotherapy, both subcutaneous and sublingual. We hope the readers can infer their own conclusions as to what came first: egg or chicken.

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3. ASTHMA AND ALLERGY AS PARALLEL CONDITIONS

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EDITORIAL
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Editorial: The Parallel March of Asthma and Allergy in Childhood: A Multi-Perspective Approach

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Keywords: allergy, allergic rhinitis, asthma, atopy, children, exercise-induced bronchospasm, food allergy, united airway disease

Editorial on the Research Topic

The Parallel March of Asthma and Allergy in Childhood: a Multi-Perspective Approach

Asthma is often associated with atopy and it can be elicited by allergens in some individuals. Frontiers in Pediatrics has dedicated a series of article on asthma and atopy. This research topic aimed to summarize current knowledge and assess various aspects that are useful for improving our understanding.

Asthma, eczema, allergic rhinitis, and food allergy are typically considered as allergic diseases. The sequence of disease progression in childhood is often referred to as the "atopic march." The connection existing between upper and lower respiratory tract allergic diseases, such as asthma and allergic rhinitis (AR), has been called United Airway Disease (UAD) (Licari et al.). Asthma and AR can be considered manifestations of a single inflammatory process. They share macroscopic pathological characteristics, similar histological appearance, with a comparable allergic response and coexisting symptoms, including cough. The interaction between nose and lung in allergic airways disease is a bidirectional process, indeed it has been proved that the treatment of AR can improve asthma symptoms (Licari et al.). Overall, the causal relationship between atopic diseases in the "atopic march" is still unclear as it is not always present in all patients and the timeline may vary. Genetics and environmental exposure have been shown important risk factors for developing asthma and atopy in childhood, probably through epigenetic mechanisms. However, gene-environment interactions have been rarely examined for both asthma and atopy in the same population and there is no consistent evidence that identical interactions are shared to asthma and atopy (Turner). On the other hand, a defect in regulatory T cells (Tregs) at birth predisposes to atopy and asthma occurrence by enhancing release of Th2 cytokines in response to allergens (Martín-Orozco et al.). Furthermore, allergen-specific Tregs reduce inflammation and remodeling in the airways (Martín-Orozco et al.).

RISK FACTORS

Asthma has been recognized to be a syndrome since it likely comprehends different conditions. Efforts have been going for a long time to classify asthma according to causes and clinical features (phenotypes) or patophysiological mechanisms (endotypes) (Guibas et al.). The early-onset asthma phenotype is strongly associated with atopy. It starts in childhood and adolescence and it is mainly Th-2 driven (Th2 endotype). Th2 endotype is characterized by atopy, elevated IgE,

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and airway eosinophilic inflammation. The early-onset asthma phenotype includes atopic asthma which represents the most common form in childhood and it is characterized by positive IgE tests for the relevant allergens whose exposure induces asthma exacerbations (Comberiati et al.). Subphenotypes of early-onset asthma include transient wheeze due to viral infections associated with no eosinophilic inflammation and viral-induced asthma. Atopic status has an influence on such variants (Guibas et al.). Children with elevated concentrations of specific IgE are at higher risk for asthma attacks provoked by viral infections (Comberiati et al.). Viral infections promote allergic airway inflammation that results in asthma attack. Thus, sensitization to aeroallergen and respiratory infections synergistically enhance the risk for asthma. In children, other phenotypes (Guibas et al.) are persistent wheeze (non-eosinophilic asthma endotype) and obesity-induced asthma characterized by a no-/low-Th2 endotype and non-atopic, that should be distinguished from decrease in FEV1/FVC due to overweight by increased airway resistance (Jones et al.). Atopy is also a main risk factor for exercise-Induced bronchospasm (EIB). Up to 40% of children with EIB have AR and 30% of these children have asthma (Caggiano et al.). Identification of allergens associated with EIB permits to adopt measures to reduce allergen exposure. This and administration of the most proper pre-medication will allow children with EIB to perform physical activity as others do.

Considering the role of allergy in asthma occurrence, it is not surprising that atopic sensitization, especially or to dog, cat, any of perennial allergens or concurrent sensitization to airborne allergens and food allergens is linked to higher risk for development of asthma in young children (Moustaki et al.). Accordingly, several score for predicting asthma occurrence have included atopic features in combination with other factors with positive results even if they are rarely used in clinical practice (Moustaki et al.).

Regarding food allergy, it is noteworthy that food allergic children are not only at increased risk for asthma onset and food-induced asthmatic episodes, but also for more severe asthma (Foong et al.).

Another link between asthma and atopy is that emerging evidences indicate that maternal prenatal stress can result in development of both atopy and asthma (Douros et al.).

PREVENTION

Avoidance of airborne or food allergens both by mothers in pregnancy and during lactation and by infants does not seem to be of benefit for primary prevention of childhood asthma (Moustaki et al.). In children with AR due to grass pollen, both types of allergen immunotherapy (AIT), subcutaneous (SCIT) and sublingual immunotherapy (SLIT) are effective in preventing onset of asthma (Tsabouri et al.). It has been proposed that asthma occurrence in offspring depends on maternal vitamin D status in pregnancy. However, prenatal levels of Vitamin D are inconsistently associated with onset of asthma in offspring. In randomized controlled clinical trials, supplementation with vitamin D in pregnant women does not reduce the risk of having asthma or whezing up to 3 years of age child (Bountouvi et al.).

A systematic review of observational studies (Castro-Rodriguez and Garcia-Marcos) on the preventive effect of Mediterranean Diet (MedDiet) consumption on atopic diseases found that in children MedDiet seems to prevent asthma/wheezing but not atopy, AR and atopic eczema. Moreover, MedDiet by the mother during pregnancy revealed some protective effect on asthma/wheeze symptoms in the offspring only up to 1 year of age. MedDiet may be of benefit because it is rich in antioxidant (Jesenak et al.). Randomized control trials on the effect of MedDiet are compulsory.

Recently, occurrence of atopy and asthma have been linked to changes in the microbiome. However, there is low evidence that administration of probiotics during pregnancy and in the newborn may prevent atopic eczema, while probiotics are not useful for the primary prevention of childhood asthma (Mennini et al.).

TREATMENT

In allergic asthma triggered by the exposure to indoor allergens, allergen avoidance may be a specific preventive measure (Cipriani et al.). Several means may help to reduce exposure to allergens, such as house dust mite, pets, cockroach, molds. Notwithstanding evidence supporting the efficacy of these measures is weak and subject of controversy, the exposure control to specific airborne allergens is widely recommended.

Oxidative stress plays a pathogenetic role in asthma. Asthma is inconsistently associated with low levels of various antioxidants. Contrasting results have been shown by studies on the effect of antioxidant supplementation on asthma (Guibas et al.).

There is a need of improving control of severe asthma in children since it is responsible of morbidity and use of about 50% of health-care funds (Martin Alonso et al.). Serious and multiple allergies, steroid-resistant eosinophilia, and airway remodeling go with severe asthma. Nowadays, the only add-on therapy licensed for use in children with severe asthma is omalizumab. Nevertheless, its use is limited in one-third of patients by higher serum IgE levels than recommended and in another one-third by clinical weakness. Therefore, pediatric severe asthma is heterogeneous of, and mechanisms underlying different phenotypes are still unknown. Two add-on treatments, monoclonal antibodies to IL-5 or its receptor, and CRTH2 antagonists, currently available for use in adults, can be attractive options for children with severe asthma. They may help to improve the persistent steroid resistant eosinophilia.

AIT appears to be effective in children with IgE-mediated asthma who do not fully respond to the conventional anti-asthmatic medications and environmental control (Tsabouri et al.). Both SCIT and SLIT reduce asthma symptoms and medication use. It is important to underline that AIT is the only treatment that may change the natural history of respiratory allergy. Severe, not controlled asthma, and medical inaccuracy were the most frequent causes of SCIT-induced adverse event. In the era of personalized medicine, further research should

be conducted to individualize AIT using recombinant antigen technology. Allergen extracts against specific proteins to which the patient is allergic or extracts with modified proteins or peptides that could increase safety/efficacy might be created.

AUTHOR CONTRIBUTIONS

CC designed and wrote the article. CM wrote the article. KP and LG-M equally designed the article, read, and made comments on the manuscript.

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REVIEW
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The Role of Sensitization to Allergen in Asthma Prediction and Prevention

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The burden of asthma in childhood is considerable worldwide, although some populations are much more affected than others. Many attempts have been made by different investigators to identify the factors that could predict asthma development or persistence in childhood. In this review, the relation between atopic sensitization as an indicator of allergy and asthma in childhood will be discussed. Cross sectional studies, carried out in different countries, failed to show any firm correlation between asthma and atopic sensitization. Birth cohort mainly of infants at high risk for asthma and case–control studies showed that atopic sensitization was a risk factor for current asthma in children older than 6 years. In general, clear relations are observed mostly in affluent Western countries, whereas in less affluent countries, the picture is more heterogeneous. For the prediction of asthma development or persistence in school age children, other prerequisites should also be fulfilled such as family history of asthma and wheezing episodes at preschool age. Despite the conductance of different studies regarding the potential role of allergen avoidance for the primary prevention of childhood asthma, it does not seem that this approach is of benefit for primary prevention purposes. However, the identification of children at risk for asthma is of benefit as these subjects could be provided with the best management practices and with the appropriate secondary prevention measures.

Keywords: allergy, asthma, atopy, prediction, prevalence, prevention, sensitization, wheezing

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INTRODUCTION

Asthma is a very common disease in childhood. In certain countries, such as the USA, it has been estimated that it is the most frequently encountered chronic condition in childhood (1). Asthma affects adults too, but in the majority of cases, the origins of the disease are in the preschool age (2). Childhood asthma is a heterogeneous clinical disorder that includes different phenotypes at different ages (3). This heterogeneity was lucidly illustrated in the Tucson birth cohort study where almost 50% of children had at least one episode of wheeze until the age of 6 years. In 20% of these children, wheezing was restricted to the first 3 years of life (transient wheezers), in 15%, wheezing appeared after the third year of age (late-onset wheezers) and, in almost 15%, wheezing was present both before 3 years, and it lasted at least until the sixth years of age (persistent wheezers) (4).

From these results, we can surmise that children with wheezing at 6 years of age are unlikely to share the same risk factors with those who never wheezed or who have outgrown wheezing episodes by the age of 6 years. Similar, though not identical, phenotypes of preschool wheezing were also identified by other epidemiological studies (5, 6). Their results corroborated that

only a subset of children with preschool wheezing will have further episodes by the age of 6 years. The above highlights the difficulties in predicting which of the preschool wheezers will eventually develop asthma as the majority will outgrow wheezing episodes by the age of 6 years. Furthermore, Saglani et al. (7) showed that eosinophilic inflammation, which is a characteristic of asthma in older children and adults, was not present in infants with recurrent wheezing and/or cough episodes even in the presence of reversible airflow obstruction. The lack of evidence for any underlying asthmatic-type inflammation and the poor agreement on definitions of different phenotypes of preschool wheezing urged the ERS Task Force in 2008 (8) to suggest not using the term asthma for preschool wheezers. Instead, they proposed the terms episodic (viral) wheeze for children who wheeze intermittently and are well between episodes, and multiple-trigger wheeze for those who wheeze both during and outside discrete episodes. These phenotypes may, however, change over time (9) and, therefore, their validity has been questioned (10).

However, regardless of what classification is used for preschool wheezers, the real challenge is to recognize the risk factors that predispose to the continuation of asthma symptoms in school-aged children and adolescents. The identification of these risk factors may allow the implementation of effective preventive and therapeutic strategies, to the extent that these factors are modifiable.

Atopy was traditionally considered a risk factor for the development of asthma; allergic march (or atopic march) is a term that has been used for many years to describe the hypothesis of progression from eczema in infancy to rhinitis and asthma in older children (11). According to the most recent definition of World Health Organization, the term "refers to the natural history of atopic manifestations, which is characterized by a typical sequence of immunoglobulin E (IgE) antibody responses and clinical symptoms, which may appear early in life, persist over years or decades, and often remit spontaneously with age" (12). Accordingly, it is considered that a substantial proportion of asthmatic patients have allergic asthma with an underlying IgE-mediated hypersensitivity mechanism and it is, therefore, called IgE-mediated allergic asthma (13). Based on this, it would be plausible to hypothesize that the identification of allergic children could lead to the identification of those at high risk for the development of asthma. Grounded on this hypothesis, many epidemiological studies have investigated whether allergy or atopy was a risk factor for asthma in childhood using either clinical surrogates such as eczema, or biomarkers such as eosinophilia, total IgE, or specific IgE antibodies to certain allergens. Other studies have tried to investigate the relationship between asthma and atopy and to clarify to what extent asthma in children can be attributed to atopy, through the parallel variation of both conditions' prevalence in different populations (14).

The aim of this review is to present the existing data on the relation of atopic sensitization—and its use as an indicator—with asthma in school-aged children, and also the role of allergen exposure for primary prevention of childhood asthma.

PREVALENCE OF ASTHMA AND ATOPY IN CROSS-SECTIONAL STUDIES WORLDWIDE

The International Study of Asthma and Allergies in Childhood (ISAAC) revealed considerable variability of asthma prevalence among different countries and different regions, and sometimes among different areas of the same country (15, 16).

In 1999, Pearce et al. (17) summarized the studies that had evaluated the prevalence of asthma and atopy in children. They reviewed seven such studies from different populations and they concluded that most of them failed to demonstrate an association between asthma and atopy prevalence. Similarly, two other groups summarized the findings of the studies that compared asthma and atopy prevalence in childhood over different time periods and observed that there was either an increase of asthma over time without; however, a parallel increase in atopy (18, 19) or, that asthma prevalence was unchanged but the atopy prevalence was increasing.

Ronchetti et al. (20, 21) conducted two systematic reviews, and their main findings were the absence of correlation between the prevalence of asthma and the prevalence of atopy, and the existence of a strong correlation between atopy in asthmatic and atopy in the non-asthmatic children; the above imply that the prevalence of atopy in asthma depends on some environmental factors that simultaneously induce atopy in asthmatic and non-asthmatic individuals.

The role of atopic sensitization in the worldwide variation of asthma prevalence was also explored by Weinmayr et al. (22) who conducted cross-sectional studies in 32 centers from 22 countries worldwide in children aged 8–12 years following the ISAAC protocol. They found that current wheeze prevalence rates were not associated with the respective rates of atopic sensitization. However, the fraction of current wheeze attributable to atopic sensitization, as this was determined by skin prick test (SPT) reactivity, was higher in affluent compared to the non-affluent countries (40.7 and 20.3%, respectively). They also observed that this fraction was strongly correlated with the gross national income.

The above data (22) indicate that the potential link between asthma and atopic sensitization differs between countries and areas; as previous studies have already shown, strong relations are observed mostly in affluent Western countries (23–26), whereas in less affluent countries, the picture is more heterogeneous picture (24, 25, 27–29).

ATOPY AS A RISK FACTOR FOR CHILDHOOD ASTHMA IN COHORT AND CASE–CONTROL STUDIES

Many studies have been designed and conducted with the aim to investigate the potential risk factors for the presence of asthma in childhood. Atopic sensitization is among the most commonly investigated risk factors for asthma in childhood. **Table 1** depicts the characteristics of relevant studies that have been published since 2001. These studies were identified through PubMed

TABLE 1 | Studies that investigated atopic sensitization as a risk factor for the development of asthma in childhood.

No.	Reference	Study population	Indicator of atopic sensitization	Age of asthma diagnosis	Measured associations
1	Alduraywish et al. (30)	Two independent birth cohorts (1) the high risk MACS cohort and (2) the population-based LISAplus cohort	(1) MACS cohort: SPTs to food and inhalant allergens at 6, 12, and 24 months (2) LISApluscohort: sIgE to food and inhalant allergens at 2 years	10–12 years	The strongest effect on asthma risk was found in both cohorts, in subjects with co-sensitization to food allergens and aeroallergens
2	Boersma et al. (31)	166 children who visited a hospital with wheezing at the age 12–48 months	sIgE antibodies to inhalants allergens at the age of 12–48 months	At least 6 years	Sensitization to inhalant allergens has a positive predictive value of 86% for asthma. It remained a strong predictor for asthma even in multivariate analysis model
3	Anderson et al. (32)	Birth cohort, 289 newborns at high risk for asthma	sIgE antibodies to inhalant allergens at the age of 2, 6, and 11 years	6 and 11 years	Sensitization to aeroallergen at 2 years triples the risk of asthma at 6 years and at 11 years
4	Gabet et al. (33)	Birth cohort, 3,860 full term healthy singletons	sIgE antibodies to food and inhalant allergens at the age of 18 months	6 years	Current symptoms of asthma were significantly more frequent in children who were mono- or pauci-sensitized or multi-sensitized
5	Amin et al. (34)	Birth cohort, 762 newborns with a parent with a positive SPT to at least 1/15 aeroallergen, living either <400 m or >1,500 m from a major road	SPTs to aeroallergens, cow's milk and hen's egg at the age of 1, 2, 3, 4, and 7 years	7 years	Sensitization to >1 aeroallergen at 12 months of age or at 3 years was more frequent among children with asthma at 7 years. The same was true for sensitization to egg, but not for sensitization to cow's milk
6	Rø et al. (35)	Subpopulation of a birth cohort, 668 children evaluated at 2 years of age from the PACT study birth cohort	sIgE antibodies and SPT to nine allergens at 2 years of age	6 years	Positive sIgE was associated with a significantly increased risk for asthma at the age of 6 years in the unadjusted for confounders model
7	van der Mark et al. (36)	A cohort of 771 children, aged 1–5 years, who visited primary care clinics during the preceding 12 months with complaints of recurrent coughing, wheezing, and/or shortness of breath	sIgE antibodies to dog, cat, HDM	6 years	Positive sIgE doubled the risk for asthma diagnosis at the age of 6 years
8	Stoltz et al. (37)	Birth cohort, 289 newborns at high risk for asthma and allergic disease development	sIgE antibodies to aeroallergens at the age of 1, 3, 6, and 9 years	6 and 8 years	At the age of 1 year, only sensitization to dog and to cat was significantly associated with asthma risk. At the age of 3 years, sensitization to any perennial allergen was associated with asthma risk
9	Llanora et al. (38)	Cohort study, 78 preschool children 2–5 years with at least one wheezing episode	SPTs to HDM	8–14 years	Children with positive SPT had a twofold higher risk for persistent wheezing at the age 8–14 years
10	Amat et al. (39)	541 infants under 36 months of age who had a history of at least three wheezing episodes	sIgE antibodies to food and inhalant allergens	13 years	Allergen polysensitization (irrespective of the type of allergen), sensitization to multiple aeroallergens and to multiple food allergens were all associated with persistent active asthma
11	Lodge et al. (40)	Birth cohort, 620 infants with a family history of asthma/eczema/allergic rhinitis/severe food allergy	SPTs to food allergens and aeroallergens at the age of 6, 12, and 24 months	12 years	Sensitization to HDM at the age of 12 and 24 months increased the odds for asthma at 12 years
12	Vial Dupuy et al. (41)	200 children who visited a pediatric pulmonology clinic with recurrent wheezing as infants (<2 years)	sIgE antibodies to food allergens and inhalant allergens	6 years	Polysensitization increased the odds for persistent asthma at 6 years of age
13	Caudri et al. (42)	Subpopulation of the PIAMA birth cohort, 848 children who were invited at the age of 3–4 years. For evaluation, they had at least one respiratory symptom suggestive of asthma	sIgE antibodies to inhalant allergens	5–8 years	A positive sIgE to any airborne allergen increased the odds for wheezing at the age of 8 years

(*Continued*)

TABLE 1 | Continued

No.	Reference	Study population	Indicator of atopic sensitization	Age of asthma diagnosis	Measured associations
14	Lødrup Carlsen et al. (43)	Nested case–control study, 265 children, 2 years old with recurrent (>2 episodes) or persistent (>4 weeks duration) doctor confirmed bronchial obstruction, and 251 controls without bronchial obstruction	sIgE antibodies to food and inhalant allergens	10 years	The probability of current asthma at 10 years of age increased with increasing levels of sIgE antibodies to a mix of allergens measured at 2 years of age. This finding was significant only for boys
15	Simpson et al. (44)	A population-based birth cohort (Manchester Asthma and Allergy Study), 1,186 participants who were recruited at birth and followed at ages 1, 3, 5, and 8 years	sIgE antibodies and SPTs to food and inhalant allergens	8 years	Multiple early atopic sensitization was strongly associated with current wheeze at the age of 8 years. This type of sensitization predicts not only the presence but also the persistence of asthma
16	Marenholz et al. (45)	1,314 children of German MAS birth cohort	sIgE antibodies to food allergens	7, and/or 10, and/or 13 years	Food sensitization increased the odds for asthma
17	Jackson et al. (46)	Birth cohort, 289 newborns with at least one parent with one or more positive aeroallergen SPT and/or a history of physician diagnosed asthma	sIgE to aeroallergens and food allergens at 1 and 3 years of age, plus SPTs to 12 aeroallergens at 5 years of age	6 years of age	Aeroallergen sensitization at the age of 1 and 3 years was associated with increased risk for asthma at the age of 6 years
18	Devulapalli et al. (47)	Nested case–control study Children aged 2 years, 265 cases with recurrent or persistent doctor confirmed bronchial obstruction and 251 controls (the same population as in study No. 14)	SPTs to food allergens and aeroallergens at the age of 2 years	10 years	Atopic sensitization at the age of 2 years did not differ between asthmatic and non-asthmatic children at the age of 10 years
19	Just et al. (48)	A cohort of 219 infants <30 months with recurrent wheezing episodes	sIgE antibodies to aeroallergens and food allergens	6 years	In univariate analysis, allergic sensitization to at least one component of tested allergens was associated with persistence of wheezing at the age of 6 years. Absence of eosinophilia in combination with absence of allergic sensitization discriminated correctly 96% of children with wheezing in remission
20	Piippo-Savolainen et al. (49)	A cohort of 83 children <2 years who were hospitalized for bronchiolitis	sIgE antibodies to aeroallergens	8.5–10 and 13.5–15 years	Early sensitization to seasonal pollens was associated with asthma at the age of 13.5–16 years
21	Eysink et al. (50)	Cohort study, 752 children 1–4 years who had visited GP complaining for cough for at least the preceding 5 days	sIgE antibodies to cat, dog, and HDM	6 years	Sensitization by the age of 4 years was a prognostic indicator of asthma
22	Arshad et al. (51)	Whole population birth cohort in the Isle of Wight, 1,456 newborns	SPTs to aeroallergens and food allergens	10 years	Asthma was associated with positive SPT at the age of 4 years
23	Kotaniemi-Syrjänen et al. (52)	A cohort of 100 infants aged 1–23 months who were hospitalized with infection-related wheezing	sIgE antibodies to aeroallergens and food allergens	5.6–8.8 years	Positive sIgE (>0.35 kU/L) to aeroallergens was associated with the risk of school age asthma. The same was true for sIgE positive test to a mixture of food allergens but with a cut off value of 0.70 kU/L
24	Illi et al. (53)	1,314 children of German MAS birth cohort	sIgE antibodies to food allergens and aeroallergens at 1, 2, 3, 5, 6, and 7 years of age	7 years	Transient sensitization was not a risk factor for asthma at the age of 7 years. Children with persistent sensitization had increased risk for asthma at the age of 7 years provided that there was a positive parental history of asthma or atopy

SPT, skin prick test; HDM, house dust mite; BHR, bronchial hyperresponsiveness; sIgE, serum immunoglobulin E (IgE); OR, odds ratio; CI: 95% confidence interval.

searching, using the following terms: allergy, atopy, SPT, IgE, RAST, sensitization, children, asthma, and prediction.

Studies referring to population of preschool children were excluded since asthma is not well defined in this age. Definition of current asthma at the age of 6–14 years was not identical among different studies. However, all studies had as a prerequisite evidence of asthma symptoms during the preceding 12 months. It should be also noted that the majority of studies included

population at high risk for asthma either because of the parental history or because the population was recruited with respiratory symptoms suggestive of bronchiolitis or wheezing episodes. Despite their heterogeneity, the majority of the presented studies pointed to the same direction, namely, that atopic sensitization of infants or toddlers was associated with increased risk for asthma later in childhood.

Asthma predictive models have also been used in order to assess the probability of a toddler to have asthma as a child. Some of these models included sensitization to allergens in their scoring system. In 2003, Kurukulaaratchy et al. (54) developed a scoring system derived from a birth cohort of Isle of Wight. Although this cohort included all the neonates that were born in 1989 irrespective of their risk level for asthma, the predictive model was a combination of recurrent chest infections at 2 years, atopic sensitization at 4 years, a family history of asthma, and absence of nasal symptoms at 12 months of age. Another predictive model that included atopic sensitization either to at least one aeroallegen or to specific food allergens was the modified asthma predictive index (API) (55), which was derived from the API (56) that was developed in 2000 by Castro-Rodriguez et al. It is, therefore, obvious that atopic sensitization *per se* has not adequate accuracy for the prediction of asthma occurrence in childhood, but it can be used in combination with other risk factors for the prediction of persistence of asthma among preschool wheezers.

It is also of interest to mention that there are studies that investigated if there was a pattern of sensitization that was associated with asthma in childhood. Illi et al. (53) found that transient early sensitization was not associated with an excess risk for asthma in childhood (7 years of age). In cases of persistent atopic sensitization, the risk for childhood asthma increased only in those children with a concomitant maternal history of asthma. However, when sensitization to food allergens was examined separately from that to inhalant allergen, it was shown that early sensitization to food allergens increased the risk for asthma development up to school age. On the contrary, early sensitization to inhalant allergens without concomitant sensitization to food allergens did not confer any excess risk for asthma development up to school age. More recently, Alduraywish et al. (30) showed that early sensitization either to food allergens or to aeroallergens was associated with increased risk for asthma in childhood. However, the strongest effect on asthma risk was observed in children with concurrent sensitization to food and inhalant allergens. In the latter study (30), there are no data regarding persistence of sensitization as available information is restricted to the age of 24 months. On the other hand, Simpson et al. (57) using a similar approach found that multiple early sensitization has the strongest association with asthma at the age of 8 years. However, they concluded that IgE antibody responses do not represent a single phenotype of atopy, but rather, several different atopic vulnerabilities, which vary in their relation with asthma presence and severity. In their study, there were no data regarding parental history of asthma or atopy and, so, it is not possible to make direct comparisons with Illi et al.'s findings.

A different pattern of atopic sensitization in relation to childhood asthma risk was identified by Stoltz et al. (37). In their birth cohort study, only sensitization to dog and cat at the first year of life or sensitization to any of the perennial allergens tested was associated with asthma risk at the age of 6 years.

Although it is well recognized that atopy does not equate to atopic sensitization (58), it seems that the latter serves as a good surrogate of atopy. Most likely, the latter constitutes a risk factor for asthma in childhood only when other prerequisites coexist such as history of infant wheezing, parental history of asthma, or parental history of atopy. Therefore, it cannot be considered a satisfactory indicator for predicting or ruling out asthma in childhood without first taking into account these factors. This comes as no surprise since asthma is a multifactorial entity dependent on genetic predispositions and environmental exposures. Based on the available evidence, wheezy toddlers with a positive family history of asthma and presence of atopic sensitization should be regarded as high risk for persistence of asthma in childhood. Similarly, in a recent systematic review, Rotriguez-Martinez et al. (59) included allergic sensitization early in life (especially polysensitization) among the factors that confer to the prediction of wheezing persistence through school age.

THE ROLE OF ALLERGY IN THE PREVENTION OF ASTHMA

Predicting the risk of childhood asthma development or persistence would be of great importance as it could facilitate the design of primary prevention measures. The last decade of the previous century, it was hypothesized that primary prevention strategies in terms of allergen avoidance for infants at high risk for asthma would be of benefit. Nowadays, it has been recognized that prevention of asthma is not so straightforward as it seemed due to the multifactorial nature of the disease.

Many different interventional studies have been conducted over the last 20 years that tested the efficacy of allergen avoidance in the prevention of asthma. A recent meta-analysis (60) examined the mono- and multifaceted intervention studies until 2011. They found that monofaceted intervention studies versus control did not lead to any reduction in childhood asthma occurrence. In contrast, multifaceted intervention studies were associated with a reduction by half of asthma diagnosis in childhood. Three intervention studies were characterized as multifaceted and were included in this meta-analysis and in two of them the asthma outcome was reported at an age older than 6 years (51, 61). Both studies included interventions regarding reduction of house dust mite and food exposure, whereas the Canadian study (61) also included measures for pet avoidance and reduction in environmental tobacco smoke. Both studies included children at high risk for developing asthma and, therefore, their results are not applicable in the general population. Furthermore, taking into consideration that asthma risk attributed to atopy varied substantially between affluent and non-affluent countries, the same intervention measures may not be of value in other population settings even in children at high risk for asthma.

The outcome is noteworthy not only for the ineffectivenes of the monofaceted interventions but also because it generated a new hypothesis that had to be tested: that early exposure to specific allergens may lead to tolerance instead of allergy. It was

shown (62) that chronic exposure to high concentrations of cat allergens can promote a modified immune response consisting of a reduction in specific IgE immunoglobulins and an increase in specific IgG immunoglobulins, especially IgG4. Similarly, Stoltz et al. (37) found that although sensitization to dog was associated with increased asthma risk, dog exposure at birth was associated with a reduced asthma risk irrespective of sensitization status to dog, at any point during the first 6 years of life. The aspect of tolerance development through early exposure has also been evident in the area of food allergy. Tolerance to peanuts was developed in infants through the early introduction of peanut in their diet instead of strict avoidance whereas high maternal consumption of peanuts during pregnancy reduced the risk of peanut allergy in the offspring (63). These observations goes in parallel with the results of a recent meta-analysis (64), which showed that maternal dietary restriction during pregnancy or lactation failed to reduce the child's risk for atopic disease. There is no evidence that any modification of the weaning procedure could help in the prevention of atopic diseases, and so, such interventions are not recommended (65).

Having taken into account all recent evidence, the German updated guidelines on allergy prevention, published in 2014 (66), do not recommend any restriction in maternal diet during pregnancy and during lactation. They also do not recommend any measures for the reduction of house dust mite exposure for primary prevention purposes. However, they recommended avoidance of exposure to tobacco smoke and other indoor air pollutants as well as indoor conditions that promote mold. They also recommended some other measures of prevention that seem to affect the risk of asthma such as avoidance of obesity and avoidance of cesarean section.

More recently, in an international report about the implications of indoor allergen avoidance in asthma prevention and management, it was also acknowledged that the results for HDM avoidance and asthma prevention were inconsistent. The authors attributed this discrepancy to the type of approach (multifaceted or monofaceted), to the initial level of indoor HDM allergen, and possibly to genetic or other environmental co-factors (67).

Therefore, based on the existing evidence, allergen avoidance cannot confer sufficiently to the primary prevention of asthma in childhood. Allergen exposure may increase the risk of atopic consequences, but the outcome is highly dependent on the dose and kind of allergen, timing of exposure, and vulnerability of the child (68). Nevertheless, it is of benefit to identify those infants who are at increased risk for the development of asthma in order to (a) intensify the implementation of measures of primary prevention according to the existing guidelines (66) and (b) provide them the best practice management, close clinical monitoring, and measures of secondary prevention.

CONCLUSION

Atopic sensitization is related with an increased risk for asthma in childhood, but the extent of this association varies considerably among different populations and there is a pronounced difference between affluent and non-affluent countries; strong relations are observed mostly in affluent Western countries, whereas in less affluent countries, a more heterogeneous picture is observed.

Atopic sensitization *per se* does not predict development or persistence of asthma in childhood although it seems that atopic sensitization is associated with development of asthma in patients with wheezing.

Although allergen avoidance had been considered as a potential preventive measure from asthma development in high risk children, the majority of intervention studies failed to show positive results.

The identification, however, of infants at high risk for the development or persistence of asthma in childhood, it remains of benefit as these subjects should be provided with the best practice management.

AUTHOR CONTRIBUTIONS

MM, co-designed the structure of the paper, wrote most of the manuscript, reviewed, and approved the final manuscript. KD: co-designed the structure of the paper, wrote some parts of the manuscript, reviewed, corrected, and approved the final manuscript. IL and ST: searched PubMed and libraries and found the available literature and wrote some parts of the manuscript.

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frontiers in Pediatrics

REVIEW
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Prenatal Maternal Stress and the Risk of Asthma in Children

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Emerging evidence indicate that maternal prenatal stress (MPS) can result in a range of long-term adverse effects in the offspring. The underlying mechanism of MPS is not fully understood. However, its complexity is emphasized by the number of purportedly involved pathways namely, placental deregulated metabolism of maternal steroids, impaired maturation of fetal HPA axis, imbalanced efflux of commensal bacteria across the placenta, and skewed immune development toward Th2. Fetal programming probably exerts a pivotal role in the end result of the above pathways through the modulation of gene expression. In this review, we highlight the current knowledge from epidemiological and experimental studies regarding the effects of MPS on asthma development in the offspring.

Keywords: maternal prenatal stress, offspring, wheezing, asthma, glucocorticoids, microbiota

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INTRODUCTION

About 40 years ago, Selye gave the first generic and comprehensive definition of stress as "the non-specific response of the body to any demand" (1). Stress represents the effects of any factor able to threaten the homeostasis of an organism; these either real or perceived threats are referred to as the "stressors" and comprise a long list of potentially adverse factors, which can be emotional or physical (2). Stressors provoke an integrated response from the central nervous system in an effort to restore or preserve homeostasis (3).

Extensive experimental animal studies and epidemiological observations allow us to surmise that events and conditions during intrauterine life can have a key role in many aspects of fetal development, affect birth outcomes, and what is more, influence subsequent child and adult health susceptibility for many common disorders with complex multifactorial etiology (4, 5). Maternal prenatal stress (MPS), in particular, may cause a series of alterations in the developing fetus, influence early life events, and have long-lasting consequences in the offspring (3, 5, 6). These alterations are mainly considered part of the fetal programming (or fetal imprinting) phenomenon. The general idea conveyed by these two synonymous terms is that there is a dynamic interplay between the genetic makeup and the environment; certain stimuli, acting during critical periods of fetal development, can trigger a series of adaptive mechanisms and reset gene expression, with profound and permanent consequences on the tissues' structure and metabolic function. The molecular mechanisms underlying fetal programming are only partially unraveled, but what is clear is that the ensuing changes have long-lasting results in the function of affected tissues and organs, and can also pass from one generation to the next (7–9). Among the compounds with programming potentials are growth factors, cytokines, and hormones, all of which can be affected by stress (8).

The aim of this review is to summarize the increasing body of epidemiological and experimental evidence regarding the effects of MPS on asthma development in the offspring. The discussed

human studies include a variety of stressors experienced during pregnancy such as, negative life events, anxiety, or depression. Similarly, animal studies have also been conducted with various types of stressors.

PRENATAL MATERNAL STRESS AND OFFSPRING OUTCOMES

Maternal prenatal stress may result in a range of long-term consequences in the offspring (3, 10–12). Research in humans has demonstrated a relation between MPS and perinatal/postnatal outcomes. Our knowledge on either the immediate or the enduring effects of MPS in the offspring derives largely from animal studies (10); however, the amount of available information from research in humans has been accumulating rapidly over the past few years. The best-studied outcomes of fetal exposure to MPS are preterm birth and low birth weight (13–15). Indeed, a systematic review—39 peer-reviewed studies—strongly indicates that MPS increases the likelihood of preterm birth (15). Potential physiological pathways include behavioral, infectious, neuroinflammatory, and neuroendocrine mechanisms. Regarding low birth weight, it has also been noted that maternal psychological and social stress during pregnancy may affect human embryo and fetus development. This is believed to be the result of a dynamic interaction between inherited and acquired genetic alterations as well as environmental factors, particularly during intrauterine life (7). It has been demonstrated that levels of placental corticotrophin releasing hormone (CRH) are associated with the rate of fetal growth and body size at birth (16). MPS is also considered responsible for a variety of functional and morphological changes of the offspring's brain, and a risk factor for conditions such as behavioral problems, learning disorders, high levels of anxiety, attention deficit hyperactivity disorder, autism, and schizophrenia (17–20). Furthermore, MPS has been associated with a higher risk for a variety of immune and metabolic alterations in the offspring such as asthma, allergic disorders, cardiovascular diseases, hypertension, hyperlipidemia, diabetes mellitus, and obesity (5, 7, 21–25).

Studies indicate that MPS may also affect the offspring's bacterial colonization (26). Recent data support that non-pathogenic commensal bacteria are present in the placenta (26). This implies an efflux of commensal bacteria from mother to fetus through the placenta, and the establishment of an early fetal microbiota (27). Studies have shown that exposure to different prenatal factors may influence the richness and variety of the offspring's gut microbiota, although clinically relevant consequences are yet to be determined (27–29). Experiments in animal models indicate that MPS can alter the offspring's bacterial intestinal colonization (30).

STRESS IN PREGNANCY

Women are more vulnerable to stress during pregnancy due to extensive hormonal and physiologic changes. MPS can be realized—in a multidimensional approach—as comprised of stressful situations (stressors), perceptions or evaluations of these stressors (appraisals), and stress responses such as subjectively experienced emotions (31, 32). Associations between MPS, conceptualized at each of these three overlying stress levels, and child outcomes, have been reported (33).

There is only a small body of literature on the prevalence of MPS, but probably its influence on maternal health has been underestimated; available evidence indicates that prevalence of MPS is relatively high. In a cross-sectional study of 1,522 ethnically and economically diverse pregnant women receiving antenatal care at a university obstetric clinic in Seattle, WA, USA, MPS was recorded as being low to moderate in 78% and high in 6% of the participants. High levels of MPS were associated with depression, panic disorder, drug use, domestic violence, and suffering from two or more medical comorbidities (33). An earlier study, which used prospectively collected data from a cohort of 14,000 women, showed that the proportion of women with probable depression was 11.8 and 13.5% at 18 and 32 weeks of pregnancy, respectively (34). The most common causes of MPS seem to be anxiety and depressive symptoms, pregnancy-related anxieties, parenting stress, and work-related stress (35). During the prenatal period, the concomitant suffering from increased levels of anxiety and depressive symptoms has been shown to be the most important risk factor for adverse birth outcomes (35). It has to be mentioned here, that although anxiety and depression represent separate psychological entities, it is difficult to be clearly differentiated as they are strongly correlated with each other (36–38). The importance of MPS has been emphasized by the American College of Obstetricians and Gynecologists who recommend regular assessment of pregnant women for psychosocial risk factors and, if needed, supportive interventions to help women cope with the psychosocial stressors (36–38).

PRENATAL MATERNAL STRESS AND IMMUNE RESPONSE—AIRWAY INFLAMMATION

There is very strong evidence, mainly from experimental studies, that MPS modulates the immune response of the offspring (3). It is therefore reasonable to hypothesize that MPS may be related to future disorders of the offspring, which develop through the dysregulation of innate and adaptive immune pathways, such as asthma (39).

The adaptive immune mechanism constitutes a critical pathway for the development of asthma, which is characterized by a Th2-dominated immune response (Il-4, Il-5, Il-13), and a down regulated Th-1 response (Il-2 and IFN-γ). Additionally, other types of T cell subsets, such as regulatory T cells, which produce Il-10 and TGF-β, as well as Th17 cells, which produce Il-17, may confer to asthma development possibly by skewing the immunological response toward the Th-2 type (40). It has been also recognized that innate immune mechanisms may also contribute to asthma development by leading toward an upregulated Th-2 response (39).

Veru et al. reviewed the animal studies that explored the relation between MPS and the offspring's immune response

(3). The review included only the studies that used exogenous/processive stressors which are considered to represent very closely the human psychosocial stress. The reviewed data indicated that MPS can lead to disequilibrium of the Th1/Th2 ratio, in favor of Th2 response. The cytokine production underlying this shift was affected by MPS exposure, though not to the same extent for each cytokine. MPS also affected, in a gender dependent way, the natural killer (NK) cytotoxicity and macrophages activity, which represent the functional activity of two essential components of the innate immunity. In general, the effect of MPS on the innate immunity function of the offspring is inhibitory, as MPS attenuates macrophages and neutrophils functions, such as spreading and phagocytosis, and decreases, at least in some models, NK cytotoxicity (41). MPS also modifies the response of T lymphocytes to mitogens and to specific antigens, but the type of modification is depended on the time of stressor application during pregnancy (41). This has been quite expected, as different periods of immune system vulnerability during gestation have been proposed on the basis of immunotoxicology data (41). These periods are depended on the maturation stage of immune system and they may be different among species.

Although there are several studies exploring the relationship of MPS and immune response of the offspring, there is only a small number of experimental studies investigating the effects of MPS on the airway inflammation in the offspring. Nogueira et al. (42) investigated the role of chronic mild MPS induced by continual changes in living and feeding conditions, on leukocyte infiltration in the airways of the rat offspring. They found that after sensitization to ovalbumin, the prenatally stressed offspring group exhibited a 50% increase of total leukocyte, eosinophil, and mononuclear cell count in the bronchoalveolar lavage (BAL), compared to the non-stressed offspring group. This difference reached statistical significance, whereas no such difference was seen in the neutrophil cell count in the BAL of the stressed and non-stressed group.

In another murine model (43), the effect of stress caused by exposure to a sound emitting rodent repellent device on pregnant mice, during the 12th and 14th days of gestation, was investigated. It was observed that airway hyper-responsiveness upon allergen aerosol challenge was significantly increased in the stressed female offspring group after sensitization to ovalbumin. A relative increase of eosinophil cell count in the BAL was also observed, indicating increased susceptibility toward airway inflammation. It should be mentioned however, that exposure to a single stressor on the 12th day of gestation, was not sufficient to induce a tendency toward airway hyper-reactivity. This observation is in line with the hypothesis that the development of immune system during gestation is characterized by windows of vulnerability. In this animal model, the ratios of Th2/Th1 cytokines in the blood and BAL of the offspring were also assessed, and the results showed a domination of Th2 cytokines in the blood. A Th2 cytokine profile was also induced in naive T cells by lung dendritic cells from prenatally stressed offspring. It was also observed that total serum IgE was significantly elevated in the stressed sensitized group, implying a degree of dysregulation of the humoral immune response.

A major mediator of the MPS action on the offspring's immune response is maternal glucocorticoids. The existing evidence supports that a fraction of the maternal glucocorticoids cross the placenta, deregulates the hypothalamic–pituitary–adrenocortical (HPA) axis of the fetus, and augments the fetal glucocorticoid production (41). In turn, elevated glucocorticoids of the fetus may lead to a predominance of Th-2 response (44). It is also known that epigenetic pathways contribute to the regulation of immune development (45). MPS may epigenetically modify key immune genes, leading to an altered immune response of the offspring. Oxidative stress (46) as well as maternal catecholamines and opioids (41) may also play the role of mediator.

It seems, therefore, that there are multiple candidate mediators of the MPS effects on the offspring's immune development. However, irrespective of the type of mediator, the main deviation of the immune response in the prenatally stressed offspring is the up-regulation of Th-2 response which may predispose in asthma development.

PATHWAYS THROUGH WHICH MPS MAY AFFECT OFFSPRING OUTCOMES

Figure 1 illustrates briefly the mechanisms discussed in the next sections.

Maternal Environment

The most important mechanism involved in the association between MPS and offspring outcomes is the hypothalamic–pituitary–adrenal (HPA). When a mother is exposed to a stressor, her HPA axis is activated and releases a number of hormones. The most important of them is cortisol; due to their highly lipophilic nature, GC can theoretically pass easily through the placental barrier and reach the fetus. In essence, however, the placenta regulates dynamically the amount of GC that will finally reach fetal circulation (47). Indeed, only a small percentage of the GC reaching placenta during periods of MPS will manage to pass into fetal circulation (approximately 10–20%) (48). Nevertheless, because of the low normal levels of fetal GC, the transfer of even this small amount can increase significantly GC concentration in the fetal circulation, suppress the fetal HPA axis, and have an impact on its normal maturation (49). Furthermore, it seems that increased maternal cortisol levels can stimulate the production of placental CRH with resultant stimulation of the fetal HPA axis (49, 50) and further increase of fetal cortisol. Glucocorticoid receptors (GRs) are ubiquitous throughout nucleated cells and so, if there is an increase in GC levels in fetal circulation, gene expression and development of many organs and tissues will probably be affected. Fetal lung maturation is known to be one of the organs highly affected by high GC concentrations (50, 51). Nevertheless, it has to be emphasized, that GC are not able on their own to fully explain the whole range of fetus and offspring impairment from MPS. Maturation of the fetal HPA and GR expression begin during late pregnancy (52–54). As a consequence, vulnerability of the fetus to excessive maternal GG before this period is limited.

In an experimental animal study (55), it was demonstrated that the offspring of mice mothers stressed during pregnancy developed airway hyperactivity after sensitization with ovalbumin.

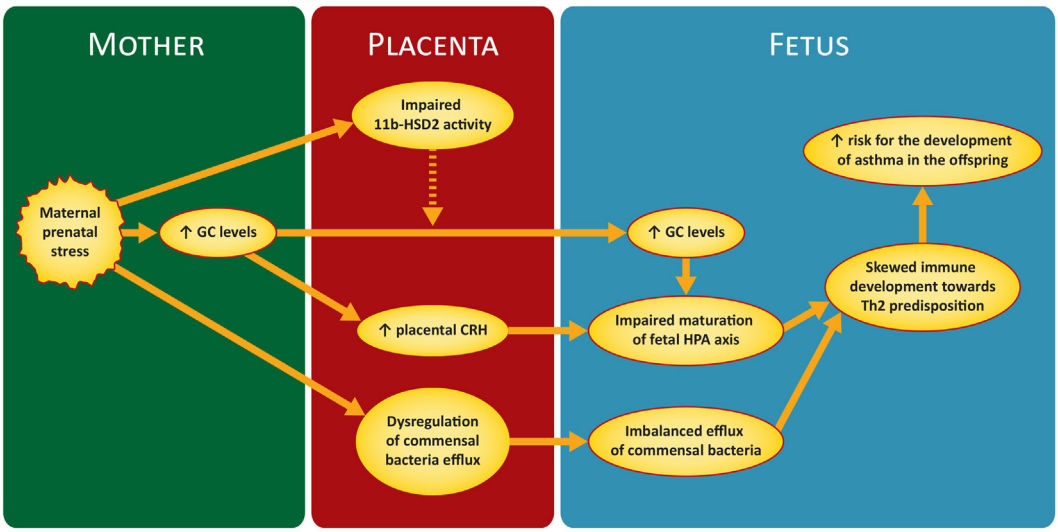

FIGURE 1 | Proposed mechanism of maternal prenatal stress in asthma predisposition in the offspring. GC, glucocorticoids; CRH, corticotrophin releasing hormone; 11 b-HSD2, 11-b hydroxysteroid dehydrogenase-type 2; HPA, hypothalamic–pituitary–adrenal.

Stressed pregnant mothers had significantly elevated corticosterone levels compared with non-stressed controls. Authors further showed that the administration of exogenous dexamethasone to pregnant mice led to offspring with asthma vulnerability. On the other hand, administration of metyrapone, which blockaded stress induced GC, abolished the asthma predisposition of the offspring. The results of this study provide experimental support on the role of MPS-related GC toward asthma predisposition in the offspring.

Placenta

Maternal prenatal stress reduces uteroplacental blood flow by trigerring a fetal stress response through fetal HPA axis activation (56). But besides that, the role of placenta in modulating and moderating fetal reactions and programming, in response to MPS, is of pivotal importance. Placenta's protective function from MPS is mainly employed by the enzyme 11-β hydroxysteroid dehydrogenase-type 2 (11β-HSD2). The enzyme's role is to attenuate the effects of excessive maternal GC by converting cortisol into cortisone, which is a much less active metabolite (57). Unfortunately, despite the unequivocal protection from the surge of maternal GC during MPS, 11β-HSD2 is far from being the ideal buffering mechanism. The levels of 11β-HSD2 increase with the progression of gestation, but fall abruptly near term (58, 59) when, as it has been aforementioned, fetus is more vulnerable to the programming potential of maternal GC. Furthermore, it has been shown, both in humans and animal models, that MPS can reduce by itself the expression and activity of 11β-HSD2 (59–61).

The protective effects of 11β-HSD2 seem to be related only with the effects of acute stress. Exposure to chronic stress can have detrimental effects on the 11β-HSD2-based protection mechanism. In an animal model, it was demonstrated that exposure to acute stressors resulted in prompt up-regulation of 11β-HSD2, whereas, in cases of chronic MPS the ability for upregulation of the enzyme's activity in response to an acute stressor was reduced by about 90% (62). Other studies have demonstrated that chronic MPS can downregulate 11β-HSD2, increasing this way the amount of cortisol that crosses the placenta (63). This downregulation of 11β-HSD2, is possibly attained through DNA methylation (60).

Concisely, 11β-HSD2 is not directly connected with the mechanisms involved in the development of asthma propensity in the offspring. However, it is probably indirectly involved since its role is to provide fetus with a protective barrier from the surge of maternal GC during MPS.

Catecholamines

Sympathetic–adrenal–medullary (SAM) system, with its response hormones adrenaline and noradrenaline, plays a central role in stress. The secretion of these hormones comes as an immediate reaction to stress, contrary to HPA that releases its effector hormones more gradually (64). Despite SAM system being an integral part of stress reactions, catecholamines have attracted limited attention regarding their role in MPS and fetal consequences. This is probably due to the fact that catecholamines are hydrophilic and apparently not able to cross the placenta barrier in physiologically significant concentrations (65). Apart from that, the vast amount of catecholamines that reach the placenta are metabolized to inactive forms through the enzymes monoamine oxidase and catechol-O-methyltransferase (66).

In studies conducted in human placental tissue and animals, it was shown that only a minor quantity of catecholamines was able to be transferred from mother to fetus (67–69). Some older evidence, however, suggests that catecholamines are able to indirectly influence fetal metabolism by affecting the uteroplacental perfusion. In an animal study, high levels of circulating maternal catecholamines led to constriction of placental blood vessels, reduction in glucose supply to the fetus, and activation of fetal catecholamine release (70). However, the evidence implying an

association between MPS and changes in fetal circulation are inconclusive, and even if this relation does exist it is transient in nature and limited to the temporary activation of the SAM system, after an acute stressful situation (65). It is clear from the above that there is paucity of data to support the implication of catecholamines in the development of asthma predisposition in the offspring and any role ascribed to SAM should be considered strictly hypothetical.

Gut Microbiota Alterations

Another conceivable indirect mechanism through which MPS may also affect the fetus, is the formation of neonate's gut microbiota. The significance of this, not yet fully confirmed mechanism, can be realized with the developmental and regulatory role of microbiota in many aspects of human physiology, including the immune system, the central nervous function, and the HPA (71–73). It seems that from the first days of life, a bidirectional communication system—the so-called "gut–brain" axis—is established between gut microbiota and the above systems; molecules such as cytokines, neuromodulators, and neurotransmitters act as messengers for this interaction (71, 73–75).

Until quite recently, the conventional belief was that fetus and its intrauterine environment remained sterile till the time of delivery (76). Although it is true that the degree of microbial colonization during and immediately after delivery is so enormous that within days the bacteria are by far more numerous than the newborn's own cells, recent studies have demonstrated that a small, though not insignificant, number of bacteria is present in the intrauterine environment. This finding implies that the foundations of human microbiota lay in the prenatally occurring bacterial efflux from mother to fetus. Bacteria most likely escape into circulation from maternal intestine, and through the blood stream, enter the amniotic fluid. Fetus swallows the amniotic fluid providing this way the fetal intestine with its first bacterial load (32).

A neonate's gut should be colonized by a diverse and balanced microbial population in order to establish a healthy microbiota (77). MPS disrupts the balance of maternal intestinal microbiota and creates a disequilibrium in the bacterial efflux to fetus and offspring (32). It has to be stressed, however, that much of the abovementioned remain largely unproved, and the development of fetal microbiota, as well as its significance, are still not entirely clear (78).

Experimental animal studies have shown that MPS alters the offspring's bacterial intestinal colonization (30, 79, 80). Recently, Jasarevic et al. (81) showed that MPS changes vaginal microbiota and this alteration is transmitted at birth to the offspring's gut microbiota. They also showed that MPS affects offspring's microbiota in a temporal and sex specific manner (82). In a clinical study of 56 women–infants pairs (83), it was found that MPS was one of the determinants of the offspring's microbiota composition. This effect reached its peak at 80 days, and it was still detectable at 110 days. High levels of MPS were associated with larger than usual numbers of Proteobacterial groups, and a relative dearth of lactic acid bacteria.

As far it regards the relation between MPS, microbiota, and asthma pathogenesis, the abovementioned findings, interpretations, and assumptions, could be incorporated in a proposed model with two interlaced pathways. In the first one, the imbalance of an infant's intestinal microbiota may deviate the development of systemic immune toward a more "asthma prone" Th2 direction. The second one lies within the concept of "Common Mucosal Immune System" (84) and suggests a "crosstalk" between mucosal compartments from different organs; microbiota driven modulations in mucosal immunity at one site, may be transferred to a mucosal surface of another site. The above proposed model can be considered as part of the more general context of hygiene hypothesis (85).

THE ASSOCIATION WITH CHILDHOOD ASTHMA

It is more than 15 years that evidence from cohort studies and animal models linked, increasingly stronger, MPS with the development of childhood asthma.

Observations from epidemiological studies challenged researchers to investigate the hypothesis, eloquently described by Barker (86–88), of the antenatal origin of many adult diseases. The field of MPS was conveniently available for the studies that followed to test this hypothesis. Among other subjects, researchers focused on the prenatal origin of asthma and allergies in children. Following studies in animal models that linked physical or psychological stress with allergy and asthma in the first decade of the 21st century, researchers worked on the early-life stress and the consequences in asthma development later on.

In 2006, a team from Charité, Berlin, Germany, described an increased vulnerability toward asthma specific clinical features in prenatally stressed offspring mice (43). The same year in Japan, researchers presented the long-term asthmogenic effects of early life psychological and physical stress in mice (89). Eight years later, Lim et al. (55) exposed mice on the 15th day of pregnancy, to an 1-h restraint stressor and assessed offspring for the development of asthma susceptibility. Increased asthma susceptibility was found only in those offspring born from stressed mothers. It appears that inflammation-related hormones during pregnancy may represent a common pathway, shared by various different, associated or not with stress, factors through which the offspring's susceptibility to develop asthma is affected.

During the same period, in the past 15 years, interesting data resulted from epidemiological studies.

Wright et al (90) conducted a prospective cohort study and reported a positive association between caregiver perceived stress by regular telephone interview and risk for wheezing episodes in children younger than 14 months of age. Few years later, a large cohort study in Canada, showed that maternal stress in early childhood, is positively associated with asthma at the age of 7 years (91). Authors used health care data in order to identify stress which was defined as "a combination of depression and anxiety" (91). The authors also found that the prevalence of asthma was dose related with the duration of exposure to stress. This interesting information was confirmed from another birth cohort, the Avon Longitudinal Study of Parents and Children (92), that assessed and scored the anxiety in pregnant women at 18 and 32 weeks of gestation and the occurrence of asthma in their offspring at

school-age. The likelihood of asthma at age 7.6 years was higher in children whose mothers had been classified (at 32 weeks of gestation) in the highest anxiety scores compared with the corresponding mothers with anxiety scores in the lowest quartiles. Additionally, after adjusting for confounders such as postnatal anxiety, they showed that there was a dose–response relationship. More recently, a group working in Canada reported their observations on 68 mothers having experienced a natural disaster during their pregnancy. They noted that the results of early-life events associated with psychological distress, on the development of allergic airway inflammation in later-life depend on both the quality and intensity of the early-life stress stimuli (93).

In the Western Australian Rhine Study, researchers looked at the association of prenatal adverse life events during pregnancy with asthma and allergy of offspring followed until the age of 14 (94). They found that prenatal adverse life events increased the likelihood for atopic diseases, which was enhanced in the absence of a maternal atopic predisposition. The risk of asthma and/or eczema was positively correlated with the number of negative life events during the second half of pregnancy. The results were more pronounced for asthma than allergic rhinitis. Similar results were reported from Sweden and Italy with children exposed to stressful life events during pregnancy (95, 96).

In a prospective cohort from the Netherlands (97) researchers assessed the associations of MPS with offspring wheezing until the age of 6 years. The study included 4,848 children and showed that psychological distress in pregnancy was associated with significantly higher risk of wheezing. The results were independent from paternal psychological distress during pregnancy or maternal and paternal psychological distress after delivery.

The relationship between MPS and asthma in the offspring seems to be influenced by the type of MPS and the age of onset of asthma symptoms. In a large Danish cohort (98), prenatal stress was retrospectively assessed using as indicator the bereavement due to the death of a close relative 12 months prior to or during pregnancy. A significant association was observed between MPS and the risk of asthma before the age of 3 years. This association, however, was not found in children 4–15 years old, unless their mothers had lost a child prior to pregnancy. In another Danish cohort (99) that included 32,271 pregnancies, an association was found between MPS and the risk of asthma and atopic dermatitis at the age of 7 years. In the latter cohort, the surrogate for MPS was the psychosocial job strain.

Strong results have arisen from a recent meta-analysis that included 10 relevant studies. Overall, the study showed that the prevalence of wheezing or asthma was higher in children whose mothers had been exposed to some kind of prenatal stress than in mothers who had not experienced such a distress (100).

CONCLUSION AND FUTURE PERSPECTIVES

Several types of MPS seem to increase the vulnerability of the offspring to asthma through interrelated pathways. Maternal GC, along with the improper activation of placental–fetal HPA axis, are considered the main mediators of this predisposition. However, there are various other pathways through which maternal stress may increase the offspring's propensity for asthma. Amongst them, MPS imposed imbalances in fetal and offspring's microbiota, is the less explored and the more promising to unravel—at least partially—the complex association between MPS and asthma. There is need for further studies with subjective and objective measures of stress, as well as objective measures of asthma, in order to elucidate the underlying signaling pathways, understand how they interact with each other, and draw concrete and meaningful clinical inferences. The natural next step—and maybe a stepping stone toward the medical communities' efforts aiming at the prevention of asthma—would be the design and development of mother-centered interventions during pregnancy focusing on how to cope with particular stressors. This may prove to be an efficient way to prevent asthma—and maybe other ailments—predisposition in the offspring.

AUTHOR CONTRIBUTIONS

KD designed the article and wrote Introduction, Stress in Pregnancy, Maternal Environment, and Placenta sections. MM wrote Prenatal Maternal Stress and Immune Response—Airway Inflammation section. ST wrote Prenatal Maternal Stress and Offspring Outcomes section. AP wrote Catecholamines and Placenta sections. MP wrote The Association with Childhood Asthma section. KP equally designed the article, read, and made comments on the manuscript.

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MINI REVIEW
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Can Getting Enough Vitamin D during Pregnancy Reduce the Risk of Getting Asthma in Childhood?

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The worldwide increase in asthma prevalence during the last decades and the re-emergence of vitamin D deficiency in many populations hinted toward an underlying association between these two conditions. Since asthma is presented with high incidence in childhood and neonatal vitamin D stores depend on maternal vitamin levels, a possible programming effect of maternal vitamin D status during gestation was suggested. Observational and longitudinal studies on this subject led to inconclusive results with glimmer of positivity. In the randomized controlled clinical trials (RCTs) that followed, increased doses of vitamin D were tested in pregnant women being at high risk of having an asthmatic child. Although, the results of RCTs showed a potential association with asthma-related phenotypes rather than asthma *per se*, the low toxicity of vitamin D supplements make it tempting to speculate that pregnant women at a high risk of obtaining a child with asthma may be benefited, especially if they are vitamin D deficient.

Keywords: vitamin D, asthma, childhood, pregnancy outcome, vitamin D supplementation

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INTRODUCTION

Epidemiological and experimental data on health impact of "multitasking" vitamin D support a protective effect of vitamin D over diseases such as cancer, cardiovascular, autoimmune, dementia, and diabetes (1, 2). Recently, randomized clinical trials have been conducted to determine optimal serum concentrations and optimal intake of vitamin D that might be beneficial to human health (3–5). The prevention of asthma development is one of the many potential health benefits of vitamin D (6). Since asthma is presented with high incidence in childhood, a possible programming effect of maternal vitamin D status during gestation has been proposed. Besides, pregnant women and infants are in high risk of vitamin D insufficiency (7–11). Several observational studies explored this issue and suggested a putative protective role of prenatal vitamin D sufficiency in the development of wheezing and asthma in the offspring [reviewed in Ref. (12)]. However, it remains unclear whether maternal vitamin D deficiency may contribute to the development of childhood asthma or it is the consequence of a generally altered biochemistry. In this mini-review article, we aim to present the current knowledge as it emerges from studies focused on the impact of maternal vitamin D status and vitamin D supplementation on the development of asthma in early childhood.

VITAMIN D LEVELS DURING PREGNANCY

The main role of vitamin D during pregnancy is to maintain serum calcium levels by promoting calcium absorption in the intestine and allowing proper function of parathyroid hormone.

It is, however, interesting that, during this period, vitamin D metabolism is shifted from the classical determinants of calcium homeostasis to other regulatory mechanisms. Although 25(OH)D circulating levels do not substantially differentiate or may be reduced compared to non-pregnant women, serum concentrations of vitamin D active metabolite, $1,25(OH)_2D$, are significantly elevated from early pregnancy (13, 14). This increased production possibly depends on 25(OH)D substrate availability (2, 15). It should be noticed that high maternal intake of 25(OH)D or exceeding vitamin D serum levels during pregnancy is not complicated from either hypercalcemia or hypercalciuria (16). Moreover, $1,25(OH)_2D$ synthesis by 1-α-hydroxylase (CYP27B1) may be triggered by other modifiers, which are increased during gestation, such as parathyroid hormone-related protein, prolactin, estradiol (17), calcitonin (18–20), and placental lactogen. Furthermore, an autocrine or paracrine placental function of upregulated CYP27B1 (21–24) and methylation of CYP24A1 gene, which induces lower levels of catabolic 24-hydroxylase, have also been suggested to contribute to the exceeding levels of $1,25(OH)_2D$ in pregnancy. Circulated 25(OH)D concentration of 40 ng/ml (100 nmol/l), more likely to be achieved by 4,000 IU D3 daily intake, has been proposed as the cutoff level to optimize maternal $1,25(OH)_2D$ levels (15).

$1,25(OH)_2D$ does not pass through the hemochorial placenta, and fetal levels of $1,25(OH)_2D$ are expected to be lower than the maternal (17). Cord blood levels of 25(OH)D correlate with $1,25(OH)_2D$ whereas this is not the case for maternal circulation (25). Contrary to $1,25(OH)_2D$, 25(OH)D and its catabolic product, $24,25(OH)_2D$, readily cross the placenta. Consequently, the infant basically depends on mother's 25(OH)D stores. Indeed, a strong correlation between maternal and cord blood 25(OH)D concentrations has been highlighted (26, 27). As a result, vitamin D deficiency during pregnancy exerts significant impact on vitamin D status of newborns and subsequently on early childhood (9, 28).

THE IMPACT OF VITAMIN D EXPOSURE *IN UTERO*

The dependence of the developing fetus on maternal vitamin D status together with the reported extra skeletal function of the hormone and vitamin D deficiency epidemic during the last years led to further investigation of the consequences of maternal vitamin D insufficiency on pregnancy outcomes (6, 29, 30). Gestational vitamin D has now been linked to a variety of diseases such as osteoporosis, autoimmune disorders, altered brain development and adult mental health, food allergies, and asthma (17, 29, 31–33). The most cited adverse outcome of maternal vitamin D deficiency is neonatal rickets, while mild hypovitaminosis D could be more pronounced in exclusively breast-fed infants, on the basis that breast milk constitutes a poor source of vitamin D compared with formulas (34–36). In the light of the possible parallel-asthma epidemic, cellular molecular studies along with epidemiological data suggest that vitamin D deficiency may have a programming effect *in utero* and promote a trajectory to develop asthma in childhood and later in life (2, 37–39). An immunodulatory role of vitamin D has also been described in both immune system and lung cell maturation, which occurs mainly during fetal and early extrauterine life (40–42).

MATERNAL VITAMIN D STATUS AND ASSOCIATIONS WITH ASTHMA IN EARLY LIFE

The putative effect of prenatal exposure to vitamin D on asthmatic phenotypes in early life has been initially investigated *via* observational studies based on either maternal dietary intake of vitamin D or 25(OH)D levels in venous maternal and/or cord blood [reviewed in Ref. (12)]. Mainly, the food-approached studies have suggested a protective role of vitamin D intake on the development of wheezing and asthma in offspring, with an attenuated effect after adjustment for multiple covariables (43–48). Despite weak associations, meta-analyses and systemic reviews replicated the potential link, warranting further investigation (49–52). Consequently, longitudinal studies measuring 25(OH)D levels, a possible more objective indicator of vitamin D status, were designed to explore the possible impact of vitamin D on asthma-like symptoms or asthma. However, these derived data failed to consistently replicate this inverse connection. A spectrum of all possible associations has accrued, demonstrating no association (53–57), an inverse (58–61), a *U*-curved (62, 63), and even a direct relationship (64, 65) between the two entities. In accordance, systematic reviews and meta-analyses on these birth cohort studies reproduced inconclusive evidence, revealing no association with asthma or wheezing (66), a *U*-shaped association with a lower risk of childhood asthma at maternal 25(OH)D levels of 30 ng/ml (70 nmol/l) (67) and a borderline significant inverse relationship (68).

Inconsistencies across studies have been attributed to a plethora of factors such as diverse study designs, missing significant follow-up, lack of a clear definition of vitamin D sufficiency in pregnancy, remarkable fluctuations in populations in different latitudes, genetic heterogeneity, the diversity of assays employed (69), low repeatability of serum 25(OH)D values (37), variety of vitamin D status determinants, multifactorial nature and phenotypical diversity of asthma, as well as imprecision in diagnosing asthma in early life. Moreover, the majority of the cohorts assessed a single measurement of 25(OH)D in cord blood or late pregnancy, which might not sufficiently reflect the virtual vitamin status during gestational dynamic process. Of note, food base approaches may be assumed as a better representative of long-term vitamin D intake, which might influence the fetus during all stages of gestation and even *ex utero*.

The inconsistent body of evidence with glimmer of positivity from observational prospective cohorts, the rather low toxicity of vitamin D, which is a relative inexpensive candidate in the attenuation of the asthma's epidemic burden on public health, were clearly indicative of the need to proceed to clinical trials. Moreover, both animal and *in vitro* experiments have demonstrated a positive impact of prenatal vitamin D exposure on aspects of lung function and the process of inflammation, which are considered to contribute to asthma pathogenesis (70–72).

The first interventional study was conducted by Goldring et al. in UK (**Table 1**). The sample consisted of women with various ethnicities. Daily vitamin consumption of 800 IU D2 during the last trimester of pregnancy did not reveal any association with lung function, airway inflammation, and recurrent or asthma-predicting wheezing at 3 years of age, in comparison with the control group (73). In a recent randomized placebo-controlled double-blind trial in New Zealand, vitamin D was administrated in unselected pregnant women from the 27 weeks of gestation and subsequently in their infants during the first 6 months of life (placebo/placebo, 1,000/400 IU, and 2,000/800 IU). Despite that there was no difference preserved in the offspring's 25(OH) D level at the age of 18months, a statistically significant lower proportion of children, belonged to vitamins D groups, were sensitized to mite antigens, as indicated by specific IgE measurements. Besides, a possible effect of vitamin D was depicted on primary care visits, where asthma was diagnosed by physicians (74). However, these clinical trials were biased by many limitations, such as small size, vitamin D administration only in the last trimester of pregnancy, possibly inadequate maternal dose administration, while authors also acknowledged that they were not primary designed to assess the specific outcome. Moreover, the first trial was not double blind and pertaining to the second one, a diagnosis of asthma at the age of 18 months was rather vague. Still, the association of vitamin D with asthma, ambiguously emerged from observational studies, could not be confirmed and established as a causative factor. Thus, further well-designed, randomized, controlled double blind trials followed to overcome the limitations of the previous studies and address the issue.

Recently, the results of two randomized controlled clinical trials (RCTs) were presented. In Denmark, Chawes et al. recruited 623 unselected women who were randomized to receive either 2,800 or 400 IU (as part of routine care) of vitamin D daily from 24 weeks of gestation until the first postpartum week. Adherence was assessed by returned pills and the follow-up rate was 94%. The primary outcome was persistent wheezing in the offspring in the first 3 years of age. A no significant 4% absolute reduction in incidence of wheezing was reported in the children whose mothers were assigned to 2,800 IU group (hazard ratio, 0.76 [95% CI 0.52–1.12]). Pertaining to secondary outcomes, the number of troublesome lung symptoms was significant lower in children of the intervention arm. However, no association was observed with asthma or other allergic diseases at 3 years of age (75). The second multicenter double-blind RCT was conducted in USA by Litonjua et al. Pregnant women on high risk to obtain children with asthma were randomized to either an interventional arm receiving 4,400 IU of vitamin D or to a control arm receiving 400 IU, as usual care, from 10 to 18 weeks of gestation until delivery. Compliance was measured by electronic medication containing caps. Ultimately, 748 children from the 876 initially enrolled mothers were followed up to 3 years of age. The cumulative incidence of asthma or wheezing was 6.1 lower in the intervention group in the first 3 years of life, but this was a borderline result, which did not meet statistical significance [hazard ratio, 0.8 (95% CI 0.6–1.0)]. Moreover, this effect was reported to be attenuated with increasing age. Of note, despite the intervention significantly increased circulating vitamin D levels in women, 1-year postpartum no difference was preserved (76).

Although the results of both RCT did not meet statistical significance, the wide confidence intervals, which include a clinically important protective effect suggest that the studies may have been underpowered to measure the primary outcomes. A recent meta-analysis, which processed these data from both these two RCT and the study of Goldring et al., suggested that prenatal daily supplement of vitamin D might have a positive effect on childhood wheezing [relative risk, 0.812 (95% CI 0.673–0.98)] (77). Given the fact that wheezing, usual viral-induced, in early childhood is a frequent cause of hospitalization and morbidity (78), further studies to confirm the possible causality are warranted.

Taken all together, vitamin D shows a potential association rather with asthma-related phenotypes than with asthma *per se*. For instance, in early childhood, wheezing is frequent transient or viral induced and 60% of these children are not expected to develop asthma (79). Therefore, it remains to be answered if a real cause-effect relationship exists or vitamin D exerts a protective role *via* other underlying mechanisms of this multifactorial disease, such as lung function or protection against viral infections. Additionally, vitamin D is a marker of UV exposure, which can modulate immunity *via* non-vitamin D related pathways (80, 81). Furthermore, as far as asthma diagnosis is ambiguous at the first 3 years of life, long-term follow-up may contribute to explore the possible relationship. However, the attenuation of the observed effect with aging (59, 74, 76) complicates this theory. Notably, a large prospective observational birth cohort with long-term follow-up until early adulthood did not reveal a protective programming effect of high maternal 25(OH)D concentration in the third trimester of pregnancy on asthma at the age of 20 years, whereas maternal 25(OH)D levels over 50 ng/ml (125 nmol/l) were associated with higher risk of asthma hospitalization during the first 25 years of life (65). Moreover, it has been proposed that studies should focus on vitamin D status in early pregnancy and supplementary administration should be initiated from the first trimester of gestation in order to observe the optimal effect on risk of childhood asthma, though evidence from Chawes et al.'s clinical trial did not confirm this notion (75). In addition, an interesting remark is that study designs and analyses do not take into consideration the starting maternal vitamin D status and dose adequacy for the different body systems. This is also an issue that should be addressed.

Irrefutably, both entities of asthma and vitamin D deficiency are multifactorial. A plethora of environmental and genetic/epigenetic factors contribute in a complex manner to a partly understood pathogenesis, while the shifted vitamin D metabolism during pregnancy is also not fully elucidated. As a result, unmeasured aspects and residual confounding factors are rather difficult to be diminished from the studies. Besides, even if the putative effect suggested by the current RCTs be strengthened by further investigations, this would only explain a little proportion of asthma epidemic. Shifting the discussion to a clinical platform, vitamin D administration during pregnancy has not yet been justified. However, given the rather low toxicity of such a supplement and based on the evidence derived of the two RCTs, pregnant women at a high risk of obtaining a child with asthma may be

TABLE 1 | Randomized control trials on vitamin D supplementation during pregnancy and asthma/wheezing.

Reference	Country and enrollment period	Study population	Intervention arms	Number/age of children at outcome assessment	Intake of intervention from/until	Effect on median vitamin D levels (ng/ml)	Outcomes of interest	Main findings
Goldring et al. (73)	UK 2007	180 mother–child pairs 4 Ethnic groups: Asian, Middle Eastern, Black and White	*Not blinded* Women: no treatment ($N = 60$) 800 IU ($N = 60$) ergocalciferol daily 200,000 IU ($N = 60$) cholecalciferol single bolus oral dose	158/3 years	Mothers: 27 weeks of gestation to delivery	Higher in cord blood of supplementary arm (control 6.8, daily dose 10.4, single dose 10 $p < 0.001$)	*Blinded* Wheezing, eczema, food allergy, rhinitis, atopy, URTI, LRTI, inhaled bronchodilator or steroids	No significant difference between groups in risk of wheezing at 3 years of age (RR: 0.86, 95% CI 0.49–1.50 $p = 0.69$) No significant difference between groups pertaining to rest secondary outcomes
Grant et al. (74)	New Zealand 2010–2011	260 mother–child pairs	*Double-blinded* Women/infant: placebo/placebo 1,000/400 IU 2,000/800 IU	185/18 months	Mothers: 27 weeks of gestation to delivery Off springs: birth to 6 months	*Enrollment*: 25 → no difference ($p = 0.19$) *Cord blood*: 27 vs 15 → higher in both supplemented groups than in placebo *6 months of age*: 40 vs 30 → higher vit D group had the highest and placebo group the lowest concentrations *18 months of age*: 24.4 → no difference ($p = 0.30$)	Secondary outcomes Sensitization to airborne allergens Primary health care visits for respiratory illnesses	Decreased proportion of children sensitized to four mites antigens Study group differences in the proportion of children with primary care visits of doctor-diagnosed asthma (11, 0, and 4%, $p = 0.002$)
Chawes et al. (75)	Denmark 2009–2010	623 mother–child pairs recruited from $COPSAC_{2010}$ cohort study	*Double-blinded* Placebo + 400 IU ($N = 308$) Cholecalciferol 2,400 + 400 IU ($N = 315$)	581/3 years	Mothers: 24 weeks of gestation to 1 week after delivery	Increase in maternal serum vitD level in treatment group (mean SD at randomization vs postpartum: 31 vs 43; control group 31 vs 29) Mean group difference 13 [95% CI 11–16, $p < 0.001$]	Persistent wheeze, asthma, URTI, LRTI, episodes of lung symptoms, SPT, specific IgE	No significant reduction in the risk of persistent wheezing per 4 ng/ml increase in maternal serum vitamin D level (HR: 0.86, 95% CI 0.89–0.99 $p = 0.03$) Reduced number of troublesome lung episodes (IRR: 0.83, 95% CI 0.71–0.97 $p = 0.02$) Upregulation or airway immune profile (principal component analysis $p = 0.04$) No effect on additional end points
Litonjua et al. (76) (VDAART)	USA 2009–2011	876 mothers at high risk of having child with asthma	*Double-blinded* Placebo + 400 IU ($N = 436$) 4,000 + 400 IU ($N = 440$)	748/3 years	Mothers: between 10 and 18 weeks of gestation to delivery	*Third trimester*: higher in supplemented group vs control (39.2 vs 26.8, mean difference 12.4; 95% CI 10.5–14.3, $p < 0.001$) *Cord blood*: significant difference preserved *1 and 3 years postpartum*: no difference	Wheezing or asthma, eczema with rash, LRTI, mean total IgE. Aeroallergens sensitization, specific IgE	No significant reduction in the incidence of asthma and recurrent wheezing by 6.1% (HR: 0.8, 95% CI 0.6–1.0 $p = 0.51$)

CI, confidence interval; HR, hazard ratio; RR, relative risk; SPT, skin prick test; RI, respiratory infection; URTI, upper respiratory tract infection; LRTI, lower respiratory tract infection.

benefited, particularly if they are deficient (82). Moreover, any intervention should be individualized to initial maternal vitamin D status and be followed up, since a *U*-curve association between vitamin D circulating levels and impact on allergy and human health has been also proposed (83, 84).

KEY ELEMENTS

- Vitamin D shows a potential association rather with asthma-related phenotypes than with asthma *per se*. Further well-designed RCTs to confirm the possible associations are warranted.
- Vitamin D supplementation during pregnancy has not yet been justified. However, given the rather low toxicity of such a supplement, pregnant women at a high risk of obtaining a child with asthma may be benefited, especially if they are vitamin D deficient. Any intervention should be individualized to initial maternal vitamin D status and be followed up.

AUTHOR CONTRIBUTIONS

AP contributed to the conception of the work, the revision, and the final approval of the manuscript. EB contributed to the design of the work, the drafting, and the final approval of text manuscript. KD contributed to the design of the work, the revision, and the final approval of the manuscript. All the authors agreed to be accountable for all aspects of the work.

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MINI REVIEW
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What Are the Effects of a Mediterranean Diet on Allergies and Asthma in Children?

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This review updates the relationship between the adherence to Mediterranean diet (MedDiet) assessed by questionnaire and asthma, allergic rhinitis, or atopic eczema in childhood. It deals with the effect of MedDiet in children on asthma/wheeze, allergic rhinitis, and atopic dermatitis/eczema, and also with the effect of MedDiet consumption by the mother during pregnancy on the inception of asthma/wheeze and allergic diseases in the offspring. Adherence to MedDiet by children themselves seems to have a protective effect on asthma/wheezing symptoms after adjustment for confounders, although the effect is doubtful on lung function and bronchial hyperresponsiveness. By contrast, the vast majority of the studies showed no significant effect of MedDiet on preventing atopic eczema, rhinitis, or atopy. Finally, studies on adherence to MedDiet by the mother during pregnancy showed some protective effect on asthma/wheeze symptoms in the offspring only during the first year of life, but not afterward. Very few studies have shown a protective effect on wheezing, current sneeze, and atopy, and none on eczema. Randomized control trials on the effect of the adherence to MedDiet to prevent (by maternal consumption during pregnancy) or improve (by child consumption) the clinical control of asthma/wheezing, allergic rhinitis, or atopic dermatitis are needed.

Keywords: allergic rhinitis, asthma, children, dermatitis, Mediterranean diet, review, wheezing

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INTRODUCTION

Diseases such as asthma, allergic rhinitis, or atopic eczema have increased in the last decades, and the highest incidence seems to occur among children (1). One of the explanations of that increase from the environmental pint of view relates to changes in diet (2). The main components of the modern diet are foods which have been highly processed, modified, stored, and transported over great distances. Furthermore, some reports show a trend to consume higher amounts of saturated fats, such as burgers, and sugar such as soft drinks (3). By contrast, the traditional diet is produced and marketed locally and is eaten shortly after harvesting (4).

One of these traditional diets is the Mediterranean diet (MedDiet). The term MedDiet refers to dietary patterns in the Mediterranean region. MedDiet is characterized by a high intake of fruits and vegetables, bread and whole grain cereals, legumes, and nuts; low-to-moderate consumption of dairy products and eggs; and limited amounts of meat and poultry. This diet is low in saturated fatty acids and rich in antioxidants, carbohydrates, and fiber. It also has a high content of monounsaturated fatty acids and $n-3$ polyunsaturated fatty acids (PUFAs), mainly derived from olive oil (from fish in some areas) (5).

In the last decade, several epidemiological studies have reported a protective effect of MedDiet on asthma, rhinitis, and atopic eczema, while others did not find any protective effect of this diet on those conditions. Therefore, before nutritional therapy could be included in guidelines devoted to preventing the inception of asthma and allergic diseases in childhood, or to improving the clinical course of those diseases, it is necessary to have a complete view of the current evidence from epidemiological studies. Then, it is time to perform randomized clinical trials (RCTs).

The aim of the present review is to update the evidence on whether the adherence to MedDiet has some effect on asthma, allergic rhinitis, and atopic eczema in childhood.

WHAT IS THE EVIDENCE SO FAR?

We conducted an electronic literature search using Medline/PubMed and EMBASE in September 2016 and extended back to 1960. The terms used to search both in titles and in abstracts were "(((Mediterranean Diet)) AND ((asthma OR wheezing)) OR ((allergic rhinitis)) OR ((atopic dermatitis OR atopic eczema)) AND ((children)))." Only original studies with human subjects were included. No restriction was made for publication language or publishing status. Also, cross-referencing from the articles found was used to complete the search.

A total of 46 studies were retrieved electronically. Among those, 24 studies were excluded for the following reasons: reviews ($n = 9$), editorials ($n = 6$), systematic reviews ($n = 4$), no inclusion of MedDiet ($n = 1$), adult population ($n = 2$), different outcome ($n = 1$), and clinical trial design ($n = 1$). Therefore, 22 original studies were included in the present review. All were observational studies (cross-sectional, cohort, or case–control studies) and assessed the adherence to MedDiet by dietary information collected using food frequency questionnaires and scoring MedDiet by means of different scores.

Effect of MedDiet Consumption by Children on Asthma/Wheeze

Fifteen studies were retrieved (**Table 1**) on this topic. We previously reported (6) eight of these studies (7–14) in a systematic review with a meta-analysis. In that review on 39,804 children, we found a negative association of "current wheezing" with the highest tertile of MedDiet score adherence (OR 0.85 [0.75–0.98]; $p = 0.02$). This result was driven by Mediterranean centers (0.79 [0.66–0.94], $p = 0.009$). A similar figure was found for "current severe wheeze" (OR 0.82 [0.55–1.22], $p = 0.330$ for all centers; and OR: 0.66 [0.48–0.90], $p = 0.008$ for Mediterranean centers). Mediterranean centers were centers <100 km from the Mediterranean coast.

However, seven studies, most of them recently published, were not included in the aforementioned review (6). Two studies were performed in Greece (17, 19), and one in each of the following countries: Mexico (15), Spain (16), Turkey (18), Brazil (20), and Peru (21). In three studies, adherence to MedDiet was significantly associated with lower asthma symptoms (17, 19, 21). Additionally, adherence to MedDiet was significantly associated with improved lung function in one study (15), but not in another one (21). By contrast, one study (16) showed higher asthma prevalence among girls with higher adherence to MedDiet. In two studies (18, 20), no significant effect of MedDiet was found. Interestingly, an inverse mediating effect of MedDiet was observed for the urban environment–asthma relation (standardized beta = −0.029, $p < 0.001$), while physical activity had no significant contribution, adjusted for several confounders (19). A direct interaction of MedDiet with maternal education was found in one of the studies (21).

Effect of MedDiet Consumption by Children on Allergic Rhinitis and Dermatitis/Eczema

With respect to the association between MedDiet consumption and allergic rhinitis and/or atopic dermatitis, 8 studies were retrieved (**Table 1**). Two studies were carried out in each of Spain (8, 25) and Turkey (26, 27), one was worldwide (13), and one in each of the following countries: Greece (9), Peru (21), and Mexico (12). High adherence to MedDiet was significantly protective for allergic rhinitis and atopy in only one study (9) as measured by skin prick test (SPT). However, no effect on allergic rhinitis was demonstrated in the other four studies (8, 21, 26, 27). Moreover, no significant effect of MedDiet was demonstrated on atopic dermatitis (25), nor on atopic sensitization as defined either by specific IgE (21) or by SPT (13).

Effect of MedDiet Consumption by Mother on Asthma/Wheeze/Allergic Diseases in the Offspring

Seven studies that explore this effect were retrieved (**Table 2**). Four studies were done in Spain (10, 28–30), one in Mexico (12), one in the US (31), and one in Greece/Spain (32). In all of them, the outcome was asthma/wheeze. In three studies, atopic eczema/atopy was included (30–32), and in two studies, rhinitis was also included (12, 30). The offspring were surveyed at different times. In three studies, it was during the first year of life (28, 29, 32), at 3 years in one study (31), at 4 years in another one (30), and at 6.5 years of life in the others (10, 12).

Adherence to MedDiet by the mother had a protective effect on wheeze during the first year of life in the offspring in one study (29), and this effect disappeared after adjusting for confounders in another study (28). In three studies (30–32), the effect of maternal MedDiet had no significant effect on wheeze. When combined maternal and child adherence to MedDiet was analyzed, only one study showed a protective effect on persistent and atopic wheezing, and atopy by SPT at 6.5 years of age (10). However, another study did not find any such association (30). Regarding atopic eczema and allergic rhinitis, adherence to MedDiet by the mother had no significant protective effect in the majority of the studies, except for one (12) in which maternal MedDiet consumption was protective for current sneeze in the offspring at 6–7 years of age.

In summary, adherence to MedDiet by children themselves seems to have a protective effect on asthma/wheezing symptoms after adjusting for confounders, but the effect is doubtful on lung

TABLE 1 | Summary of studies reporting the association between adherence to MedDiet and asthma/wheezing, allergic rhinitis, and dermatitis/eczema in children.

Reference	Country	Sample	Study design	MedDiet score	Outcomes	Primary results: effect of MedDiet	Adjusted confounders
Sanchez-Solis et al. (7)	Spain	683 (6–8 years)	C-S	Mod.Psal 1[b]	Clinical significant asthma	adjOR = 0.78 [0.61–0.97]	Percent body fat
Chatzi et al. (9)	Greece	690 (7–18 years)	C-S	KidMed[c]	Current wheezing, ever wheezing, wheezing ever with atopy Allergic rhinitis ever, allergic rhinitis ever with atopy, current allergic rhinitis	Not associated with current and ever wheezing adjOR = 0.34 [0.18–0.64], $p < 0.001$ for allergic rhinitis ever; adjOR = 0.39 [0.13–0.97], $p < 0.05$ for allergic rhinitis ever with atopy; adjOR = 0.49 [0.24–0.99], $p < 0.05$ for current allergic rhinitis	Age, sex, BMI, parental asthma, number of older siblings
Garcia-Marcos et al. (8)	Spain	20,106 (6–7 years)	C-S	Mod. Psal 2[a]	Current occasional asthma, current severe asthma Current rhinoconjunctivitis	adjOR = 0.90 [0.82–0.98] for current severe asthma in girls adjOR = 0.98 [0.93–1.03] for current rhinitis in girls, adjOR = 0.99 [0.95–1.03] for current rhinitis in boys	Older and younger sibling, maternal smoking
Chatzi et al. (10)	Spain	460 (6.5 years)	C-S	KidMed[c]	Persistent wheeze, atopic wheeze	Not associated with either asthma outcomes	Sex, maternal and paternal asthma, maternal social class and education, BMI, total energy intake
Castro-Rodriguez et al. (11)	Spain	1784 (4.1 ± 0.8 years)	C-S	Mod.Psal 1[b]	Current wheeze	adjOR = 0.54 [0.33–0.88]	Several factors[e]
Romeiu et al. (15)	Mexico	158 asthmatic (9.6 years) 50 non-asthmatic (9.3 years)	Cohort	Mod. Trich[d]	Inflammatory response (IL-8), lung function (FEV1, FVC)	Positively associated with FEV1 ($p = 0.045$) and FCV ($p = 0.018$) and was modifier for the effect of ozone on FVC ($p = 0.02$)	Sex, BMI, previous day min. temperature, corticoid use, chronological time
De Batlle et al. (12)	Mexico	1,476 (6–7 years)	C-S	Mod. Trich[d]	Ever asthma, ever wheezing, current wheezing Rhinitis ever, current rhinitis, current sneezing, current itchy-water eyes	adjOR = 0.60 [0.40–0.91] for ever asthma; adjOR = 0.64 [0.47–0.87] for ever wheezing adjOR = 0.64 [0.36–1.15] for rhinitis ever; adjOR = 0.87 [0.65–1.18] for current rhinitis; adjOR = 0.71 [0.53–0.97] for current sneezing; adjOR = 0.96 [0.64–1.45] for current itchy-watery eyes	Sex, maternal education, exercise, current ETS at home, maternal asthma and rhinitis
Nagel et al. (13)	20 countries	50,004 (8–12 years)	C-S	Mod. Psal 2[a]	Ever asthma, current wheeze, atopic wheeze, BHR SPT	adjOR = 0.95 [0.94–0.99] for ever asthma; adjOR = 0.97 [0.94–0.99] for current wheezing; adjOR = 1.05 [0.96–1.14] for BHR No association with positive SPT (adjOR = 1.00 [0.95–1.04])	Age, sex, ETS, parental atopy, exercise, siblings
Gonzalez-Barcala, et al. (16)	Spain	14,700 (6–7 and 13–14 years)	C-S	Mod. Psal 2[a]	Prevalence and severity ever asthma	adjOR = 2.26 [1.2–4.2] among girls aged 6–7 years	BMI, parental smoking, maternal education
Suarez-Varela, et al. (25)	Spain	20,106 (6–7 years)	C-S	Mod. Psal 2[a]	Atopic dermatitis	adjOR = 1.03 [0.99–1.08], $p = 0.071$	Gender, obesity, ETS first year of livelife, siblings, exercise
Arvaniti et al. (14)	Greece	700 (10–12 years)	C-S	KidMed[c]	Ever asthma, asthma symptoms, ever wheeze, exercise wheeze	Lower ever asthma, any asthma symptoms, ever wheeze, and exercise wheeze (all $p < 0.005$)	Age, sex, BMI, physical activity status, energy intake
Grigoropoulou et al. (17)	Greece	1,125 (10–12 years)	C-S	KidMed[c]	Ever asthma (symptoms)	adjOR: 0.84 [0.77–0.91]	Age, gender, BMI, physical activity status, and energy intake

(*Continued*)

TABLE 1 | **Continued**

Reference	Country	Sample	Study design	MedDiet score	Outcomes	Primary results: effect of MedDiet	Adjusted confounders
Akcay et al. (18)	Turkey	9,991 (13–14 years)	C-S	Mod. Psal 2[a]	Asthma diagnosis by physician	No significant association	Not adjusted
Tamay et al. (26)	Turkey	10,984 (13–14 years)	C-S	Mod. Psal 2[a]	Physician-diagnosed allergic rhinitis	No difference on physician-diagnosed allergic rhinitis	Not adjusted
Tamay et al. (27)	Turkey	11,483 (6–7 years)	C-S	Mod. Psal 2[a]	Physician-diagnosed allergic rhinitis, current rhinoconjunctivitis, lifetime rhinitis, current rhinitis	OR = 0.97 [0.94–0.99], $p = 0.007$ for physician diagnosed allergic rhinitis, OR = 0.97 [0.94–0.99], $p = 0.01$ for current rhinoconjunctivitis, OR = 0.97 [0.96–0.98], $p < 0.001$ for lifetime rhinitis. But after being adjusting no association with any outcome was found	Exercise frequency, hours spent on watching TV/computer, foods
Alphantonogeorgos et al. (19)	Greece	1,125 (10–12 years)	C-S	KidMed[c]	Ever asthma (symptoms)	Low adherence associated with more asthma (rural area $p = 0.04$, urban area $p < 0.001$). Mediation through the MedDiet (beta = −0.029, $p < 0.001$) reduced the harmful effect of urban living	Age, gender, parental history of atopy
Silveira et al. (20)	Brazil	268 persistent and 126 intermittent asthmatic (3–12 years)	C-S	Mod. Psal 2[a]	Persistent (mild, moderate or severe) vs. intermittent asthma	No difference for persistent vs. intermittent asthma (57 vs. 52%, $p = 0.40$)	Not adjusted
Rice et al. (21)	Peru	287 asthmatic and 96 controls (9–19 years)	C-C	Mod. Psal 1[b]	Asthma diagnosis by physician, ACT, lung function	OR = 0.56 [0.36–0.90], $p = 0.02$; adjOR = 0.55 [0.33–0.92], $p = 0.02$, for asthma. No difference in ACT, nor FEV1. Interaction with maternal education (higher MedDiet score with more education)	BMI, sex, age, maternal education
					Allergic rhinitis, atopy (specific IgE)	No association with any allergy outcome was found	

ACT, asthma control test; adjOR, adjusted odds ratio; BMI, body mass index; C-C, case control; C-S, cross-sectional; ETS, environmental tobacco smoke; FVC, forced vital capacity; FEV1, forced expiration volume in the first 1 s; Ig, immunoglobulin; IL, interleukin; MedDiet, Mediterranean diet; OR, odds ratio; SPT, skin prick test.

[a]*Modified from Psaltopoulou (23) (min–max score: 0–20).*

[b]*Modified from Psaltopoulou (23) (min–max score: 0–36).*

[c]*Modified from KIDMED (24).*

[d]*Adapted from Trichopoulou (25).*

[e]*Age, birth weight, livestock during pregnancy, cesarean, antibiotic and acetaminophen consumption during the previous 12 months, rhinoconjunctivitis, dermatitis, paternal and maternal asthma, maternal age and education level, current paternal and maternal smoking, vigorous physical activity, and cats at home in the last 12 months.*

TABLE 2 | Summary of studies reporting the association between adherence to MedDiet during pregnancy and asthma/wheezing/allergic disease in offspring.

Reference	Country	Sample of (mother/ offspring)	Study design	MedDiet score	Asthma, dermatitis, and allergic rhinitis outcomes	Primary results: effect of MedDiet	Adjusted confounders
Chatzi et al. (10)	Spain	460	C-S	KidMed[b]	Persistent wheeze and atopic (SPT) wheeze at 6.5 years	A high MedDiet Score during pregnancy was protective for persistent wheeze (adjOR = 0.22 [0.08–0.58]), atopic wheeze (adjOR = 0.30 [0.10–0.90]), and atopy (adjOR = 0.55 [0.31–0.97]) at age 6.5 years	Sex, maternal and paternal asthma, maternal social class and education, BMI, and total energy intake at age 6.5 years
De Batlle et al. (12)	Mexico	1,476	C-S	Mod. Trich[c]	Ever asthma, ever wheezing, current wheezing, rhinitis ever, current rhinitis, current sneezing, and current itchy-watery eyes at 6–7 years	No associations were found between mothers diet score during pregnancy and asthma or allergic rhinitis outcomes in children in the crude or adjusted analyses, except for current sneezing = 0.71 [0.53–0.97]	Sex, maternal education, exercise, current tobacco smoking at home, maternal asthma and maternal rhinitis
Castro-Rodriguez et al. (28)	Spain	1,409	Cohort	Mod.Psal 1[a]	Any wheeze at first year	MedDiet ($p = 0.036$) and olive oil ($p = 0.002$) during pregnancy were significantly associated with less wheezing. Only olive oil consumption remained associated (adjOR = 0.57 [0.4–0.8], $p = 0.002$)	Sex, exclusive breastfeeding, day care, eczema, maternal asthma, smoking during pregnancy, siblings, mold stains on household wall, and preterm birth
Lange et al. (31)	US	1,376	Cohort	Mod. Trich[c]	Recurrent wheeze, doctor diagnosis of asthma and atopy at 3 years	OR = 0.64 [0.43–0.95], adjOR = 0.98 [0.89–1.08] for recurrent wheeze. No association with doctor's diagnosis of asthma, or atopy	Sex, maternal race, maternal education level, household income, maternal and paternal history of asthma, presence of children at home, maternal pre-pregnancy, BMI, breast feeding duration, passive smoke exposure
Chatzi et al. (32)	Spain and Greece	Spain: 1,771; Greece: 745	Cohort	Mod. Trich[c]	Wheeze and eczema at 12 months	Not associated with risk of wheeze and eczema	Not adjusted
Pellegrini-Belinchón et al. (29)	Spain	1,164	Cohort	None	Recurrent wheeze at first year	adjOR = 0.436 [0.297–0.640]	Nursery, eczema, maternal asthma, smoking in third trimester
Castro-Rodriguez et al. (30)	Spain	1,001	Cohort	Mod.Psal 1[a]	Current wheeze, dermatitis and allergic rhinitis at 4 years	MedDiet score adherence by mother and by child at year 4 did not remain a protective factor for any outcome	Many environment factors[d]

adjOR, adjusted odds ratio; BMI, body mass index; C-S, cross-sectional; MedDiet, Mediterranean diet; OR, odds ratio; SPT, skin prick test.

[a]*Modified from Psaltopoulou (22) (min–max score: 0–36).*

[b]*Modified from KIDMED (23).*

[c]*Adapted from Trichopoulou (24).*

[d]*Age, birth weight and height, cesarean, antibiotic and acetaminophen consumption during the previous 12 months, oral contraception use, parental asthma, parental rhinitis, parental dermatitis, maternal age and education level, current paternal and maternal smoking, breastfeeding, siblings, pets at home during pregnancy, mold stain, day care, type of fuel, TV video and physical activity at 4 years, air pollution, and colds during first year of life.*

function and bronchial hyperresponsiveness. By contrast, the vast majority of the studies showed no significant effect of MedDiet on preventing allergic diseases (atopic eczema, rhinitis, or atopy). Finally, studies on adherence to MedDiet by the mother during pregnancy showed some protective effect on asthma/wheeze symptoms in the offspring only during their first year of life, but not after. Only very few studies showed a protective effect on wheezing, current sneeze, and atopy. No study has shown any protective effect on atopic dermatitis.

The results of the protective effect of MedDiet on asthma/wheezing symptoms in children found in the present review expand the results reported in systematic reviews with meta-analyses previously carried out by our group (6) and by others (33). The present report also confirms the protective effect of MedDiet consumption by the mother on asthma or allergic diseases in their offspring, as was described in a different review published by our group (34) and by others (35, 36), suggesting some epigenetic effect of diet. However, no systematic review on the effect of MedDiet on other allergic diseases, i.e., rhinitis or atopic eczema has been previously published. In the present study, no effect was shown in seven out of eight studies.

HOW MedDiet MIGHT WORK?

In the past decades, one of the most important environmental changes worldwide has been that of diet patterns. Due to changes

in dietary fat intake (by increasing $n-6$ PUFAs and decreasing $n-3$ PUFAs), through an increased production of prostaglandin (PG) E2 might have contributed to the increase in the prevalence of asthma (37). PGE2 suppresses T-helper (Th) 1 and increases Th2 phenotype, thus reducing IFN-gamma. Th2 phenotype, in which there is an increase of IgE isotype switching, is associated with asthma and atopic diseases. On the other hand, diets low in antioxidants may have increased the susceptibility to develop asthma (38). In this case, the proposed mechanism involves the decline of lung antioxidant defenses, resulting in increased oxidant-induced airway damage.

Several of those studies reported on the beneficial effect of single foods rich in $n-3$ PUFA or antioxidants on asthma or allergic diseases. A meta-analysis concluded that increased consumption of vegetables and fruits, zinc, and vitamins A, D, and E, is related to lower prevalence of asthma and wheeze in children and adolescents (36). However, most of the studies included in that meta-analysis fail to account for the interactions between nutrients (39). Yet, since our normal eating behavior is to follow certain diet patterns that contain many specific foods, it seems reasonable to put the focus on food patterns or diets.

Mediterranean diet is rich in both antioxidants and *cis*-monounsaturated fatty acids. Moreover, cereals (whole grain) are rich in vitamin D, phenolic acids, and phytic acid. Moreover, fruits, vegetables, and legumes are rich in vitamins C and E, carotenoids, selenium, and flavonoids (5). Additionally, olive oil for cooking and dressing salads is an important part of MedDiet. The main active components of olive oil are oleic acid, phenolic derivatives (hydroxytyrosol, tyrosol, oleuropein, and ligstroside), and squalene, all of which have been found to exhibit a marked antioxidant activity. Also, oleuropein and hydroxytyrosol (its hydrolysis product) are among the most potent antioxidants (40). One study has shown that, after a multivariate analysis, olive oil consumption by the mother during pregnancy, but not MedDiet, remained as a protective factor (aOR = 0.57 [0.4–0.9]) for wheezing during the first year of life in the offspring (28). Therefore, it seems reasonable to think that a higher adherence to MedDiet or olive oil may have some protective effects on asthma and allergic diseases in childhood. The ability of this diet to counteract oxidative stress might have an effect on asthma inception (41).

WHAT IS NEEDED?

All studies included in the present review were observational (cross-sectional, cohort, and case–control) studies. All but one of them used three different MedDiet scores: one modified from Psaltopoulou (22), one modified from KIDMED (23), and another one adapted from Trichopoulou (24). Seventeen studies come from Mediterranean countries (nine from Spain, five from Greece, and three from Turkey). While these observational studies have reported potentially beneficial associations with MedDiet on asthma/wheezing, it is unknown whether an intervention to promote the MedDiet could reduce the prevalence of asthma and allergic diseases in children.

Currently, there are no RCTs testing the hypothesis that adherence to MedDiet could decrease the risk of asthma and allergic disease in children. One pilot RCT in pregnant Scottish women at high risk of having asthmatic or allergic offspring is ongoing (42). It aims are to establish recruitment, retention, and acceptability of the dietary intervention and to assess the likely impact of the intervention on adherence to a MedDiet during pregnancy through seeking a reduction in the incidence of asthma and allergic problems (42). In adults, only one 12-week open-label trial was published (43). In that small study, 38 asthmatic adults were randomized to receive either 41 h of dietician services or only 2 h of services, or just one dietician session and recipes (control). The study achieved its primary outcome of altering the eating habits of participants in the high-intensity intervention toward a MedDiet pattern. The study had not enough power to detect clinical endpoints; however, non-significant improvements in asthma-related quality of life, asthma control, or spirometry were observed in the intervention groups (43).

CONCLUSION

Adherence to MedDiet by children seems to have a protective effect on asthma/wheezing symptoms, but not on allergic rhinitis, eczema, or atopy. Adherence to MedDiet by the mother during pregnancy might have some protective effect on asthma/wheeze symptoms in the offspring only during their first year of life, and few studies have shown some protective effect on current sneeze and atopy, but none on eczema. Randomized control trials on MedDiet adherence to test the real usefulness on primary prevention (by maternal consumption during pregnancy) or on clinical improvement (by patient consumption) of asthma/wheezing, allergic rhinitis, and dermatitis in childhood are needed.

AUTHOR CONTRIBUTIONS

JC-R contributed to the study concept, literature search, data collection, and manuscript writing. LG-M contributed to the literature search, data collection, and manuscript writing.

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Probiotics in Asthma and Allergy Prevention

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Interest in probiotic research and its potential benefits in infant foods are relatively recent but significantly increasing. The evolution of the knowledge in the last 20 years demonstrated that alterations in the microbiome may be a consequence of events occurring during infancy or childhood, including prematurity, cesarean section, and nosocomial infections. Several pieces of evidence prove that a "healthy" intestinal microbiota facilitates the development of immune tolerance. Interventional studies suggest that probiotics could be protective against the development of many diseases. Nevertheless, many factors complicate the analysis of dysbiosis in subjects with food allergy. Comparison in-between studies are difficult, because of considerable heterogeneity in study design, sample size, age at fecal collection, methods of analysis of gut microbiome, and geographic location. Currently, there is no positive recommendation from scientific societies to use pre- or probiotics for treatment of food allergy or other allergic manifestations, while their use in prevention is being custom-cleared. However, the recommendation is still based on little evidence. Although there is valid scientific evidence *in vitro*, there is no sufficient information to suggest the use of specific probiotics in allergy and asthma prevention.

Keywords: probiotics, asthma, allergy, prevention, children

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INTRODUCTION

The 2001 FAO/WHO definition of probiotics ("live microorganisms that, when administered in adequate amounts, confer a health benefit on the host") has been widely adopted by regulatory agencies, such as Codex alimentarius, the European Food Safety Authority (EFSA), scientists, industry, and consumers. Everyone agrees that a specific probiotic strain should have been investigated in properly controlled studies to confer a specific benefit before claiming the existence of such benefit. If this is not fulfilled, the only allowed claim would be "contains probiotics." Studies using probiotics or prebiotics have been generally designed as exploratory and were not sufficiently designed to fulfill the criteria for substantiation of a health claim under the current regulation by EFSA (1).

In order to recommend specific probiotics or a mixture of probiotic strains for allergy prevention, they must prove to reduce the risk of later allergies when given to the pregnant or breast-feeding mother or directly to the infant.

Interest in probiotic research and its potential benefits in infant foods is quite recent, but significantly increasing. According to a recent bibliometric analysis, the total number of documents published on probiotics in pediatrics over the period 1994–2014 was 2817. Research production

on probiotics in pediatrics showed a 90-fold increase during the study period. Approximately 22% of articles originated from USA and has the greatest share (2). The top 10 cited articles over the past two decades revealed that the majority of most important articles focused on the role of probiotics in the treatment of allergy and diarrhea in children. In **Table 1**, we summarize the main mechanisms of action of probiotics.

PROBIOTICS IN PEDIATRICS

In the last 20 years, it became clear that events occurring during infancy or childhood, including prematurity, cesarean section, and infections, influence the microbiome. Microbiome alterations have been associated with infantile colic, necrotizing enterocolitis, asthma, atopic diseases, diabetes, mood disorders, and autism spectrum disorders (3).

Interventional studies suggest that probiotics could prevent or reduce the severity of some of these diseases, but the biological mechanisms—and the optimal intervention for each—remain poorly understood.

PROBIOTICS FOR ALLERGY PREVENTION

A "healthy" intestinal microbiota facilitates the development of immune tolerance (4, 5). Earlier studies showed that gut-associated lymphoid tissues (GALT), including Peyer's patches, are poorly developed or absent in germ-free mice (6, 7). It was shown that the introduction of *Bacteroides fragilis* into the lower gut of germ-free mice in the neonatal period could lead to a redevelopment of GALT and induction of tolerance (8). It was also proven that the inability to establish an effective immune tolerance early in life increases the host's risk of developing allergic and inflammatory diseases (9). For example, mice raised in a sterile environment show reduced immunoglobulin A and interleukin (IL)-10 producing T regulatory (T_{reg}) cells and are unable to develop oral antigenic tolerance (7, 10, 11). Segmented filamentous bacteria and Clostridium species, particularly clusters IV and XIVa, promote the development of IL-17-producing T cells and T_{reg} cells, respectively (12, 13). Furthermore, the gut microbiota of food allergic mice—but not of tolerant ones—transmitted susceptibility to food allergy when transferred into germ-free mice (14).

Many factors complicate the analysis of dysbiosis in subjects with food allergy. Comparisons between studies are difficult, because of heterogeneity in study design, sample size, age at fecal collection, methods of analysis of gut microbiome, and geographic location (15). Nevertheless, evidence of gut dysbiosis in food allergy is evolving with time, aided by increasing availability of new techniques. Studies relying on bacterial cultures showed that infants allergic to cow's milk had higher total bacteria and anaerobic counts (16), but this finding was not consistent across studies (17) and no association could be established between culturable gut bacteria and sensitization to food, including milk, casein, egg, peanut, and hazelnut (18).

The hypothesized mechanisms by which the commensal microbiota influences the outcome of the allergic response are manifold (19). Intestinal bacteria can modulate the innate lymphoid cells, directly acting on T_{regs} through their toll-like receptors (TLRs). Commensal microbiota promotes the differentiation of induced T_{regs} (iT_{reg}) from naïve $CD4^+$ T-cells by a T_{reg} intrinsic, TLR- and myeloid differentiation primary response gene 88 (MyD88)-dependent mechanism (20, 21).

Another mechanism by which the commensal flora promotes tolerance is the production of short chain fatty acids (SCFAs), generated by bacterial fermentation of dietary fibers. SCFA act on T cells *via* a G-protein-coupled receptor (GPR43) and protect mice from intestinal inflammation by expanding colonic T_{reg} cells (22). SCFAs also promote the generation of intestinal T_{reg} cells from naïve $CD4^+$ T cells by T-cell intrinsic epigenetic mechanisms (23). Butyrate, a SCFA known as histone deacetylase inhibitor, increases Foxp3 protein acetylation conferring increased stability and enhanced suppressive function on *de novo* generated intestinal iT_{reg} cells (24). A high fiber diet protects against allergic airway inflammation by altering the composition of the flora, leading to increased Bacteroidetes and decreased Firmicutes, and resulting in increased circulating levels of SCFAs (25).

TABLE 1 | Mechanisms of action of Probiotics.

Microbiological action	Epithelial action	Immunological action
– Modulation of the composition of the microbiota – Competitive adhesion to the receptors with the prevention of pathogens invasion – Production of bacteriocin with prevention of growth of pathogens	– Modulation of the epithelial cell barrier – Expression of the tight junction proteins – Short chain fatty acids with improvement of epithelial barriers and anti-inflammatory action	– Innate immunity modulation (maturation dendritic cells) – Modulation of Th1/Th2 rate – Increase of number and activity of T regulatory cells

PROBIOTICS FOR PREVENTION OF ASTHMA AND ECZEMA

In general, preventive strategies for asthma and allergic disorders have been proposed in 2014 (26):

(1) General health education: avoidance of tobacco smoke exposure during pregnancy and after birth.
(2) Primary prevention for infants at higher risk. Several longitudinal birth cohort studies have clearly demonstrated an increased risk of allergic manifestations if one or two parents are or have been affected themselves.
(3) Secondary prevention strategies for children who have already developed allergic sensitization or the first manifestations of allergic diseases; those strategies aim to reduce the incidence of clinical manifestations, such as rhinitis, food allergy, or asthma.

Pre-clinical studies have shown that modifying the microbiota could modulate the global immune response of the host, thus reducing sensitization and allergic inflammation (7, 11). Many studies have suggested the hypothesis that pre- and probiotics might be protective for asthma.

The inhalation of allergens stimulates the innate immune system to release cytokines which promote antigen expressions on $CD4^+$ T-cells and activate the antigen-presenting cells and the T cells to produce Th2 responses (27, 28). Th2 cytokines (IL-4, IL-5, IL-9, and IL-13) induce asthma-like changes in the airways and lung parenchyma, as airway eosinophilia, pulmonary lymphocytosis, mastocytosis, alternative macrophage activation, and epithelial cell proliferation with goblet cell hyperplasia. Previous studies have shown that matrix metalloproteinases, members of a family of enzymes that cleave extracellular matrix proteins, are implicated in many inflammatory conditions (29). Specifically, in asthma, MMP9 levels are significantly increased (30). Treatment with LGG has been shown to decrease MMP9 expression in lung tissue and to inhibit inflammatory cell infiltration. In addition, in OVA-sensitized mice, LGG reduced OVA-specific IgE levels in serum, suppressed the airway hyper-responsiveness to methacholine and decreased the number of infiltrating inflammatory cells and Th2 cytokines in bronchoalveolar lavage fluid and serum (31). Similar results have been reported with other probiotics (32).

Specifically, in pediatric asthma, LGG was reported to reduce the concentration of exhaled nitric oxide among 4- to 7-year-olds (33), but these results could not be replicated (34).

Early administration of *Lactobacillus reuteri* to infants did not result in a reduction of asthma [RR 1.16 (0.33–4.10)], nor did *Lactobacillus rhamnosus* HN001 [RR 0.95 (0.62–145)] or *Lactobacillus paracasei* spp. *paracasei F19* [RR 1.05 (0.39-2.81)] (35–37).

Better results have been obtained with probiotic bacteria based on *in vitro* modulation of cytokine production. *Bifidobacterium bifidum*, *B. lactis*, and *Lc. lactis* were shown to have a good IL-10-inducing capacity and to exert a significant inhibition of Th2-related cytokines IL-5 and IL-13 (38–40). Administered perinatally in a selected combination, they reduced the development of eczema up to the age of 2 years. Their beneficial effect does not reach the age of 6 years and does not lead to primary prevention of asthma.

A systematic review of randomized trials assessing the effects of any probiotic administered to pregnant women, breast-feeding mothers, or infants demonstrated that probiotics could reduce the risk of eczema in infants (41). The certainty in the evidence is low or very low because of the risk of bias, inconsistency and imprecision of results, and indirectness of available research.

As underlined in two recent reviews, replication of the promising results in collaborative well-coordinated multicentre harmonized studies with multidisciplinary expertise in pediatrics, immunology, and microbiology would, thus, be of great importance to enable future evidence-based implementation (42).

A more prolonged gut microbiota management could achieve a long-lasting impact (43, 44).

GUIDELINES RECOMMENDATIONS: OVER TO SCIENTIFIC SOCIETIES

- The European Academy of Allergy and Clinical Immunology (EAACI) stated in its food allergy and anaphylaxis guidelines on primary prevention of allergy, that "there is no evidence to recommend prebiotics or probiotics or other dietary supplements based on particular nutrients to prevent food allergy" in at risk groups and in the general population (grade of recommendation B) (45).
- The Nutrition Committee of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) concluded 2011 after a systematic literature review on the effect of infant formula supplemented with prebiotics or probiotics on the preventive effect on allergy, that "there is too much uncertainty to draw reliable conclusions from the available data" (46).
- The World Allergy Organization (WAO) suggested 2015 on their guidelines on the prevention of allergy to consider using probiotics in:
  (a) women pregnant with children with high risk for allergy,
  (b) women who breastfeed infants at high risk of developing allergy, and
  (c) infants at risk of developing allergies, because there is a net benefit resulting in primary prevention of eczema (47).

CONCLUSION

There is no positive recommendation from any scientific community to use specific probiotics for the prevention of food allergy or other allergic manifestations (48), but their use in prevention as a whole class has widespread in clinical practice (49, 50). We are more open to the use of probiotics than in the past, but the recommendation is based on little evidence. Although there is valid scientific evidence *in vitro*, there is no sufficient information to suggest that the use of probiotics is effective in preventing allergy and asthma. At this point, it seems necessary to understand more precisely the microbiota composition of healthy humans. Only by identifying the specific changes, we would realize that the "ideal probiotic," able to prevent or fight specific dysbiosis of specific disease. Future studies will take stock of state-of-the-art methods for the evaluation of the microflora to better define the indications, the probiotic strains, and the type of prebiotic used.

AUTHOR CONTRIBUTIONS

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REVIEW
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Allergen Avoidance in Allergic Asthma

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Allergic asthma is the most frequent disease among the chronic respiratory disorders in pediatric age with an important social impact. In the last years, many efforts have been made to identify effective preventive approaches to get a better control of symptoms and to obtain the best future outcomes for the patients. In patients with allergic asthma triggered by the exposure to indoor allergens, the avoidance is the first intervention to prevent the appearance or the worsening of bronchial symptoms. This review article summarized the most recent evidence from literature about the efficacy of specific control interventions for the most important allergens. Even if a wide spectrum of interventions has been suggested and may help to reduce exposure to trigger allergy for sensitized patients suffering from respiratory allergy, evidence supporting the efficacy of these approaches is still weak and subject of controversy. However, the exposure control to specific airborne allergens is still widely recommended and may be effective as part of a holistic approach to reduce the severity of allergic respiratory symptoms in sensitized individuals.

Keywords: allergen, asthma, avoidance, exposure, childhood, mite, mold, pets

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INTRODUCTION

Allergic asthma is a chronic respiratory disease that affects millions of people worldwide with a significant burden to patients and the community; its prevalence varies among countries from 1 to 18% (1, 2). Over the past decades, a great interest has been focused on recognizing effective treatments and preventive approaches to reduce the burden of asthma, especially early in life and in the pediatric population. Current research are in fact directed to understand the factors related to the development and worsening of asthma and to find new strategies to reduce exacerbations, its epidemiological and socioeconomic impacts, and to improve the quality of life.

Asthma often occurs in patients sensitized to indoor allergens (e.g., mites and pets). Data from German MAS birth cohort study showed that schoolchildren sensitized to perennial allergens with high exposure early in life are more prone to develop an impaired lung function at school age than children without sensitization or sensitized to indoor allergens but with a low exposure in the first years of life (3). This study highlighted the importance of avoidance of allergen source as a fundamental intervention in the management of allergic patient.

In patients with allergic asthma triggered by the exposure to particular allergens, the avoidance is the first measure to prevent the appearance or the worsening of bronchial symptoms.

Therefore, since a key factor for the development of respiratory allergy is the exposure to airborne allergens, many avoidance strategies have carried out to reduce allergens exposure to avoid the elicitation of symptoms.

The aim of this review article is to focus on the most effective interventions for reducing exposure to the most common airborne allergens in patients with allergic asthma. Primary prevention of allergic sensitization and allergic asthma, which includes also percutaneous sensitization and sensitization of oral and the gastrointestinal tract falls outside the purpose of this review.

References were identified by searches of PubMed and UpToDate and restricted to English articles. Search terms used were "pediatric asthma," "allergen avoidance," "pollen avoidance," "pets avoidance," and "molds avoidance." We included only meta-analysis, randomized controlled trials, reviews, and systematic review articles pertaining to humans.

The Guilty: Focus on Airborne Allergen Sources

- *House dust mites (HDM)* represent the typical indoor perennial allergens. *Dermatophagoides pteronyssinus* (European HDM), *Dermatophagoides farinae* (American HDM), and *Blomia tropicalis* represent the most important HDM species. The most notable mite allergens are Der p 1 and Der f 1, but recently many other molecules have been characterized and studied in patient sensitized to mite allergens (4). HDM belong to arthropods, in particular to the class of arachnids and their average size is about 0.33 mm. They found sustenance from skin cells that flake off and warm and humid environments facilitate their survival. HDM life can be 6–8 weeks long and females are able to produce 40–80 eggs during their life (5). The life cycle takes ~3–4 weeks. Fecal particles contain a complex mixture of allergenic mite-derived proteins, endotoxin, enzymes, and mite and bacterial DNA, all of which can be immunostimulatory (6, 7). The highest dust mite concentrations are in mattresses (8), but they may be found also in house dust, bedding, upholstered furniture, carpets, and curtains. Their growth and reproduction are facilitated by modern house insulation, higher temperatures, and in particular in presence of high indoor humidity. Dust mite infestation is far less common in arid and high-altitude climates, such as the mountain states and southwestern USA.
- *Pets* (furry animals) are a common source of allergens (9) and sensitization to cats' and dogs' main allergens is well known to be associated with severe asthma in childhood (10, 11). The major cat allergen Fel d 1 (secretoglobulin) is found in cats' skin and fur mainly as product of sebaceous and salivary glands (9), whereas dog allergens are mainly found in its hair, dander, and saliva, Can f 1 (lipocalin) represents the major allergen (9).
- *Cockroaches*, such as *Blatella germanica* (German cockroach), are able to shed or excreted tiny protein particles with allergenic properties and, therefore, they can act as indoor allergens. *B. germanica* and its derived allergens are particularly important in the cities of North America as highlighted in some case–control studies and provocation studies (12–14). A study of 476 children found that the combination of specific skin test positivity and exposure to cockroach allergen was associated with significantly higher rates of hospitalization, compared to when this combination was absent (0.37 vs 0.11 hospitalizations/child/year) (15).
- *Pests*, such as mice and rats, may represent a source of allergens, by secreting major allergens, such as Mus m1 and Rat 1, in their urine. Rodents' main allergens represent relevant allergenic source in schools, labs, as well as in the domestic environments (16, 17). Mus m 1 is the major mouse allergen, and it has been detected in mouse dander, urine, and hair (18) and as well as furry animals' allergens can be present even in homes without mice (19).
- *Molds* represent both indoor (perennial) and outdoor (seasonal) allergens. Indoors, molds can be found in any moist, dark place, while outdoors they results from vegetation degradation. Mold floats easily in the air. The high peak of outdoor spores can be identified in the mid-summer; dry air spores, such as those of *Alternaria* and *Cladosporium*, peak especially in the afternoon hours under low humidity. Mold spores need a relative humidity >65%, a temperature between 50 and 90°F (10–32°C), and organic matter as their nutrient base to grow.

Fungal spores may elicit both seasonal and perennial allergic symptoms. Alternaria is the most prevalent mold in dry, warm climates, and it is commonly found in soil, seeds, and plants. *Cladosporium* is the most commonly identified outdoor fungus and also the most prevalent spore in temperate regions, mainly found in decaying plant material.

Aspergillus may be frequently detected in dust, but also in fertilizer masses and vegetation. *Penicillium* may be present in dust, grains, and other foods. All of these molds induce allergic respiratory diseases, such as rhinitis, allergic asthma, and hypersensitivity pneumonia. Evidence showed a link between allergic sensitization and exposure to *Alternaria* and severe asthma (20–23).

- *Pollens'* function in natural mechanisms is to transfer gametes of flowers through current of air or insect spreading (8, 24). Pollens reach the peak concentration in the main countries from spring to summer, while in tropical countries, the pollination period may be longer (8, 24). Mean tree pollens diameter varies from 20 to 50 μm, and single plant can produce up to a million of pollen grains daily. Pollens are released above all in the morning, reaching the peak concentration by the afternoon (25). In Europe, allergic patients with hay fever and/or asthma are often sensitized to grass pollen allergens. A single pollen diameter varies from 10 to 100 μm (26). Most of pollen allergens are removed by the nasal mucosa and upper tracheobronchial tract, while the submicronic pollen-derived bioaerosols (<5 μm) easily reach the lower respiratory tract (27). Microscopic particles (0.5–2.5 μm) named "bioaerosol" are released by pollen ruptures, in example on contact with water and contain major allergens (27, 28).

Avoidance Measures against Indoor Allergens

An important and widely investigated intervention to obtain a good management of patient with asthma is the control of indoor allergens exposure (29). A wide spectrum of interventions has been suggested and may help to reduce exposure to trigger allergy for sensitized patients suffering from respiratory allergy (**Table 1**).

TABLE 1 | Interventions to eliminate or reduce airborne allergens in the indoor environment.

House dust mite
Use bed-encasing for mattress and duvet/pillows (pore diameter <10 μm)
Wash bedding weekly in hot water and dry in a heated drier
Remove dust mite reservoirs (i.e., toys and stuffed animals, carpets)
Reduce indoor humidity (less than 50%)
Vacuum with a HEPA filter bag
Remind that chemicals to kill mites or denature proteins have a modest effect
Pets
Remove pet from bedrooms and everywhere the child spends a lot of time
Clean accurately upholstered furniture, walls, and carpet
Remove upholstered furniture and carpet if possible
Encase the mattress and pillows with bed encasing (pore diameter <6 μm)
Keep the pet clean with frequent washes
Use a HEPA air filter
Cockroach and pests
Inspect to detect hiding places debris (grease, kitchen) and identify food sources
Store food in sealed containers
Exterminate with pesticides or bait traps
Remove clutter and seal holes or cracks in the home
Indoor molds[a]
Reduce indoor humidity (less than 50%)
Remove contaminated carpets, wallpaper, and woodwork
Treat washable surfaces with detergent and water and then dry completely
Repair water leaks
Pollens
Keep windows closed
Bath to remove allergens from hair and body
Consider to use HEPA air filtration
Consider same principles for outdoor mold avoidance

HEPA, high-efficiency particulate air.
[a]Level of evidence is largely based on experts opinion.
Modified from Platts-Mills (30) and Baxi and Phipatanakul (8).

HOUSE DUST MITES

The rationale of avoidance intervention for HDM is that by reducing or containing the mite population, patient exposure to mite allergens is also reduced, resulting in fewer symptoms. Although an intuitive strategy, avoidance is not supported by robust evidence of efficacy and is still the subject of controversy, mainly because of the impartiality of inclusion criteria in reviews and because the actual contribution of indoor environmental factors is difficult to demonstrate scientifically (31–34). Despite this, avoidance is still widely recommended to reduce the severity of allergic respiratory symptoms in sensitized individuals and may be effective as part of a holistic approach combining avoidance of tobacco smoke, improved education, and regular assessment (35, 36).

Which Avoidance Measures Should Be Recommended to HDM-Sensitized Children with Asthma?

Neither the mites nor their debris can be seen under normal circumstances, therefore education of patients regarding dust mites avoidance may be difficult.

Control measures should be based on allergen exposure monitoring performed according to well-defined and validated methods. It's imperative at this purpose to report the presence of the sources of allergens. The choice of optimal procedures to monitoring depends on the setting and allergens' source. Indoor airborne allergen levels may be assessed in settled dust or in an air sample. Bed, carpet, or sofa is the favorite sources to collect dust sample by using a vacuum cleaner with a collection device. The presence of allergens can be detected and quantified with an ELISA test, even if this method might not provide accurate measurement of inhaled allergens. Successful protocols for allergen avoidance are multifaceted (29). Not surprisingly, there is a high degree of variability between studies on avoidance.

1. Physical barriers should be recommended?

Frequent vacuum cleaning and acaricides have been proved to be not sufficiently effective in reducing HDM exposure, while specific *physical barriers*, in particular pillow and mattress encasing, have been demonstrated to be more useful at this purpose (strength of evidence B). The exchange of humidity without transfer of allergens is the most important property of bed encasing, providing a barrier effect between the human body and HDM in the mattress.

Different special covers are available on the market; those made of plastic, made of permeable synthetic fibers, non-woven synthetics, and finely woven with variable pore size (<10 μm block dust mite allergens; 6 μm also block mites and cat allergens) represent special covers for HDM avoidance (37). In a prospective study, Halken et al. recruited 60 asthmatic children sensitized to HDM who received pillow and mattress encasings or sham encasings (38). After 12 months of treatment, they found that HDM allergen levels were decreased and that the use of inhaled steroids was lower than before treatment (38). Other trials did not show clinical benefit in asthmatic patients by using HDM covers alone (39, 40). Therefore, the use of physical barriers, such as pillow and mattress encasing, should be recommended to decrease exposure to mite allergens, if possible as part of a comprehensive avoidance plan.

2. Is it useful to remove carpets, upholstered furniture, and drapes from the house?

Efforts could be made to *restrict the presence of carpets*, upholstered furniture, and drapes in the environment of the dust mite allergic patients, in particular in the rooms where the patient spends the greatest amount of time, first of all in the bedroom. There is a lack of evidence to support this recommendation alone, but these interventions may be useful as a part of a comprehensive intervention plan.

3. Should patient be advised to control humidity in their house?

Humidifier use should be avoided, while *dehumidifiers* can be used, although these do not generally filter the air as air conditioners do (strength of evidence B).

4. And what to say about aggressive cleaning?

Dry heat and steam treatments are two possible interventions that can eliminate HDM and reduce exposure to mite allergens.

Washing sheets, pillowcases, mattress pads, and blankets weekly effectively reduces mite counts (41). Some useful advices to reduce HDM levels can be to wash the bedding weekly (strength of evidence B) and using a heated drier, in fact hot water (>130°F) is able to kill mites while cold water may reduce HDM concentrations by 90% (41).

Among the so called *acaricides*, benzil benzoate and tannic acid are the most known; chemicals products showed to have modest effects on reducing mite allergen (strength of evidence B), therefore their use is not recommended.

5. Which are the benefits of combined avoidance interventions?

Several controlled trials have successfully documented a decrease in mite allergen for 6 months or more following combined avoidance interventions (39, 42–47).

Platts-Mills et al. showed a significant improvement in patients with asthma and sensitized to HDM after a long-term dust mite avoidance (48). In this trial were enrolled nine patients who used a hospital room as their bedroom for 12 months and their clinical symptoms and medication use were evaluated at the end of that period: two participants stopped to use all asthma medication and five participants no longer needed inhaled steroids. Symptoms and peak flows improved and five patients showed an eightfold increase in their bronchial provocation tests (48).

A recent meta-analysis was performed to evaluate the effectiveness of allergen avoidance in the prevention of allergic symptoms in previously sensitized patients and newborns that have the potential to develop allergies (49). A total of 14 RCTs were identified among all the articles published from January 1980 to December 2012 about allergen avoidance, of which six RCTs were of previously sensitized patients. The examined allergen exposure reduction was mainly obtained through combined intervention to reduce indoor allergens (removal of pets and molds, air filtration systems and vacuum cleaners, special mattress and pillow covers, cockroach killing). In some trials, allergen counts (i.e., cat dander, mite) were recorded, but their concentration at sampled sites did not always correspond to participant's exposure. Furthermore, even little amount of allergen can lead to bronchial symptoms (50); this was the reason because authors did not considered the absolute allergen levels to evaluate the efficacy of allergen avoidance measures. This meta-analysis demonstrated that exposure reduction to known allergen sources did not improve lung function (FEV1, PEF) in previously sensitized patients (49).

A study on asthmatic adolescents with exclusive sensitization to mites, demonstrated that bed encasing are effective in reducing bronchial hyperreactivity if compared with placebo and acaricides (benzyl benzoated) (45).Twenty general practices in two different English cities performed a RCT of 335 children aged 6–16 years suffering from allergic asthma and/or rhinitis to compare the effectiveness of allergen-specific interventions vs usual care (51). Specific allergen avoidance strategies (for tree and grass pollens, pets, HDM, or molds) were provided according to allergy history and SPT. After 1 year, patients receiving specific allergy intervention showed fewer nasal symptoms and a higher QoL index, while no significant changes were observed in bronchial symptoms, health-care utilization, or number of days with impaired daily activities (51).

In conclusion, a comprehensive approach to avoid exposure to HDM [including education, encasings, removing carpets and other mites reservoirs, upholstered furniture, drapes, keeping the humidity below 50%, and vacuuming with a high-efficiency particulate air (HEPA) filter every week] is thought to offer the greatest benefit in reducing mite exposure (strength of evidence A).

6. Which are the new perspectives in the future of HDM avoidance?

Recently, new systems to avoid HDM exposure during night have been investigated; *laminar airflow systems* connected with special filters seemed particularly effective in severe, uncontrolled asthma (52). A German multicenter study included 30 patients among children and adult asthmatic patients with uncontrolled moderate-to-severe disease who were treated with an add-on temperature-controlled laminar airflow (TLA) (53). Data from 4 to 12 months of TLA use showed that the addition of TLA to the patients' regular medication significantly reduced the number of exacerbations and the utilization of hospital resources and improved asthma symptoms as confirmed by a significant increase of the Asthma Control Test index (53).

The GINA guidelines acknowledge that measures should be implemented wherever possible to prevent the development of asthma and asthma symptoms and exacerbations. However, considering that mite allergens may trigger asthma symptoms, the GINA guidelines conclude that no single avoidance measure is likely to reduce exposure to mite allergens, but also that an integrated approach to avoidance cannot be widely recommended (54, 55).

The evidence-based guidelines for asthma management revised for the National Asthma Education and Prevention Program in 2007 recommends allergen avoidance as part of the management of asthma in patients with known allergen sensitivity (56), while in contrast, a recent meta-analysis concluded that dust mite avoidance is "of no use" in the treatment of asthma.

It is important to note that over half of the reported trials of dust mite avoidance have failed because the proposed measures did not reduce allergen exposure for a sufficient period of time (47, 57). A sustained intervention for at least three to 6 months was necessary to demonstrate clinical benefit. Thus, it should be suggested not only to adopt appropriate avoidance measures, but also to effectively sustain these interventions over time; patients should be advised that symptoms are expected to improve gradually (30).

PETS

Cats' and dogs' allergens are ubiquitous and have been found also in places where pets are not present: a study performed by Bollinger et al. showed that Fel d 1 was detectable in the dust samples from 38/40 homes without cats with a median concentration of 258 ng/g (58). Also clothing are a relevant reservoir of cat allergens: in schools a significant difference in the mean concentrations of cat allergen in the air was found between classes with many and few cat owners (59). In the same study, cat-allergic children who attended classes with more than 18% of schoolmates with

a cat at home showed a ninefold increased risk of exacerbation of asthmatic symptoms after the beginning of school than those attending classes with few cat owners (59). In sensitized patients, the exposure to pets is related to an impairment in the lung function. In the Asthma Control Evaluation study which enrolled 546 inner-city adolescents, elevated specific IgE to cat were linked with higher FE_{NO} concentrations, poor lung function, and higher eosinophilia and with an increased risk of exacerbations (60). In another study enrolling 374 adolescents, FE_{NO} levels in pediatric patients with allergic sensitization to cat resulted statistically significant higher in patients with a cat or other furry animals at home than in patients without pets in their indoor environment (61).

Which Avoidance Measures Should Be Recommended to Pets-Sensitized Children with Asthma?

The advice to families of asthmatic children sensitized to furry animals (cat and dog) is to decrease overall exposure.

1. Should patients remove pets from their homes?

The most effective measure recommended by the AAAAI and the ACAAI is to *remove the pet from the home* (strength of evidence A) (62).

Otherwise, since most families are recalcitrant in removing the animals for affective reasons, specific measures to reducing allergen exposure with the pet still living in the home have been assessed and reviewed (strength of evidence C) (8, 30, 62). Despite of this, parent should be advised that a pet in the house spread a so large concentration of allergens that the clinical benefit and effectiveness of the proposed measures is yet to be proven (62).

2. Which are the benefits of keeping the pet (at least) outdoor?

This measure is effective, but even when cats are removed from the house, allergens persist for many weeks. Wood et al. analyzed the consequence of cat removal in household-dust samples, and they found that allergen concentration begin to decrease several weeks after cat removal, achieving levels similar to control houses without cats into 24 weeks (63). These data may explain the exacerbation of symptoms experienced by subjects allergic to cats after moving into houses which had previously hosted cats (30).

3. Should we recommend the use of air filters?

Air filtration reduces indoor levels of airborne allergens. In particular *HEPA air cleaners* are effective in reducing dog and cat allergen concentrations (strength of evidence B), while duct cleaning was not found to be effective (strength of evidence D) (62). Van der Heide et al. conducted a crossover study on 20 asthmatic children sensitized to cat and/or dog and with a pet in the home, who significantly improved their lung function 3 months after the placement of air cleaners in living rooms and bedrooms (64). Systematic reviews on the benefit of air filters and air cleaners suggested that allergic patients should choose one of the following options: portable room air cleaners with HEPA filters, especially during sleeping, or in case of home with heating, ventilation, air-conditioning systems a regular maintenance schedules and the use of HEPA filters (65, 66).

4. Which are the advantages of physical barriers?

Woven microfiber bed encasings can block Fel d 1 passage if they are made with pore size lower than 6 μm and may be useful after removing the pet from the home. Meanwhile, the benefit of this measure is still unclear (strength of evidence C) (62). In contrast, non-woven microfiber encasings act as reservoir of allergen and are unsuitable for allergen avoidance (strength of evidence C) (62).

5. Should patients use specific cleaning approaches in their houses?

Chemical treatments (e.g., tannic acid) act modifying the allergen structure. Tannic acid is a protein-denaturing agent and its effect on pets' allergen reduction has been longtime investigated. Despite it has shown a capability to denature Fel d 1 and a subsequent 80% reduction in allergen if applied on furniture or carpets, no evidence of improvement in respiratory health was demonstrated (strength of evidence C) (67). Solution of sodium hypochlorite at 0.05% may be effective in inactivating indoor allergens and reducing allergen exposure. Despite of this, its frequent domestic use (>4 times/week) can be irritant for the respiratory system, increasing lower respiratory tract symptoms (strength of evidence C) (62, 68).

Regular use of *high-efficiency vacuum cleaners* can reduce indoor Fel d 1 and Can f 1 exposure, although the health effects are uncertain (strength of evidence B) (62). The impact of high-efficiency and standard vacuum-cleaners use on indoor allergens levels (mite, cat, and dog) was analyzed in a controlled trial showing lower allergen levels and clinical improvement in the peak expiratory flow rate, FEV1, and bronchodilator use after a 12-month period in homes cleaned with HEPA filters vacuums compared with vacuums with standard filters (69).

Finally, the use of *dry heat* should not be recommended to reduce exposure because Can f 1 and Fel d 1 are very thermostable allergens, in fact allergen concentrations are only partially reduced after 60 min of heating treatment at 140°C, and, respectively, 50 and 70% of allergen remains after treatment (strength of evidence C) (70).

6. Should pets be washed regularly?

The effect of *washing pets* on allergen exposure has been studied (71). Washing pets once or twice a week can reduce the concentration of Fel d 1 or Can f 1 (72) exposure but the effectiveness of this measure in term of better control of asthma symptoms has not been demonstrated (strength of evidence B) (62). Otherwise, washing cats less often does not generate any clinical benefit in patients, since airborne allergen concentration gets back to basal levels within 3 days (71).

7. Do the so-called "hypoallergenic breeds" really exist?

Up to now, no scientific evidence supports the existence of *hypoallergenic cats or dogs* (strength of evidence C) (30). Some

breeds of cats (e.g., Siberian cat) have shown lower lever of Fel d 1, but no subsequent lower airborne dispersion of Fel d 1 within the domestic setting has been shown (30). Similarly, as shown in a recent study, no evidence support the classification of certain dog breeds (e.g., Labradoodle, Poodle, Spanish Waterdog, Airedale terrier) as "hypoallergenic" (73). Investigators compared the levels of Can f 1 in hair samples and in the indoor environment of hypoallergenic and non-hypoallergenic breeds and found no differences in Can f 1 levels in the homes of the two groups (73).

The application of the above mentioned measures is essential to control exposure to pets allergens and to improve respiratory health (strength of evidence C) (62).

COCKROACHES

Despite the measures necessary to reduce exposure, the first interventional trials were generally unsuccessful in reducing symptoms (74, 75), maybe because patients could be exposed to high levels of multiple allergens, especially those living in poor conditions, so intervention focused to remove only one allergen can have a poor global impact.

Which Avoidance Measures Should Be Recommended to Cockroaches-Sensitized Children with Asthma?

Current recommendations to avoid cockroaches allergens include placing multiple baited traps or poisons, eliminating potential food sources, and removing reservoirs of cockroach debris and standing water (76). The allergen settles quickly and does not remain airborne, so air filtration systems are not helpful in reducing cockroach allergen exposure.

PESTS

An American study on inner-city school-age children with asthma examined dust samples from bedrooms to detect the presence of pests allergens and found that the allergen Mus m 1 was present in all the samples (77). In addition, also schools are a source of mice allergen exposure and levels of mice allergen in inner-city schools are often higher than in homes (16). Among dust samples from four different urban schools in the northeastern of US, up to 90% of them contained significant levels of mouse allergens and 68% of them had levels of mouse urinary protein greater than 0.5 μg/g (78). High exposure is considered as a risk factor for asthma morbidity: sensitization to mice and concomitant high levels of domestic exposure to mouse allergens were found to be associated with more physician visits, emergency department (ED) accesses, and hospitalizations in asthmatic preschool-aged children (strength of evidence B) (79). In addition, infants with household exposure to mice were more prone to develop wheezing during the first 12 months of life and in later years (80).

Which Avoidance Measures Should Be Recommended to Mice-Sensitized Children with Asthma?

Mouse allergen avoidance needs multiple interventions. Multifaceted approach in the homes over a 5-month period was found to be effective in decreasing significantly mouse allergen concentration (81). Moreover, these measures were found to be effective also to control asthmatic symptoms in sensitized children (82).

The main specific control measures include education of patients, cleaning, to cover residues of food and waste, the use of air filters, to repair holes and cracks in indoor environments, and using low-toxicity pesticides and traps (strength of evidence C) (30, 81). Often also professional exterminate on is needed to reduce mouse allergen levels (strength of evidence A) (76).

Indoor Molds

Since molds are present in environments, the absolute level of spore contamination can be used to make decisions about costly remediation of indoor environments. However, mold spore or mold-specific allergen levels should be interpreted with caution, as these are rarely quantitative and they are often generated using samples that may not be representative of the entire environmental area of concern.

Which Avoidance Measures Should Be Recommended to Indoor Mold-Sensitized Children with Asthma?

Today, strategies for elimination are not based on scientific evidences.

Reducing humidity by increasing ventilation, covering cold surfaces such as water pipes with insulation, and increasing the air temperature to reduce surface humidity are inexpensive actions that can help discourage mold expansion (83).

A systematic review of 61 published observational studies concluded that exposure to visible mold was associated with increased risk of wheezing and asthma (84). Furthermore, strong evidence has been provided by a birth cohort study, which found that exposure during infancy to measured mold species, especially *Aspergillus fumigates*, *Aspergillus ochraceus*, *Aspergillus unguis*, and *Penicillum*, was associated with subsequent childhood asthma (85). A randomized controlled trial in adults with asthma evaluated the effect of removing indoor mold, applying a fungicide, and installing a fan in the loft. In this study, 164 homes housing and 232 asthmatic patients were randomly assigned to undergo cleaning with detergent and fungicide and installation of an attic fan or to have this intervention performed 1 year later. Patients experienced a reduction in the medication use (41% decreased in the intervention group vs 17% increased among control patients) and an improvement of their breathing symptoms of asthma and rhinitis (52 vs 0% in the intervention and control groups, respectively), but without statistically significant evidence of benefit (86). Another similar randomized trial in asthmatic children also found modest benefit (87).

Avoidance Measures against Outdoor Allergens

Pollens and molds counts are assessed directly (i.e., grains of pollen or mold spores per cubic meter) over a 24-h period, their daily monitoring during a seasons is an important information to control asthma and allergies. The most relevant airborne allergens may be carried by wind for long distances and in several

directions, so the complete allergen avoidance cannot be obtained in the real life.

POLLENS

Pollen outdoor exposure and climate variability may induce asthma attacks or exacerbate symptoms in patients with specific sensitization to pollens. In particular, thunderstorms are considered as severe risk factors in asthma exacerbation because rain washing airborne pollen grains and the derived bioaerosols can represent a trigger factor for asthma exacerbations, explaining the so called "thunderstorm asthma epidemics" (27, 28). In Australian cities, up to a 10-fold increase in ED access due to asthma attacks was recorded within 24 h after a thunderstorm (88). The same phenomena happened in London in 1994 when 40 patients presented with asthma exacerbations within 24 h after a thunderstorm (89). Moreover, pollen may increase sensitivity to other airborne allergens, mainly due to its protease enzymes that increase the permeability of the epithelial membranes, disrupting their transmembrane adhesion proteins (90).

Which Avoidance Measures Should Be Recommended to Pollen-Sensitized Children with Asthma?

Pollens level is difficult to be controlled in the outdoor environment, so avoidance measures are mainly focused on the allergen control in the domestic setting.

Efforts should be focused to reducing indoor exposure and include closing windows and doors during high counts; using air conditioning and HEPA filters in the car and in the home and bathing after being outdoor during pollen to remove allergens from the hair and the body (**Table 1**) (8).

Should Patients Follow Specific Recommendations When They Get Outside during Pollen Season?

Patients should stay inside during thunderstorms and in the midday and afternoon, when pollen counts are highest. In addition, they should wear glasses or sunglasses and a face mask over the nose and mouth during mowing. If possible vacationing in a different ecosystem during pollen season might be suggested (8).

OUTDOOR MOLDS

Allergic sensitization to *Alternaria* has been identified as a risk factor for severe asthma symptoms and also for epidemic asthma (20, 21). An American study performed by Targonski et al. showed that asthma deaths in Chicago increased of two times when the concentration of *Alternaria* spores were higher than 1,000 spores per cubic meter (22). Dales et al. analyzed the relationship between the number of visits for asthma in the ED of a children's hospital and concentrations of pollens and fungal spores during a 5-year period (91). The concentration of fungal spores significantly correlated with the number of ED visits. Sears et al. performed a study in a group of New Zealand children followed from birth to 13 years. They demonstrated that allergic sensitization to HDM, cat, dog, and indoor mold were significantly associated with current asthma, while grass sensitivity did not show a similar association (23).

Which Avoidance Measures Should Be Recommended to Outdoor Molds-Sensitized Children with Asthma?

Even if outdoor pollen or mold spore level is difficult to control, the amount of allergen that gets inside home can be object of quantification. During those period when outdoor counts is high, some useful advices may be to close windows and doors and have frequent baths to remove all allergen residues on hair and body (**Table 1**).

CONCLUSION

Over the recent years, a wide spectrum of interventions has been suggested to reduce the exposure to trigger allergy in sensitized patients suffering from respiratory allergy. Although often intuitive strategies, avoidance interventions for the main allergens are not supported by robust evidence of efficacy and are still subject of controversy. Specific control interventions could be effective as part of a holistic approach, so that allergen avoidance is still widely recommended in the most recent guidelines (54, 55) to reduce the severity of allergic respiratory symptoms in sensitized individuals.

AUTHOR CONTRIBUTIONS

FC and EC reviewed literature about the selected topic and collaborated in drafting the manuscript. GR examined and corrected the final version of the manuscript. All the authors read and approved the final draft of the manuscript.

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REVIEW
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Gene–Environment Interactions—What Can These Tell Us about the Relationship between Asthma and Allergy?

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Asthma is a common condition, which is associated with atopy and allergic conditions including hay fever, eczema, and food allergies. Asthma and atopy are both complex conditions where genetic and environmental factors are implicated in causation. Interactions between genetic and environmental factors, likely *via* epigenetic mechanisms, are widely thought to be important in determining the risk for developing asthma and atopy. The nature of the relationship between asthma and atopy is unclear and the answer to the question "does atopy cause asthma?" remains unknown. This review explores the relationship between asthma and atopy from a gene–environment interaction perspective and tackles the question "are similar gene–environment interactions present for asthma and atopy?" The main finding is that gene–environment interactions are described for asthma and atopy in children but these interactions are seldom sought for both asthma and atopy in the same population. In the few instances where a gene–environment interaction is related to both asthma and atopy, there is no consistent evidence that similar interactions are common to asthma and atopy. Many plausible gene–environment interactions for asthma and atopy are yet to be explored. Overall, from the gene–environment interaction perspective, there is absence of evidence to better understand the complex relationship between asthma and atopy.

Keywords: asthma, atopy, child, eczema, environment, gene, mechanism

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INTRODUCTION

Childhood asthma is a common condition, which affects approximately 1 million children in the UK (1) and six million children in the US (2). Treatment for asthma is available to treat and prevent symptoms, but at present there is no cure for asthma and, therefore, there is a pressing need to understand why some individuals develop asthma. Approximately 50 years of research has yielded a considerable amount of information, which allows us to understand some aspects of asthma pathogenesis.

Asthma has long been associated with atopy [(3), p. 63–70] and is regarded by some individuals as an atopic condition, i.e., one caused by atopy. Asthma is understood to be a complex condition where genetic and environmental factors both contribute to causation, and twin studies suggest that as much as 70% of asthma causation may be explained by hereditary factors [(4), p. 8–14]. The search for "the" asthma gene was called off many years ago with the realization that asthma is a polygenic conditions

where approximately ten genes each make a modest contribution to risk [(5), p. 68–74]. There are several environmental exposures, which are associated with childhood asthma and these include exposure to second hand smoke (SHS), inhaled chemicals, mold, ambient air pollutants, some deficiencies in maternal diet, and respiratory viruses (6). Recent work suggests that the relationship between environmental exposures and asthma may change over time; for example, the relationship between SHS and asthma has become slightly stronger over time, perhaps as children become more susceptible (7). Many non-communicable diseases, such as asthma, have both a genetic predisposition and environmental triggers. The gene–environment relationship is nicely captured in the phrase "genetics loads the gun and the environment pulls the trigger."

Atopy, defined here as production of Immunoglobulin E specific to a common environmental exposure, is a highly prevalent phenomenon in modern children. Some children who are atopic have no symptoms [(8), p. 580–587] and the prevalence of childhood atopy is hard to detect due to it being clinically silent in some individuals, but is likely to be in excess of 30% in Western populations. The prevalence of atopic conditions such as eczema and hay fever is more easily identified due to the presence of symptoms and is close to 30% in many populations despite the use of self-reported diagnosis captured often by different definitions [(9), p. 733–743; (10), p. e008446]. Twin studies of eczema [(11), p. 535–539] and hay fever [(12), p. 2177–2182] suggest that hereditary factors explain up to 80% of causation of these atopic conditions.

The nature of the relationship between asthma and atopy is unclear. While many children with asthma are also atopic and have eczema, hay fever, or food allergies, there are many more children with atopy than with asthma [(9), p. 733–743; (10), p e008446]. In the largest community study of asthma and atopy in the UK, approximately 50% of 6 year olds with asthma (as evidenced by wheeze) were atopic (as evidenced by skin prick positivity) [(13), p. 974–980], i.e., many young children with asthma symptoms are not atopic. The "atopic march," where at a population level, the prevalences of food allergy, eczema, asthma, and hay fever peak at increasing ages [(14), p. 99–106], has been cited as evidence to support a causal relationship between atopy and asthma but at an individual level, this "march" is very rarely seen [(15), p. e1001748]. While it is possible that atopy may lie on a causal pathway toward asthma, the reverse may also be true (i.e., asthma may lead to the development of atopy) and a third possibility remains that asthma and atopy are independently caused by some other process. The present review is one in a series, which explores the nature of the relationship between asthma and atopy from a number of perspectives. The focus of this review is to review the relationship between asthma and atopy from the gene–environment perspective. Specifically, the hypothesis tested here is: the same gene–environment interactions are associated with both asthma and atopy.

To test this hypothesis, a 2007 review of gene–environment interactions for asthma [(16), p. 1032–1035] was summarized and the literature published after 2007 describing gene–environment interactions for asthma was reviewed. The literature describing gene–environment interactions for atopy and atopic conditions was also summarized. This was not an exhaustive or systematic review, instead, the aim was to identify a number of gene–environment interactions for asthma and for atopy and determine whether there were any common interactions. Papers were included, which described gene–environment interactions for severity of asthma and atopy. Epigenetic mechanisms are covered elsewhere in this series and the interested reader is referred there.

METHODOLOGICAL ISSUES FOR GENE–ENVIRONMENT INTERACTIONS

The study of gene–environment interactions for asthma and atopy in childhood is challenging for a number of reasons, which are discussed more fully elsewhere [(17), p. 1229–1240]. The reader should be aware of the following issues before considering the evidence:

1. Definitions of asthma and atopy differs between studies, which makes comparison challenging. For asthma, definitions include self-reported symptoms, current, or "ever" doctor diagnosed asthma and also objective measurements of respiratory physiology, e.g., FEV_1. For atopy, definitions might include self-reported current or "ever" eczema, hay fever, and food allergy symptoms, and objective measures such as skin prick reactivity and total IgE.
2. Measuring environmental exposures is a challenge and many different methodologies might be applied to the same exposure. Often, exposure is by subjective report, which is known to be potentially unreliable, e.g., exposure to tobacco smoke.
3. Studies require a large sample size to avoid false positive finding and also to detect small effect sizes. Many studies are underpowered. Publication bias means that relatively small studies where associations are seen are published whereas similar sized studies where no associations are seen are not accepted (or even submitted) for publication.
4. Studies require replication in more than one population to be considered generalizable.
5. How are genetic factor(s) of interest selected and related to which environmental exposure(s)? Searching for plausible interactions between candidate genes and environmental exposures can be justified based on current knowledge but this confines research to what is already known.
6. New analytical approaches are required, which can consider large numbers of single-nucleotide polymorphisms (SNPs) and environmental exposures, often which are measured at different ages in the same individual.

GENE–ENVIRONMENT INTERACTIONS FOR ASTHMA

The 2007 non-systematic review of gene–environment interactions for asthma [(16), p. 1032–1035] found that the majority of the literature had been published since 2000 and was focused in two areas: first, interactions between oxidant exposures (primarily SHS) and variants in genes coding for antioxidant defenses

[especially the family of antioxidant enzymes collectively called glutathione-S transferase (GST)]; and second, interactions between exposures to bacteria or bacterial products and variants in genes coding for components of the adaptive and innate immune system (e.g., CD14). See **Figure 1**.

Since 2007, interactions between oxidant exposures and variants in GST continue to be described, **Table 1**. Two papers reported on associations between variants coding for the antioxidant protein glutathione S-transferase P1 (GSTP1) and SHS exposure [(18), p. 125; (19), p. 226–232] and neither found an association although one [(18), p. 125] was able to describe increased risk for asthma among those exposed to SHS and low dietary vitamin E, who also were genetically predisposed to oxidant stress. Two other studies related SNPs in the gene coding for GSTP1 and exposure to dampness [(20), p. e30694] and air pollution [(21), p. e52715]; one study described complicated gene–gene and gene–environment interactions for dampness (but not several other exposures) and asthma [(20), p. e30694] while the second described a small increased risk for asthma among those genetically predisposed to oxidant stress and exposed to nitrogen dioxide (NO_2) [(21), p. e52715]. In addition to variants in genes coding for GST, a study from Hungary also observed a twofold increase in asthma risk for children with rare SNPs, which might reduce host antioxidant defenses but only on exposure to high ambient NO_2 concentrations [(22), p. 25–33].

Exposure to products of tobacco smoke is a well-known risk factor for childhood asthma (6) and interaction with genetic variants, which reduce GST have already been discussed, but interactions with variants in other genes might also be important. The ORMDL3 gene is associated with asthma in many populations and is found in the 17q21 region, so not surprisingly, gene–environment interactions have been sought between this area of the genome and exposure to products of tobacco smoke. Two papers [(24), p. 1985–1994; (23), p. 94–97] examined the relationship between many SNPs in the 17q21 region and SHS exposure. One paper found evidence of an interaction between mutant variants and early exposure to SHS and early onset asthma in young adults [(24), p. 1985–1994]. The second paper found no evidence between a single SNP and antenatal exposure to products of tobacco smoke but the mutant variant was associated with a modest increase in risk for early wheeze in association with exposure to pets [(23), p. 94–97]. A third study, from Mexico, reported an unexpected interaction between SNPs in the gene coding for tumor necrosis factor and increased smoking among non-asthmatic children [(26), p. 616–622]. A fourth paper described an interaction between maternal smoking and genetic variant in the IL-1 receptor antagonist for childhood asthma [(25), p. 502–508]. Smoking is an exposure, which is often under reported and which is confounded by many variables including lifestyle and domestic environment, so although not infrequently implicated in gene–environment interactions for asthma, the nature of the relationship cannot be assumed to be causal.

Genetic interactions with house dust mite (HDM) have been sought in two studies [(28), p. 885–92.e2; (27), p. 229–237]; both studies described associations between increased HDM exposure and variants in genes coding for factors associated with the inflammatory response and increased risk for asthma or for respiratory physiological changes associated with asthma. One of these studies was not able to replicate findings in all the populations studied [(28), p. 885–92.e2]. The final exposure considered in gene–environment interactions for asthma in this review is exposure to a farming environment. A study of five populations [(29), p. 138; Jan-144] was not able to replicate interaction between farm exposure and a number of candidate genes for asthma.

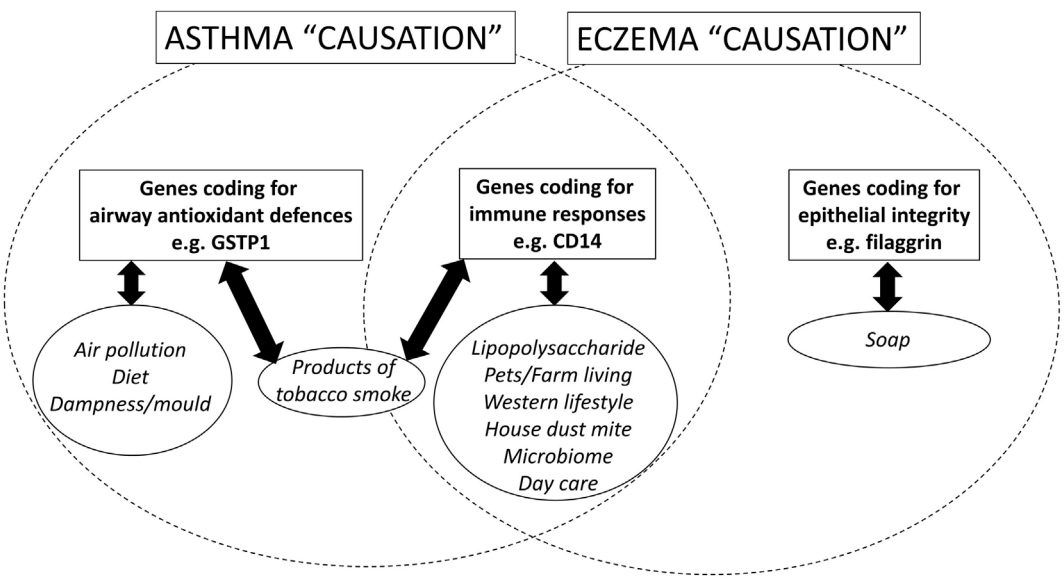

FIGURE 1 | A schematic diagram, which shows the candidate genetic and environmental factors for asthma and eczema (a surrogate for atopy) in children.

TABLE 1 | Summary of examples of where gene–environment interactions for asthma or asthma outcomes have been sought.

Reference	Genetic variant	Environmental exposure(s)	Outcome reported	Study participants	Association with atopy?	Comments
Brauner et al. [(23), p. 94–97]	17q21 locus (rs7216389)	Antenatal and infant exposure to tobacco smoke and furred pets	Recurrent wheeze (>3 parent reported episodes) at 18 months	101,042 Danish infants	Not reported	Individuals homozygous for the mutant variant and exposed to pets postnatally were at increased risk for recurrent wheeze compared to homozygous wild type [OR 1.6 (1.0, 2.4)]. No interaction was seen for tobacco smoke
Bouzigon et al. [(24), p. 1985–1994]	36 SNPs in 17q21 region	Tobacco smoke exposure in early life (early life not defined)	Doctor diagnosed asthma	1,511 participants in 372 French families, mean age 31 years	No association present for IgE and blood eosinophil counts	Interaction between SNP, early smoking exposure, and early onset asthma
Ramadas et al. [(25), p. 502–508]	3 SNPs in the IL1 receptor antagonist gene	Maternal smoking during pregnancy	Asthma by 10 years	921 participants in a UK birth cohort	Not reported	rs2234678 was associated with a fourfold increased risk for asthma for those whose mothers smoked during pregnancy
Wu et al. [(26), p. 616–622]	6 SNPs in the gene coding for tumor necrosis factor alpha and lymphotoxin A	Tobacco smoke	Asthma and atopy	596 4- to 17-year-old Mexican children	Not replicated for atopy	Two variants in TNF gene (rs1800629 and rs 361525) were associated with increased risk for asthma among non-exposed children
Lee et al. [(18), p.125]	Glutathione S-transferase P1 (GSTP1) (rs1695)	Tobacco smoke and vitamin intake	Doctor diagnosed asthma (ever)	1,124 South Korean children (including 110 with asthma), mean age 9 years	Not reported	Those with low vitamin A intake and exposed to tobacco smoke and homozygous for the GSTP1 mutant variant had increased risk for asthma [OR 4.4 (95% CI 1.6, 12.5)] compared to those with the same genotype but not exposed
Munoz et al. [(19), p. 226–232]	IL-13 (rs20541 and rs1800925), GSTP1 (rs1695), and CPY1A1 (rs1048943)	Tobacco smoke	Asthma	201 children from Mexico	Not reported	No evidence of gene–environment interaction for asthma apparent
Bunyavanich et al. [(27), p. 229–237]	Purinergic receptor (P2Y12) involved in leukotriene cascade. 19 SNPs considered	House dust mite (HDM)	Lung function at 9 years of age (including FEV_1, BDR and PC_{20})	422 children (mean age 8 years) and 1,266 parents in childhood asthma management program study (USA)	Not reported	5 SNPs tested were associated with lung function and among those exposed to 10 μg/g HDM, homozygous for the rare genotype for 3 of these SNPs were associated with reduced FVC or increased PC_{40} among those exposed to higher HDM concentrations compared to children homozygous for the wild type
Sordillo et al. [(28), p. 885–92.e2]	IL9 SNP (rs2069885) identified in the discovery cohort	HDM	Asthma severity	4 cohorts involved. A discovery cohort, three replication cohorts [including the CAMP study (27), 229–237]	Not reported	Children with mutant variant were at increased risk for asthma attack after exposure to increased HDM (≥10 μg/g dust) in two of the three replication cohorts. The magnitude of effect was approximately a threefold increase
Ege et al. [(29), p. 138; Jan-144]	GWAS (500,000 SNPs) and seven candidate genes	Farm exposure	Doctor diagnosed asthma	1,708 children aged 5–13 years in Germany, Switzerland, Poland, and Austria	Two SNPs previously linked with farm-related exposures were associated with asthma but not atopy	No interactions were found for common SNPs or the seven candidate genes. Not unexpectedly, given the number of SNPs tested, there were interactions with rarer SNPs, which are likely to be false positives
Eder et al. [(30), p. 1117–1124]	7 SNPs in gene coding for Capsase recruitment domain protein (pattern recognition receptor)	Farm exposure	Doctor diagnosed asthma, parent/self-reported wheeze, and hay fever, total IgE	668 children in Germany	Interaction seen for atopy and asthma.	The minor allele of one SNP (rs2075817) as associated with reduced risk for atopy, hay fever, and asthma associated with atopy but only in children living on farms (odds ratio typically fourfold lower)

(*Continued*)

TABLE 1 | Continued

Reference	Genetic variant	Environmental exposure(s)	Outcome reported	Study participants	Association with atopy?	Comments
Su et al. [(20), p. e30694]	GSTP1 (antioxidant gene rs1695), INSIG2 (insulin-related gene), and IL4Ra (rs 1805010)	Indoor dampness	Doctor diagnosed asthma (ever)	1,545 Taiwanese children (including 235 with asthma) mean age 13 years	Not reported	Analysis identified complicated interaction between three of the 17 variants tested and indoor dampness. There were no associations between genetic variants and with other exposures (including antenatal exposure to products of cigarette smoke, pets, cockroach, carpet use)
Hwang et al. [(21), p. e52715]	GSTP1 (rs1695)	Ambient air pollution (averaged over 3 years prior to recruitment)	Doctor diagnosed asthma (ever)	3,825 Taiwanese children (including 295 with asthma) typically aged ≤12 years	Not reported	Same cohort as reference [(20), p. e30694]. Risk of asthma was increased among children homozygous for the mutant variant (val105) per quartile of fine particulates exposure [OR 1.5 (1.0, 2.3)]
Ungvári et al. [(22), p. 25–33]	12 SNPs in gene coding for NFE2L2 (gene product important to antioxidant defenses)	Exposure to high concentrations of NO_2	Doctor diagnosed asthma	651 children and young adults from Hungary 307 with asthma, mean age of asthmatics 11 years and 22 years for controls	Not reported	Among cases only, rare alleles of 2 SNPs (rs 258882 and rs6721961) were approximately twice as frequent among those with high compared to low exposure

SNP, single-nucleotide polymorphism; rs, reference SNP identifier number (this gives a unique identifier to each SNP); OR, odds ratio.

TABLE 2 | Summary of examples of where gene–environment interactions for atopy, eczema, hay fever, or food allergy have been sought.

Reference	Genetic variant	Environmental exposure(s)	Outcome reported	Study participants	Association with asthma?	Comments
Ege et al. [(29), p. 138; Jan-144]	GWAS (500,000 SNPs) and seven candidate genes	Farm exposure	Atopy (type specific IgE > 0.35 kU/L)	1,708 children aged 5–13 years in Germany, Switzerland, Poland, and Austria	One SNP previously linked with farm-related exposures was associated with atopy but not asthma	No interactions were found for common SNPs or the seven candidate genes. Not unexpectedly, given the number of SNPs tested, there were interactions with rarer SNPs, which are likely to be false positives
Bottema et al. [(35), p. 593–602]	IL13 and CD14 (9 SNPs tested)	Tobacco smoke and pet exposure	Total IgE	3,062 children from three cohorts in the Netherlands assessed to age 8 years	Not reported	Minor alleles for 2 CD14 SNPs (rs2569190 and rs2569191) were associated with lower IgE concentrations for those exposed to pets and higher for those not exposed to pets. The magnitude of effect is not stated. Minor alleles for 2 SNPs in IL13 gene were associated with increased IgE concentrations but without interaction
Penders et al. [(36), 231–6.e1-5]	14 SNPs in the toll-like receptor-4 and CD14 genes	Higher burden of stool *E. coli* at 1 month of age	Total IgE- and parent-reported eczema at 2 years	957 children from a Netherlands birth cohort	Not reported	Evidence of gene × gene × environment interaction (TLR4 rs10759932 × CD14 rs2569190 × increased *E. coli* exposure) for elevated IgE
Zhang et al. [(37), p. 621–630]	24 SNPs in 11 immunity-related genes and four IgE response genes	Westernized versus Eastern lifestyle	Current reported eczema and rhinitis	858 children from Finland ("Western") and Russia ("Eastern")	No association with asthma and wheeze	SNPs were associated with rhinitis (rs1800896), eczema (rs227306), or elevated IgE (rs324015) among those "exposed" to Western environment. Magnitude of effect not stated

(*Continued*)

TABLE 2 | **Continued**

Reference	Genetic variant	Environmental exposure(s)	Outcome reported	Study participants	Association with asthma?	Comments
Biagini Myers et al. [(38), p. 430–437]	CD14 (among 7 SNPs tested)	Dog exposure	Diagnosed eczema at two and 3 years of age	762 children from the USA, mean age 3 years	Not reported	Children who were not homozygous for the C variant for −159C/T (rs 2569190) were at reduced risk for eczema [OR 0.4 (0.2, 0.8)] and further reduced if there was a dog in the house [OR 0.4 (0.1, 0.9)]. No associations were seen for exposure to second hand smoke, house dust mite, visible mold
Suzuki et al. [(39), p. 1408–11.e1]	2 SNPs in CD14 gene (rs2569190 and rs5744455) and one in the IL-4 receptor alpha gene (rs1805010)	Day care attendance at ≤2 years of age	IgE	473 children from Japan, mean age 9 years	Not reported	There were interactions between day care attendance and the IL-4Rα variant and also CD14 (rs5744455) for reduced IgE. Children with both the Val/Ile IL-4Rα variant and the CC or CT CD14 variant had the lowest IgE in association with day care attendance

GWAS, genome-wide association study; SNP, single-nucleotide polymorphism; rs, reference; SNP identifier number (this gives a unique identifier to each SNP); OR, odds ratio.

GENE–ENVIRONMENT INTERACTIONS FOR ATOPY

At first inspection, there seems to be very little to suggest that there may be common gene–environment interactions for both asthma and atopy. First, genome wide association studies have identified different loci for genes associated with asthma and eczema (a surrogate for atopy). Second, while gene–environment interactions for asthma focus on variants in genes coding for host antioxidant mechanisms, gene–environment interactions for eczema (an atopic condition) are focused on genes associated with epithelial integrity [(31), p. 3–21]. However, GWAS studies [(32), p. 1154–1162] and candidate gene studies [(33), p. 704–714] do find some common areas of the genome and specific variants associated with both asthma and atopy and these regions/genes code for components of the immune system, for CD14, IL4, IL4R, IL13, see **Figure 1**. Arguably, the most well-recognized gene–environment interaction for atopy is the "endotoxin switch" where individuals carrying with the CC genotype for CD14-159/T (rs 2569190) are at increased risk for atopy at lower endotoxin exposures, but this risk reduces as endotoxin exposure rises [(34), p. 386–392]; those homozygous for CC are at increased risk for non-atopic wheeze at higher endotoxin exposures [(34), p. 386–392].

Compared to gene–environment interactions for asthma, there appear to be considerably fewer publications, which describe gene–environment interactions for atopy; for this review, five papers [(29), p. 138; Jan-144; (35), p. 593–602; (36), p. 231–6.e1–5; (37), p. 621–630; (38), p. 430–437] and one letter [(39), p.1408–11.e1] were identified, **Table 2**. All the publications describe associations between variants in genes whose products are part of the adaptive or innate immune system. Variants in the gene coding for CD14 are described in three papers [(35), p. 593–602; (36), p. 231–6.e1–5; (38), p. 430–437] of which two describe interactions with pet exposure [(35), p. 593–602; (38), p. 430–437]. Compared to the literature describing gene–environment interactions for eczema, considered for atopy *per se* is rather sparse. While an interaction between CD14 variants and pets for IgE or eczema is plausible, this need replication in other populations.

CONCLUSION

Gene–environment interactions were the "new kids on the block" during the first 10 years of this century, and during this time, there were many publications and regular review articles. Since 2010, there has been a notable reduction in the number of published original research articles and reviews of gene–environment interactions for asthma and atopy (or eczema to be more precise). The shift of focus away from gene–environment interactions may be partly explained by disappointment in the relatively few interactions described and their apparently small effect size. Technological developments may also have shifted scientific thinking and gene–environment-wide interaction studies (GEWIS) may be the "new new kids on the block" [(40), p. 227–230]. An example of a GEWIS is reported in this review [(29), p. 138; Jan-144].

So what is the evidence that there are common gene–environment interactions for asthma and atopy? One paper describing gene–environment interactions for asthma identified in the earlier review [(16), p. 1032–1035] described an interaction between a variant coding for toll-like receptor 2 (rs 4696480) and living on a farm for both asthma and atopy [(30), p. 1117–1124], but this could not be replicated in other populations subsequently [(29), p. 138; Jan-144]. The present review did identify three studies where a relationship between the same gene–environment interaction was sought for both asthma and atopy but no common interaction was found [(24), p. 1985–1994; (26), p. 616–622; (37), p. 621–630]. There is, therefore, *absence of evidence* to robustly answer the question "are the same gene–environment interactions associated with both asthma and atopy?" but there is some

evidence of absence that the same gene–environment are not related to asthma and atopy.

Studying the relationship between asthma and atopy is not easy due to issues, which include definitions and the coexistence of the two conditions in many individuals in Western populations. The association between asthma and atopy (as evidenced by eczema) may change over time for a population (7), which adds to the challenge in better understanding the nature of the relationship. A paper that is based on epidemiology studies, and which will be frequently cited in this series of reviews, suggests that perhaps as much as 50% of asthma may be attributable to atopy [(41), p. 268–272]. In some countries, many children with asthma are non-atopic [(42), p. 409–416], so the relationship between asthma and atopy is apparently irrelevant for some individuals.

Ultimately, gene–environment interactions are likely to be important to the development of both asthma and atopy but, at this time, do not give a useful insight into the nature of the relationship between asthma and atopy. Other perspectives, for example, intervention studies, may be more helpful in understanding the inter-relationship between asthma and atopy. Some interventions are linked to reduced eczema in preschool children but not asthma [(43), p. 1178–1184; (44), p. 807–813] while other interventions achieve reductions in asthma and eczema into and beyond school age [(45), p. 1046–1051; (46), p. 49–55].

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REVIEW
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Regulatory T Cells in Allergy and Asthma

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The immune system's correct functioning requires a sophisticated balance between responses to continuous microbial challenges and tolerance to harmless antigens, such as self-antigens, food antigens, commensal microbes, allergens, etc. When this equilibrium is altered, it can lead to inflammatory pathologies, tumor growth, autoimmune disorders, and allergy/asthma. The objective of this review is to show the existing data on the importance of regulatory T cells (Tregs) on this balance and to underline how intrauterine and postnatal environmental exposures influence the maturation of the immune system in humans. Genetic and environmental factors during embryo development and/or early life will result in a proper or, conversely, inadequate immune maturation with either beneficial or deleterious effects on health. We have focused herein on Tregs as a reflection of the maturity of the immune system. We explain the types, origins, and the mechanisms of action of these cells, discussing their role in allergy and asthma predisposition. Understanding the importance of Tregs in counteracting dysregulated immunity would provide approaches to diminish asthma and other related diseases in infants.

Keywords: regulatory T cells, allergy, FOXP3, asthma, immune system, inflammation, tolerance, children

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REGULATORY T CELLS (TREGS): TYPES AND PHENOTYPIC CHARACTERISTICS

Regulatory T cells are a specific $CD4^+$ T cell population involved in peripheral tolerance by inhibiting autoreactive $CD4^+$ T cells that have eluded negative selection in the thymus and controlling inflammation in diverse biological processes, such as infection, metabolic disease, tissue repair, cancer, and hypersensitivity reactions (1, 227). Tregs suppress inflammation by upregulating immunosuppressive molecules and tissue homing receptors and repressing genes, preventing the acquisition of pro-inflammatory functions (2). At least five subtypes of Tregs have been identified and classified based on the expression of the transcription factor FOXP3. The group of $FOXP3^+$ Tregs includes thymus-derived Tregs (tTregs) and peripheral regulatory T cells (pTregs). The $FOXP3^-$ Tregs group includes Tr1, Th3, and $CD8^+$ Tregs.

$FOXP3^+$ Tregs: tTregs and pTregs and Their Phenotypic Markers

Two types of $CD4^+FOXP3^+$ Tregs have been described. A major population of Tregs of thymic origin, called thymus-derived Tregs (tTregs), also known as natural Tregs (nTregs), which mediate tolerance to self-antigens (3), and a second population that arises extrathymically in secondary lymphoid tissues when naive T cells (Tconv) encounter antigens and differentiate under the influence of TGF-β (4). These peripheral regulatory T cells (pTregs) are mainly present in the gastrointestinal

tract and in the lungs during chronic inflammation, with specificities directed against microbial antigens or environmental allergens (5). Probably due to their different origins, FOXP3$^+$ tTreg and pTreg cells are characterized by a non-overlapping T-cell receptor (TCR) repertoire. Based on that, a division of roles has been suggested in which tTreg would regulate immune responses developed against self-antigens and pTreg cells would regulate immune responses against "non-self" infectious or innocuous antigens (6, 7).

At the moment, there are no exclusive markers for Tregs, although it has been described that Tregs express several molecules that altogether characterize them and allow their identification in comparison to T conventional or effector T cells (Teff).

Thus, CD25 was the first marker associated with Tregs; nevertheless, this protein is also present in recently activated T cells. As a consequence, CD25 expression can be used only to differentiate Tregs from Tconv. In naive mice, Tregs show constitutive expression of CD25, while in humans, Tregs exhibit very high levels of CD25, and activated T cells show intermediate expression of this molecule (8, 9).

FOXP3, a member of the forkhead transcription factor family, was identified as a Tregs-specific transcription factor in mice (10, 11) and in humans (12). More than 90% of murine Tregs express this transcription factor, while naive and Teff do not present detectable levels of this molecule. Similarly, most human CD4$^+$CD25^{high} Tregs express FOXP3, but, contrary to the results observed in mice, human Teff express intermediate levels of FOXP3 upon activation for a short period of time (13), introducing serious doubts regarding the specificity of FOXP3 as an exclusive marker for human Tregs (14). Moreover, FOXP3 plays a decisive role in Treg cell lineage establishment and function (10, 11). With regard to that, a mutation in the *FOXP3* human gene is responsible for the human syndrome known as immunodysregulation, polyendocrinopathy, and enteropathy X-linked syndrome (IPEX), or X-linked autoimmunity and allergic dysregulation syndrome (XLAAD), equivalent to the murine syndrome known as Scurfy (10, 15–17). Murine and human diseases are characterized by low levels of circulating Tregs, suggesting a critical role for *Foxp3* and *FOXP3* for appropriate Treg differentiation in both species, respectively. Although 60–70% of patients with IPEX have mutations in FOXP3 and produced normal levels of IL-10 (18), other studies (19, 20) have described that certain IPEX patients lacked expression of CD25 (IL-2 receptor alpha chain) and showed defective IL-10 production after *in vitro* stimulation of their Tregs (20). These data suggest fundamental and non-overlapping roles for both Tregs (FOXP3$^+$ and IL-10$^+$) in the control of autoimmune and allergic disorders (9, 21).

FOXP3 gene expression is regulated by epigenetic modifications of conserved non-coding sequences (CNS) presented in four elements. Regarding that, it is known that pTreg cells are less stable than tTreg cells and can lose FOXP3 expression and produce cytokines, such as IFN-γ and IL-17, under inflammatory conditions (22). This lack of stability can be explained by the methylation status of the CNS2 region of the *FOXP3* gene, which is stably hypomethylated in tTreg cells, but is incompletely demethylated in pTreg cells (23, 24).

In addition to CD25 and FOXP3, tTreg and pTreg cells express similar levels of shared Treg cell markers, such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), glucocorticoid-induced TNFR-related protein (GITR), inducible T cell Costimulator (ICOS), and CD103. However, many of those markers are also upregulated by activated CD4$^+$ T cells under inflammatory conditions, and their expression does not allow discrimination between these two populations (25). In order to distinguish between tTreg and pTreg cells, the use of Helios and Neuropilin-1 (Nrp-1) has been proposed since the expression of such markers is higher in tTreg compared with pTreg cells (26–28). Finally, thymic-derived Tregs can be differentiated into two subpopulations based on the degree of FOXP3 expression and the presence or absence of CD45RA (29). These populations are "*CD25^{++}CD45RA$^+$ (FOXP3^{lo}) resting Treg cells (rTreg cells) and CD25^{+++}CD45RA$^-$ (FOXP3^{hi}) activated Treg cells (aTreg cells), which represent different stages of Treg cells differentiation and are both suppressive in vitro*" (29).

As already mentioned, it seems that Tregs display a certain level of functional plasticity since they have the capability to perceive cytokines in their milieu and respond to them with the expression of a subset of appropriate genes; this functional plasticity is critical for the regulation of the peripheral immune response. In fact, Tregs are widespread in non-lymphoid tissues, skin, lungs, and intestine, since barrier tissues contain large populations of specialized Tregs that utilize several of the same molecules to reach the same sites as their effector cell counterparts. Thus, specific transcription factors activation and the expression of several chemokine receptors such as CCR4, CCR6, CXCR3, and CXCR10 have been suggested to characterize four different subsets of human tTregs (30), which colocalize and control specific Th subsets (Th1, Th2, Th17, Th22) expressing identical chemokine receptors. Supporting this idea, it has been shown that Treg cell homing to the skin requires the expression of CCR4 by Tregs and of ligands for P- and E-selectin by cutaneous vascular endothelial cells. In fact, Tregs with deficiency in one of these molecules expression fail to correctly control immune responses in the skin (31, 32).

Generation of Thymus-Derived Regulatory T Cells (tTreg)

It is well established that thymocyte destiny depends on the binding affinity/avidity of the TCR to its self-peptide–MHC ligands on antigen-presenting cells (APC). Thus, although thymocytes expressing a TCR with low affinity for self-peptide–MHC complexes are positively selected and differentiate into conventional T cells, thymocytes that bind self-peptide–MHC complexes with high affinity undergo negative selection and experience cell death in order to remove potentially self-reactive T cells. A third possibility is the existence of thymocytes expressing TCRs with intermediate avidity for self-peptide–MHC, which may differentiate into cells with a regulatory function (tTregs) (33).

Together with the interaction of the TCR with self-peptide–MHC, additional signals are required for thymic induction of Tregs, such as costimulation through CD28 or the signaling provided by the IL-2R-γ_c cytokine family. Thus, deficiency of tTregs was observed in CD80/CD86 and CD28 knockout animals (34). Accordingly, the absence of the Treg population from

the thymus and periphery was observed in IL-2R-γ_c knockout animals (35) as well as the emergence of autoimmunity in animals lacking IL-2Rβ, which could be repaired by the transfer of Tregs from control mice (36, 37). Furthermore, in IL-$2^{-/-}$ or $CD25^{-/-}$ mice, the expression of FOXP3 in thymocytes was drastically reduced and these animals developed fatal autoimmunity disorders (35).

Regulatory T cells may also be induced to differentiate at a double-positive ($CD4^+CD8^+$) developmental stage (38), since a momentary reduction of $FOXP3^+$ thymocytes in newborn mice was observed upon elimination of TGF-βRI in the murine thymocytes at a double-positive ($CD4^+CD8^+$; DP) development stage (39). These results support the hypothesis that thymic Treg selection is enhanced by TGF-β signaling. In this regard, TGF-β signaling has been demonstrated to control apoptosis of self-reactive thymocytes through a Bim-dependent mechanism, raising the Treg precursor cells (40). In addition to the above data in mice, studies on the development of human thymic Tregs have shown that an important percentage of $FOXP3^+$ single-positive (SP) cells derive from $FOXP3^+$ DP thymocytes (41).

In summary, the majority of the Tregs originate in the thymus at the CD4 SP stage and intermediate signal strength TCR, IL-2, CD28, and TGF-β are needed for FOXP3 upregulation during the process of Treg cell generation. Additionally, Tregs may be differentiated at a previous double-positive stage in the presence of TGF-β.

Generation of Peripheral Regulatory T Cells (pTreg)

Experiments based on the transfer of purified $FOXP3^-$ $CD4^+$ T cells into lymphopenic mice followed by the subsequent analysis of the TCR repertoire of the $FOXP3^+$ T cells generated, compared with that of cells which remained $FOXP3^-$ demonstrated that "*TCR repertoires were only partially overlapping by $FOXP3^+$ Tregs and $FOXP3^-$ non-Treg $CD4^+$ T cells present in control mice*" (42, 43). Furthermore, it was found that the TCRs from Tregs located in the colon were different from those expressed by Tregs from other tissues, and a subset of these colonic TCRs was specific for antigens associated with the gut microbiota (44). The above results suggest that while the differentiation of thymic Tregs is induced by intermediate affinity interactions of TCRs with self-peptide–MHC molecules, the development of peripheral Tregs is probably promoted by encounters with foreign antigens, e.g., foods, gut microbiota, and allergens.

Nevertheless, it has been suggested that the TCRs expressed by pTreg cells probably exhibit high affinity, as shown by the observation that "*rare high-affinity antigenic peptides allow for most efficient FOXP3 induction upon stimulation of a cognate transgenic TCR displayed by peripheral $CD4^+$ T cells in comparison with a less efficient pTreg generation by low-affinity peptide variants*" (45). Additionally, it seems that insufficient activation of T cells promotes the induction of FOXP3, since CTLA-4 expression is essential for the induction of FOXP3 *in vitro* by a mechanism dependent on TGF-β presence (46), while CD28 has the contrary effect (47, 48). Thus, *in vitro* and *in vivo* studies suggest that FOXP3 induction and pTreg cell generation require high-affinity TCR signaling together with suboptimal costimulation (high CTLA-4 and low CD28 signaling) (40), and the process is helped by the presence of high amounts of TGF-β (47). Signaling through TGF-βR seems decisive for the expression of FOXP3 in most peripheral $CD4^+$ T cells (49).

The pTreg cell generation requires the combined action of soluble factors, such as TGF-β and IL-2, in the microenvironment and the presentation of the antigens by appropriate APCs. Furthermore, the presence of all-transretinoic acid (ATRA) in the Tconv environment synergizes with TGF-β, and this effect is great enough to promote pTreg generation even when a high costimulation is being produced. This is particularly evident in lung tissues where resident macrophages ($CD45^+CD11c^+$MHCclass $II^{low}F4/80^+$) constitutively expressing TGF-β and retinoic acid are the main subset of cells driving pTreg cell induction from naive $CD4^+$ Tconv cells (50).

The data discussed so far indicate that pTreg cells generation is influenced by a specific type of TCR signaling, and costimulation, and through cooperation with other signals, such as TGF-β, IL-2, and ATRA. These conditions suggest that pTreg cell differentiation could be restricted to precise locations such us mucosal surfaces where they may regulate immune responses to harmless antigens such as commensal microbiota and prevent allergic inflammation. Supporting these ideas, "*studies using Rag-1-deficient T-B monoclonal mice sufficient or deficient in FOXP3 demonstrated that TCR transgenic pTreg cells were sufficient to establish mucosal tolerance and control allergic inflammation induced by the model antigen recognized by the TCR*" (5).

Additionally, several studies suggest a role of microRNAs (miRNAs) in the generation and function of Tregs (51, 52). Specifically, miR155 was recently described as a player in Treg cell differentiation and was shown to induce upregulation of several Treg cell-associated genes when transferred to conventional T cells (51, 53). It has also been reported that an acquired decrease of the endoribonuclease Dicer, involved in the generation of miRNAs (54), induces spontaneous autoimmunity in a disease model (52). The progression to autoimmune disease was associated with a marked decrease in Dicer and an increased miR-155 expression which can upregulate CD62L in Tregs. Furthermore, Singh et al. (55) found three miRNAs (miR-15b/16, miR-24, and miR-29a) that regulated the induction of Tregs from naive $CD4^+$ T cells, with miR-15b/16 having the highest effect. Important genes regulated by miR-15b/16 were Rictor and mTOR, which encode components of the mTOR signaling pathway involved in controlling the generation of Tregs.

In summary, the requirements to promote induction of pTregs include high-affinity interaction of TCRs with peptide: MHC complexes; suboptimal activation of dendritic cells (DCs); mucosal administration of peptide; signaling by cytokines, such as TGF-β and IL-2; and regulation by the appropriate miRNAs (49, 51–53, 55, 56).

$FOXP3^-$ Tregs: Tr1, Th3, and $CD8^+$ Tregs

In addition to $FOXP3^+$ Tregs residing in lymphoid and non-lymphoid tissues, there are other tissue-residing Tregs with unclear origins. They could have a thymic origin and migrate to peripheral tissue and proliferate in response to inflammation or they could develop from $CD4^+CD25^-$ Tconv as a consequence of antigen recognition in the tissue.

Type 1 regulatory T cells (Tr1) are a population activated in the periphery after antigenic stimulation in the presence of IL-10 (57, 58). These cells are characterized by the expression of CD4, CD226, lymphocyte-activation gene 3 (LAG3), and CD49b as their specific markers (59). The Tr1 Tregs produce a large amount of the "*cytokines IL-10 and TGF-β, some IL-5, low levels of IFN-γ, and IL-2, but no IL-4*" (57). The secretion of the immunosuppressive cytokine IL-10 is the main mechanism by which Tr1 cells are thought to regulate the immune response (60).

T helper type 3 cells (Th3) is a subset of Tregs that differentiate in the periphery upon interaction with a specific antigen and mediate suppression by secreting the cytokine TGF-β. These cells "suppress the proliferation and activation of Th1 cells and the development of autoimmunity in the mouse model of multiple sclerosis" (61). Thus, Th3 cells may have a role in controlling autoimmunity and allergy in humans (62), although their role in the maintenance of immune tolerance in humans has yet to be clearly defined (60).

An immunoregulatory function has also been attributed to some $CD8^+$ Treg subsets, and blocking this immunosuppressive action may induce autoimmune reactions. The most frequent phenotype for $CD8^+$ Tregs is $CD25^+CD28^-$ (63, 64), although the expression of other markers has been described and includes CTLA-4, CD122, CD38-4, GITR, CD8αα, and CD103. This Treg subset has been observed in tonsils but is infrequent in peripheral blood. The action mechanisms of these cells include suppression by cell contact; the release of regulatory cytokines such as IL-10 and TGF-β; and the promotion of anergy in APCs (60, 65).

IMMUNE MATURATION AND ALLERGY/ ASTHMA INCIDENCE

In addition to genetic and environmental factors, proper immune system maturity during the first years of life is fundamental to avoid allergic asthma (AA). In fact, a high percentage of asthmatics (ninety percent) are diagnosed by 6 years of age, suggesting that the influence of intrauterine milieu and early life events such as atopic diseases and wheezing illnesses induced by respiratory viral infections are highly determinant for the development or not of asthma during childhood (66, 67). In this sense, abnormal regulatory T-cell function and/or numbers have been pointed to as the main cause of AA incidence and it has been observed that Tregs are already defective in the umbilical cord blood of newborns at a genetic risk of allergy (67, 68).

Asthma: Definition and Classification

Asthma is the most frequent childhood chronic inflammatory airway disease worldwide and is characterized by reversible airflow obstruction. The prevalence of asthma in children ranges between 5 and 20%, with approximately 300 million people suffering from this disease (69, 70).

Asthma can be classified as mild, moderate, or severe according to National Asthma Education and Prevention Program, Global Initiative for Asthma (GINA), or American Thoracic Society (ATS) guidelines (71, 72). Nevertheless, asthma is an extremely heterogeneous disease that develops in many clinical forms or phenotypes with distinct pathogenic mechanisms and is induced by diverse sensitizers such as allergen exposure, viral infection, oxidative stress, and air pollution, amongst others. Today, clinical asthma is mainly divided into two main phenotypes: AA and non-allergic asthma (NA). AA is characterized by airway hyperreactivity (AHR), eosinophilic airway inflammation, increased total and specific IgE levels and blood eosinophil counts, elevated mucus production, and reversible airway obstruction and remodeling (73). Th2 cells initiate AA, and the characteristic cytokines are produced by both Th2 cells (IL-4, IL-5, and IL-13) and type 2 innate lymphoid cells (ILCs) (IL-5 and IL-13). These molecules induce IgE class switching by B-cells (IL-4), eosinophils infiltration (IL-5), and hyperplasia of goblet cells (IL-13) (74).

Allergic symptoms usually start in childhood upon sensitization of the airways to common allergens such as dust mites, animal dander, or tree pollens (**Figure 1**, Step 1). The immune mechanisms involved are divided into two phases. First, a sensitization and memory phase develops (**Figure 1A**). Second, the effector phase occurs and includes the immediate and late responses (**Figure 1B**) (75). Throughout the sensitization phase, the clonal expansion and development of allergen-specific Th2 cells and the production of the cytokines IL-4 and IL-13 take place (**Figure 1**, Steps 2–4). These cytokines induce B-cells class-switching to allergen-specific IgE antibodies, which bind to the high-affinity receptor for IgE (FcεRI) that is expressed on mast cells and basophils (**Figure 1**, Steps 4–5). The initiation of the effector phase takes place after a new encounter with the same allergen that binds to the IgE–FcεRI complex causing cross-linking and as a consequence triggering the activation of mast cells and basophils, which release the anaphylactogenic molecules contained in their cytosolic granules (**Figure 1B**, Step 6). These molecules trigger the symptoms of the immediate reaction (**Figure 1B**). Late-phase reactions are generated by the prolonged presence of the allergen, which initiates a specific Th2 cells activation, with the production of cytokines such as IL-4, IL-5, IL-9, IL-13, and IL-31. These are critical to sustaining specific IgE levels, the arrival of inflammatory cells to tissues, eosinophilia, mucus release, and smooth muscles contraction (76, 77). Such events can conclude with the more severe symptoms of allergy, such as asthma, rhinitis, dermatitis, and less frequently systemic anaphylaxis. Additionally, recently identified cytokines such as IL-25, thymic stromal lymphopoietin (TSLP), and IL-33, secreted by injured epithelial cells among others, have been associated with the development of the Th2 response (78, 79). Furthermore, additional T-cell subsets contribute to the allergic airway disease, such as IL-9-producing Th9 cells but also Th1 and Th17 cells (80). Finally, it has been found that neutrophils infiltrate airways after recruitment by certain allergens and release inflammatory mediators contributing to asthma development (81).

Influence of Intrauterine Milieu in the Neonatal Immune System and Risk of Asthma Development

The intrauterine milieu is critical for an appropriate immunological maturation of the fetus but could be influenced by environmental

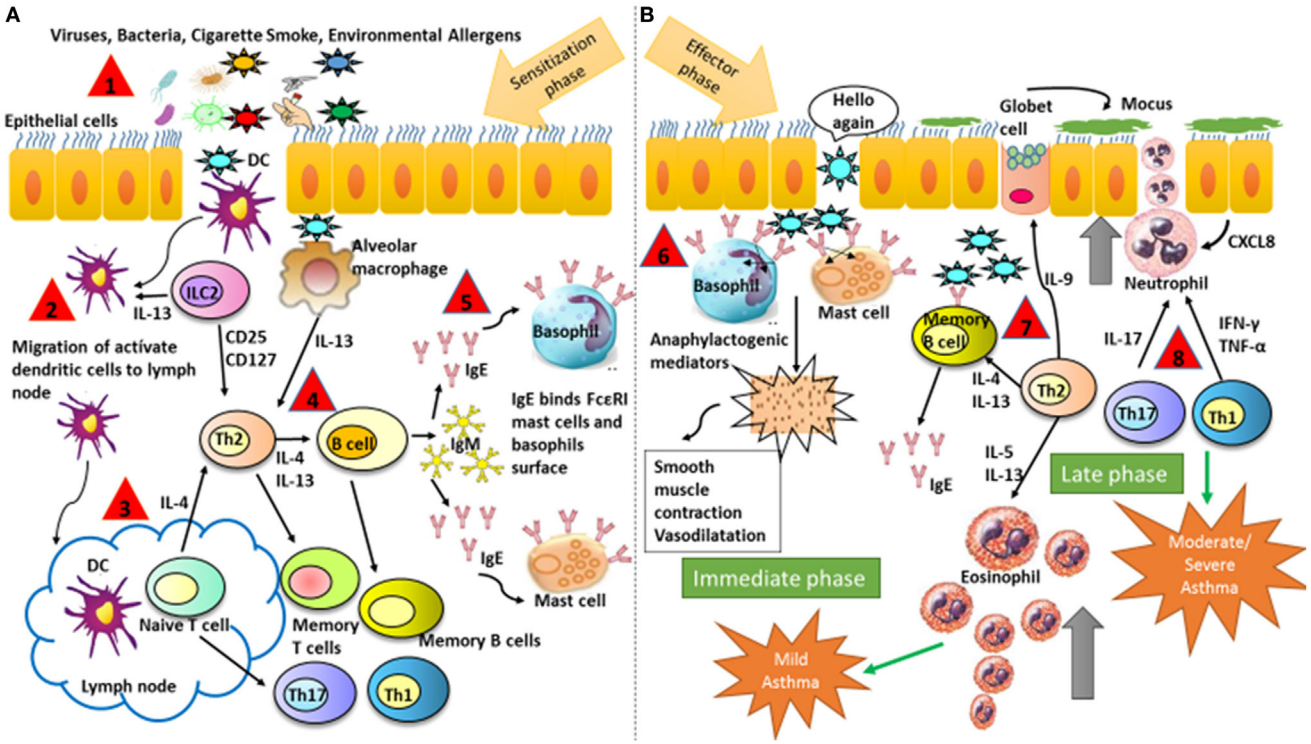

FIGURE 1 | **Schematic illustration representing major mechanisms that contribute to the development of asthmatic symptoms**. **(A)** Sensitization and memory phase. The sensitization phase is initiated when the allergen is introduced in the organism for the first time. At the end of this phase, the immune system is prepared for a new contact with the same allergen. **(B)** Effector phase includes the immediate and late responses. The effector phase is initiated after a new contact with the same allergen that binds to IgE on the surface of basophils and mast cells. The followed numbers describe the consecutives steps in asthma: (1) antigen entry, (2) migration of dendritic cells from the alveolar space to the lymphatic node, (3) antigen presentation to naive T cell and maturation into Th2, (4) production of Th2 cytokines and antibodies production, (5) isotype switch from IgM to IgE, (6) IgE binds to FcεRI on mast cells and basophils and inflammatory mediators are released, and (7) and (8) response mediated by memory B-cells and Th cytokines with recruitment and activation of eosinophils and neutrophils. DCs, dendritic cells.

factors. Thus, it has been shown that maternal conditions such as diet, microbiota, inflammation, and environmental antigens can be decisive for an adequate immune development during pregnancy and during the postnatal period. Therefore, allergy/asthma can represent an early consequence of inappropriate immune regulation (82).

The development of the fetal immune system and organs such as lungs and airways take place during the intrauterine period and they are more vulnerable to environmental influences. Microbial exposures during this period are particularly important. Thus, based on the hygiene hypothesis, it may be possible that microbial antigen transfer from the mother to the progeny starts during the pregnancy (83–85) dispensing a first supply of immune stimulation. Epidemiological research has also shown that "a high microbial environment during pregnancy induces greater protection from allergy than postnatal exposure alone" (82, 86). Thus, maternal exposure to microbial compounds during pregnancy is coincident with increased expression of certain Toll-like receptors (TLR2 and TLR4) and the CD14 molecule on peripheral blood cells, suggesting that this type of exposure during the prenatal period might prevent childhood sensitization (86). For example, maternal exposure to farm animals during fetal development is related with an improvement in the function and number of Tregs within umbilical cord blood and a reduction in the secretion of Th2 cytokines or lymphocyte proliferation upon innate stimulation (39). Additionally, experiments conducted in mice have demonstrated that exposure to endotoxin during pregnancy avoids future sensitization and lung inflammation episodes induced by allergens in the offspring (87). Moreover, a mother tolerant before pregnancy can transfer this immunological tolerance to her descendants, suggesting that the immunological status of the mother has a great influence in the immune response of the offspring to allergens (88). In fact, it has been shown that during fetal development, the tTreg levels in venous blood change depending on pet exposure levels and the atopic condition (65). In addition to infection and exposure to allergens, it has been demonstrated that pollution and diet influence the development of disease during early infancy (89). Thus, enhanced asthma symptoms in children have been associated with poor maternal intake of vitamins D, E, and zinc during fetal development (90). Specifically, vitamin D has been associated with $FOXP3^+$ and $IL\text{-}10^+$ Tregs generation and survival in humans and mice (91). Furthermore, it has been shown that diet-related factors can control the epigenetic mechanisms that modify the risk of allergic disease (89). Thus, a maternal diet high in folates, choline, methionine, vitamin B12,

or exposure to cigarette smoke may promote DNA methylation, inhibit gene transcription, and foster asthmatic symptoms (92, 93). These studies have shown that the regulatory cytokines IL-10 and TGF-β participate in the dysregulated immunity of the lung.

Postnatal Maturation of the Immune System and Risk of Asthma Development

High susceptibility to infections and/or the emergence of atopic and/or asthmatic manifestations in children have been linked to the functional immaturity of the immune system influenced by the degree of exposure to several immunostimulatory factors during embryonic development and during the early infancy (76). In general, the immune system that is still immature at birth and triggering most TLR ligands in their leukocyte subsets produces less type1 IFN, IL-12, and TNFα but increased amounts of IL-1, IL-6, IL-10, and IL-23 in comparison to the same adult cells; in general, the newborn innate response presents Th2- and Th17-biased immunity while Th1 response is scarce. This fact highlights the fundamental role of antigen exposure during early infancy for an adequate maturation of the immune system. Developmental deficiency in the circulating DC compartment has been implicated in atopic diseases in children (94), with attenuated capacity for IFN-γ and IL-12 production (95, 96) and with a disequilibrium in the balance between cytokines secreted by Th-cells (pro-inflammatory versus regulatory) (97). Furthermore, children at an elevated risk of atopy usually display decreased responses to certain vaccines (98). This could be explained by an immune dysregulation during the intrauterine period and/or an absence of exposure to infectious and/or environmental microbes during childhood. As a result, children may experience a failure in their protective Treg responses to allergens (99, 100). Additionally, it is possible that the lack of deviation from an allergen-specific Th2 immune response toward a Th1 may play a role (101). During childhood, such mechanisms may be alternatively activated by different types of microorganisms, albeit infectious or innocuous. For example, helminthic infections are related to IL-10 production and diminish the risk of allergic disorders (102) and have been shown to induce Tregs in an animal model of allergen challenge, thus preventing development of airway inflammation (103). A murine model has demonstrated that a heat-inactivated suspension of *Mycobacterium vaccae* also protected against airway inflammation *via* IL-10 and TGF-β production (104). However, the preventive effect of a livestock exposure may be through TLR-mediated immune bias toward Th1 responses to antigens present in the farm environment (105). In relation to that, it has been shown that the immunosuppressive role of $CD4^+$ $CD25^+$ Tregs may be regulated by TLR signaling during the course of the immune response. TLR signaling may influence the balance between $CD4^+$ Th and Tregs and, as a consequence, orchestrate the subsequent immune response. In fact, a significant decrease in $CD4^+$ $CD25^+$ Tregs has been described in TLR2-deficient but not TLR4-deficient mice in comparison with control mice. Other data suggest that DC maturates *in vivo* upon binding of their TLR ligands, which subsequently regulate the development of the Teff (106).

In addition, the intestinal microbiota modulates the newborn Th2-biased immunity by promoting a Th1-cell response (107). Colonization of the newborn with the gut microbiota begins right after birth and is regulated by specific mucosal DCs, which bind antigens and favor T effector or Tregs differentiation. These DCs are characterized by a high expression of TLRs and costimulatory molecules upon interaction with TLR ligands, by production of the immunosuppressive cytokine IL-10 and absence of pro-inflammatory factors (108). It can be hypothesized that this specific control of TLR responsiveness is a mechanism to prevent unwanted inflammatory responses.

Interestingly, infection with a specific type of bacteria has also been associated with Treg development. Thus, infection of newborn mice with *Helicobacter pylori* protects from the progression of allergic airway symptoms and has been related with an accumulation of Tregs in the lung (109). Furthermore, DCs exposed to the bacteria were impaired in their maturation after lipopolysaccharide stimulation and induced the expression of FOXP3 in Tconv (110).

Finally, one study demonstrated that environmental antigens were transferred from the mother to the lactating mice *via* breast milk. As a consequence of this, protection from AA and tolerance mediated by Tregs and TGF-β production were observed in such newborn mice. These data propose a mechanism explaining breast feeding-induced tolerance in neonates and support the role of maternal factors on the regulatory responses that affect the predisposition to allergic disorders (67, 111).

ROLE OF TREGS IN ALLERGY AND ASTHMA

Importance of Tregs in Allergic Diseases

Although the population is continuously being exposed to a wide range of allergens, not everyone develops allergic sensitization, and not all atopic individuals are asthmatic. This fact could be explained by genetic susceptibility to both atopy and asthma and differences in the maturation of the immune system and organs/tissues affected by allergic diseases. With regard to immunological sensitization, it has been shown that under certain conditions non-atopic healthy individuals develop Th2-cell responses to common allergens. In this sense, the control of potentially harmful T cells requires the use of an active immunosuppressive mechanism by Tregs. Defective or overwhelmed suppression by Tregs could explain the development of allergic airway inflammation in asthma (105). Accordingly, the development of allergic reactions can result from decreased induction, impaired function, or both, of allergen-specific Tregs in genetically allergy-prone subjects. On the contrary, in another study, Treg numbers were higher in asthmatic versus healthy children, and Tregs of children with AA show sufficient suppression of Th1/Th2 cytokines; whereas Tregs from infants with NA do not. These results suggest that the high number of Tregs in certain patients with AA might still not been sufficient to control the disease or additional mechanisms, such as deficiency in innate immune regulation, may be relevant for persistent inflammation (70, 73).

Nevertheless, studies on human newborn Tregs have found an association between decreased regulatory function at birth and the development of allergic diseases. For example, a defect was detected in the suppressive function of newborn Tregs in a child who was later diagnosed of egg allergy (68), and an inverse relation has been found between postnatal deficiencies in Treg numbers and/or function and development of allergy phenotypes during childhood (112). Regarding that, it has been shown that "*whereas the turnover and suppressor function of non-atopic infant's Treg cells appears to increase with age, there is a delay in this process in atopic infants*" (113).

The importance of neonatal pTregs in asthma prevention is based on the observation that the cytokines IL-4 and IL-6 inhibit FOXP3 expression in naive $CD4^+$ T cells. As a consequence, the generation of Tregs should be less efficient when it goes in parallel with conventional T-cells activation due to the presence of an allergen. However, if pTregs can be induced at early times, the tolerance mechanisms would promote expansion of the pTreg population (114).

One study showed deficient $CD4^+CD25^{hi}$ T cell numbers and function and decreased FOXP3 (mRNA) in the lungs of asthmatic children in comparison to healthy controls (115). Another study has shown that patients with asthma have normal numbers of $CD4^+CD25^{hi}$ and $CD4^+CD25^{hi}$ $FOXP3^+$ Tregs in peripheral blood compared to healthy individuals, although the expression of the FOXP3 protein was attenuated (116). Conversely, inhibition of Th2-cell responses to allergens has been described by Tregs in healthy donors. Thus, depletion of $CD4^+CD25^+$ T cells from PBMC of healthy donors increased the proliferation and Th2 cytokines release in response to allergens as compared to whole PBMC cultures (117). In addition, passive transfer of allergen-specific Tregs can attenuate chronic airway inflammation induced by the allergen. Importantly, $CD4^+CD25^+$ Tregs also inhibited airway remodeling, and this might occur through an early decrease of the profibrotic cytokine TGF-β in lung (118).

Activation and Recruitment of Tregs

An intense research focus concerns the study of the specific mechanism of Tregs generation and location in the airways of asthmatic mice and humans. The results of several studies suggest a role for ICOS-L-expressing DCs and the presence of the immunosuppressive cytokine IL-10 (119), although other researchers hypothesized that plasmocytoid DCs contribute decisively to the development of Tregs and accumulation in the airways (120). It has recently been reported that "*siglec-F^+ alveolar macrophages were found to be the major APC driving the differentiation of $FOXP3^+$ Treg cells in the lungs of mice following allergen inhalation, in a process requiring TGF-β and the retinal dehydrogenases, RALDH-1 and RALDH-2*" (121). The means by which Tregs migrate to the allergic lung tissue and lymph nodes of mice implicate the expression of certain chemokine receptors such as CCR4 and CCR7, respectively (122). Supporting this, studies have demonstrated that CCL17 and CCL22, both ligands for CCR4, have a role in the accumulation of $CD4^+CD25^+$ Tregs to the airway tissue throughout allergen response (118). The $CD103^+$ conventional DC subset of the lungs (123, 124) is involved in the release of the CCR4 ligands, leading to the development and recruitment of Tregs in that location (125). Importantly, maintenance of T regulatory cell function required a continued allergen presence. In fact, discontinuous allergen exposure led to a reduction in Treg function and an increase of pathological symptoms (126). It would appear that Tregs control migration of effector cells to inflamed tissues and line up appropriate immune responses at different stages after antigen challenge.

Mechanisms of Suppression by Tregs in Allergic Processes

Regulation is a general process that uses many strategies to attenuate inflammation, and the specific mechanism triggered depends on the tissue and associated milieu where the antigenic challenge occurs. Thus, Treg cell suppressive functions are similar when controlling autoimmunity or allergy and their action could be mediated by multiple mechanisms that involve either the release of suppressive cytokines (IL-10, TGF-β, and IL-35) (127–129) and cytolytic molecules [granzymes (Gzm) A and B] (130) or the downmodulation of APC through expression of inhibitory molecules such as CTLA-4 (CD152) and LAG-3 (CD223) (131); deprivation of trophic cytokines (IL-2 through CD25) (132); modulation of metabolic pathways (CD73 and CD39) (133); and modulation of the expression of specific transcription factors and receptors. Tregs function can also be regulated by endogenous danger signals or alarmins released by epithelial cells at the mucosal barrier. Colonic Tregs express the IL-33 receptor (ST2), allowing them to respond to the cytokine IL-33 produced by epithelial cells as a result of tissue damage. After IL-33 binding to their ST2 receptor, Tregs respond by amplifying their regulatory functions and restraining intestinal inflammation (28, 134). One of several combined mechanisms could be used by Tregs to regulate activation of the different cell types involved in the allergic response including B-cells, ILC2 cells, mast cells, eosinophils, neutrophils, $CD4^+$ and $CD8^+$ T cells, NK cells, NKT cells, monocytes, and DCs (135) (**Figure 2**).

Suppression of Type 2 ILC2 by Tregs

Innate lymphoid cells are a population of mucosal innate cells characterized by a lack of antigen specificity (absence of T- and B-cell receptors) and by shared developmental origin and phenotypic traits with T cells. ILC2 produce large amounts of Th2 cell cytokines and are linked to allergic disorders, such as asthma, chronic rhinosinusitis, and atopic dermatitis (28).

It has been demonstrated that peripherally induced Tregs effectively suppress the production of the ILC2-driven, proinflammatory cytokines IL-5 and IL-13, both *in vitro* and *in vivo* by blocking of ICOS:ICOS-L interaction on ILC2 cells. Inducible T cell Costimulator (ICOS) is a receptor expressed by ILC2 cells, Tregs, and others. It is well known that ICOS:ICOS-L interactions on ILC2 cells have a role in cell function and survival and are involved in controlling Th2 cytokine release, airway hyperreactivity (AHR), B-cell differentiation, and IgE class-switching in mice (136, 137). A recent study by Maazi et al. (138) demonstrated that the expression of ICOS and ICOS-L in ILC2s from human peripheral blood was increased by *in vitro* culture in the presence of IL-2 and IL-7 but not of IL-33. It has been demonstrated that

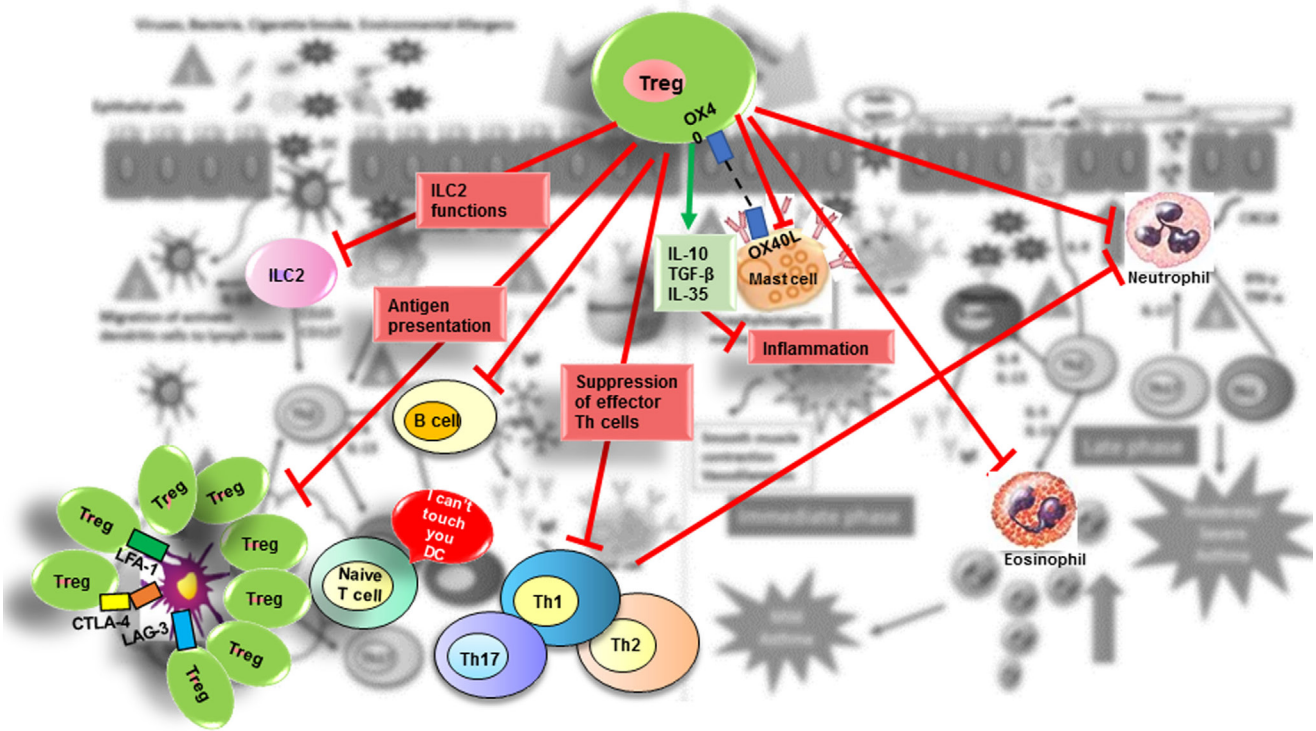

FIGURE 2 | Suppressive role of Treg in allergic diseases. Tregs contribute to the control of allergic diseases through several pathways. Red arrows indicate suppressive effects that Treg exert directly or indirectly on effector cells (ILC2 cells; mast cells; basophils; DC; B-cells; effector Th2, Th1, and Th17 cells; neutrophils). The green arrow indicates suppressive molecules produced by Tregs.

cell contact is required for suppression of ILC2 mediated by Treg, TGF-β, and IL-10. Thus, *in vitro* stimulation of human ILC2s with IL-2, IL-7, and IL-33 and subsequent blockade of ICOS:ICOS-L interactions decreased the release of IL-5 and IL-13 cytokines (139). Additionally, it has been shown that human pTregs suppress syngeneic human ILC2s *via* ICOS-L to control airway inflammation in a humanized ILC2 mouse model (140) (**Figure 2**).

Suppression of Mast Cells by Tregs

Mast cells are essential to initiate the immediate phase of allergic reactions (**Figure 1**, Step 6), and their degranulation initiates the triggering of allergic symptoms (141). It has recently been demonstrated that the symptomatic phase of allergic disorders can be controlled by constitutive FOXP3$^+$ Tregs in mice (142), although mast cells increased the IL-6 secretion. This inhibition by Tregs is produced *via* direct cell-to-cell contact between OX40 expressed on Tregs and OX40 ligand on mast cells, which leads to increased intracellular levels of cyclic AMP (cAMP) and results in blockage of extracellular Ca^{2+} (**Figure 2**). However, the suppression of IL-6 secretion by mast cells seems to be controlled *via* TGF-β (143). *In vitro* studies have shown that "*IL-4 and TGF-β1 had balancing effects on mast cell survival, migration and FcεRI expression, with each cytokine cancelling the effects of the other. Dysregulation of this balance may impact allergic disease and be an objective of targeted therapy*" (65, 144).

Suppression of APCs by Tregs

Dendritic cells are key initiators and master regulators of the allergen-specific immune response by processing and presenting antigens to Tconv and secreting cytokines that control T cell differentiation to a certain effector type. Tregs could directly act on DCs by downmodulating their surface expression of CD80/CD86 and subsequently blocking generation of an allergen-specific Th2 cell immune response. Suppression of DCs appears to be mediated through LAG-3, CTLA-4, leukocyte function-associated antigen 1 (LFA-1), and other molecules (**Figure 2**).

Thus, Tregs may express LAG-3, a homologous of the CD4 molecule that acts as a coreceptor of the MHCII complex, although with higher affinity. The binding of MHCII and LAG-3 reduces the maturation and costimulatory capacity of DCs and, as a consequence, diminishes their capacity for antigen presentation to Tconv (131, 145, 146). Activated human T cells express MHCII molecules, and interaction of Tregs *via* LAG-3 on Teff might also induce immunosuppression (147).

Additionally, murine and human Tregs exhibit a constitutive expression of the coinhibitory molecule CTLA-4, which is detected on the surface of Teff upon activation (148). The deficiency of the *CTLA-4* gene produces serious autoimmune disorders similar to those induced by defective *FOXP3*, demonstrating that CTLA-4 is essential for Treg function (149). CTLA-4 binds to the same ligands CD80/86 as CD28 (T cell costimulatory antigen) but with a higher binding affinity. The interaction between CD28

and its ligands CD80/86 on DCs is required for T-cell activation. However, CTLA-4 binding to the same ligands blocks such activation and induces the generation of anergic T cells. CTLA-4 may also suppress or decrease the surface expression of CD80/86 molecules, decreasing the activation of Tconv. Furthermore, the interaction of CTLA-4 with CD80/86 in Tregs can promote the generation of indoleamine 2,3-dioxygenase (IDO) that catalyzes degradation of the essential amino acid tryptophan to kynurenine, provoking the starvation of Teff and cell cycle arrest. Additionally, IDO induces pTreg generation (150). Also, CTLA-4 may be involved in the reduced glutathione synthesis observed in murine DCs, which promotes a Redox milieu unfavorable for the proliferation of conventional T cells (151). In relation to that, several studies have shown that certain polymorphisms in the *CTLA-4* gene are significantly associated with susceptibility to autoimmunity (152).

Regulatory T cells can control Tconv activation by reducing their interaction with DCs (153). In fact, it has been described that the aggregation of $CD25^{hi}$ Tregs around DCs, *via* CTLA-4, downregulated CD80/86 molecules expression (149, 154). Thus, there is a competition between Tregs and Tconv for the interaction with DCs reducing their capability to activate Teff (105). This downregulation of CD80/86 expression by Tregs is also partly dependent on the adhesion molecule LFA-1, thereby indirectly impeding the activation of Tconv cells by APCs *in vitro* (155, 156) and *in vivo* (149, 157) (**Figure 2**).

Another surface molecule involved in immunosuppression is T cell immunoglobulin and ITIM domain (TIGIT), which is highly expressed on Treg and Teff. A protein named Poliovirus receptor (PVR, NECL5, or CD155), highly expressed on DCs and others, was identified as a high-affinity coreceptor for TIGIT. Upon interaction of Treg cell with DCs through TIGIT, secretion of the suppressive cytokines IL-10 and TGF-β by DCs was observed (158).

Neuropilin-1 is another molecule expressed by Tregs and which elongates their contact with DCs reducing the presentation of antigen to Tconv. These results were confirmed by using an anti-Nrp-1 antibody to abrogate Treg-mediated suppressive activity (159, 160). However, as other $CD4^+$ cells express Nrp-1 (161) it is possible that the anti-Nrp-1 antibody was interfering with cell activation rather than Treg function (146).

Finally, ICOS:ICOS-L interactions between mostly plasmacytoid DCs and Tconv could result in their differentiation into IL-10-secreting Tregs (28).

Suppression of Th by Tregs

During the activation process, T cells follow several differentiation pathways, acquiring specific properties and functions. Th cells could differentiate into Th1 (IFN-γ-secreting cells), Th2 (IL-4/IL-5-secreting cells), Th17 (IL-17-secreting cells), and other subsets. Th1 cells are specialized in the elimination of intracellular microorganisms; Th2 cells are required for fighting extracellular pathogens; and Th17 cells protect against extracellular fungal and bacterial pathogens and have a role in autoimmune tissue injury. Regarding that, it has been shown that Tregs can avoid allergy by suppression of the effector Th1, Th2, and Th17 cells (162, 163) (**Figure 2**). Nevertheless, such results are not clear since it has been published that TGF-β1 secreted by $FOXP3^+$ Tregs is necessary to block Th1 and support Th17-cell generation (164). On the other hand, it has been suggested that Tregs induced by nitric oxide can suppress Th17 but not Th1 cell development and function (165). Furthermore, it has been published that Tregs can also block the synthesis of the cytokine IFN-γ without inhibiting Th1 cell differentiation (166) and that $FOXP3^+$ Tregs induce Th17 cell differentiation *in vivo* through IL-2 modulation (167). Additionally, it has been found that the cytokines IL-6 and TGF-β promote the generation of pathogenic Th17 cells (65, 168). Further studies should be performed to clarify the importance of Tregs in the suppression of specific Th subsets.

Recent evidence suggests the existence of a balance between Tregs and Th17 cells during the first stages of naive T cell development. Thus, the presence of IL-6 and TGF-β promotes the differentiation of Tconv into Th17 cells. However, without IL-6 in the milieu, T cells differentiate into Tregs. Additionally, IL-21 has a role in the generation of the Th17 subset and inhibits FOXP3. Given the contribution of Th17 cells in asthma, the inhibition of the Th17 population by Tregs is crucial to maintaining the immune homeostasis (146).

Furthermore, it has been observed that Tregs suppress the TCR-mediated proliferation and IL-2 release of Tconv cells (132). The mechanism by which Tregs suppress murine (169) or human (168) Tconv proliferation can be directly mediated by immunosuppressive factors or by a contact-dependent action. Tregs can also block Tconvs indirectly by controlling the activation of APCs as previously described (14).

Another mechanism to control immune activation would be effector cell-death induction by Tregs. In fact, it was observed that human tTregs express Gzm A and kill activated $CD4^+$ T cells and other cells by perforin-dependent mechanism (170). Another study reported a partially Gzm B-dependent inhibition of Tconv proliferation by murine Tregs *in vitro*, although perforin was not involved (130). A further example of suppression of T effector cells by Tregs was demonstrated *in vitro* and *in vivo* in a model of transplantation in mice with the involvement of the death receptor TRAIL (171).

At the same time, high-level IL-2R expression on Tregs, indispensable in Treg cell homeostasis (172), could deprive Teff of IL-2 and inhibit their proliferation (173). Nevertheless, there is a controversy about the role, if any, of the decreased availability of IL-2 because of its consumption by Tregs that exhibit high expression levels of the CD25 molecule and may depend on the specific setting and stimulation conditions of the cells (14).

Suppression of Eosinophils and Neutrophils by Tregs

Eosinophils are secondary effector cells involved in the pathogenesis of allergy (**Figure 1B**). It has been reported that Tregs can inhibit their function (174), and a negative correlation has been found between the percentage of $FOXP3^+$ cells in bronchoalveolar lavage fluid (BALF) from tolerant mice and the number of eosinophils detected in that fluid (65, 175). The mechanism by which Tregs inhibit eosinophils activation is mediated by IL-10 released by Tr1 cells (176, 177).

Additionally, Tregs might directly control allergic responses by induction of neutrophil apoptosis and/or by promoting an immunosuppressive phenotype on these cells (**Figure 2**) that generate IL-10, TGF-β1, IDO, heme oxygenase-1 (HO-1), and the suppressor of cytokine signaling 3 molecule (SOCS3) (178). These anti-inflammatory neutrophils may inhibit Th17 cell induction, which depends on the presence of both TGF-β1 and IL-6 (179). Additionally, phagocytosis of apoptotic neutrophils by macrophages and DCs results in a reduction in IL-23 release by these cells, which in turn leads to lower IL-17 secretion by $CD4^+$ T cells (180). Hence, cooperation of activated Tregs with neutrophils might result in inhibition of Th17 response, providing an important control of inflammatory responses. Supporting this idea, one study (181) has shown that the reduced capacity of weanling, as compared with neonatal, mice to develop inducible bronchus-associated lymphoid tissue (iBALT) in response to LPS can be reversed by the elimination of Tregs. This was associated with a high expression of IL-17A and CXCL9 and with increased neutrophilic inflammation in the lungs.

Suppression of B-Cells by Tregs

Activated Tregs may directly suppress effector B-cells through the release of Gzm and perforin (182). As a consequence of this, Tregs can control IgE production and the posterior mast cell-mediated inflammation. In fact, the large amounts of IL-10 and TGF-β that are secreted by Tregs drastically inhibit IgE release, although a simultaneous increase in the production of IgG4 and IgA by B-cells has been observed. This isotype unbalance has also been reported in individuals naturally exposed to large allergen doses. Thus, beekeepers with multiple stings and patients with chronic helminthic infections have tolerance mediated by IL-10 and increased levels of antigen-specific IgG4 (183). Recently, the identification of IL-10-producing B regulatory cells with immunosuppressive function has been reported; these cells may also control the inflammatory reactions mediated by T cells (184) and may participate in the generation of peripheral $CD4^+CD25^+$ cells by inducing the development or elongating the survival of such cells (146).

Suppression by Tregs through Expression of Effector T Cell-Specific Transcription Factors

Several researchers have suggested that as with conventional $CD4^+$ helper T cells, Tregs display various phenotypically and functionally diverse subsets and that their location in different tissues is critical for their ability to interact with and regulate the different subsets of Teff (82). Tregs may modulate a Th cell subset specifically by expressing the characteristic transcription factor as well as adhesion and chemoattractant receptors of said specific subset that would target them to the same tissues and inflammatory sites (185). These comprise chemokine receptors such as CXCR3, CCR8, and CCR6, involved in the migration of cells to locations of Th1-, Th2-, or Th17-mediated inflammatory responses, as well as other general receptors such as CCR2 and CCR5 (185, 186). In this regard, it was shown that upregulation of T-bet in a Treg subset upon IFN-γ secretion was required for the control of inflammatory responses mediated by Th1 cells (187). Similarly, the expression of IRF-4 (associated with Th2 and Th17 cells) in Tregs was essential for the inhibition of immune responses mediated by Th2 cells (188). Moreover, the presence of the transcription factor STAT3, characteristic of Th17 cells, in Tregs was critical for the suppression of the intestinal inflammation associated with Th17 cells (189). In accordance with this, the absence of GATA-3, transcription factor associated with Th2 cells in Tregs, led to autoimmunity, defective FOXP3 expression, and elevated cytokine levels specific of Th1, Th2, and Th17 cells (190). In fact, Tregs lacking GATA-3 expression usually transform into a Th17 subset. In relation with that, Bcl6, which acts by suppressing GATA-3 transcriptional activity independently of IL-4 and STAT6, is crucial for the control of Th2 inflammation by Tregs (191–193). The mechanism used by Tregs to suppress each subset of Teff might include deprivation of limiting factors since Tregs accumulate at the location of the immune response. Accordingly, IRF-4 or STAT3-deficient Tregs lack suppressive function *in vitro*. In contrast, the presence of certain transcription factors in Tregs might inhibit FOXP3 expression with the subsequent blocking of Tregs suppressive function. Thus, STAT3 is a transcription factor induced by the release of various cytokines that downregulate Tregs and FOXP3, such as IL-6, IL-23, or IL-27 (192, 194). In fact, the lack of STAT3 in T cells in a model of induced colitis promoted the development of Treg and diminished the symptoms of the disease (195). Probably, the different $CD4^+$ T cell subsets have the ability to experiment high levels of plasticity between them, although there is controversy about the stability of the Tregs *in vivo* (22, 196). Nevertheless, several studies indicate an evident plasticity between Th17 cells and pTregs (197), and supporting this idea, a transitory stage with simultaneous expression of RORγt and FOXP3 has been detected (198). In the context of a specific cytokine environment, the release of IL-17 in murine and human Tregs, which might maintain immunosuppressive activity, was described (199, 200).

Suppression by Tregs *via* Ectoenzymes

During the immune response, extracellular ATP acts as a danger signal and may exert its effects on DCs. Cell damage induces the release of the intracellular ATP since the nucleotide is present within the cells in high concentration. "*Extracellular ATP can be sensed by purinergic P2 receptors such as CD39. This molecule is the main ectoenzyme in the immune system, hydrolyzes ATP or ADP to AMP and is expressed by B cells, DCs, all mouse Treg cells, and about 50% of human Treg cells*" (201). Thus, another anti-inflammatory mechanism that may be used by Tregs could be catalytic inactivation of extracellular ATP by CD39 (201). Supporting this idea, CD39 knockout Tregs showed decreased suppressive capacities *in vitro* and *in vivo* (133). In fact, CD39 expression was suggested to identify a highly suppressive human Treg subset (202), and inhibition of Tconv proliferation by this subset could be partially abolished by suppression of ectonucleotidase activity (203). CD73 is another ectoenzyme, also expressed by Tregs, that degrades AMP to adenosine (204). Adenosine binds to the A2A receptor and may suppress DCs and/or Teff, e.g., by increasing cAMP (205). *In vivo*, signaling through A2A receptor might lead to anergy and induce pTreg development (206). In conclusion, adenosine seems to contribute

to the regulatory function of certain Treg subsets. Thereby, cAMP is transferred through gap junctions from the Tregs into Teff where it activates protein kinase A that suppresses proliferation and IL-2 release by triggering the activation of inducible cAMP early repressor (ICER) (207).

Suppression by Tregs *via* Cytokines Secretion: TGF-β, IL-10, and IL-35

Despite the critical role of TGF-β and IL-10 in various *in vivo* models, the specific contribution of immunosuppressive cytokines in Treg-mediated regulation is still poorly understood (**Figure 2**).

Role of TGF-β

TGF-β is a pleiotropic cytokine that directly prevents proliferation of T- and B-cells and also induces cell death of immature or naive B-cells. In addition, this cytokine inhibits macrophage proliferation and function, acts such as a chemoattractant for eosinophils and can suppress allergen-specific IgE release. Furthermore, TGF-β participates in Treg function and supports the generation of pTregs. TGF-β may promote pro-inflammatory Th17 responses. Thus, TGF-β favors the conversion of Teff into FOXP3$^+$ Tregs in the periphery (208). However, in the presence of IL-6, TGF-β sustains the differentiation of Th17 from Tconv (177). *In vivo* studies have demonstrated the existence of a TGF-β-dependent mechanism for Treg-mediated immunosuppression (164). In this respect, it has been shown that TGF-β1 specifically expressed by Tregs plays a role in the regulation of allergic responses. In fact, TGF-β is involved in a negative feedback mechanism to regulate airway inflammatory responses, repair asthmatic tissues, and induce fibrosis in human subjects (177). Nevertheless, the effects of TGF-β in patients with allergic disorders appear to be complex, with evidence of both disease inhibition and promotion. Tregs can release large quantities of soluble or membrane-bound TGF-β and the partial neutralization of TGF-β reversed the *in vitro* inhibition of murine and human T-cell proliferation (128, 209, 210), supporting the hypothesis that TGF-β secreted by Tregs regulates inflammatory responses. However, other researchers found no such connection between TGF-β release and suppression of T cells by Tregs (211, 212). Furthermore, neutralizing antibodies against TGF-β did not block suppressive activity *in vitro* and *in vivo*, and effector T cell responses were not inhibited by supernatants from cellular suppression assays (213). Furthermore, Tregs from TGF-β-deficient mice maintain their suppressive function (214). Probably, the contribution of TGF-β to the immunosuppressive function of Tregs might depend on the location of the immune response and the characteristics of the effector cells involved in the process.

Role of IL-10

IL-10 is a cytokine synthesized by a diverse number of cell types, including B-cells, monocytes, DCs, natural killer cells, and T cells. The requirement for the release of IL-10 by Tregs in the control of allergic reactions has been demonstrated. In fact, inhibition of allergic airway inflammation has been shown by adoptively transferred allergen-specific Tregs (118, 215). Supporting the concept that IL-10 produced by Tregs plays a fundamental and non-redundant role in the induction of immune tolerance in patients with allergic airway disorders, studies by several researchers show that Treg cell-specific deletion of IL-10 promoted allergic airway inflammation (195). IL-10 has immunosuppressive functions and can modulate the activity of several cell subsets involved in allergic reactions, such as mast cells (216), Th2 T cells (217), eosinophils, and DCs (149). Specifically, it decreases pro-inflammatory cytokine release and Th1 and Th2 cell response, probably due to their effects on APC. Direct effects on T-cell function have also been demonstrated. T-cell activation requires antigen-specific recognition by the TCR and the signaling through costimulatory molecules such as CD28 and ICOS. On these cells, the tyrosine kinase Tyk-2, associated with the IL-10 receptor acts as a recruitment site for Src homology domain 2-containing protein tyrosine phosphatase 1 (SHP-1), which is a negative regulator for T-cell activation. After IL-10 binds to its receptor, Tyk-2 phosphorylates SHP-1 (117, 149), which immediately binds to and dephosphorylates the CD28 and ICOS costimulatory receptors, producing the inhibition of downstream signaling (218). Accordingly, T cells from SHP-1$^{-/-}$ mice exhibited increased activation upon CD28 and ICOS binding compared with control mice, which was not attenuated by IL-10 supply. Activation of mast cells and eosinophils, the two effector cell types involved in the early and late phases of the allergic response, were inhibited by IL-10. Data from studies in murine models and human subjects have indicated that IL-10 contributes to the immune homeostasis in lung tissue (28).

Role of IL-35

IL-35 is a cytokine composed of two different subunits: the Epstein–Barr virus-induced gene 3 (EBI3) and a subunit of IL-12 (p35, IL-12α). It was identified as an anti-inflammatory and immunosuppressive cytokine produced mainly by Tregs (219). According to that, Tregs lacking one of the two subunits of IL-35 had decreased suppressive capability *in vitro* and *in vivo* in an intestinal bowel disease (IBD) murine model. Furthermore, EBI3$^{-/-}$ and IL-12α$^{-/-}$ mice have Tregs with attenuated suppressive capacity, which supports the role of IL-35 in Treg-mediated immunosuppression. In contrast to mice, human Tregs do not constitutively express IL-35 (220), although this cytokine may contribute to human regulation. In fact, the treatment of Tconv (human or murine) with IL-35 promoted Tregs-mediated suppression and did not require IL-10, TGF-β, or FOXP3 (221). A specific role of IL-35 in allergic responses has been described. Thus, IL-35 protein and mRNA levels in allergic asthmatics were shown to be lower than in healthy controls (222). In addition, the number of FOXP3$^+$ Tregs and IL-12p35$^+$ T cells in patients with AA was also found to be decreased (222). Furthermore, the production of IL-17, allergic airway hyperresponsiveness and the frequencies of macrophages, neutrophils, lymphocytes, and eosinophils in BALF increased in mice that were deficient in EBI-3 (223). Finally, it has been demonstrated that IL-4, IL-5, and IL-13 in BALF were inhibited by the administration of plasmid DNA encoding recombinant single-chain IL-35 or adenovirus expressing IL-35 (224) These findings strongly support a fundamental role for IL-35 in the control of allergic responses.

Suppression by Tregs *via* Transfer of miRNAs

microRNAs have recently been described as critical regulators of Treg development and function. These are small double-stranded RNAs that negatively regulate gene expression at a post-transcriptional stage (225). Furthermore, an exosomal pathway has been described that can capture miRNAs from certain cells and transfer them to other cells (226), providing a mechanism for cell communication. In this sense, Okoye et al. (53) have observed that Tregs are capable of releasing miRNA-containing exosomes. Specifically, Tregs released and transferred Let7d to Th1 cells, regulating Th1 cell proliferation and IFN-γ release. Furthermore, generation of miRNA and the release of exosomes by Tregs were both required for suppression of Th1 cell activation *in vivo* and for the prevention of systemic inflammatory disorders. Supporting this new mechanism of control by Tregs, it has been demonstrated that exosomes isolated from Tregs can inhibit Teff. However, this suppressive effect was not as potent as that of Tregs, suggesting that exosome transfer and other mechanisms are necessary for optimal regulation (53). Additionally, miR-21 expression has been associated with Tregs deficient in Bcl6 which exhibit a defective ability to control Th2 inflammation by limiting the transcriptional activity of GATA-3 (193).

In summary, the mechanisms of Treg cell-mediated suppressive function open up the possibility that Tregs capture and deliver different miRNAs and other molecules to different cells at different times depending on the specific situation.

FUTURE PERSPECTIVES

Although many efforts have been made to uncover the mechanisms that control allergic responses, several aspects remain to be clarified.

First, special interest should be paid to the prevention of allergic diseases through the study of intrauterine and postnatal factors that influence the predisposition of individuals to suffer such diseases. In this sense, the mechanisms by which TLRs modulate the differentiation and immunosuppressive ability of thymic and peripheral Tregs during the different stages of their development deserve special attention.

Second, important questions have arisen regarding the controversy on the plasticity of Tregs. It remains to be further clarified whether the expression of transcription factors specific for each Th subset promotes the acquisition by Tregs of selective migratory characteristics and/or certain specialized suppressive capacities to effectively regulate the inflammatory response induced by each subset of Teff. Additionally, the mechanisms by which certain Tregs subsets target specific effector T cell populations with more efficient suppressive results than others should be explored.

Third, the contribution of each particular suppression mechanism to the maintenance of self-tolerance and immune homeostasis should be studied. It is necessary to elucidate whether a general suppressive mechanism used by any type of Treg at any tissue exists or, conversely, different mechanisms are used depending on the location, type of antigen, Treg subsets, and conditions of the immune responses.

Success in analyzing these cellular and molecular events, *in vitro* and *in vivo*, in rodents and in humans can reveal which factors are important in the proper differentiation of Tregs, in their plasticity, and which suppression mechanisms mediated by Tregs are adequate targets for an effective control of the immune responses.

AUTHOR CONTRIBUTIONS

MN-M produced the figures, collaborated in the writing, and performed critical reading of the manuscript. JM-G organized references, collaborated in the writing, and performed critical reading of the manuscript. EM-O wrote the paper and supervised figures and references.

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Oxidative Stress and Bronchial Asthma in Children—Causes or Consequences?

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Bronchial asthma is one of the most common chronic inflammatory diseases of the airways. In the pathogenesis of this disease, the interplay among the genes, intrinsic, and extrinsic factors are crucial. Various combinations of the involved factors determine and modify the final clinical phenotype/endotype of asthma. Oxidative stress results from an imbalance between the production of reactive oxygen species and reactive nitrogen species and the capacity of antioxidant defense mechanisms. It was shown that oxidative damage of biomolecules is strongly involved in the asthmatic inflammation. It is evident that asthma is accompanied by oxidative stress in the airways and in the systemic circulation. The oxidative stress is more pronounced during the acute exacerbation or allergen challenge. On the other hand, the genetic variations in the genes for anti-oxidative and pro-oxidative enzymes are variably associated with various asthmatic subtypes. Whether oxidative stress is the consequence of, or the cause for, chronic changes in asthmatic airways is still being discussed. Contribution of oxidative stress to asthma pathology remains at least partially controversial, since antioxidant interventions have proven rather unsuccessful. According to current knowledge, the relationship between oxidative stress and asthmatic inflammation is bidirectional, and genetic predisposition could modify the balance between these two positions—oxidative stress as a cause for or consequence of asthmatic inflammation.

Keywords: bronchial asthma, oxidative stress, oxidative damage of biomolecules, chronic inflammation, childhood

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INTRODUCTION

Bronchial asthma (BA) represents the most common chronic respiratory disease in children, and its prevalence is constantly increasing especially in the developing countries (1). The disease is characterized by chronic ongoing airways inflammation accompanied by structural (so-called remodeling changes—epithelial fragility, goblet cell hyperplasia, enlargement of submucosal mucus glands, hypertrophy and hyperplasia of airway smooth muscles, airway wall thickening) and functional changes (specific and non-specific hyperresponsiveness, quantitative and qualitative changes in mucus production) in the airway wall. BA develops as a consequence of the interaction among genes (disease determining and modifying genes), intrinsic (e.g., hormonal changes, immune dysregulation) and extrinsic (e.g., allergens, pollutants, infections, physical and environmental factors) factors. The intrinsic and extrinsic factors are able to modify the gene expression directly or indirectly through epigenetic changes (2, 3).

Recently, many attempts were made to classify and stratify the diseases into different phenotypes or endotypes, which are characterized by specific patterns and aspects with respect to the development of inflammation. Phenotype means a cluster of characteristics that define asthma and its subsets. Till date, several asthmatic phenotypes have been determined and characterized based on the triggers (e.g., allergen-induced asthma, non-allergic asthma, infections-exacerbated asthma, aspirin-exacerbated respiratory diseases, exercise-induced asthma) or clinical presentation (e.g., transient wheezing, non-atopic wheezing in toddlers, exacerbation-prone asthma) (4). Another classifying approach is represented by the endotypes of asthma, which attempts to characterize asthma subset according to the pathophysiological mechanisms involved in the development and persistence of asthmatic inflammation in the airways (5). There were several differences between children and adults regarding BA pathophysiology and clinical manifestations (6). The interplay between immune and non-immune cells leads to inflammation, which is characterized by various levels of clinical expression and symptoms. During the pre-exacerbation, the acceleration of the inflammatory cascades results in the asthma exacerbation of different severities (7). The persistent and intermittently exacerbated inflammation is accompanied by overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can also contribute to the promotion and persistence of the airway inflammation (8).

OXIDATIVE STRESS AND BA DEVELOPMENT

Oxidative Stress in Health and Disease

Oxidative stress was shown to be an important component of the aging processes (9) and many other pathological conditions and processes. Oxidative stress results from an imbalance between the production of pro-oxidants (e.g., ROS or RNS) and antioxidant defense mechanisms in the body. The endogenous sources of ROS are cell organelles (mitochondria, peroxisomes, endoplasmic reticulum), various enzymes and enzymatic complexes (e.g., cytochrome P_{450}, NADPH oxidases, nitric oxide synthase, xanthine oxidase), immune and non-immune cells (especially phagocytes, activated eosinophils and neutrophils, monocytes and macrophages, airway epithelial and smooth muscle cells, endothelium), and others (e.g., heme proteins, reactions of metal ions). On the other hand, there are also many exogenous sources of ROS, such as cigarette smoke, ultraviolet light, ionizing radiation, pollutants, ozone, organic solvents, metals, and some medicaments (e.g., chemotherapeutic agents). Both endogenous and exogenous sources of ROS can play an important role in the pathogenesis and worsening of various inflammatory conditions, especially through continual accumulation of the oxidative changes in biomolecules (8, 10–13).

Mechanisms of Oxidative Stress in BA

Many authors showed that BA is significantly associated with increased oxidative stress expressed by the increased markers of oxidative damage. Based on the development and persistence of oxidative stress, several crucial aspects and mechanisms can be identified (11):

- Overproduction of ROS and RNS in chronic inflammation (13, 14).
- Deficiency of intrinsic (e.g., glutathione) or extrinsic (e.g., vitamins and natural antioxidants in diet) antioxidant substances (15–17).
- Decreased activity or dysfunction of antioxidant enzymes (18, 19).
- Over-activity of pro-oxidative enzymes (20–22).

Production of highly reactive oxygen species leads to the progressive damage of various biomolecules (nucleic acids, lipids, proteins, saccharides) with the functional and structural consequences. Under physiological condition, antioxidant defense mechanisms are able to eliminate and repair these changes. However, unregulated overproduction of ROS, during inflammation of various origins, leads to accumulation of the changes without sufficient repair. Oxidative damage of the biomolecules influences the signaling pathways, enzymatic functions, gene expression, and many other essential biological processes. Structural changes are contributing to the chronic remodeling of the tissues. Another possible contribution to the oxidative damage of biomolecules in addition to ROS is RNS. These are formed from the reaction of nitric oxide with oxygen or ROS. RNS could modify the thiol groups (–SH groups; formation of nitrosothiol) or change the tyrosyl residues to nitrotyrosine. This process usually leads to the inactivation or dysfunction of the modified proteins (10, 23).

Oxidative Stress and BA—Molecular Consequences

Oxidative stress represents a substantial component of BA development and persistence. Chronic inflammation leads to the overproduction of ROS and RNS. During the allergen challenge or other situations (i.e., infection, pollutants, physical endurance), the pronouncement of inflammatory cascades is accompanied by the accelerated production of ROS/RNS. This could contribute to the structural changes in the airways, support the remodeling processes, decrease the sensitivity to the anti-inflammatory and anti-asthmatic treatment, and worsen the clinical course of the diseases. Uncontrolled asthma with the sinusoidal acceleration of ROS/RNS production pronounces the pathophysiological aspects of the inflammation and dysfunction of the biomolecules and is clinically associated with remitting–relapsing course of the disease (**Figure 1**) (8, 12, 23).

Many studies showed that the cells formatting airway inflammation in BA are an important source of ROS in these patients (23). Oxidative damage of the biomolecules could have both functional and structural consequences. It plays an important role in the development, persistence, and consequences of BA of all phenotypes or endotypes, since inflammation is crucial in pathogenesis of all the asthmatic forms and subtypes. ROS and RNS, insufficient function of antioxidant defense mechanisms and oxidation of the biomolecules have many consequences, such as enhanced release of arachidonic acid

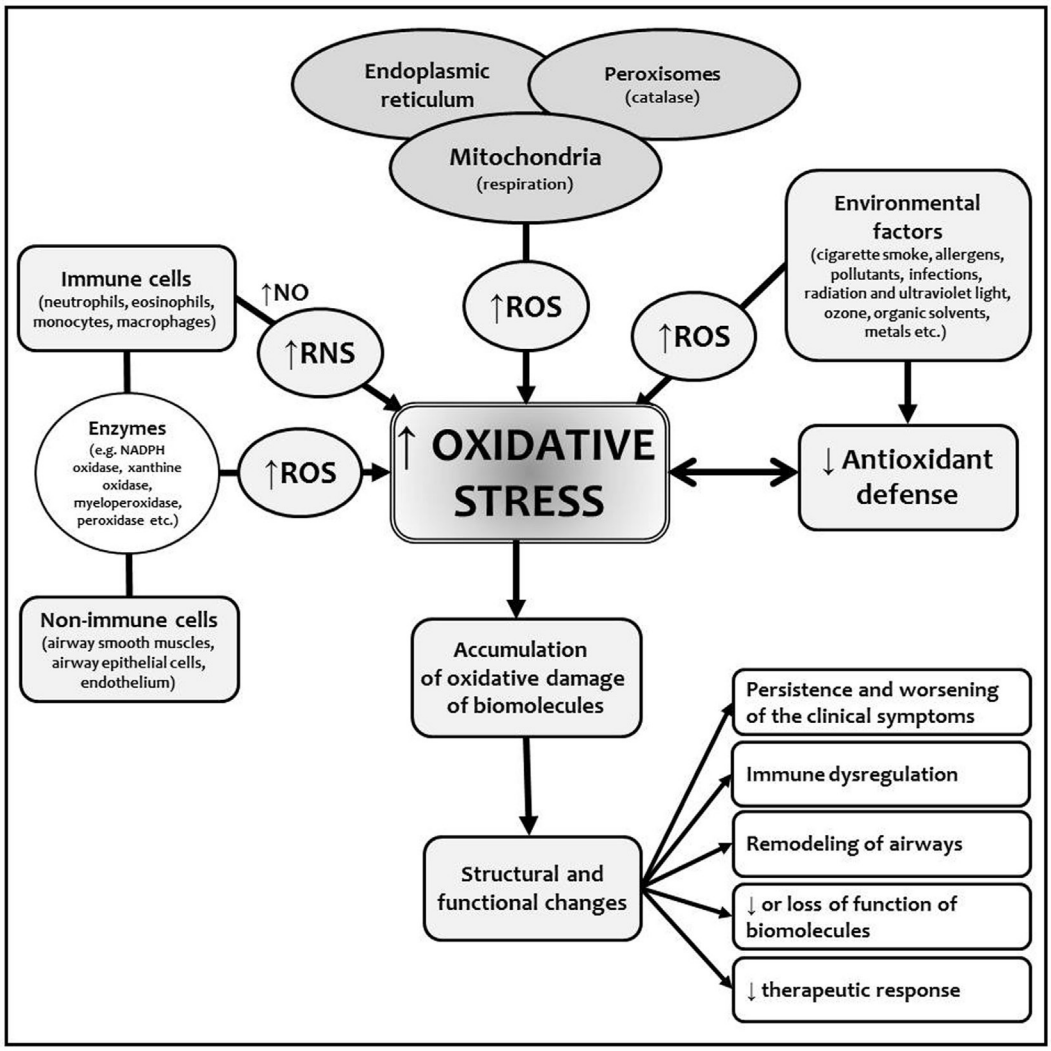

FIGURE 1 | Origin of ROS and RNS and oxidative stress in bronchial asthma (BA). Chronic inflammation in BA is characterized by the overproduction of ROS/RNS as a consequence of increased activation of immune and non-immune cells in cellular inflammatory infiltrate. Various exogenous factors promote and amplify the inflammation and also increased the formation of reactive species *via* many mechanisms (e.g., induction of mitochondrial dysfunction, DNA repair mechanisms damage). Chronic inflammation decreases the capacity of endogenous antioxidant defense mechanisms, which are not able to compensate overproduction of ROS/RNS. This leads to the accumulation of the changes in biomolecules with structural and functional consequences. Abbreviations: NO, nitric oxide; RNS, reactive nitrogen species; ROS, reactive oxygen species.

from cell membranes and formation of inflammatory markers, hyperreactivity and contraction of airway smooth muscle, increased vascular permeability with airway edema, increased bronchial hyperresponsiveness and mucus secretion, increased synthesis of pro-inflammatory cytokines and chemoattractants, induced release of tachykinins and neurokinins with augmentation of neurogenic inflammation, and impaired response to bronchodilators (**Figure 2**) (8, 11, 16).

Exposure to environmental allergens has been shown to stimulate overproduction of ROS/RNS, resulting in the damage of DNA (nuclear and mitochondrial), proteins, and lipids with decrease or even loss of their physiological functions. Moreover, accumulation of oxidative damage causes the structural changes in the airways tissues (16). It was also shown that environmental allergens, such as house dust mite-allergens, possess direct cytotoxicity and lead to the increased oxidative damage, and DNA double-strand breaks in asthmatic lungs (24). Moreover, oxidative damage of DNA and histones could lead to epigenetic changes, such as the diminished responsivity to anti-asthmatic drugs, e.g., corticosteroids (21). On the other hand, the ROS production still presents an important part of adaptive and protective mechanisms.

Markers of Oxidative Stress in BA

Due to low concentrations of ROS, their extreme reactivity, and very short lifetime, direct measurement of ROS is very complicated and, therefore, various indirect markers of oxidative damage, such as markers of lipid peroxidation (e.g., malondial-

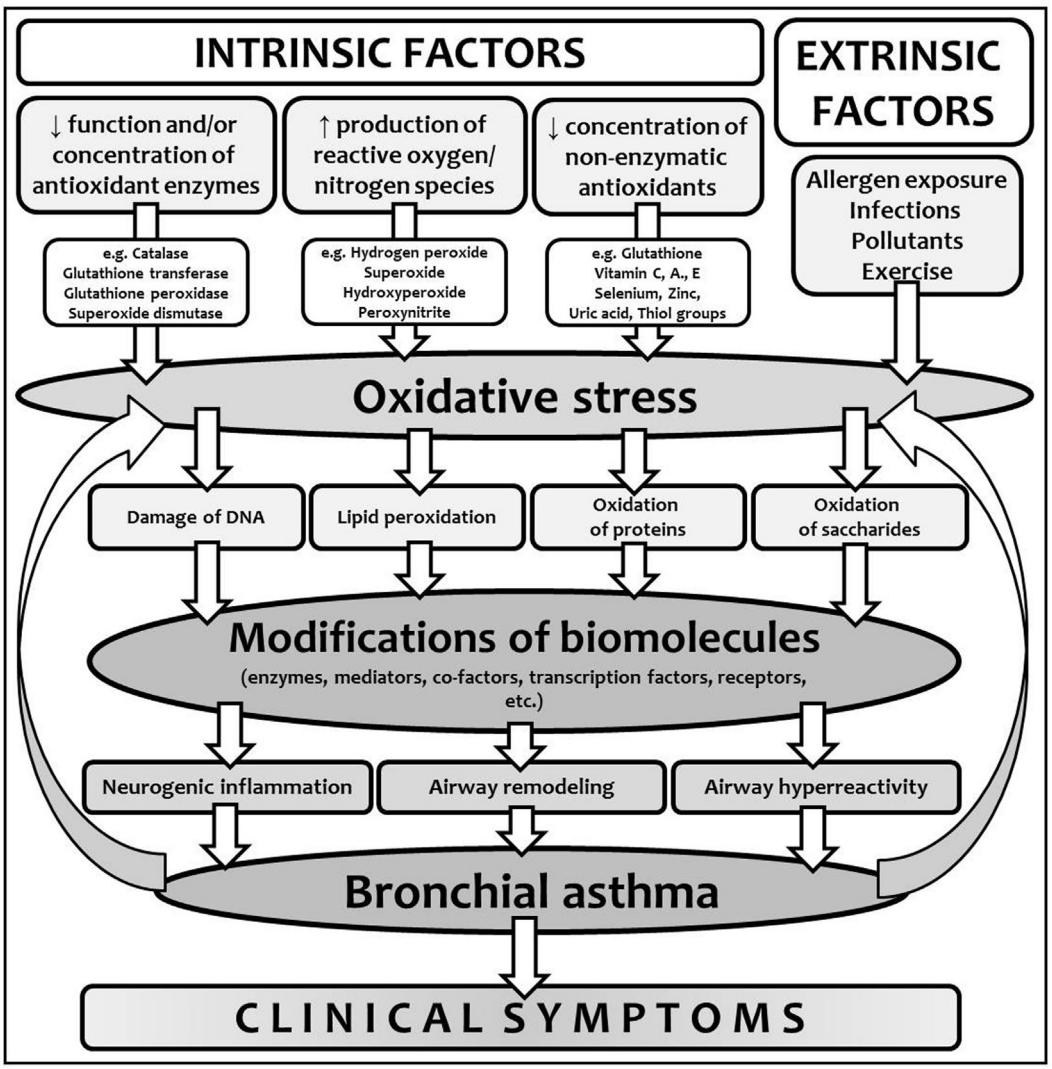

FIGURE 2 | Oxidative stress in the context of bronchial asthma development. Interplay between intrinsic and extrinsic factors leads to the production of reactive oxygen species (ROS) and in certain conditions, due to the imbalance between the production of ROS and antioxidant defense, oxidative stress and modification of biomolecules develops. Due to the modifications of various biomolecules with different functions, particular components of chronic inflammation lead to asthma development and clinical symptoms onset. Moreover, non-controlled or partially controlled and regulated inflammation is another important source of ROS, which closes the vicious circle.

dehyde, 8-isoprostane, thiobarbituric acid-reactive substances (TBARS), acrolein, hexanal, heptanal, nonanal, 4-hydroxyhexanal, 4-hydroxynonenal), protein oxidative damage (e.g., decreased content of free thiol groups, nitrotyrosine, nitrosothiols), DNA damage (e.g., 8-hydroxy-2-deoxyguanosine), or general total antioxidant capacity of plasma could be used (25). Oxidative stress and its manifestations have been shown to be present both in the airways (bronchoalveolar lavage fluid, airway tissue, epithelial lining fluid, exhaled breath condensate, sputum, saliva) and in the systemic circulation (25–30). Airway oxidative stress in BA could be attributed to various sources of ROS, such as exposure to environmental pro-oxidants, airway infiltration of inflammatory immune cells, metabolic dysregulation, and reduced capacity of antioxidant mechanisms (12). Increased production of ROS during airway inflammation is more pronounced after the allergen challenge (31). It was suggested that the assessment of oxidative stress by-products can be used for asthma severity monitoring (16).

It was shown that oxidative stress markers in BA are associated with worse clinical control, worse disease severity, or reduced lung function (32). The amount of ROS is directly correlated with the degree of bronchial hyperreactivity (14). The most impressive changes can be observed during acute exacerbation of asthma, in asthma with concomitant allergic rhinitis or in poorly controlled asthma. Changes in the concentration of the antioxidants and the markers of oxidative damage can be determined in the peripheral blood, serum, plasma, bronchoalveolar lavage fluid, lung, and bronchial tissues biopsies and even in the

exhaled breath and its condensate (25–30, 33–37). Clusterin represents a sensitive cellular biosensor of oxidative stress with antioxidant properties and is associated with the clearance of cellular debritus and apoptosis. Its serum concentration is elevated in patients with severe asthma and is inversely correlated with lung functions (38).

Interestingly, umbilical cord blood-derived basophils from the neonates born to mother with atopic asthma showed increased markers of oxidative stress, decreased activity of glutathione peroxidase, and increased production of interleukin 4. This could contribute to the development of allergic hyperreactivity in children at risk of asthma (39).

ANTIOXIDANT STRATEGIES FOR ASTHMA TREATMENT

Based on the observation from the previous studies that BA is associated with decreased antioxidant protection due to changes in the functions of antioxidant enzymes of decreased concentrations of non-enzymatic antioxidants, the use of different antioxidants could be an interesting mode of supportive and complementary anti-asthmatic therapy (10, 28).

Epidemiological data suggest that antioxidants have a significant effect on the incidence and severity of BA. It was shown that BA and airflow limitation are associated with deficiency of various antioxidants, such as carotenoids, retinol, coenzyme Q10, and vitamin C, D, and E (17, 40–45). In many studies, total antioxidant status of the serum in asthmatics was lower when compared with healthy controls (28, 46). Conversely, the antioxidant deficiencies were not confirmed in other studies (47).

In several studies, the supplementation with a nutraceutical of antioxidants and anti-inflammatory compounds (e.g., curcumin, zinc, selenium, vitamin D) was associated with reduction of airway inflammation, as documented by a decrease in fractional exhaled nitric oxide (FENO) (48) or prolonged time to exacerbation (49). Supplementation of coenzyme Q10 reduced the dosage of oral corticosteroids in steroid-dependent asthmatics (50). Conversely, consumption of broccoli sprouts did not improve eosinophilic inflammation, inflammatory or oxidative stress markers, or other clinical features of asthma among atopic asthmatics despite a marked increase in the serum levels of sulforaphane, which is a potent inducer of antioxidant enzymes (51). Recently, porous antioxidant polymer microparticles were developed as a potential therapeutic system for BA. They possess antioxidant activity and could be used as a carrier for anti-asthmatic drugs, e.g., corticosteroids (52). However, other studies did not confirm the general benefits from antioxidant supplementation in asthma management (53). There are no clinical trial data to support the use of antioxidants to prevent asthma or allergy development (54). Other than corticosteroids, which possess antioxidant and anti-inflammatory properties, other anti-asthmatic drugs, e.g., montelukast, did show conflicting results regarding their capacity to improve total antioxidant status and decrease oxidative damage (55, 56).

Tobacco smoke exposure was confirmed to be an important risk factor for bronchial hyperreactivity development. It increases the oxidative stress with its further consequences. Moreover, it was shown that toxic chemicals in tobacco smoke are able to induce epigenetic changes with increased expression of various pro-inflammatory genes (57). Therefore, prenatal and postnatal exclusion of tobacco smoke exposure could be used as an important preventive approach against allergic sensitization and inflammation development with the attenuation of oxidative stress (58). Prenatal supplementation of vitamin C and E improved pulmonary functions of newborns of smoking mothers (59), but in a large placebo-controlled study, this effect was not confirmed (60).

Oxidative stress is a dynamic process with very tiny border between protective and harmful effects and with problematic predictability of the threshold after which disease ensues and, therefore, this could explain a general inconsistence in the clinical trials with antioxidant supplementation in asthma management. Although the supplementation of various antioxidants appears to be a promising adjuvant therapy for asthma, various studies did not confirm the significant benefits over standard therapy. The potential of antioxidant therapy could be improved by taking into consideration individual characteristics of each particular asthmatic (e.g., the presence of various polymorphisms in the genes for antioxidant enzymes) and environmental risk factors, instead of treating oxidative stress in the airways broadly (12, 41).

SUMMARY

In our research, we studied and analyzed the possible role of oxidative damage in the development of BA in children from several aspects. We studied the markers of oxidative stress in exhaled breath [exhaled carbon monoxide (eCO), nitric oxide] and in the peripheral blood (concentration of free thiol groups as a marker of protein oxidation and concentration of TBARS as a marker of lipid peroxidation) and also analyzed the selected single nucleotide polymorphisms in the genes for two important antioxidant enzymes, such as polymorphisms of two important antioxidant enzymes, theta isoform of glutathione transferase (gene *GST-T1*) and catalase (gene *CAT*).

Examination of exhaled breath seems to be very practical tool for investigation and clinical management of BA both in children and in adults. Till date, several molecules were detected in exhaled breath of asthmatics, but the most commonly used marker of airway inflammation is the FENO. In the group of our asthmatic children, we clearly showed positive correlation between the levels of FENO and the concentration of total IgE in serum and peripheral blood eosinophils. Atopic asthmatics, children with concomitant allergic rhinitis and asthmatics yielded higher levels of FENO during acute exacerbation when compared with non-atopic asthmatics, children without allergic diseases of upper airways and during the stable, clinically controlled disease, respectively (34). Although FENO is preferentially considered to be the marker of allergic and eosinophilic airway inflammation, on the other hand, it can also reflect the increasing oxidative stress (61). Moreover, nitric oxide could also react with oxygen or ROS to form RNS, such as peroxynitrite, which contribute to the increased oxidative

damage under pathological conditions. Therefore, FENO could also be used as an indirect marker of oxidative stress. The eCO is another non-invasive marker detectable in exhaled breath under different pathological conditions of respiratory tract. Exhaled CO is produced by heme oxygenase 1, whose activity can be increased by different factors, such as ROS. Therefore, eCO can serve not only as an indirect inflammatory marker but also as a marker of oxidative stress (15). We showed that eCO is higher in asthmatic children when compared with healthy subjects. Acute exacerbation of BA is accompanied by a significant increase in eCO compared to clinically controlled disease. The level of eCO is higher in atopic asthmatics when compared with non-atopic asthmatics and asthma associated with allergic rhinitis. Moreover, we studied the correlation between selected markers of oxidative damage of biomolecules and eCO levels. We found a significant negative correlation between the concentration of free thiol groups and eCO in atopic asthmatics and during acute exacerbation of asthma. Since we were not able to find such correlation in non-atopic asthmatics, in controlled asthma and in healthy subjects, it can be assumed that eCO could be used as an indirect marker of oxidative stress in different respiratory tract diseases, such as BA (35).

As discussed above, oxidative stress and oxidative damage of the biomolecules represents an important part of chronic inflammation in the airways. We confirmed decreased concentration of free thiol groups (marker of protein oxidation) in asthmatic children compared to healthy subjects. Atopics showed significantly decreased concentration of –SH groups compared to non-atopic asthmatics. We also evaluated a marker of lipid peroxidation—concentration of TBARS. Asthmatics yielded higher concentration of TBARS than healthy controls, especially of atopic phenotype and during acute asthmatic exacerbation (18, 62). Besides the markers of oxidative damage of proteins and lipids, we also focused on the analysis of the selected polymorphisms of two important antioxidant enzymes—theta isoform of glutathione transferase (gene *GST-T1*) and catalase. These two enzymes represent essential mechanisms in the protection against oxidative damage, because they utilize a wide range of products of oxidative damage as substrates. Children with asthma had higher prevalence of the *GST-T1* null genotype compared with healthy controls and these genotypes increased the risk for asthma development by 3.17 folds. Interestingly, atopic asthmatics had a lower prevalence of *GST-T1* gene null genotype than non-atopics (62). Regarding the selected polymorphism -262 C/T in catalase gene (*CAT*), the TT genotype was more frequent in asthmatics than in healthy children (OR = 5.63). This genotype correlated positively with the concentration of –SH groups and negatively with the content of TBARS (18).

CONCLUSION

Oxidative stress represents an important part in the pathogenesis of asthma, but on the other hand, it is only a part in the complex mosaic of BA development. Contribution of oxidative stress to asthma pathology remains at least partially controversial, since antioxidant interventions have proven rather unsuccessful. According to current knowledge, it is not possible to definitely resolve whether oxidative stress is the reason or a consequence of chronic inflammation in asthmatic airways. Since the therapeutic use of antioxidants was not generally proven in clinical studies for asthma, the appropriate selection of asthmatic patients with the potential to benefit from antioxidant therapy needs further investigation. Future research should be focused on the detection of the individual asthmatics, in which application of a particular antioxidant strategy could modify the clinical course and support the standard medication. Moreover, the development of effective antioxidant therapy (new antioxidants or modification in the existing anti-asthmatic molecules with increased antioxidant properties) with complex biological effects could improve the clinical management of asthmatic and allergic patients. Another issue in the context of preventive allergology would be the development of preventive antioxidant strategies that would help in the global burden of allergic diseases.

AUTHOR CONTRIBUTIONS

All the authors equally contributed to the concept of the work, performed literature search, written the text, revised it critically, and approved the final version of the manuscript to be published.

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Asthma and Obesity in Children Are Independently Associated with Airway Dysanapsis

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Background: An increase in the prevalence of overweight and asthma has been observed. Both conditions affect negatively lung function in adults and children. The aim of this study was to analyze the effect of overweight and asthma on lung function in children.

Methods: We designed a case–control study of healthy and asthmatic subjects nested within an epidemiological asthma prevalence study in children between 8 and 16 years of age. The effect of asthma and overweight on lung function was assessed by impulse oscillometry and spirometry obtained at baseline and 10–15 min after salbutamol.

Results: 188 children were recruited, 114 (61%) were asthmatics and 72 (38%) were overweight or obese. Children with asthma and overweight had a higher FVC (+1.16 *z* scores, $p < 0.001$) and higher FEV_1 (+0.79 *z* scores, $p = 0.004$) and lower FEV_1/FVC (−0.54 *z* scores, $p = 0.008$) when compared to healthy controls. Compared to normal weight asthmatics, the overweight had higher FVC (+0.78 *z* scores, $p = 0.005$) and lower FEV_1/FVC (−0.50 *z* scores, $p = 0.007$). In the multivariate analysis, overweight was associated with an increase of 0.71 and 0.44 *z* scores in FVC and FEV_1, respectively, and a reduction in FEV_1/FVC by 0.40 *z* scores ($p < 0.01$ for all). Overweight had no effect on maximal flows and airway resistance at baseline, and this was not modified by inhalation of a bronchodilator. Asthma was also associated with higher post-BD FVC (0.45 *z* scores, $p = 0.012$) and FEV_1 (0.35 *z* scores, $p = 0.034$) but not with FEV_1/FVC and $FEF_{25–75\%}$. Two-way analysis of variance did not detect any interaction between asthma and overweight on lung function variables before or after bronchodilator.

Conclusion: Our results suggest that asthma and overweight are independently associated with airway dysanaptic growth in children which can be further scrutinized using impulse oscillometry. Overweight contributed more to the reduction in FEV_1/FVC than asthma in children without increasing airway resistance. Spirometry specificity and sensitivity for obstructive diseases may be reduced in populations with high prevalence of overweight. Adding impedance oscillometry to spirometry improves our understanding of the ventilatory abnormalities in overweight children.

Keywords: lung function, asthma, children, overweight, obesity

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INTRODUCTION

Asthma and obesity are common chronic diseases that affect the physical, social, and mental health of individuals. Obesity, characterized by the accumulation of body fat, predisposes to several health risks in children (1, 2).

In Brazil, as well as in many countries, the prevalence of obesity in children and adolescents has increased in recent decades (3). Data from Brazilian databases show that 33% of children between 5 and 9 years are currently considered overweight, and 16% of boys and 11% of girls were considered obese (4).

In the same period, there was an increase in the prevalence of asthma in children and adolescents in Brazil (5, 6) with substantial increase in morbidity and health costs (7). Quite consistently, some studies suggest a link between obesity and asthma (8, 9). However, the causal relationship remains controversial and studies exploring the mechanisms involved are required to clarify this association (10–12).

Interestingly, both conditions can negatively affect lung function. In adults, the relationship between overweight/obesity and pulmonary function seems to be well established and most studies detected a reduction in FVC and FEV_1 in subjects with overweight and obesity (13–15). However, in children, the relationship between overweight and lung function is less clear. Systematic reviews have shown a significant reduction in lung volumes (16), and an increased FVC and FEV_1 in overweight and obese children (17, 18). Although several studies did not report lower flows (19, 20), some studies have detected a deleterious effect on lung volumes and flows with increased body mass index (BMI) in children (21–24). This pattern of disproportionate but physiologically normal lung growth characterized by an increase in expired volume that is not accompanied by a comparative increase in maximal flows was termed dysanaptic growth (25). More recently, the pooled analysis of several cohorts from healthy and asthmatic children and adolescents confirmed the association of overweight or obesity to higher baseline FVC, TLC, and FEV_1 and lower maximal flows and FEV_1/FVC (26). These authors also report an association between the presence of dysanapsis and respiratory morbidity in asthmatic children. Still, most studies show results from baseline lung function only, and it is possible that some degree of bronchoconstriction is present, particularly in children with more persistent asthma. Bronchoconstriction, if present, would amplify the mismatch between FVC and FEV_1.

In this context, where the preferred parameter to detect obstruction (i.e., FEV_1/FVC ratio) is similarly affected by either asthma or overweight, the analysis of airway resistance would be an important alternative tool to evaluate whether the observed reduced ratio is an expression of a true obstructive disorder or simply a variant of normality. The aim of this study was to analyze the specific effects of overweight and asthma on lung function in school age children, comparing the results by spirometry and impulse oscillometry, before and after bronchodilation.

MATERIALS AND METHODS

Subjects

We designed a case–control study nested within an epidemiological investigation of asthma prevalence in 8–16 years old children attending public schools in Porto Alegre, Brazil. Briefly, parents responded a respiratory health questionnaire and asthma was defined as a positive answer to all the following questions: "Has your child ever had asthma?" and "Did your child have wheeze in the previous year?" and "Did you use asthma medications in the previous year" (for this question a checklist of specific asthma medications was shown to mothers). Asthmatic children identified by the questionnaire were invited to participate in a lung function study. Classmates without asthma, according to the same questionnaire, were invited to participate in the study as healthy controls. The exclusion criteria were a history of cardiovascular or immune deficiency conditions, presence of other chronic respiratory diseases, recent asthma or rhinitis exacerbations, and diagnosis of an acute respiratory infection or use of oral steroids in the previous month.

Anthropometric Measurements

Children were weighed with a calibrated scale after removing heavy clothing and shoes, with feet in parallel, head in the midline and arms along the body. Weight was recorded to the nearest 0.1 kg. For the measurement of height, a stadiometer (Altura Exata®, Belo Horizonte, Brazil) was used. The measurements of height and weight were used to calculate BMI. *z* scores for BMI and percentiles were calculated using the British 1990 Growth Reference Centiles (27). The classification for normal weight, overweight, and obesity was based on sex- and age-specific cutoff points adopted by the International Obesity Task Force (28). This classification uses centile curves that at age 18 years pass through the widely used BMI cutoff points of 25 and 30 kg/m^2 for overweight and obesity, respectively. For example, in an 11-year-old child, the BMI cutoff point for overweight is 20.55 for males and 20.74 for females.

Asthma Classification

Asthma severity was defined by GINA (29) classification standards, based on the treatment required to achieve control of the symptoms. Asthma control was also assessed by a validated Brazilian Portuguese version of the Childhood Asthma Control Test (c-ACT) (30, 31).

Lung Function Tests

Pulmonary function tests were performed in the Laboratory of Respiratory Physiology by trained professionals, in a quiet environment at room temperature, using an incentive Koko spirometer (Ferraris, USA), and an Impulse Oscillometry System (IOS, CareFusion, Yorba Linda, CA, USA). The order of the lung function tests was IOS, spirometry, bronchodilator, IOS, and spirometry. All evaluations were performed at baseline and 10–15 min after four puffs of salbutamol using a spacer (AeroChamber™), in accordance with the guidelines of the American Thoracic Society and European Respiratory Society (32).

Spirometry

The spirometry was performed in accordance with the guidelines published by the ERS/ATS (32). At least three acceptable and reproducible maximal flow–volume curves were obtained

before and after using a bronchodilator (Salbutamol, 400 μg). Results were transformed to *z* scores according to international reference values (GLI 2012) that adjust for height, age, ethnicity, and sex (33).

Impulse Oscillometry

Measurements were performed and analyzed in accordance with ERS/ATS guidelines (34). Subjects were asked to close their lips around the mouthpiece and cheeks were supported. A nasal clip was used during all the measurements. If artifacts were detected the measurement was repeated until three acceptable curves were obtained. Measurements of respiratory impedance were made at 5 and 20 Hz and the mean values of resistance (R5 and R20) and reactance (X5) from the best three trials were used for the analysis. Results were transformed to *z* scores from reference values (35).

Statistics and Ethics

Demographic data are presented by descriptive statistics. Continuous data are summarized by arithmetic means and SD, or by median and quartiles. Qualitative and quantitative variables were described, respectively, by mean and SD or median/range or frequency/percentage. Groups were compared by the Student's *t*-test. Correlation between BMI and lung function variables was tested by the Pearson test. Two-way analysis of variance (ANOVA) and Bonferroni *post hoc* test were used to compare lung function variables in subgroups stratified by overweight and asthma. The effect of overweight and asthma on lung function was evaluated by multiple linear regressions using lung function expressed as *z* scores as the dependent variables, and overweight and asthma as dichotomic independent variables. All tests were two sided and significance was set at the 95% level, and the analyses were performed on SPSS, version 22.0 (SPSS, Chicago, IL, USA). The study was approved by the Human Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Brazil. Parental written informed consent was obtained from all participants.

RESULTS

The demographics of the subjects enrolled in the study are presented in **Table 1**. Of the 188 children, 97 (52%) were females, 114 (61%) had asthma, 49 (26%) were classified as overweight, and 23 (12%) as obese. The BMI *z* score ranged between −2.2 and 3.95. Due to the small number of obese children, all statistical analyses were performed combining all the 72 overweight and obese subjects. The range for age and height were 8.3–14.8 years and 122–171 cm, respectively. Most of the subjects (127/188, 67%) were classified as white. There were no statistical differences between children with asthma and controls regarding anthropometry and ethnicity. Among 114 asthmatic children, reliable information regarding asthma severity and asthma control were obtained in 101 (89%) and 100 (88%), respectively. Children with asthma were classified as having intermittent 39 (39%) or mild persistent asthma (41, 41%) and the remaining 21 (21%) with persistent moderate asthma. None had severe persistent asthma. Regarding asthma control 89 (89%) had partially or uncontrolled asthma in the previous 12 months.

TABLE 1 | Clinical and demographic characteristics of the sample.

	Healthy controls, *n* = 74 (39%)	Asthma, *n* = 114 (61%)
Ethnicity (% white)	57 (77)	70 (61)
Sex (% male)	33 (45)	58 (51)
Age (years)	11.17 ± 1.19	11.10 ± 1.10
Weight (kg)	43.69 ± 10.60	44.52 ± 13.29
Weight (*z* score)	0.78 ± 1.00	0.86 ± 1.21
Height (cm)	146.97 ± 8.35	146.55 ± 8.46
Height (*z* score)	0.36 ± 0.92	0.33 ± 0.94
Body mass index (BMI)	19.98 ± 3.46	20.49 ± 4.72
BMI (*z* score)	0.86 ± 1.06	0.90 ± 1.37

Baseline and Post-BD Lung Function

The results of lung function tests are presented in **Table 2**, stratified by asthma and by BMI grade. There was no association between asthma severity or asthma control (c-ACT) and BMI or lung function. At baseline, children with asthma had significantly lower $FEF_{25–75\%}$ and FEV_1/FVC and higher R5 and R20 and lower reactance X5 ($p < 0.01$ for all analysis). FEV_1 was similar in asthmatic and healthy controls. Children with asthma had significantly higher bronchodilator response in FEV_1 and $FEF_{25–75\%}$; 6.6 versus 3.3% ($p < 0.01$) and 23.3 versus 12.2% ($p < 0.001$), respectively. Children with and without asthma had similar reduction in R5 and R20 with bronchodilator.

Stratifying the subjects by BMI grade we detected a significant positive effect of overweight on FEV_1, FVC, and a reduction in FEV_1/FVC ($p < 0.01$ for all). These differences when compare to non-overweight children were maintained after bronchodilator. Being overweight was not associated with lower maximal flows or higher airway resistance. Overweight children had comparable bronchodilator response in FEV_1, $FEF_{25–75\%}$, R5, R20, and X5 when compared to normal weight children.

Stratified Analysis of Post-BD Lung Function

To explore the effects detected in the previous analysis the sample was stratified by asthma and overweight in four subgroups: healthy controls, controls overweight, asthma, and asthma overweight. The analysis was performed on lung function post-bronchodilator to minimize the effect of bronchial tone on the results and is shown in **Figure 1**. Results are from two-way ANOVA with *post hoc* Bonferroni pairwise analysis. Children with asthma who were overweight had a FVC that was 1.16 *z* scores higher than healthy controls, equivalent of +362 mL or +13.6% ($p < 0.001$). They also had higher FEV_1 (+0.79 *z* scores, 9.6%, $p = 0.004$) and lower FEV_1/FVC (−0.54 *z* scores, $p = 0.008$) values when compared to healthy controls. Compared to normal weight asthmatics, the overweight had higher FVC (+0.78 *z* scores, $p = 0.005$) and lower FEV1/FVC (−0.50 *z* scores, $p = 0.007$). The

TABLE 2 | Baseline and post-bronchodilator lung function of 188 school age children stratified by asthma and by body mass index (BMI) classification.

	Stratified by asthma		Stratified by BMI classification	
Baseline	**Healthy controls (n = 74)**	**Asthma (n = 114)**	**Normal weight (n = 116)**	**Overweight (n = 72)**
FEV_1	−0.023 ± 0.906	−0.030 ± 1.051	−0.175 ± 0.907	0.211 ± 1.083**
FVC	−0.014 ± 0.898	0.311 ± 1.096*	−0.074 ± 0.889	0.596 ± 1.117**
FEV_1/FVC	−0.093 ± 0.746	−0.560 ± 0.884**	−0.238 ± 0.828	−0.600 ± 0.872**
$FEF_{25–75\%}$	0.006 ± 0.912	−0.485 ± 1.029**	−0.248 ± 0.990	−0.362 ± 1.047
R5	0.467 ± 0.722	0.852 ± 0.794**	0.636 ± 0.786	0.804 ± 0.783
R20	0.296 ± 0.543	0.643 ± 0.646**	0.542 ± 0.632	0.450 ± 0.625
X5	−0.168 ± 0.927	−0.775 ± 1.930**	−0.482 ± 1.760	−0.623 ± 1.419
Post-BD				
FEV_1	0.188 ± 0.786	0.543 ± 1.134*	0.232 ± 0.901	0.682 ± 1.151**
FVC	0.056 ± 0.879	0.529 ± 1.278**	0.075 ± 0.965	0.781 ± 1.312**
FEV_1/FVC	0.155 ± 0.700	−0.005 ± 0.770	0.204 ± 0.709	−0.182 ± 0.747**
$FEF_{25–75\%}$	0.368 ± 0.797	0.309 ± 0.909	0.375 ± 0.803	0.263 ± 0.960
R5	0.067 ± 0.563	0.305 ± 0.639*	0.191 ± 0.676	0.244 ± 0.522
R20	0.112 ± 0.401	0.381 ± 0.495**	0.310 ± 0.478	0.222 ± 0.475
X5	0.364 ± 0.746	−0.026 ± 1.949	0.066 ± 1.925	0.223 ± 0.859

All variables expressed as z scores.
FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; $FEF_{25–75\%}$, forced expiratory flow at 25–75% of FVC; R5, resistance at 5 Hz; R20, resistance at 5 Hz; X5, reactance at 5 Hz.
Values expressed as mean ± SD.
$p < 0.05$.
**$p < 0.01$.*

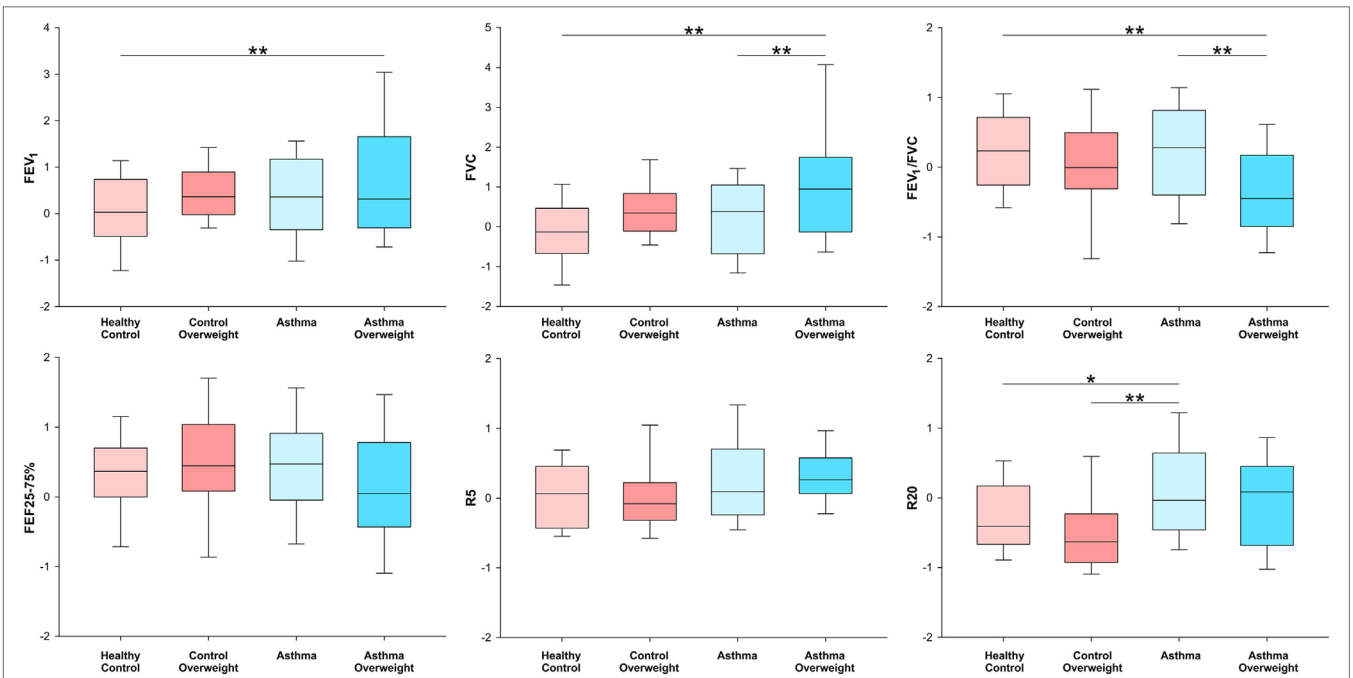

FIGURE 1 | Box-plots showing median and interquartile ranges for FEV_1, FVC FEV_1/FVC, $FEF_{25–75\%}$, R5, and R20 stratified in four subgroups by the presence of overweight and asthma. *$p < 0.05$ and **$p < 0.01$ for analysis of variance with *post hoc* Bonferroni pairwise analysis.

magnitude of the effect of overweight in asthmatic subjects, after adjusting for height, age, and sex was +234 mL (8.7%, $p < 0.01$) in FVC and a reduction of 3.4% in FEV_1/FVC ($p < 0.01$) when compared to normal weight asthmatic children. Normal weight asthmatics had higher post-BD R20 compared to healthy controls (+0.27 z scores, $p = 0.013$) and overweight controls (+0.38 z scores, $p = 0.003$).

Multivariate Analysis of the Effect of Overweight and Asthma on Lung Function

Figure 2 and **Table 3** show the magnitude of the effect of overweight and asthma on lung function estimated by multivariable linear regression, with healthy controls as the reference group. The presence of overweight was associated with an increase of 0.7 z scores in FVC, equivalent to 210 mL or 7.5% ($p < 0.001$).

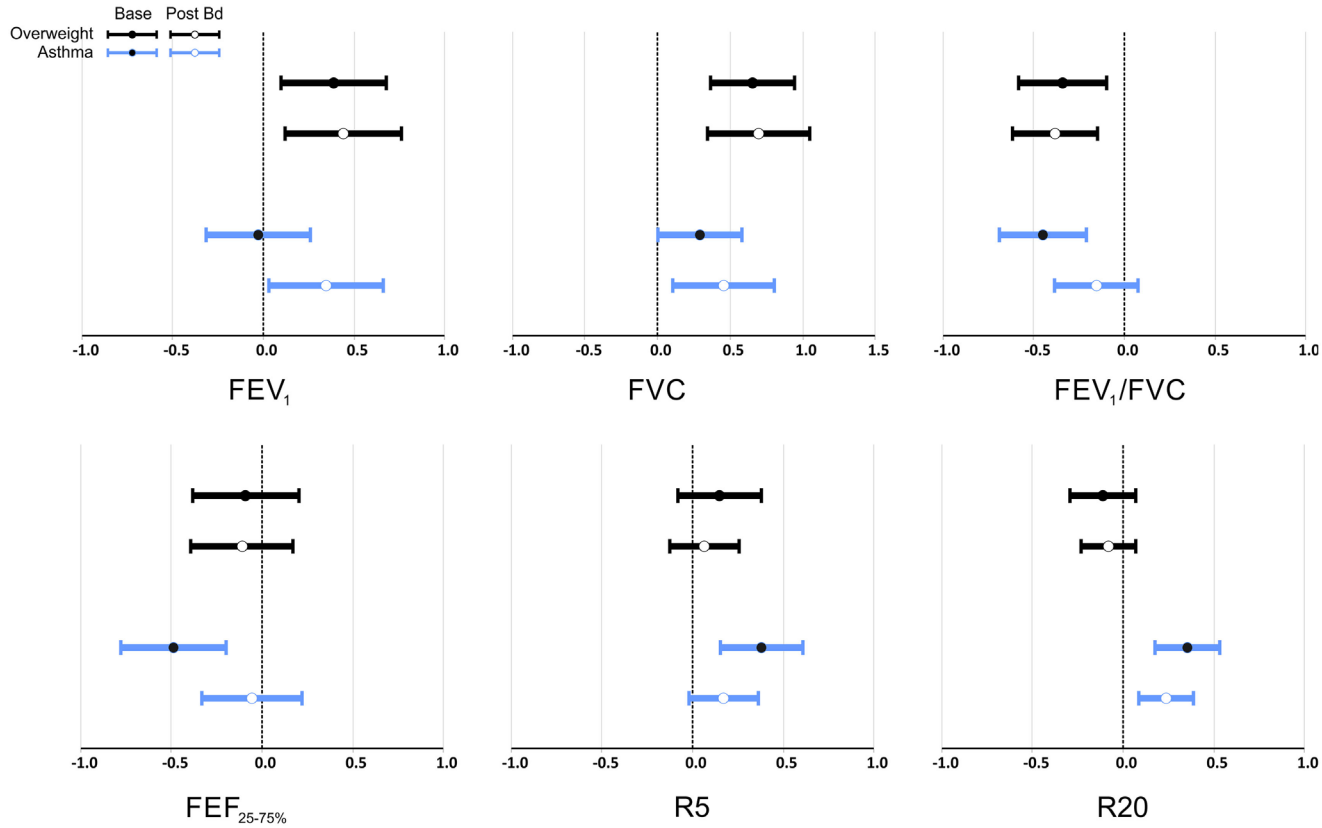

FIGURE 2 | Multivariate analysis of the effect of overweight and asthma on lung function pre- and post-bronchodilator expressed as *z* scores as the dependent variables, and overweight and asthma as dichotomic independent variables. Data is presented as estimated mean and 95% confidence interval of the difference to healthy controls. FEV_1, FVC, FEV_1/FVC, and $FEF_{25-75\%}$ expressed in *z* scores (33) that adjusts for height, age, sex, and ethnicity; R5 and R20 expressed in *z* scores (35) that adjusts for sex and height.

FEV_1 was also significantly higher with overweight, but the magnitude of the difference was smaller in comparison to FVC: +0.39 at baseline and +0.44 *z* scores post-BD ($p < 0.01$ for both). In addition, overweight had a significantly impact on pre- and post-BD FEV_1/FVC ($p < 0.001$) reducing the ratio by 0.34 and 0.40 *z* scores, respectively. Overweight had no detectable effect on maximal flows and airway resistance at baseline and this was not modified by inhalation of a bronchodilator.

Asthma was also associated with significantly higher FVC, 0.29 *z* scores ($p = 0.047$) at baseline, and 0.45 *z* scores ($p = 0.012$) after bronchodilator. After bronchodilator FEV_1 was 0.35 *z* scores higher in asthmatics ($p = 0.034$), but not at baseline.

At baseline, asthma was associated with significantly lower FEV_1/FVC (−0.45 *z* scores, $p < 0.001$), $FEF_{25-75\%}$ (−0.40 *z* scores, $p = 0.001$), and higher R5 and R20 (0.38 and 0.35 *z* scores, $p < 0.001$ for both). Reactance was also lower at baseline in children with asthma (−0.6 *z* scores, $p = 0.014$); this difference was reduced and no longer significant after bronchodilator as well as the differences of FEV_1/FVC, and $FEF_{25-75\%}$. After bronchodilator, children with asthma maintained a higher R20 (0.24 *z* scores, $p = 0.002$). Two-way ANOVA did not detect any interaction between asthma and overweight on lung function variables before or after bronchodilator.

DISCUSSION

Our results suggest that both asthma and overweight are associated with airway dysanaptic growth in children which can be further scrutinized using impulse oscilometry. The assessment of lung function by spirometry and forced oscillations, at baseline and after bronchodilator, allowed the analysis of lung growth and airway growth separately and without the confounding influence of bronchial tone. Our results point toward an independent positive effect of BMI and asthma on FVC and FEV_1, with a negative effect on FEV_1/FVC in school age children. However, the discrepancy promoted by overweight and expressed by a lower FEV_1/FVC is not associated with smaller airways and is also not modified by bronchodilator. Interestingly, after reducing bronchial tone, it becomes clear that the contribution of overweight is substantially bigger than the contribution of asthma in lowering the ratio FEV_1/FVC (−0.40 versus −0.13 *z* scores, respectively). One could interpret these findings as additional evidence that overweight promotes a lung growth that is different from normal, with a disproportional increase in lung parenchyma (FVC) in comparison to airway (maximal flows and airway resistance). The same pattern of large lungs is seen in swimmers (36), divers (37), and other such athletes, as well as subjects living in high

TABLE 3 | Multivariate analysis of the effect of overweight and asthma on lung function.

	Baseline						Post-BD					
FEV_1	**Estimated difference**	**SE**	**Lower bound**	**Upper bound**	***p*-Value**	**R^2**	**Estimated difference**	**SE**	**Lower bound**	**Upper bound**	***p*-Value**	**R^2**
Overweight	0.387	0.147	0.097	0.678	0.009	0.036	0.442	0.164	0.119	0.765	0.008	0.073
Asthma	−0.027	0.147	−0.316	0.262	0.855		0.346	0.162	0.026	0.667	0.034	
FVC												
Overweight	0.655	0.146	0.366	0.944	<0.001	0.119	0.706	0.180	0.352	1.061	<0.001	0.127
Asthma	0.291	0.146	0.004	0.578	0.047		0.450	0.178	0.099	0.801	0.012	
FEV1/FVC												
Overweight	−0.339	0.123	−0.582	−0.097	0.006	0.107	−0.400	0.118	−0.634	−0.167	0.001	0.078
Asthma	−0.449	0.122	−0.690	−0.208	<0.001		−0.135	0.117	−0.367	0.096	0.250	
$FEF_{2575\%}$												
Overweight	−0.089	0.148	−0.381	0.204	0.550	0.058	−0.127	0.143	−0.409	0.155	0.375	0.006
Asthma	−0.486	0.148	−0.777	−0.195	0.001		−0.041	0.141	−0.320	0.239	0.774	
R5												
Overweight	0.148	0.115	−0.078	0.375	0.198	0.066	0.066	0.096	−0.125	0.256	0.497	0.024
Asthma	0.378	0.114	0.152	0.604	0.001		0.171	0.095	−0.017	0.360	0.075	
R20												
Overweight	−0.110	0.091	−0.290	0.070	0.229	0.080	−0.079	0.076	−0.229	0.070	0.297	0.066
Asthma	0.352	0.091	0.173	0.531	<0.001		0.238	0.075	0.089	0.386	0.002	
X5												
Overweight	−0.110	0.243	−0.589	0.369	0.652	0.034	0.207	0.272	−0.330	0.744	0.448	0.019
Asthma	−0.602	0.242	−1.078	−0.125	0.014		−0.424	0.269	−0.956	0.108	0.118	

All variables expressed as z scores.

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; $FEF_{25–75\%}$, forced expiratory flow at 25–75% of FVC; R5, resistance at 5 Hz; R20, resistance at 5 Hz; X5, reactance at 5 Hz.

altitudes (38), and these are findings that have no association with respiratory disease. These observations recommend caution to label a lower FEV_1/FVC as a true obstructive pattern in overweight children. Another implication is that a lower FEV_1/FVC observed in overweight asthmatic children compared to normal weight asthmatic does not necessarily represent worse clinical respiratory disease.

This is the first study that addresses the impact of overweight and asthma in children comparing two methods that elicit different aspects of lung function, such as spirometry and forced oscillations, before and after bronchodilator. Our findings are in line with studies that reported increased FVC in overweight children (39) but contrast with findings in adults where TLC, FVC, and FEV_1 were typically reduced (40). However, the effect of weight on lung function seems to be modified by the duration and severity of obesity (41). In addition, an inverse U-shaped association between BMI and FVC in children have been proposed (42). The most plausible explanation for the absence of these ventilatory abnormalities in our sample of school age children is the small number of subjects with obesity.

The finding of increased FVC in children with asthma is interesting and has been reported previously (43, 44). The magnitude of the increase in baseline FVC in our study is similar to that reported for boys in an older study (44) by the order of 3%, and this effect was smaller than the 6.6% reported by Strunk et al. (43). The post-bronchodilator FVC was not reported in these studies, precluding any comparison. Since this increase in FVC is not observed in overweight adults, this finding suggests that it is a developmental abnormality that normalizes, or even reverses by the end of somatic growth as suggested by studies in young adults. Our observation of increased post-BD FEV_1 is intriguing since most studies have reported lower values in children with asthma when compared to healthy controls (45, 46). Our interpretation is that the potential reduction in airway caliber due to inflammation and remodeling in asthma was offset by the increase in FVC. In our sample, most of the children with asthma were classified as intermittent or mild persistent, unlikely to have significant and persistent reduction in maximal expiratory flows.

Our results do not establish that asthma and overweight as either the cause or the effect of the increase in lung volume, only its cooccurrence. A follow-up study would provide a more accurate description of these associations. Other factors associated with overweight may be relevant for the observed uneven, dysanaptic lung growth. A higher BMI during childhood is associated with higher inspiratory capacity (19), maximal inspiratory pressure (47), and reduced leg length-to-height ratio (48), all presenting some potential to promote disproportional increases in FVC. These factors are not considered in the current predicted equations and could contribute for underestimation of the FVC.

The results of our study suggest that spirometry, which is widely used in the detection and management of asthma, may have lower specificity and sensitivity for asthma in a predominantly overweight society. The observed 13.6% increase in FVC, 7.6% in FEV_1, and the decrease of 3.5% in FEV_1/FVC in overweight asthmatics is clinically relevant and would have an impact in spirometry, both at diagnostic level and severity classification.

With the increasing prevalence of obesity in children, we will see not only an excess of diagnoses of obstructive disease due to low FEV_1/FVC but also a delay to recognize a significant reduction in FEV_1 and FVC due to the use of reference equations not adjusted for body weight. Our data suggest that airway resistance is not increased by overweight and its measurement could be more reliable to assess obstructive diseases in children. Increase in airway resistance was observed in overweight and obese adult subjects (49) but not in preschool children (50). The only study in children that reported an increase in airway resistance with increase in BMI was obtained in children with a history of severe bronchiolitis in the first year of life (51). Our results confirm previous review that airway resistance by IOS can add valuable information in the assessment of respiratory diseases in children (52).

Our study has some limitations that deserve mention. First, the small number of subjects of the study and particularly, the small number of obese and moderate asthmatics subjects enrolled may have reduced power to independently detect the impact of both conditions on lung function. This narrow spectrum was expected from a community-based sample, and it did somehow limit the possible explorations of data in our exploratory study. External validation of the findings, particularly the lack of association of lung function and overweight with clinical outcomes in the asthma group, are needed. In addition, other important variables could not be included in the study; perhaps the most relevant is total lung capacity and residual volume, assessed by plethysmography.

In conclusion, our study demonstrated that both overweight and asthma independently promote an increase in FVC and FEV_1 with a resulting decrease in FEV_1/FVC. The results support the concept that overweight promotes an unequal lung growth and that its magnitude is such that it is likely clinically relevant: FVC is 13.6% higher in children with asthma and overweight when compared to healthy controls and 8.7% higher when compared to children with asthma and normal weight. Overweight contributed more than asthma to the deficit in FEV_1/FVC without increasing airway resistance. Our results suggest that the effect of overweight is mostly in increasing lung size without any perceptible effect on airway resistance; the effect of asthma is both an increase in lung size and in airway resistance. Our results suggest that spirometry specificity and sensitivity for obstructive diseases may be reduced in populations with high overweight and obesity prevalence. Adding measurements of airway impedance by impulse oscillometry to spirometry may improve our understanding of the true clinically relevance of ventilatory abnormalities in overweight children.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of "Comitê de Ética em Pesquisa – CEP" with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the "Comitê de Ética em Pesquisa da PontifÍcia Universidade Católica do Rio Grande do Sul."

AUTHOR CONTRIBUTIONS

MJ and RS designed the study. RS, CR, EI, RM, PP, and MF oversaw all clinical aspects of the study (IRB approval, consenting, sample collection). MJ, CR, JH-F, and EI obtained lung function. CR, RM, and EI developed the cloud-based database. MJ, MF, and CR analyzed the data. All authors wrote and reviewed the manuscript.

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REVIEW
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Mechanisms Mediating Pediatric Severe Asthma and Potential Novel Therapies

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Although a rare disease, severe therapy-resistant asthma in children is a cause of significant morbidity and results in utilization of approximately 50% of health-care resources for asthma. Improving control for children with severe asthma is, therefore, an urgent unmet clinical need. As a group, children with severe asthma have severe and multiple allergies, steroid resistant airway eosinophilia, and significant structural changes of the airway wall (airway remodeling). Omalizumab is currently the only add-on therapy that is licensed for use in children with severe asthma. However, limitations of its use include ineligibility for approximately one-third of patients because of serum IgE levels outside the recommended range and lack of clinical efficacy in a further one-third. Pediatric severe asthma is thus markedly heterogeneous, but our current understanding of the different mechanisms underpinning various phenotypes is very limited. We know that there are distinctions between the factors that drive pediatric and adult disease since pediatric disease develops in the context of a maturing immune system and during lung growth and development. This review summarizes the current data that give insight into the pathophysiology of pediatric severe asthma and will highlight potential targets for novel therapies. It is apparent that in order to identify novel treatments for pediatric severe asthma, the challenge of undertaking mechanistic studies using age appropriate experimental models and airway samples from children needs to be accepted to allow a targeted approach of personalized medicine to be achieved.

Keywords: severe therapy-resistant asthma, mechanisms, eosinophils, innate cytokines, therapies, remodeling, children

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INTRODUCTION

Severe asthma is thought to be rare in children, affecting approximately 2–5% (1) of all patients; however, we have little idea of the actual size of the problem (2). The proportion of health-care resources utilized by patients with severe disease is disproportionate to prevalence, whereby, they use up to 50% of all health-care costs for asthma (2). Improving control for patients with severe asthma is, therefore, a significant unmet clinical need. Pediatric asthma is a heterogeneous disease, and within that, severe asthma is also recognized to be heterogeneous with numerous clinical, pathological,

and physiological phenotypes (3). It is apparent that, in order to identify novel treatments for pediatric severe asthma, the mechanisms that mediate the disease in children need to be investigated so that a targeted approach of personalized medicine can be achieved. However, mechanistic data in childhood studies are rare, partly because obtaining airway samples from children is a challenge and also because there is a reluctance to generate age-specific experimental models. This review will summarize the current data that give insight into the pathophysiology of pediatric severe asthma and will highlight potential targets for novel therapies (**Figure 1**). Avenues for future research and approaches that will enable mechanistic studies to be undertaken more readily in children will also be discussed.

DIAGNOSIS OF SEVERE ASTHMA IN CHILDREN

In order to accurately identify the mechanisms mediating severe pediatric asthma, it is essential that the diagnosis is correct. Objective measures supporting the key pathophysiological features of asthma including reversible airflow obstruction, airway hyperresponsiveness, chronic airway inflammation, and the presence of confirmed wheeze and breathlessness are essential in confirming the diagnosis. The ERS/ATS guidelines for the diagnosis and management of severe asthma include children (4) and must be adhered to in the assessment of these patients. In particular, the guidelines stipulate it is important that children

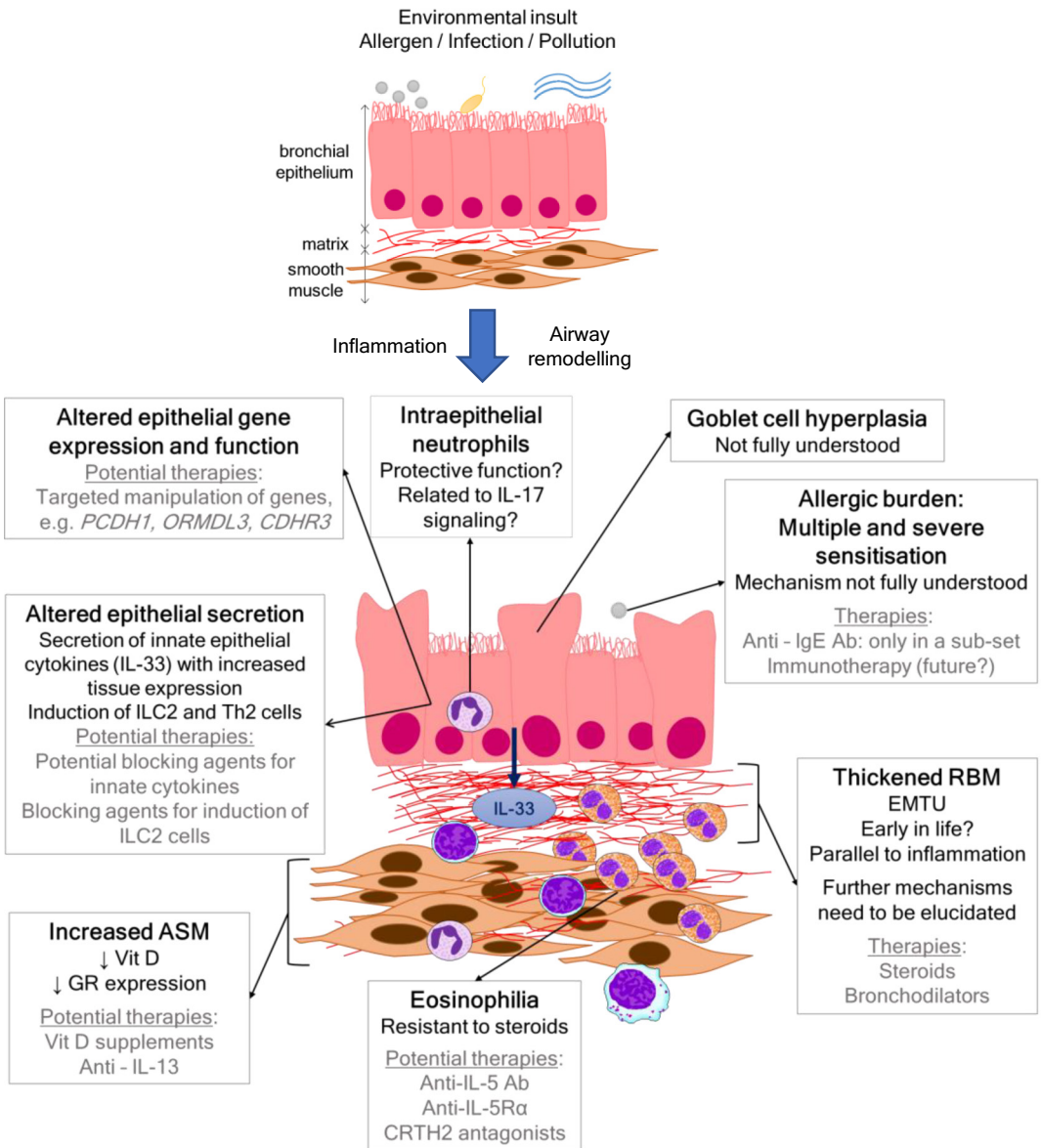

FIGURE 1 | Scheme showing airway wall features, mechanisms, and current and/or potential therapies in pediatric severe asthma.

on maximal maintenance treatment and poor control are not automatically labeled as having severe asthma. The umbrella term used to describe children who have poor control despite maximal prescribed treatment [GINA steps 4/5, or maintenance inhaled steroids ≥800 mcg daily budesonide and long-acting beta-agonists (LABAs)] is problematic severe asthma (5). Within this is a subgroup with difficult asthma, in whom, underlying modifiable factors such as poor adherence to treatment, explain persistent symptoms and poor control (6). After modifiable factors have been optimized and addressed (7), there remains a group of children with good adherence and persistent poor control, these are patients with true severe asthma (5), and will form the focus of the data discussed in this review.

PATHOLOGY OF SEVERE ASTHMA IN CHILDREN: INFLAMMATORY PHENOTYPES

Airway Eosinophilia: Utility As a Therapeutic Target?

Studies that have included children with true severe asthma have shown that the airway pathology is characterized by luminal [bronchoalveolar lavage (BAL)] and tissue (endobronchial biopsy) eosinophilia (8). This eosinophilic airway inflammation persists despite systemic steroids in the majority of patients (9). Whether there is an association between airway eosinophil number and atopy (8) or not (10) is still uncertain. Although there is no disputing the steroid resistant eosinophilia that characterizes pediatric severe asthma (8), we have little idea of the functional impact of eosinophilia on disease manifestation. There is little clinical correlation between airway eosinophilia and symptom control or lung function. Murine experimental studies have shown that eosinophil-deficient mice have a similar phenotype to wild-type mice and, therefore, eosinophils play little role in the development of house dust mite (HDM)-induced allergic immunity (11) or airway hyperresponsiveness (12). It, therefore, appears that the eosinophilia does not contribute to altered lung function or daily symptom control. However, targeting eosinophilic inflammation using a monoclonal antibody to interleukin (IL)-5 has shown a significant reduction in exacerbations in adults with severe asthma (13). But interestingly, the subgroup that benefited most had both eosinophilic disease and frequent exacerbations (14). This suggests a specific impact of eosinophils in promoting exacerbations, but has not been confirmed mechanistically. Few studies that have targeted airway eosinophils have been undertaken in children with severe asthma. Disappointingly, one pediatric study that compared the effect of titrating maintenance inhaled steroid therapy according to sputum eosinophils or to clinical guidelines and symptoms-based management showed no benefit of the eosinophil-guided strategy in reducing exacerbations (15). This was in contrast to a prior study undertaken in adults (16). A possible explanation for the lack of effect in children may be that there is marked within patient variability in airway eosinophils over time which is independent of clinical disease status or changes in treatment (17). A systematic review of studies in children, which have used exhaled nitric oxide as a non-invasive surrogate marker for eosinophilic inflammation to guide treatment have shown some benefit in reducing exacerbations, but no effect on daily symptom control or maintenance treatment (18). The current evidence suggests that targeting airway eosinophils is most likely to be successful in children with a frequently exacerbating phenotype and may be less effective in those with persistent symptoms, and there is unlikely to be any benefit on improving lung function. To date, trials of the efficacy of monoclonal antibodies that target either IL-5 or its receptor have not been undertaken in children with severe asthma, but given the prevalence of airway eosinophilia in children, this seems an obvious avenue to pursue. Mepolizumab is an anti-IL-5 humanized monoclonal antibody that reduces circulating eosinophils. In adults and adolescents (aged 12–17 years), exacerbations decreased without improvement in FEV_1 nor quality of life with Mepolizumab (13). However, only a very small number of adolescents were included. Studies specifically assessing efficacy in children with severe asthma have not yet been undertaken. However, no differences in adverse effects were observed in the adolescent group enrolled in the phase 3 trial compared to the overall population (13, 19). An ongoing clinical trial (20) studying the pharmacological properties of subcutaneous administration of Mepolizumab in children aged 6–11 years with severe eosinophilic asthma will help to determine safety in younger children. Recently, another anti-IL-5 humanized monoclonal antibody, reslizumab, has been approved by the European Medicines Agency as add-on therapy in adults with uncontrolled severe eosinophilic asthma (blood eosinophil count ≥400 cells/μl). It decreased exacerbations, improved lung function and quality life (21). It will be assessed in patients aged 12 years and older with severe eosinophilic asthma (22). Benralizumab targets the receptor for IL-5 (IL-5Rα) and in a phase III study in patients aged 12 years and above with severe uncontrolled asthma, it was well tolerated and depleted blood eosinophils, reduced exacerbation rates, and improved lung function (23). However, none of the trials that have been undertaken to assess the efficacy of blocking IL-5 in severe asthma have assessed efficacy in children alone. Therefore, at present, we do not know whether the data from the studies in adults can be extrapolated to pediatric severe disease. This is an obvious gap in our knowledge that needs to be addressed especially because of the marked pulmonary eosinophilia that characterizes pediatric severe asthma.

An important point to consider when defining biomarkers that may help to identify patients most likely to benefit from treatments that target eosinophilic inflammation is the relationship between peripheral blood eosinophils and airway eosinophils. When adherence to maintenance high-dose inhaled steroids has been optimized, and those with true severe asthma have been identified, there may be little relationship between compartments, whereby, elevated airway eosinophils may persist despite a normal blood eosinophil count (24). In adult studies, a cut-off of 0.3×10^9 cell/l (25) for blood eosinophils gave approximately 75% sensitivity and specificity for sputum eosinophilia. Therefore, there is no single peripheral or non-invasive biomarker that can be used to represent airway eosinophilia, and a composite measure is likely to be most helpful.

DENDRITIC CELLS (DCs)

The antigen-presenting cells of the lung, DCs, capture allergens reaching the airway epithelium, process them into peptides, and load them onto the major histocompatibility complexes class II. In contrast to gut and skin, airways are immunologically immature at the time of birth (26), and DCs are not present in the airways at birth but stimuli such as microbes or pollutants can activate pattern-recognition receptors (PRRs) on epithelial cells that produce cytokines and chemokines attracting immature pre-DCs (27, 28). Activation of epithelial PRRs also results in release of cytokines, such as IL-25 (29), IL-33 (30), or thymic stromal lymphopoietin (TSLP) (31), and danger signals, such as uric acid (32), which further activate DCs. Activated DCs migrate to draining lymph nodes where along with costimulatory molecules will bind and activate T cell receptors (TCRs) on the surface of naïve CD4 T cells (33).

Two subpopulations of DCs have been identified: myeloid or conventional DCs (cDCs) or DC1 (CD11c+ CD123dim+ in cytometric analysis) and lymphoid or plasmacytoid DCs (pDCs) or DC2 (CD11c− CD123high+) (34). Apart from the cDCs, mouse studies have reported that a different subset of DCs, monocyte-derived DCs, orchestrate the pro-inflammatory environment in the airways by secreting chemokines that attract inflammatory cells during allergen challenge (35). Both mDC and pDCs take up inhaled allergen and present it to T cells in animal models of allergic airways disease (36). Depletion of pDCs during allergen challenge resulted in allergic airways disease whereas adoptive transfer of pDCs before sensitization prevented disease, suggesting a protective role of pDCs that could be applied in clinic (36).

Activated DCs can form tight junctions with the airway epithelium and detect inhaled allergens without disturbing the epithelial barrier (37) and have upregulated chemokine receptors and costimulatory molecules so they have more capacity to migrate to the lymph nodes and stimulate naïve T cells (38). In a study including 50 atopic children and 40 healthy controls, serum OX40L levels were higher in children experiencing acute severe asthma exacerbations and during stable severe persistent asthma compared to mild/moderate exacerbations and mild or moderate persistent asthma, respectively, and this correlated positively with blood eosinophil counts (39). It has been reported that sputum from asthmatic children treated with inhaled steroids contain increased airway DCs with reduced expression of the costimulator CD86, suggesting that either asthma or steroid therapy may impair DC trafficking and/or maturation reducing the pro-inflammatory responses (40). Flow cytometry analysis of DCs in cord blood of neonates from allergic and non-allergic parents and in peripheral blood of allergic and healthy children has allowed the identification of a new DC population CD11c− CD123dim+ named "less differentiated" DCs (ldDCs). This population was the predominant DC population in cord blood and decreased with age. It was also increased in children with atopic dermatitis whereas was decreased in asthmatics receiving high-dose inhaled corticosteroids. So it was proposed that ldDC could be involved in the severity of allergy/asthma. No differences in DC populations were found in cord blood from neonates with low versus high risk for allergic disorders (41). In contrast, blood pDCs were increased in both atopic and non-atopic asthmatic adults (42).

Although targeting DCs may be an attractive approach in the treatment of asthma, there are currently no specific therapies, either from experimental studies, or being tested in clinical trials that target DC numbers or function. It is still necessary to understand the complex cellular and molecular pathways involved in altering pDC function in pediatric asthma before therapeutic applications can be considered.

AIRWAY EPITHELIUM: INTERACTIONS BETWEEN INNATE AND ADAPTIVE IMMUNITY IN PEDIATRIC SEVERE ASTHMA

The airway epithelium is the first site of contact between the host and environment. Allergens, viruses, and other environmental exposures directly stimulate and interact with the epithelium. It has a role not only as a physical barrier but also contributes to the development of the immune response and maintenance of inflammation. Therefore, it is not surprising that the airway epithelial barrier is altered in asthma (33, 43). In recent years, genetic studies of bronchial epithelial cells have discovered several genes, such as protocadherin 1 (*PCDH1)* (44), *periostin (POSTN)*, *serpin family B member 2 (SERPINB2)*, and *chloride channel accessory 1 (CLCA1)* (45), associated with asthma phenotypes. Of these, *PCDH1* was very specifically associated with childhood asthma (46). Consequently, it has been suggested that many of the different pathological mechanisms underlying asthma phenotypes may originate in the airway epithelium (47).

Allergens, microorganisms, and allergen-derived protease activities not only activate DCs but also airway epithelial cells through the activation of toll-like receptors, which leads to secretion of cytokines and danger signals. These signals can be propagated through the dysregulation of the epithelial–mesenchymal trophic unit (EMTU), which is the bidirectional interaction between epithelium and mesenchyme involving the release of growth factors and cytokines, resulting in the amplification of inflammation and structural changes (remodeling) (43, 48). It is thought that the drivers of remodeling may be recruited CD34+ fibrocytes located at areas of collagen deposition and in BAL acting as myofibroblasts (49, 50), but it has also been proposed that they could stimulate the differentiation of resident mesenchymal cells (50). Epithelial cells can also transdifferentiate into fibroblasts/myofibroblasts by epithelial–mesenchymal transition (51), but this has not been proven in asthma (52).

INNATE EPITHELIAL CYTOKINES AND TYPE 2 LYMPHOID CELLS IN PEDIATRIC SEVERE ASTHMA

Innate immunity is being increasingly recognized as being an equal contributor to asthma pathogenesis as adaptive immunity. Upon exposure to environmental stimuli (allergens, infection, and pollution), the activated epithelium releases cytokines, such

as IL-25, IL-33, or TSLP and danger signals, such as uric acid, which contribute to the onset of innate immune mechanisms resulting in disease initiation and propagation. DCs (as discussed above), mast cells (MCs) (53), type 2 innate lymphoid cells (ILC2) (54), and basophils (55) are all induced by the release of the innate epithelial cytokines. Consequently, targeting IL-25, IL-33, and TSLP is an interesting therapeutic approach for severe asthma and is actively being pursued.

Specifically, in pediatric severe asthma, we have shown increased expression of the innate epithelial cytokine IL-33 in the bronchial tissue and an association with increased levels and both airway remodeling and steroid resistance (56). More recently, we have shown that a specific sub-phenotype of patients with severe asthma and fungal sensitization have even higher levels of IL-33 in both BAL and biopsy (57). It is now also apparent that the downstream effector cells that are induced by IL-33, ILC2 cells are increased in the airways of children with severe asthma compared to non-asthmatic controls (58). Interestingly, a specific association between type 2 ILCs and severe asthma has also been demonstrated in adults (59). The ILCs present in BAL from pediatric patients were characterized by lineage negative markers (absence of the T cell antigens) and presence of the type 2 receptor CRTH2. In contrast to Th2 cells, they were a rare cell population, making up only 0.2% of lymphoid cells. Of note, however, both cell types did express CRTH2. Although increased numbers of both ILC2s and Th2 cells have been demonstrated in pediatric severe asthma, their functional and clinical relevance remains unknown, since there were no clear correlations between cell numbers in BAL and symptoms or lung function (58).

Asthma has been typically considered a Th2 disorder since the predominant inflammatory phenotype is eosinophilic as observed in BAL and endobronchial biopsies from children with severe disease (8). Although studies have investigated T lymphocytes in peripheral blood from children, data relating to the airway inflammatory phenotype have been lacking. We have recently shown that children with severe asthma have increased numbers of CD4+ T cells in BAL compared to non-asthmatic controls, and that these cells make up approximately 40% of all airway CD3+ lymphocytes and express the CRTH2 receptor (58). Interestingly, when we had previously quantified CD4+ cells in endobronchial biopsy, numbers were not significantly different to non-asthmatic controls (8), suggesting that there may be differences in the luminal and tissue compartments.

Mechanistically, naïve T cells in draining lymph nodes differentiate to Th2 cells *via* IL-4-mediated activation of STAT6 and GATA3. Th2 cells migrate to the airway mucosa and secrete the Th2 cytokines IL4, IL-5, and IL-13. But, detection of Th2 cytokines in severe asthmatic children remains controversial (3, 8). It has been generally accepted that IL-5 mediates the recruitment of eosinophils by the expression of epithelium-derived chemokines named eotaxins [CC-chemokine ligand 11, CCL24, and CCL26]. It also promotes bone marrow development and mobilization of eosinophil precursors. Children with STRA have airway remodeling and eosinophilic inflammation, but in the absence of detectable levels of Th2 cytokines, without neutrophilia nor MC infiltration (8).

MAST CELLS

Cross-linking of FcεRI following MC exposure to allergen can result in MC activation, which is characterized by degranulation and production/secretion of preformed histamine, lipid mediators, enzymes (proteases, hydrolases, cathepsin G, and carboxypeptidase), and cytokines (including tumor necrosis factor, IL-4, IL-5, IL-6, IL-13, 3 CCL3, IL-33, and granulocyte-macrophage colony-stimulating factor) (60). The arachidonic acid-derived mediators are prostaglandin (PG)D_2, leukotriene (LT) C_4, and platelet-activating factor, which can induce bronchoconstriction, mucus secretion, and edema (61). The wide repertoire of cytokines has several effects ranging from IgE synthesis to neutrophil and eosinophil activation to fibroblast growth. Similarly, the secreted enzymes have wide effects, such as degradation of allergens, enhanced airway smooth muscle (ASM) contractility, and enhanced IL-33 activity (60). But the role of MCs in asthma is not based solely on its products but also on their strategic location. Whereas in healthy airways MCs are predominantly located near blood vessels and within the lamina propria (62), MCs tend to relocate to the airway epithelium (63), submucosa (64), submucosal glands (65), and ASM (66) in asthma. However, these data are all from adult studies. The contribution of MCs to pediatric asthma is less well known. Several studies have reported that MC frequency was similar in the subepithelium (8, 10, 67, 68) and in the ASM (8, 69) between wheezing or asthmatic children and controls. In a recent study in biopsies of severe asthmatic children, it was reported that ASM MC numbers were associated with the number of severe exacerbations and eosinophilia, but not with remodeling or lung function (70).

Despite the apparent importance of MCs in the pathology of severe asthma, to date, therapies that are MC stabilizers such as cromolyn sodium and nedocromil that inhibit MC degranulation have proven very disappointing in the clinic. MC predominance in the airway submucosa and epithelium has been associated with severe asthma in adults because of increases in PGD_2 levels, which is produced mainly by MCs but also Th2 cells, macrophages, and eosinophils (64). PGD_2 binds to smooth muscle cells leading to vasodilatation and bronchoconstriction and can also bind the chemoattractant receptor-homologous molecule expressed on TH2 lymphocytes (CRTH2) inducing Th2 cytokine production and further promoting activation of MCs and PGD_2 production in asthma (64).

PGD_2 RECEPTOR 2 (CRTH2) ANTAGONISTS IN SEVERE ASTHMA: A NOVEL THERAPEUTIC APPROACH

A recent novel class of drug that is undergoing phase II studies in adults and seems attractive for pediatric severe asthma is the CRTH2 antagonists (71). CRTH2 is present on MCs, but also on eosinophils, Th2 cells, and ILC2. Given the overwhelming evidence that pediatric severe asthma is associated with severe atopy, is eosinophilic, and associated with significantly increased numbers airway of ILC2, the strategy of blocking the CRTH2 receptor is very appealing. A randomized, parallel double blind

placebo-controlled trial of a CRTH2 antagonist in adults with persistent, moderate-to-severe asthma, and an elevated sputum eosinophil count showed a reduction in sputum eosinophils in the active group, and no associated significant adverse effects (72). Another potential advantage of these compounds is that they can be administered orally. However, clinical efficacy is yet to be proven. Small phase II trials have suggested efficacy in achieving an improvement in symptoms and lung function (73), but they did not target the population that had eosinophilic or type 2 high diseases. It is increasingly apparent that as more and more add-on therapies become available and may potentially be utilized in children, the need to identify the right drug for the right patient phenotype will be essential (74).

REGULATORY T CELLS

After TCR engagement, activation of T cells can be suppressed by regulatory CD4+CD25+ T cells (T_{reg}) (75). Therefore, T_{reg} cells can control allergen-specific immune responses and low numbers or dysfunctional T_{reg} cells may contribute to allergic disease and asthma. However, few studies have investigated the role of T_{reg} cells in pediatric severe asthma. Low T_{reg} cells in blood and sputum as well as impaired suppressive function during exacerbations have been reported in severe refractory asthmatic adults compared to healthy controls (76). In contrast, another small study comparing numbers of T_{reg} cells in BAL from moderate to severe asthmatic adults compared to mild asthmatics reported that T_{reg} cells were increased in the severe group (77). In asthmatic children, T_{reg} cells were lower in BAL (78) and blood (79). Peripheral T_{reg} cell levels were lower compared to healthy controls, especially in the acute phase and in the severe group. Th1/Th2 ratio correlated positively with T_{reg} cells and negatively with disease severity (79).

T_{reg} cells can act through perforin-mediated cytolysis as well as IL-10 and TGF-β. IL-10 is a potent anti-inflammatory cytokine expressed by several cell types, including T cell subpopulations. IL-10 suppresses the production of inflammatory cytokines, the DC-mediated antigen presentation to T cells as well as the function of MCs and eosinophils (80). In addition, IL-10 inhibits IgE and favors IgG4 to IgE (81). Lower levels of IL-10 are produced by macrophages and mononuclear cells from asthmatics (82, 83). Defective IL-10 expression has been associated with increased steroid resistance in children with severe asthma (84), and vitamin D enhances the frequency of both IL-10+ and Foxp3+ T_{reg} cells in children with severe asthma (85). In a translational setting, these data suggest that vitamin D supplementation may be effective in enhancing the frequency of T_{reg} cells in pediatric severe asthma.

Other studies have suggested that TGF-β, rather than IL-10, may be more important and serve as a biomarker of asthma control in atopic asthma (86). TGF-β is a pleiotropic cytokine with numerous functions that are vital in maintenance of pulmonary homeostasis, such as inhibiting Th2 and Th1 cell responses or inhibiting IgE production (87). In children, polymorphisms in TGF-β2 have been associated with atopic asthma (88). PCR analysis of bronchial and nasal epithelial cells concluded that TGF-β2 was differentially expressed in pediatric asthmatics compared to atopic non-asthmatics and healthy children (89). However, a much better understanding of the complex TGF-β signaling network in pediatric severe asthma is required before specific molecules can be targeted in a valid clinical study.

AIRWAY REMODELING: MECHANISMS AND THERAPEUTIC TARGETS

Children with severe asthma have evidence of all of the structural airway wall changes (remodeling) that are apparent in adults. They have increased thickness of the reticular basement membrane (RBM) (8, 90), increased ASM (8, 91, 92), goblet cell and submucosal gland hyperplasia (93), and evidence of angiogenesis (67). Of these changes, increased bronchial ASM has been closely related to worse lung function and greater bronchodilator reversibility (91, 92, 94).

Relationships between Inflammation and Remodeling

It is often proposed that remodeling occurs as a consequence of chronic airway inflammation. Payne et al. (90) compared RBM thickness in 19 children with difficult asthma prescribed high-dose inhaled steroids (6–16 years) and 10 age-matched non-asthmatics children with healthy, steroid-naive asthmatic adults, and life-threatening asthmatic adults. RBM thickness was not associated with severity, asthma symptoms, age, or airway inflammation. Fedorov et al. (95) compared bronchial biopsies between non-asthmatic, moderate, and severe asthmatic children (5–15 years) and showed excess deposition of interstitial collagen in the RBM occurred early in life but did not correlate with submucosal eosinophils and suggested that RBM thickness is established early in life due to an abnormal EMTU. Both studies proposed that remodeling is dissociated from eosinophilic inflammation. The dissociation between airway remodeling and eosinophilic inflammation has been demonstrated in a mouse model in which HDM-induced airways remodeling was equivocal in eosinophil-deficient and wild-type mice (96). Mechanistic data from a neonatal mouse model of inhaled HDM exposure have shown that remodeling is unlikely a consequence of inflammation, but that both processes occur in parallel (97). Remodeling can, therefore, develop in the absence of an inflamed airway with just excessive bronchoconstriction (98), and thus, there is an urgent need for therapies that can target structural changes alone, as many children with severe asthma remain symptomatic with significant airway hyperresponsiveness in the absence of inflammation (99).

Airway Smooth Muscle

The importance of targeting ASM remodeling as a therapeutic approach is made apparent by the very consistent association with increased ASM and worse lung function in both adult and pediatric studies. Increasing ASM has also been associated with lower serum vitamin D levels and worse asthma control in children (94). Mechanistically, a relationship between increased airway remodeling and a vitamin D-deficient diet has also been shown in a neonatal mouse model of HDM-induced allergic airways disease (100). These data suggest studies that focus on

investigating ASM function in pediatric severe asthma are likely to be helpful in discovering novel therapeutic targets. In addition, that vitamin D supplementation to achieve normal serum levels in children with severe asthma is an important consideration as it may minimize remodeling.

There is evidence that ASM function is specifically impaired in adult severe asthma, and that the mechanism is related to glucocorticoid resistance, whereby glucocorticoid receptor expression is reduced with impaired nuclear translocation (101). In contrast to ASM, few functional consequences have been reported in association with the thickness of the subepithelial RBM. Moreover, increased thickness is not an isolated finding in asthma, although the degree of thickening is greater in severe asthma, this feature may also be present in children with cystic fibrosis (102) and adults with COPD (103). Thus, it is difficult to know the impact that therapies, which target increased RBM thickness may have on disease manifestation.

IMPORTANCE OF ALLERGY IN PEDIATRIC SEVERE ASTHMA: MECHANISMS AND ANTI-IgE ANTIBODY THERAPY

More than 85% of children with severe asthma are atopic, defined by serum IgE antibodies and a positive skin prick test to common aeroallergens (8). One of the key clinical features that allows distinction between children with difficult asthma (poor control with poor adherence) and severe therapy resistant asthma (poor control despite good adherence) is significantly more severe asthmatics were polysensitised to several allergens, and more patients had food allergy (104). However, perhaps the most important distinctive feature of severe asthma is when atopy is quantified, rather than assessed as simply being present or not (105, 106). Children with severe asthma have a much worse and higher allergic burden (107). This suggests allergic sensitization plays a critical role in the pathogenesis of severe asthma in children (108). The role of allergy in pediatric severe asthma needs to be understood to help identify underlying mechanisms of disease progression, which will impact both on the choice of add-on therapies for these patients, but also on the discovery of novel therapeutics. In this regard, the one therapy that has been approved for use in children with severe asthma is the recombinant DNA-derived humanized monoclonal antibody against IgE (omalizumab), which works by reducing the quantity of cell-bound IgE, downregulation of high-affinity IgE receptors FcεRI on MCs, basophils, and DCs, and prevention of mediator release from effector cells (109, 110). Decreased sputum and bronchial eosinophils, as well as T cells were observed in adult bronchial biopsies after omalizumab (109). In a study (111) involving 334 children aged 6–12 years with moderate-to-severe atopic asthma treated with beclomethasone dipropionate and omalizumab or placebo, omalizumab reduced number of exacerbations as well as the frequency of exacerbations when withdrawing ICS. Another smaller 16-week study in children with severe asthma reported that omalizumab allowed a significant reduction in daily prednisolone dose and improved control and life quality (112).

However, the current licensed indication requires serum IgE levels to be within a set range, the maximum being 1,500 IU/ml. At least one-third of children with severe asthma have an IgE greater than 1,500 IU/ml because of severe and multiple allergies (8). In addition, approximately one-third of children who are eligible and are given a trial of treatment do not have a clinical response (113, 114). Therefore, there is still a subgroup of children with very severe disease and marked morbidity for whom currently no licensed add-on therapies are available. Interestingly, given the burden of monthly, or two weekly injections posed by omalizumab, and that specific subgroups, adolescents in particular, who are at high risk of asthma death, but more likely to be non-compliant with maintenance therapy, an approach of giving omalizumab prior to the Autumn increase in asthma exacerbations has been efficacious (115). Omalizumab therapy was associated with improved IFN-α responses to rhinovirus. However, changes in allergen-stimulated cytokine responses in peripheral blood T cells, or changes in T regulatory cells were not seen (116). Given the clinical benefit, this suggests the effects of omalizumab are unlikely *via* an impact on T cell responses, and more likely *via* other immune effector cell types such as MCs (116).

ALLERGY IN PRESCHOOL WHEEZE: A POSSIBLE WINDOW FOR ASTHMA PREVENTION?

Preschool children with wheezing disorders may or may not progress to develop asthma. Airway inflammation can be assessed in BAL and endobronchial biopsies from children with severe wheezing. BAL from wheezing children contains increased lymphocytes, polymorphonuclear cells, and macrophages/monocytes as well as LT B4, C4, PG E2, and the potentially epithelial-derived 15-hydroxyeicosatetraenoic acid were all increased (117). Bronchial biopsy studies in infants under 2 years with severe wheeze have reported an absence of RBM thickening and eosinophilic inflammation (118). However, when older children at a median age of 3 years with severe recurrent wheezing were compared to non-wheezing controls, they had increased airway eosinophils and RBM thickness (119). Birth cohort studies have repeatedly shown that the most prominent risk factor for progression of preschool wheeze to asthma is early allergen sensitization (120). Also, in a similar manner to older children with severe asthma, the risk of developing asthma and the greatest reduction in lung function is in those preschool wheezers who have both early and multiple allergic sensitization (121, 122). Unfortunately, targeting eosinophils with early inhaled steroids is not disease modifying (123), and this is explained by the mechanistic data that shows an absence of eosinophils does not impact the phenotype of allergic airways disease (96). However, given the definite association between early allergic sensitization and progression of preschool wheeze to asthma, several alternative interventions have been proposed to achieve disease prevention (124). One of these, to investigate the role of omalizumab in preschool wheeze to achieve disease modification is currently being tested in a clinical trial (125).

EMERGING THERAPIES FOR SEVERE ASTHMA

According to the ATS/ERS guidelines, severe asthmatics have poor control despite treatment with high-dose inhaled or oral corticosteroids combined with LABAs. A summary of the emerging add-on therapies that are being trialled for severe asthma is provided below. However, it is important to remember that, of these, only omalizumab is currently licensed for use in children with severe asthma.

Muscarinic Antagonists

These drugs act as bronchodilators by non-specifically antagonizing the muscarinic acetylcholine receptor and inhibiting smooth muscle cell contraction and mucus secretion. Short-acting muscarinic antagonists (SAMAs), such as ipratropium bromide, can be used in severe asthmatic children and adults during asthma exacerbations (126) and to reduce β-agonist doses in order to avoid side effects, but they are less effective than inhaled beta-agonists (127). In 1998, Qureshi et al. already reported that adding this drug to a combined therapy of albuterol and corticosteroids decreased hospitalizations for severe asthmatic children (aged 2–18 years). More recently, a meta-analysis reported that the combination of SAMAs and SABAs in children during exacerbation improves lung function and reduces the risk of tremor and the risk of admission (128).

Longer-acting muscarinic antagonists are an interesting option as controller medications. Inhaled Tiotropium (Spiriva Respimat®) was first indicated in COPD treatment. Currently, it is approved as an add-on maintenance bronchodilator in adults with asthma taking ICS/LABAs and who have experienced at least one severe exacerbation in the previous year. It was shown to improve lung function and symptoms in uncontrolled moderate-to-severe asthmatic adults and reduced the risk of exacerbations in those treated with ICS/LABAs (129, 130). US FDA has recently approved it as an asthma maintenance treatment in children aged 6 years and over (131). However, it has not been approved in Europe. Its efficacy as an add-on therapeutic in pediatric severe asthma remains unknown. Pediatric trials that will allow selection of patients that are most likely to benefit are needed.

Immunomodulators

Molecular-based therapies, which allow treatment according to the predominant inflammatory phenotype, are of particular interest in severe asthmatic children who do not respond to standard therapy. Apart from specificity, they provide long-term control and allow a reduction in ICS and oral steroid dose. An important aim in the utility of novel therapeutics for children is not just as add-on treatments that will allow disease control, but also as steroid sparing therapies to minimize the significant adverse effects of high-dose corticosteroids. Several antibody-based treatments are available.

Neutralizing IgE: Omalizumab

The mechanism of action and specific utility of omalizumab in pediatric severe asthma has been discussed in the section on allergy in children above.

In the UK, omalizumab is indicated as an add-on therapy to improve control in adults and children aged 6 years and over with severe persistent confirmed IgE-mediated asthma, and who need continuous or frequent treatment with oral corticosteroids (defined as four or more courses in the previous year) (132). The predominant benefit is a reduction in exacerbations. However, limitations for children include the upper limit of serum IgE for which it can be prescribed and at least one-third do not have a clinical response.

Blocking IL-5 Signaling: Mepolizumab

Mepolizumab is an anti-IL-5 humanized monoclonal antibody that reduces circulating eosinophils. It is indicated as an add-on to standard therapy in severe refractory eosinophilic asthma in adults when the blood eosinophil count is ≥300 cells/μl in the previous 12 months and ≥4 exacerbations needing systemic corticosteroids in the previous 12 months need for continuous oral corticosteroids equivalent of more of prednisolone 5 mg/day over the previous 6 months (133). Exacerbations in adults decreased without improvement in FEV_1 nor quality of life. The data for efficacy in children are currently lacking, and clinical trials that address this unmet need are urgently needed.

Blocking Th2 (IL-13 and IL-4) Signaling: Lebrikizumab, Dupilumab, and Pitrakinra

Lebrikrizumab, a humanized monoclonal antibody against IL-13, has been evaluated for severe asthmatic adults. Initial studies showed Lebrikizumab treatment resulted in improvement in lung function in adults with uncontrolled asthma taking ICS, especially in those with higher levels of serum POSTN. There was also a decrease in exacerbation rates in those patients with higher blood eosinophils and IgE levels (134). However, subsequent confirmatory studies in adults have been disappointing (135) and it is not yet indicated for adult severe asthma.

Animal studies have shown that IL-4 can induce IL-13-independent AHR and goblet cell hyperplasia, suggesting dual inhibition of both IL-4 and IL-13 could suppress these events (136). Dupilumab is a human monoclonal antibody against the IL-4 receptor α chain (IL-4Rα) blocking downstream signaling *via* both the IL-4 and IL-13 receptors. In 2013, Wenzel and colleagues performed a 12-week study (phase 2a) to asses Dupilumab in persistent moderate-to-severe asthmatic adults with high blood (≥300/μl) or sputum (≥3%) eosinophils (Th2 high disease) (137). Dupilumab was effective in reducing exacerbations, increasing lung function and reduced Th2-associated inflammation, as well as allowing a reduction and/or stopping of maintenance dose of ICS while maintaining improvement of asthma. More recently, in a 24-week study (phase 2b) in uncontrolled persistent asthmatic adults, injection with Dupilumab every 2 weeks as an add-on therapy to medium-to-high-dose ICS and LABAs led to improvement of FEV1, reduction in exacerbations, and better asthma control regardless of baseline eosinophil count (138). During the treatment patients with at least 300 eosinophils/μl at baseline had an increase in blood eosinophils, so the next clinical trial (139) excluded patients with high eosinophils and also included children (from 12 years). Efficacy of this antibody in uncontrolled persistent asthmatic children (aged from 6 to 12 years) will be assessed in a clinical trial starting on 2017 (139). Pitrakinra is

a recombinant human IL-4 antibody containing mutations that allow it to prevent the assembly of IL-4Rα with either IL-2Rγ or IL-13Rα (140). In a recent study, a dose–response for asthma exacerbations was identified in a specific subgroup according to the SNPs genotype in *IL4RA* gene (141).

Blocking Th17 Signaling: The Role of IL-17 and Neutrophils in Pediatric Severe Asthma

In adults with severe asthma, high levels of IL-17A have been reported in sputum, BAL fluid, and peripheral blood (142–144), and this was associated with increased disease severity (145). In children, IL-17A has been reported to induce neutrophilic airway inflammation and promote steroid resistance (146). Levels of IL-17A in sputum, nasal wash, and plasma as well as levels of circulatory T cells expressing IL-17 were studied in children with moderate asthma, and it was suggested that IL-17 could be associated with asthma severity (147). In contrast to the data from studies in adults, we have shown levels of IL-17A are not elevated in either the BAL or endobronchial biopsies of children with severe asthma compared to non-asthmatic controls (148). However, we did show increased expression of IL-17Rα in the airway submucosa and epithelium of children with severe asthma. Furthermore, numbers of neutrophils were also similar in the BAL and submucosa of children with severe asthma and controls (8, 148). However, there was a subgroup of patients with increased numbers of neutrophils only within the epithelium, and these patients had better symptom control improved lung function, symptom control, and were prescribed lower dose maintenance inhaled steroids. Therefore, unlike adult severe asthma, neutrophils might be beneficial in pediatric severe asthma pathophysiology. Therefore, the role of neutrophils and IL-17 is not completely understood in children with STRA and requires further investigation before therapies that are either anti-neutrophilic or block IL-17 are tested. Interestingly, Brodalumab, a human anti-IL-17 receptor monoclonal antibody, has shown no benefit in adults with moderate-to-severe disease (149).

Anti-TSLP Antibody: AMG157

The monoclonal antibody AMG157 blocks the binding of TSLP with its receptor and has been shown to reduce bronchoconstriction and attenuate the early and late phase response in allergic asthmatic adults (150). It also reduced blood and sputum eosinophil counts and FeNO before allergen challenge, suggesting that TSLP may be an important upstream regulator of type 2 inflammation in the airways. The efficacy of AMG157 is currently being assessed in adolescents (12–17 years old) with mild-to-moderate asthma (151) and will be assessed in uncontrolled severe asthmatic adults (152). The efficacy of blocking TSLP in children with severe asthma remains to be seen. There is no evidence to date that levels of TSLP are elevated in the airways of children; therefore, a direct extrapolation of findings from adult studies to pediatric studies may not be beneficial.

Anti-PGD_2

Prostaglandin D2 is a lipid inflammatory mediator produced by cyclooxygenases and PGD_2 synthases mainly in MCs, but also in Th2 cells, macrophages, and much less in eosinophils and basophils. They subsequently bind to D prostanoid 1 and chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) receptors triggering anti-inflammatory effects as well as pro-inflammatory effects through PGD_2-CRTH2 pathway, which is upregulated in asthma (153). This is a particularly attractive therapeutic for children and has been discussed in detail above in the section on airway inflammation in pediatric severe asthma.

FUTURE DIRECTIONS

We have discussed two add-on treatments that are currently available for use in adults that mechanistically are attractive therapeutic options for children with severe asthma and need to be pursued. Monoclonal antibodies to IL-5 or its receptor, and CRTH2 antagonists, both of which may help to ameliorate the persistent steroid resistant eosinophilia and elevated Th2 and ILC2 cells that are apparent. Although both types of interventions should be efficacious, we know that omalizumab, the currently licensed add-on treatment for children, does not work or cannot be used in about 50%, so similarly, it is likely that the other two therapeutics will also only work in a subgroup. In contrast to adult studies, given the current mechanistic data from children, it seems much less likely that antibodies that target IL-13, IL-4, or IL-17 will be beneficial in children. This is because these data showing elevated levels of these mediators in pediatric airways are scarce. A molecular target for which therapeutic agents are not currently available, but would certainly be worth pursuing for children, is IL-33. This is because it is elevated in children, is relatively steroid resistant, promotes airway remodeling (56), and is more specifically associated with the sub-phenotype of severe asthma with fungal sensitization (57).

It is essential to remember that pediatric severe asthma is markedly heterogeneous, and in contrast to adult disease, our current understanding of the underlying sub-phenotypes and endotypes is very limited. This is because it is a rare disease, but also because mechanistic studies that use either age appropriate experimental models or airway samples from children are a challenge to undertake. We know that there are distinctions between the factors that drive pediatric and adult disease, most importantly, pediatric disease is present in the context of a maturing immune system and during lung growth and development. Given the acknowledged heterogeneity and the relatively small number of patients that are affected, it is essential that we now undertake multicenter, national and if possible, international unified studies to assess the efficacy of novel therapeutics and to investigate the mechanisms of action of these drugs in children. Only such an approach will allow us to understand the mechanisms mediating disease, and to identify important endotypes, which will allow us to stratify patients and ensure add-on treatments are accurately targeted to achieve effective personalized medicine.

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AM and SS contributed equally to this work.

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REVIEW
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Relationship of Allergy with Asthma: There Are More Than the Allergy "Eggs" in the Asthma "Basket"

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Asthma and allergy share a similar and very close course, especially through childhood. Considerable research effort has been put in untangling these associations; however, it is now becoming obvious that this is an exceedingly difficult task. In fact, each research breakthrough further perplexes this picture, as we are steadily moving toward the era of personalized medicine and we begin to appreciate that what we thought to be a single disease, asthma, is in fact an accumulation of distinct entities. In the context of this "syndrome," which is characterized by several, as of yet poorly defined endotypes and phenotypes, the question of the link of "asthma" with allergy probably becomes non-relevant. In this review, we will revisit this question while putting the emphasis on the multifaceted nature of asthma.

Keywords: asthma, endotype, phenotype, wheeze, pediatrics, classification, allergy

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INTRODUCTION

Asthma is a chronic pulmonary inflammatory disease wherein the innate and adaptive immune systems cooperate with epithelial cells to cause airway hyperresponsiveness (AHR), mucus overproduction, airway wall remodeling, and bronchoconstriction. Clinically, it is characterized by recurrent episodes of wheezing, breathlessness, and chest tightness. It affects more than 10% of the population in many westernized countries and more than 300 million people worldwide (1). It has a great impact in childhood and is the leading cause of school absenteeism in the United States, causing approximately 50% of children to miss at least one school day yearly (1).

Asthma is seen as an allergic disease; this assertion, although well documented, is probably an oversimplification. In fact, up to the last decade, our view of asthma as a single disease was likely oversimplified. Although main characteristics of asthma are airflow obstruction, bronchial hyperresponsiveness, and underlying inflammation, it is rare that all these characteristics can be found in all patients of large cohorts. Asthma recently started to be recognized as a "syndrome," a complex condition with variability in its pathophysiology, severity, natural history, comorbidities, and treatment response (2). Therefore, an important question in the last decade is whether asthma is a single disease with a variable presentation, or several "linked" diseases that share salient clinical features (3, 4). It is now becoming clear that the latter is probably true and that the diagnostic label "asthma" likely encompasses many different disease variants (phenotypes) with different etiologies and underlying pathophysiological mechanisms.

It is this complexity of asthma that makes it difficult to discern its link with allergy (atopy). These are indeed two intertwined conditions and are closely associated in the minds of most clinicians. However, several asthma subtypes may in fact have little to do with atopy. This is where the narrative

about the asthma endotypes and phenotypes, and our need to move into a new era of personalized medicine, comes into place. About a decade ago, the word "phenotype" entered the field of asthma research and management (2). In scientific language, the word "phenotype" refers to "the observable properties that an organism displays in the context of a certain disease, which are caused by the interactions of the organisms genotype with the environment." In simpler terms, a particular phenotype (and in this case an asthma phenotype) is defined and told apart from the other asthma phenotypes by its prominent/unique clinical characteristics (5). Once the discussion about the phenotypes was afoot, it became glaringly obvious that these subtypes of asthma were often underpinned by discreet pathophysiological processes, giving rise to the concept of "endotypes" and increasing asthma complexity. Hence, the term "endotype" defines a specific biological pathway that underpins the clinical observations, which constitute a phenotype (2, 6).

When one looks from this perspective, it is easy to see the reasons for the difficulty to clarify the link of allergy/atopy to asthma; not least important of these reasons being that asthma may in fact include several different clinical conditions underpinned by several different pathophysiological processes. In this review, we will try to look into this asthma/atopy relationship from the perspective of the complex multifaceted disorder, that is asthma, and will attempt to individually link some of its subparts with atopy (**Figure 1**). This is, however, unlikely to give us a clear picture

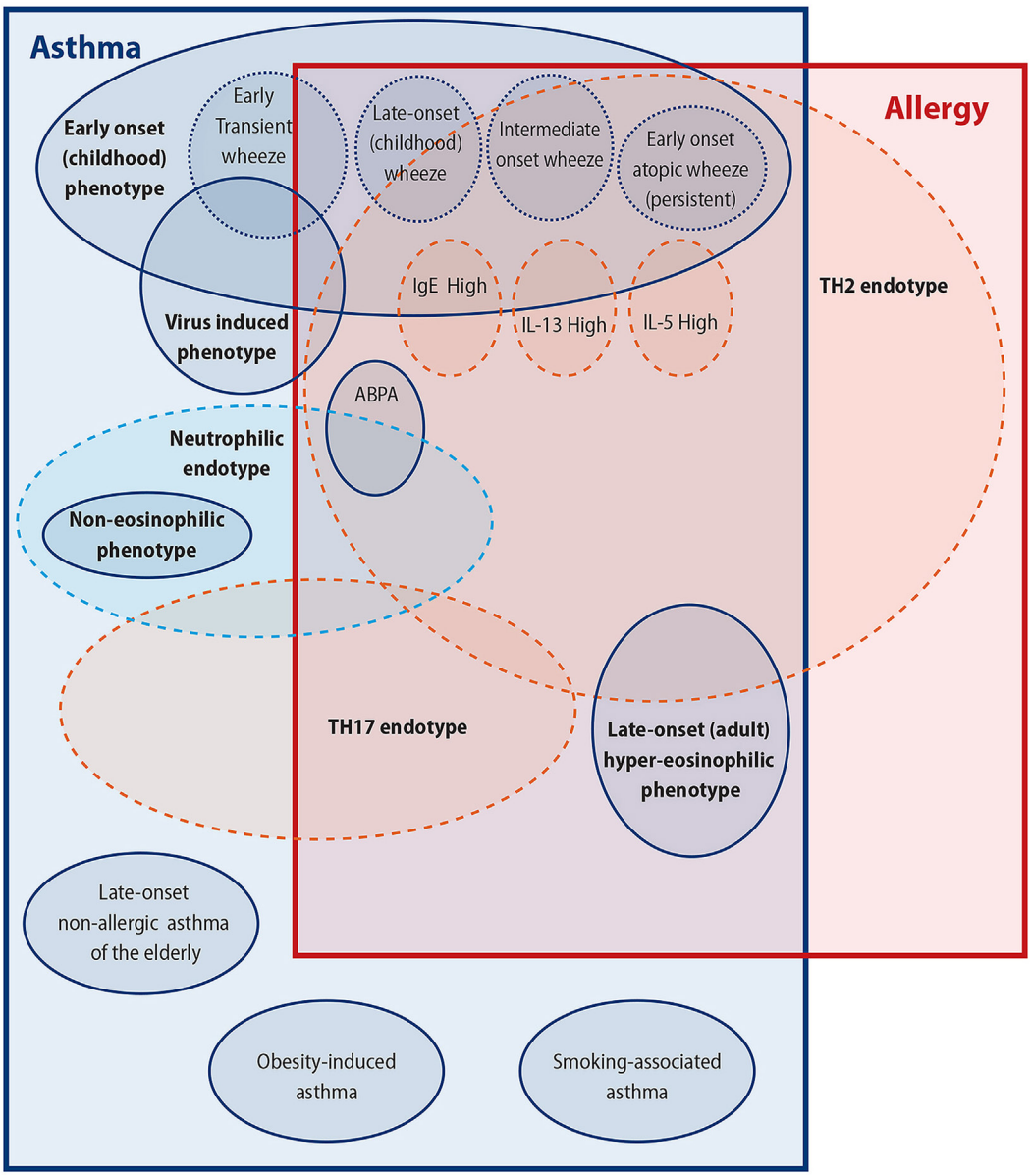

FIGURE 1 | Attempting to untangle the asthma–allergy associations. There are more than the allergy "eggs" in the asthma "basket."

of the relationship of asthma with allergy; in our opinion, this relationship is oversimplified and is therefore likely to be wrong as was our view of an oversimplified asthma disease for many decades now.

ASTHMA ENDOTYPES AND ALLERGY

The expression "endotype" refers to "an asthma subtype defined by a distinct functional or pathophysiological mechanism" (2), it is a widely used and discussed term, and little consensus exists about endotype numbers and characteristics. Currently, standardized diagnostic/management approaches based on endotypes are being sought (2), and some are being used in the clinical setting (e.g., omalizumab for IgE-high allergic asthma), but the majority of asthma cases are still being treated with the one-size-fits-all management, which was established decades ago.

Th2 Endotype

This is one of the widest and probably the best-defined asthma endotypes thus far. It is characterized by a type 2 immune response that involves Th2 cells, B cells, basophils, eosinophils, mast cells, major cytokines secreted from immune cells [interleukin (IL)-4, IL-5, IL-9, and IL-13], and others secreted from epithelial cells (IL-25, IL-31, IL-33, and TSLP) (7). This is the endotype underpinning allergic asthma and is strongly linked to atopy, IgE production, and eosinophilic inflammation (8). This is arguably one of the most important endotypes in childhood, as it is closely associated with the early-onset asthma (EoA) phenotype that usually starts during childhood and early adolescence. This phenotype, described in detail in the next paragraphs, consists of several other subphenotypes, has a high prevalence, and often persists into adulthood. This endotype is indeed prevalent in adulthood, seeing that about 50% of *mild* asthmatics have an endotype that is associated with eosinophilia, mast cell activation, development of allergen-specific IgE, and Th2 cytokine production (9). Several subendotypes might exist within this endotype, such as the IL-5-high, IL-13-high, or IgE-high (10).

Given this endotype's dependence on Th2 cytokines, it is unsurprising that it responds to therapies targeting IL-4 and IL-13, particularly in allergen-challenge models (8, 11, 12). Indeed, IL-4/13 blockade is most efficacious in patients with Th2-related asthma, especially with peripheral eosinophilia (13–15). Anti-IL-5 treatment in some patients has been associated with lower frequency of acute asthma, a steroid-sparing effect, and improved lung function (16–18). Regulators of Th2-type cytokines (such as IL-25, IL-33, and TSLP) and inhibitors of TSLP are gaining ground (19). However, one must recognize that a major pathogenic pathway such as the Th2 endotype is complex and heterogeneous, with several determinants that have non-linear dynamic interactions (10, 20). Therefore, currently, there is only one routinely used biologic therapy for Th2 asthma, omalizumab, an anti-IgE molecule; In any case, this further documents the close link of this endotype with allergy (19). There is now considerable experience with this drug, which works best in atopic patients with severe, inadequately controlled disease (21).

Non-Th2 Endotype

Approximately half of asthmatic patients have Th2-no/Th2-low endotype (15), yet much less is known about this heterogeneous group. Some agents of interest are the cytokines IL-17, IL-1b, TNF-a (22), and a chemokine receptor (CXCR2), which are associated with neutrophilic inflammation (10). From the clinical point of view, these patients show less airway obstruction and hyperreactivity than Th2-high asthmatics. Importantly, these are generally non-atopic-patients, and there is little to no evidence of allergy in childhood or beyond (23).

Two major mechanisms leading to non-type 2 asthma have been postulated: (i) activation of the IL-17-dependent pathway and (ii) innate immune response dysregulation bringing about neutrophil inflammation (20).

Th17 Endotype

Th17 cells are characterized by the production of IL-17A, IL-17F, and IL-22. They develop in response to transforming growth factor-b and IL-6 production and are dependent on the expression of transcription factor RORgt. Accumulating evidence suggests a role for TH17 cells in asthma, especially severe steroid-resistant asthma. Increased IL-17A+ cells can be found in lung biopsies of patients with severe asthma compared to those with mild (24). In both adults and children, serum IL-17A is significantly higher in severe asthmatics compared to mild ones (25), and it has been linked to remodeling and AHR. This asthma endotype has little relation with atopy.

Neutrophil Endotype

Neutrophil inflammation is well established in mouse models of asthma, where it has been linked to the development of airway hyperresponsiveness (AHR) and remodeling. In an experimental model of Th1/neutrophil-predominant asthma, TNF-a reduced the responsiveness to steroids, whereas its neutralization restored steroid responsiveness (26). In humans, patients with non-allergic asthma demonstrated considerable neutrophil inflammation induced by IL-17-shifted pro-inflammatory immune reactions (7). A role for a dysregulated innate immune response in neutrophilic asthma has been proposed; this is characterized by altered gene expression of toll-like receptors, and increased expression of genes linked to IL-1b and TNF-a/nuclear factor-kB (27). Enhanced neutrophil chemotaxis/survival in the airways and impairment of anti-inflammatory mechanisms could further underlie this endotype. Obviously, there is little room for an important atopy component in this endotype.

Mixed Th2/Th17 Endotype

There exists a mixed Th17/Th2 endotype in asthma, as Th2 cells can differentiate into dual-positive Th2/Th17 cells (28). These cells were identified in the bronchoalveolar fluid of asthmatic patients (29).

The relationship between the Th17 and Th2 responses is highly complex. IL-17 produced in response to injured epithelium could enhance the production of IL-4 and IL-13 from Th2 cells (7). Conversely, IL-4 and IL-13 may amplify Th17 responses by upregulating CD209a expression on dendritic cells (7). In any event, this mixed endotype has seen little research,

and although it is likely associated with allergy, this remains to be elucidated.

ASTHMA PHENOTYPES AND ALLERGY

Early-Onset Asthma

The early asthma phenotype is prone to eczema development in early childhood (30). Eczema implies that this phenotype is mostly Th2 driven. A Th2 association in EoA is described (31, 32), but cases with low IgE and limited response to inhaled steroids suggest that there are forms of EoA not related to Th2, exemplified by virus-induced wheeze (33).

Early-onset asthma can be classified into several subphenotypes with varying association to atopy. The Tucson population-based birth cohort study retrospectively subclassified preschool wheeze into three groups: "transient wheeze," "early persistent wheeze," and "late-onset asthma" (34). Transient wheeze is thought to be caused by viral infections. Viral and allergy mechanisms could also cooperate to orchestrate the development of both transient wheeze and future allergic asthma (35). Early infection with respiratory syncytial virus has been found to increase the susceptibility to allergic asthma *via* the IL-4 receptor pathway (36). Another subphenotype is "early-onset allergic asthma" (represented in the Tucson study by "early persistent wheeze"), the classic form of persistent asthma that has a childhood onset and bares allergic features, including allergen sensitization and allergic rhinitis. Airway eosinophilia is common in early-onset allergic asthma, and a TH2-dominant inflammatory process is believed to underlie it. Allergy involvement is vital as inhalation of a specific allergen triggers bronchoconstriction and inflammatory cell influx. The efficacy of omalizumab, and the studies on IL-4/IL-13 modifiers, implies a central role of IgE and TH2 cells/cytokines in this subphenotype (2).

Other subphenotypes of this variant have also been recognized. Four subtypes have been identified by unsupervised cluster analyses on 161 subjects in the pediatric asthmatic cohort from the Severe Asthma Research Program (SARP) (37). Cluster 1 consists mainly of mild, later onset, and less atopic asthma with normal lung function. The other clusters represent the early-onset, atopic asthma subphenotype, with, however, variable severity and lung function. These clusters were similar to the ones seen in the adult SARP analyses (11, 38), where the subphenotype of "early-onset, atopic asthma" represented the majority of cases reported. Other cases included "obesity-induced asthma" and "late-onset (adult) non-atopic asthma" of varying subphenotypes.

Other well-known phenotyping attempts include The Avon Longitudinal Study of Parents and Children, which collected data on wheeze at multiple time points from birth to age 7 years, for 6,265 UK children (39). A distinct new phenotype was identified, "intermediate onset wheeze" (onset at 4–6 years of age), which showed the strongest associations with atopy. Late-onset wheeze was also strongly associated with cat, house dust mite, and grass pollen sensitization. "Early-onset atopic asthma" (which in this cohort was likely represented by the "persistent wheeze" cases) had an onset of 6–18 months of age and was also strongly associated with atopy (39). These findings regarding the wheeze subphenotypes and their relation with atopy were similar to those from analyses of the Dutch Prevention and Incidence of Asthma and Mite Allergy study, a multicenter birth cohort that enrolled 4,146 pregnant women (40). Other findings from a population-based longitudinal cohort that enrolled 1,650 preschool children in the UK were largely similar although the authors failed to detect an "intermediate onset wheeze" subset and noted a "non-atopic, persistent wheeze" subset (41). This subset was also detected in an independent population-based cohort by the same authors (validation cohort) (42) and could partly represent the "late-onset wheeze" subset reported in the other studies.

Early transient wheeze is largely underpinned by the virus-induced asthma phenotype. Virus-induced asthma is a very common phenotype in children, and it has been very well described as the "September epidemic" (43). Although "virus-induced asthma" is—as the name of the sub-phenotype indicates—"transient," virus infections can also alter the course of preexisting asthma or can modify the immune system, hence increasing susceptibility to allergen sensitization and/or asthma in childhood (44). Indeed, wheezy episodes associated with rhinovirus are a strong predictor of asthma (45). This is another testament to the close association of the pediatric asthma subphenotypes with allergy, as even this variant, which is mainly induced by viruses, can be affected by individual atopic status.

Adult-Onset (Severe) Hypereosinophilic Asthma

Adult-onset asthma with highly elevated numbers of eosinophils is associated with Th2 cytokines and Th2 inflammatory cells like eosinophils, mast cells, and basophils. Eosinophil numbers between 150 and 300 eosinophils per milliliter have been used in asthma trials to define hypereosinophilic asthma, but full consensus is still lacking. The patients often show severe reactions after cyclooxygenase inhibitor intake. However, regardless of the eosinophil basis, the link of this phenotype with the Th2 endotype is not clear (46). This is another poorly defined phenotype as there is a group of individuals who demonstrate strong eosinophilic inflammation but a paucity of symptoms (47). Compared to early-onset Th2 asthma, adult-onset asthma is characterized by the presence of raised eotaxine-2/CCL24 levels; eotaxine-2/CCL24 is a potent proeosinophilic chemokine, which might be the cause of the raised eosinophil count (48). The link of this phenotype with atopy is not clear, but given the eosinophil component, one can assume that some association may exist. Such a link is also supported by the responsiveness of patients with hypereosinophilic, adult-onset asthma to IL-5-targeted therapies (16).

Late-Onset Non-allergic Asthma of the Elderly

Asthma in the elderly, above the age of 65, is another phenotype (49). Elderly patients with asthma tend to be more symptomatic, with more pronounced airway obstruction, frequent hospital admissions, and a significantly higher mortality (50). They also have frequent comorbidities, which contribute to the severity of the disease. While asthma is prevalent in these ages [6.9% of people above the age of 65 in USA (51)], it is significantly

underdiagnosed. This phenotype can be divided into persistent asthma that started early in life and newly diagnosed asthma. Neutrophilic airway inflammation is more prevalent among elderly patients, while atopy and elevated IgE levels are less frequent, but this largely depends on the "new-onset *vs* persistent asthma from a younger age" distinction.

Obesity-Related Asthma

Another no-/low-Th2 endotype has been identified in obese asthmatics (52). Obesity-induced asthma is characterized by lack of atopy, female predominance, and late onset (53). It is generally more severe and harder to control as it is less responsive to standard controller therapies. Also, large observational studies have demonstrated that obesity is associated with wheezing even in non-asthmatic individuals (54). Asthma is significantly overdiagnosed in these patients, who do not gain benefit from anti-inflammatory asthma treatment. Non-asthma wheezing has complicated research into the obesity-related phenotype, and it still remains to be elucidated whether this phenotype is underpinned by several endotypes or a single endotype with defined inflammatory pathways. In any case, atopy probably has little involvement here.

Non-Eosinophilic Asthma

Non-eosinophilic asthma is a well-documented and prevalent asthma phenotype, as approximately half of asthma patients have no evidence of eosinophilic inflammation (55). Neutrophilic inflammation has been identified in several studies as a hallmark of this variant (e.g., the SARP cohort). Furthermore, non-eosinophilic asthma is associated with innate immune dysfunction and increased expression of several biomarkers, including neutrophil elastase, TLR2 and 4, IL-1β, IL-8, MMP-9, and TNF-a (56). As expected, the response of non-eosinophilic asthma to inhaled corticosteroids is weak, and its management can be challenging. No consensus exists about the neutrophil cell count threshold that could define neutrophilic asthma. A relationship of this phenotype with smoking is discussed. It is unlikely that this phenotype is strongly connected to atopy (57).

Smoking-Associated Asthma

In a recent cluster analysis, the phenotype of smoking-related asthma was described (51). This group consisted of mainly male adults (66%) who are non-atopic. They had preserved postbronchodilation spirometry but more pronounced respiratory symptoms. Cigarette smoking is a well-known aggravating factor of asthma symptoms and associated burden. Most likely, coexistence of early changes of chronic obstructive pulmonary disease (COPD) also contributes to the clinical presentation of this phenotype, as it has been demonstrated that clinical symptomatology of COPD could precede significant pulmonary function decline. Therefore, this phenotype probably corresponds to asthma-COPD overlap syndrome.

Allergic Bronchopulmonary Aspergillosis (ABPA)

Allergic bronchopulmonary aspergillosis is a well-described hypersensitivity condition following colonization of the airways by *Aspergillus fumigatus*. This asthma endotype is characterized by a mixed pattern of neutrophilic and eosinophilic airway inflammation, elevated *Aspergillus*-specific IgE and IgG (58). Given this pathophysiological mechanism, ABPA could be considered an allergic endotype.

CONCLUSION

We are entering an era where precision medicine is gaining considerable ground, especially where complex diseases come into play. These diseases ideally exemplified by asthma had been tackled for decades with a one-suits-all management, only to be noted now that this approach leaves much to be desired in terms of efficacy, in a large portion of patients. This group that fails to respond to the conventional standardized approach accounts for more than 50% of asthma-related health-care utilization and is at increased risk of death due to asthma (7). Therefore, it is imperative that such patients who may benefit from a more personalized therapeutic approach are recognized (22).

In this context, categorization of patients *via* the use of endotypes, phenotypes, and distinct biomarkers will replace the rough cut "one-size-fits-all" approach. From this viewpoint, we feel that the current view of the allergy/asthma link is too generalized. Asthma, as discussed above, is indeed linked to allergy from a broad perspective and especially in the pediatric age, but such oversimplifications do not appear to further serve us. Asthma has a huge heterogeneity due to individual genetic and epigenetic variability and discrete environmental exposures, which are dependent on regional characteristics, varying climatic conditions, and population distributions (7). This complexity is further increased when looking at the highly variable severity of the symptoms, which has in fact been used as a criterion for further subphenotyping of asthma (severe, mild, treatment resistant, etc). Different patients can have differing disease severities, even within the same endotype (2), wherein treatment response can also vary greatly, e.g., in allergic asthma response to medication can be influenced by both the degree of allergic reactivity and allergen exposure, and their complex interactions (2).

All this evidence suggests that sweeping generalizations in regard to the asthma/atopy link are probably inappropriate, as "In asthma we must embrace the concept of a complex endotype consisting of several sub-endotypes" (10). In conclusion, in this "basket," that is asthma, there are several "eggs" of atopy, but a closer look will reveal several other eggs that we have yet to identify.

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REVIEW
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Exercise-Induced Bronchospasm and Allergy

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Sport is an essential part of childhood, with precious and acknowledged positive health effects but the impact of exercise-induced bronchoconstriction (EIB) significantly reduces participation in physical activity. It is important to recognize EIB, differentiating EIB with or without asthma if the transient narrowing of the airways after exercise is associated with asthmatic symptoms or not, in the way to select the most appropriate treatment among the many treatment options available today. Therapy is prescribed based on symptoms severity but diagnosis of EIB is established by changes in lung function provoked by exercise evaluating by direct and indirect tests. Sometimes, in younger children it is difficult to obtain the registration of difference between the preexercise forced expiratory volume in the first second (FEV1) value and the lowest FEV1 value recorded within 30 min after exercise, defined as the gold standard, but interrupter resistance, in association with spirometry, has been showed to be a valid alternative in preschool age. Atopy is the main risk factor, as demonstrated by epidemiologic data showing that among the estimated pediatric population with EIB up to 40% of them have allergic rhinitis and 30% of these patients may develop adult asthma, according with atopic march. Adopting the right treatment and prevention, selecting sports with no marked hyperventilation and excessive cooling of the airways, children with EIB can be able to take part in physical activity like all others.

Keywords: exercise-induced bronchospasm, asthma, atopy, allergy, sport, children

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INTRODUCTION

As known physical activity is fundamental for growth and long-term development in children, it has been shown to induce positive physiological and psychological effects, an improvement in cardiovascular, respiratory, and muscular systems. Furthermore, children undertaking physical training frequently modify their diet, with a reduced risk of overweight and obesity; in so doing, physical inactivity is considered to be an independent risk factor for various chronic diseases of adulthood (1). The terms "exercise-induced asthma" (EIA) and "exercise-induced bronchoconstriction" (EIB) are often used interchangeably. A consensus between the American Academy of Allergy, Asthma and Immunology, the American College of Allergy, Asthma and Immunology, and the Joint Council of Allergy, Asthma and Immunology used the term "EIB with asthma" for EIB with clinical symptoms of asthma and "EIB without asthma" for an acute airflow obstruction without asthma symptoms (2). A joint Task force of European Academy of Allergy and Clinical Immunology and European Respiratory Society described EIB as the reduction in lung function happening after exercise, as observed in exercise test, while defined EIA as symptoms of asthma occurring after exercise (3). In fact

EIB is a temporary contraction of respiratory muscle after exercise that happens frequently in subjects without diagnosis of asthma, especially athletes, changing with the intensity of exercise and the environment (4). EIB is a distinct form of airway hyperresponsiveness, which is defined as the tendency of airways to constrict more easily and more forcefully than normal airways in response to a wide variety of bronchoconstrictor stimuli (5). Asthmatic subjects without anti-inflammatory treatment are at risk to have an asthma attack induced by exercise, up to 75–80% (6), but even people with no diagnosis of asthma may develop reduction in lung function after exercise, possibly such as a significant risk factor for the development of asthma (7). Consequently, EIB can occur in the presence or absence of asthma (4) and even if normally the physiologic response to exercise typically products in bronchodilation (8), subjects without asthma diagnosis may suffer from EIB.

PREVALENCE

The prevalence of EIB ranges from 5 to 20% in the general population to even 100% in people with uncontrolled asthma. This huge variability depends not only on the criteria used for diagnosis, because there is not a gold standard (4), but also on the population samples studied. EIB is, in fact, reported to be particularly frequent (up to 45%) in children (9). It has been observed that exercise-induced wheezing in <5 years in childhood has been reported to be a strong predictor of persistent asthma in adulthood (10). Children and adolescents are more frequently affected than adults (11) and in the Oslo birth cohort study "Environment Childhood Study" 36.7% of 10-year-old children with a diagnosis of asthma showed EIB, with a positive exercise test, while 8.6% had a positive EIB test in the entire population-based birth cohort (12). An estimated 12% of the pediatric population has EIB and 30% of these patients may develop adult asthma (13). EIB reflective of the underlying asthma condition is reported in up to 90% of subjects with identified asthma but EIB can occur in individuals without a clinical history of asthma (14). Athletes particularly suffer from EIB without known asthma (15, 16), more frequently those with risk factors such as lung injury secondary to prematurity (17) or neonatal chronic lung conditions (18). However EIB is more commonly reported when asthma is associated to heavy exercise like in competitive athletes. Recent data in literature describe a prevalence of 10% of EIB in school children (19), according with results reported in two studies 15 years apart, estimating EIB from 17% (20) to 7.7% (21) or in 15% of a large pediatric study (22). But these assessments vary depending on the population and method of diagnosis in fact by using a free running test with peak flow monitoring the prevalence in one primary school population was 7.4% (23). In a pediatric Algerian population EIB prevalence has been described in up to 45% (24), up to 40% of individuals with allergic rhinitis. In fact among various risk factors for EIB is needed to identify history of allergy. The risk factors affecting the prevalence of EIB are multifactorial, including the presence and severity of asthma, family history of asthma or atopy including inhalant allergy, age, ethnicity, sex status, intensity and duration of exercise, presence of respiratory infection, atmospheric, and economic conditions, including an urban or rural setting and poverty (25, 26).

MECHANISM OF EIB

Physical exercise is one of many non-pharmacologic and non-immunologic stimuli that can produce episodes of airway obstruction in patients with asthma. In asthmatic subjects the immune environment is tilted toward the Th2 side of the T helper cell axis as, for example, severity correlates IL-3, -4, -5, -9, and -13. Levels of IL-4 mRNA and protein are higher in asthmatic airway cells and its activation of STAT6 results in airway hyperresponsiveness, mucin production and goblet cell hyperplasia. IL-4 and IL-13 increase the number of NKT cells in the airways, increase B-cell IgE production and IL-13 has been implicated in airway remodeling (27, 28). While the number of mast cells and eosinophils is abnormally high in people with currently active asthma, mast cells are found in high density in healthy non-asthmatic subjects (29). The mediators include prostaglandins and histamine that contribute to the onset and severity of the EIB, and cysteinyl leukotrienes that sustain the presence of EIB and retard recovery of forced expiratory volume in the first second (FEV1). Sensory nerves are also likely to be involved, in that they respond both to a change in osmolarity and to cysteinyl leukotrienes (30). Physical activity is the second leading cause of acute airway obstruction and ranks only behind viral upper respiratory tract infections in this regard (14). Classical mechanisms behind EIA and EIB include the so-called osmolar (or airway drying) and vascular (or "thermal") hypothesis (31). Both hypotheses are based on the marked increased ventilation during physical activity, leading to increased water and heat loss through respiration. Increased water loss increases the osmolality of the extracellular fluid lining the bronchial mucosa, causing water to move extracellularly possible through the water channels, aquaporins, and bronchial epithelial cells to "shrink," with an increase in intracellular ion concentration (17) and release of inflammatory mediators from mast cells, eosinophils, neutrophils, and other inflammatory cells including newly formed eicosanoids (32, 33). The epithelium may serve as a key regulator of the balance of eicosanoids in the airways by activating the release of bronchoconstrictive eicosanoids in inflammatory cells in close contact and by alterations that reduce the synthesis of the protective PGE2 (34). So the main factor is now thought to be the inflammation induced by changes in airway osmolarity, and both osmolar and thermal mechanisms may work together under conditions of significant heat loss involving airway rewarming after cooling of the airways as the initiating mechanism. During normal tidal breathing, the nose functions like a rebreathing organ with warming up and humidifying the inspired air. The respiratory heat loss increases with increasing exercise intensity due to the increased ventilation. If the inhaled air is cold, the respiratory heat loss with the resulting cooling of the airways is further enhanced (35, 36). The cooling of the airways results in reflex parasympathetic nerve stimulation causing bronchoconstriction through the vagal nerve (37). At first, it is notable that a reflex vasoconstriction of bronchial venules to conserve heat occurs, but when exercise ends, the increased ventilation ceases, as does the cooling stimulus, causing a rebound vasodilatation of the peribronchial venules. The resulting smooth muscle constriction due to nerve stimulation and mucosal edema due to vasodilatation in susceptible individuals reduces the size of

the bronchial lumen with increased airways resistance (37). Even in bronchoalveolar lavage fluid, it is possible to find increased peptidoleukotriene concentrations because of the bronchial epithelial damage with eosinophil and neutrophil influx due to exercise (29).

ATOPY AND ENVIRONMENTAL FACTORS

Environmental factors such as temperature of inhaled air, the humidity and intensity of exercise have a significant effect on the induction of bronchoconstriction. In a study made with the aim to understand if school also could be a significant site of allergen exposure for children in terms of environmental factors, such as atmospheric conditions and the presence of allergens, both potentially predictive of exercise-induced symptoms during physical education, it was showed an effect of environmental factors, like humidity and barometric pressure, and environmental allergens, in particular it was observed of cat allergens, on the occurrence of the EIB and cough in schoolchildren (38). Atopy and upper airway diseases are also known to influence EIB. A study, made on adult population, mean age 22.8 years, with the objective to identify differences between EIB alone and EIB with asthma, did not reported significant difference in total IgE, atopy rate, and house dust mite sensitization rate but it was observed an increased sensitization rate to outdoor molds in EIB-positive patients (39). Evidence supports airway hyperresponsiveness and decreased lung function from chronic exposure to air pollutants during exercise. The increased development of atopic dermatitis (AD) in infancy and subsequent allergic rhinitis and asthma in later childhood is known as the atopic march (40). The progressive atopy is dependent on various underlying factors such as the presence of filaggrin mutations as well as the time of onset and severity of AD. A dysfunctional skin barrier was suggested as a site for allergic sensitization to antigens and colonization of bacterial super antigens. This induces systemic Th2 immunity that predisposes patients to allergic nasal responses and promotes airway hyper reactivity. AD is a major risk factor for the development of asthma, and children with AD have an increased odds ratio of developing asthma compared to children without AD in several longitudinal studies. The main risk factors for progression and persistence of asthma are IgE sensitization and early onset and severity of AD. Epidemiologic studies illustrate strong associations between rhinitis and asthma (41). Studies on the prevalence of asthma in patients with rhinitis vary considerably, but it has been reported to be as high as 80% (41). Many patients with allergic rhinitis have lower airway hyperreactivity or bronchial hyperresponsiveness. Allergic rhinitis as a risk factor for developing asthma has been supported by several studies (42). Ciprandi et al. (43) showed that nasal symptoms, airflow, and markers of inflammation (eosinophils, Th2 cytokine levels) directly correlate with lower airway markers including FEV1. It was found that approximately 75% of subjects with asthma report rhinitis; patients with rhinitis have increased risk for asthma and lower airway reactivity compared to patients without rhinitis (44); furthermore it was observed that risk for asthma increases from 2.0% in subjects without rhinitis to 18.8% in subjects with allergic rhinitis when exposed to either pollen or animal dander. Exercise may trigger allergic respiratory, systemic and skin disorders (1).

SYMPTOMS

A large variety of symptoms could be present: shortness of breath, enhanced breathing effort, chest tightness, cough, wheezing, decreased performance, increased fatigue, chest pain, and chest tightness (18). Exertional dyspnea in children may be a presenting symptom but rarely in isolation as this usually is suggestive of an alternate diagnosis of deconditioning or vocal cord dysfunction (VCD) or cardiopulmonary disorders. Symptoms that are more apparent on inspiration may indicate exercise-related laryngeal obstruction (45). VCD occurs during exercise, while EIB usually occurs afterward but may overlap, and the two entities may coexist with VCD and asthma. In the majority of studied children with asthma, the time to maximal bronchoconstriction after exercise is short, suggesting that the onset of EIB occurs during exercise (46). Symptoms typically appear within a few minutes after the start of exercise and may continue for 10 or 15 min after the end of workout. Anyone can experience these symptoms, especially if out of shape, but with EIB, they are more severe than would be considered normal (47, 48). Even non-specific symptoms of stomachache or sore throat in children may be indications of EIB (49).

DIAGNOSIS

The diagnosis of EIB is established by changes in lung function provoked by exercise, not on the basis of symptoms neither on therapeutic trials without diagnosis (4). In the clinical practice, often an exercise challenge can be indicated in subjects with suggestive symptoms but showing to have normal to near-normal spirometry both before and after bronchodilator (4). This challenge can occur with free running, treadmill running or cycling, or with exercise surrogates such as mannitol (18), particularly in patient with known asthma for reason of safety (4). The objective tests of bronchial responsiveness are divided into "direct tests" [methacholine (MCH), histamine] and "indirect tests" [exercise, mannitol, adenosine 5-monophosphate, non-isotonic aerosols, and hyperpnea (EVH)]. The MCH test is widely used. MCH acts as an analog of acetylcholine, directly stimulating the cholinergic receptors in the airways' smooth muscle. It has a high sensitivity but a low specificity for active asthma and a low sensitivity to recognize EIB (29, 50). Mannitol test can reproduce the "osmolar" mechanism of EIA/EIB, through the osmotic action of this agent, demonstrating sensitivity and specificity comparable to the MCH for the diagnosis of EIA/EIB (29, 51, 52). Another effective test, made by ventilating dry air with CO_2 for 6 min through a low-resistance circuit at a rate higher than that usually realized during maximum exercise, is the EVH test (19). Test is positive when a $\geq$10% sustained reduction in FEV1 is achieved. However, at the moment specialists prefer to detect serial lung function measurements after a specific exercise to identify EIB and to quantify the severity of the disorder. Normally the value assessed is the FEV1 because more reproducible and more selective than peak expiratory flow rate. The airway response is reported as the percent fall in FEV1 from the baseline value. The exercise challenge requires pulmonary function monitoring. The ideal setting should be characterized by dry air preferably with relative humidity <20%

and temperature <22°C. Anyway at the moment, we there is not a firm consensus for the conditions under which exercise should be performed (4). The challenge is performed with 2 min to ramp up to at least 85% of the maximum heart rate or 95% for children or athletes, maintaining this heart rate for 6 min (53) and monitoring pulmonary functions every 5 min until 30 min after challenge (15, 19, 29). The difference between the preexercise FEV1 value and the lowest FEV1 value recorded within 30 min after exercise is expressed as a percentage of the preexercise value. EIB diagnosis requires a ≥10%fall in FEV1 within 30 min of challenge (**Figure 1**). The severity of EIB can be graded as mild, moderate, or severe if the percentage fall in FEV1 from the preexercise level is >10% but, 25%, >25% but, 50%, and >50%, respectively (22). The surrogates for exercise testing, developed because easier to implement than exercise challenge, such as eucapnic voluntary hyperpnea or hyperventilation, hyperosmolar aerosols, including 4.5% saline, and dry powder mannitol (22) were established before the widespread use of inhaled corticosteroids (ICSs). Today, a decline in the FEV1 of ≥30% in a person taking inhaled steroids is considered severe EIB. Exercise challenge testing induces high levels of ventilation ideally by a rapid increase in exercise intensity over 2 to 4 min. Most protocols involve running while breathing dry air (10 mg H_2O/L) with a nose clip in place. A valid test need the achievement of an optimal ventilation, greater than 21 times the resting FEV1, for 4 to 6 min of exercise, after which serial measurements of lung function are executed. Alternative diagnostic tests comprise inhalation of hyperosmolar saline, eucapnic voluntary hyperpnea of dry air and inhalation of dry powder

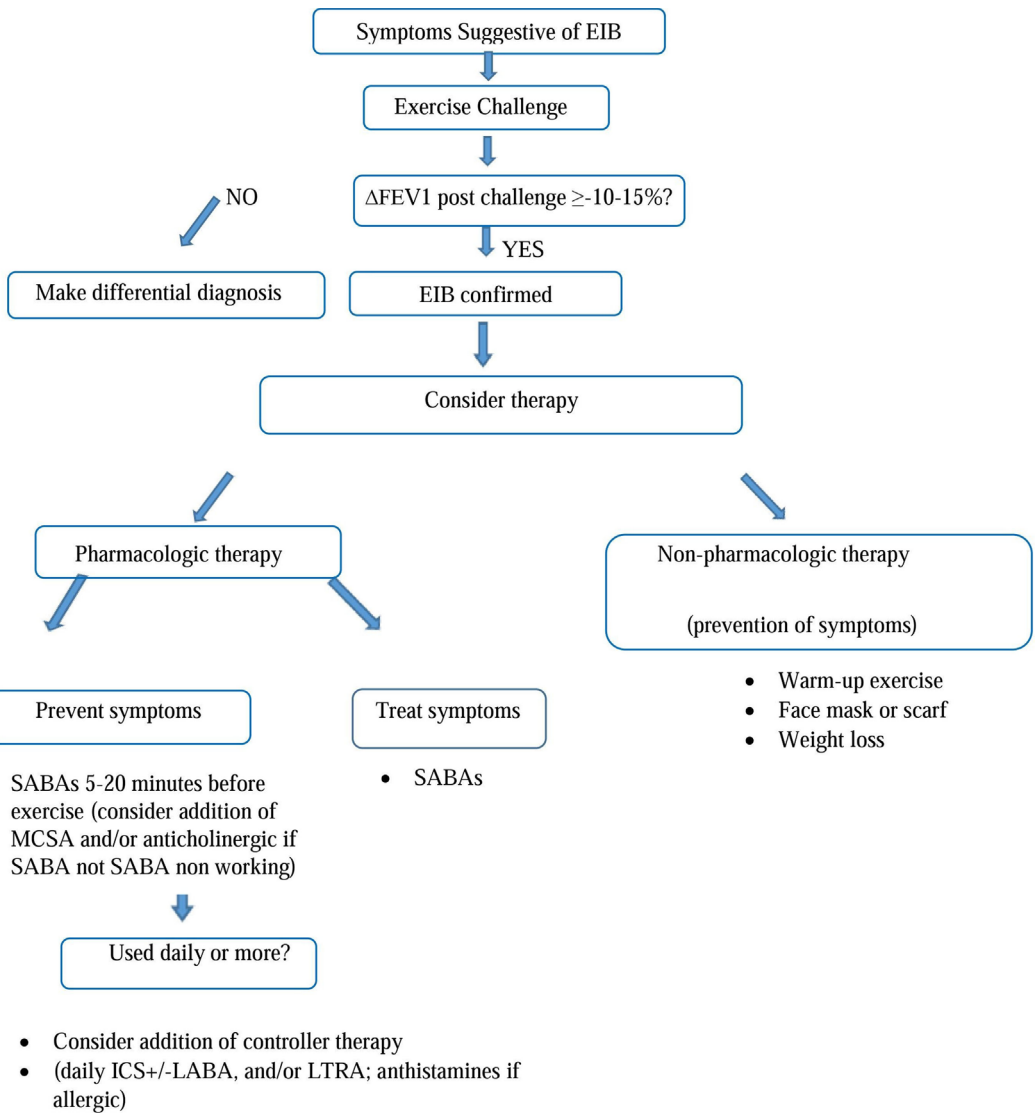

FIGURE 1 | Algorithm for diagnosis and treatment of EIB. [Figure adapted by author from Parsons et al. (22). This reference provides the most contemporary state-of-the-art American Thoracic Society (ATS) guidelines on EIB]. Abbreviations: EIB, exercise-induced bronchoconstriction; FEV1, forced expiratory volume in the first second; SABA, short-acting b2-agonists; MCSA, mast cell stabilizing agent; ICS, inhaled corticosteroids; LABA, long-acting b2-agonists; LTRA, leukotriene receptor antagonists.

mannitol (54). Is possible to diagnose EIB in 3–6 years of age children by measurement of FEV0.5 and airway resistance using the interrupt technique in 5–12 years old (15, 19, 29). In fact among bronchoprovocation tests in preschool children, interrupter resistance (RINT) has been used in association with spirometry to evaluate the presence of EIB. 36% of children (18 of 50) showed the presence of broncho-obstruction after exercise, showing that RINT may be a valid alternative method, definitely easier to run than spirometric examination, in these cases (55). Differential diagnosis is needed with other respiratory diseases (**Table 1**), distinguishing inspiratory stridor alone from inspiratory stridor with or without expiratory wheezing (4). For example is essential to differentiate EIB from such as the exercise-induced VCD, that should be consider when the respiratory distress is inspiratory and it occurs with inspiratory respiratory stridor during maximum exercise. This illness may co-occur with VCD. To differentiate EIB/EIA from other lung illnesses is essential to prescribe the best therapy as asthma treatment has no effect upon this and others pneumological diseases (1).

TREATMENT

We should classify EIB treatment in pharmacological and non-pharmacological therapy. Non-pharmacological therapy includes maneuvers to improve air condition during exercise by pre warming and humidification, making warmup before the exercise, improving general physical conditioning such as weight loss if necessary (22). Preventing water loss by using a face mask may promote humidification and attenuate EIB (4). Nowadays need validation both dietary supplementation with omega-3 fatty acids and ascorbic acid and measures to reduce sodium intake appearing inconclusive in reducing EIB (4). Many therapeutic options are available to prevent EIB. For example, inhalation of β2 agonist at recommended dose immediately before exercise effectively prevents the symptoms by stimulating β2 receptors on mast cells inhibiting release of contractile mediators and inducing relaxation of the airway smooth muscle like an antagonist of such mediators (15). Principal pharmacologic treatments are short-acting b2-agonists (SABAs) and long-acting b2-agonists (LABAs), ICSs, and leukotriene receptor antagonists (LTRAs). Inhaled anticholinergic agents (ipratropium) and antihistamines furthermore should be useful to control symptoms. For patients with EIB, the official American Thoracic Society (ATS) clinical practice guideline suggests administration of an inhaled SABA before exercise (48). The SABA is typically administered 15 min before exercise. A controller agent is generally added whenever SABA therapy is used daily or more frequently. In patients with EIB who continue to have symptoms despite using an inhaled SABA before exercise, or who require an inhaled SABA daily or more frequently, because of serious side effects, ATS recommend against daily use of an inhaled LABA as single therapy. The potential duration of protection afforded by a β2 agonist is 4–6 h for SABAs, like albuterol or terbutaline, and twice as long for LABAs, such as formoterol or salmeterol. However, tolerance may be developed if these drugs are taken daily (56), with reduction of duration of protective effect approximately to 2 h for SABA and 6 for LABA. Probably the tolerance is due to the downregulation of β2 receptors on the mast cell with poor efficacy in preventing mediator release. Furthermore because of the tolerance EIB may have a more rapid onset and an incomplete and slow answer to a SABA (4, 57). ATS recommendations provide administration of ICS not only before the exercise but daily. In fact ICS should be taken for 2–4 weeks to see the maximal improvements. ICSs alone or in combination with other drugs can improve the EIB control in terms of severity and symptoms frequency but they do not prevent the tolerance from LABA daily therapy (4). Even if the effective role of leukotriene antagonists are not defined, montelukast can be used daily or intermittently (4) to prevent EIB, as observed efficacy at 2, 12, and 24 h after a single oral dose of 10 mg (58). Montelukast is reported to reduce the% fall in FEV1 by 40–60% and to reduce the recovery's time of FEV1, without tolerance induction (59). Combined therapy of antihistamine with montelukast can be useful and more effective than a single drug but the combination is not generally recommended (15).

TABLE 1 | Exercise-induced asthma: differential diagnosis [modified from Del Giacco et al. (1)].

Diagnosis	Patients	Symptoms	Test
Exercise-induced asthma	Children, asthmatic or not, during physical activities	Dyspnea, wheezing, cough, thoracic pain	Spirometry before and after exercise, refer to a sport medicine specialist
Exercise-induced vocal cord dysfunction	Asthmatic children and children active in sports	Symptoms occur during maximum effort. Symptoms disappear when exercise is stopped unless the patient continues to hyperventilate. The dyspnea is of inspiratory type. There are audible inspiratory sounds from the laryngeal area and no signs of bronchial obstruction	Exercise test with maximal exercise load, 6–8 min duration
		No effect of pretreatment with inhaled bronchodilator	Direct laryngoscopy during exercise test
Exercise-induced hyperventilation	Children and adolescent active in sports, children in general	Hyperventilation with respiratory dyspnea and increased end-tidal CO_2	Case history, observation during dyspnea
Exercise-induced anaphylaxis	Children and adolescent active in sports	Shortness of breath accompanied by pruritus, urticarial and low blood pressure	Allergy skin test, identify possible dietary triggers
Chronic lung diseases	Children affected by difficult to treat asthma, cystic fibrosis etc	Exercise limitation due to reduced lung function	Maximal exercise stress test with oxygen consumption, lung function test

In terms of stabilization of mast cells, sodium cromoglycate and nedocromil sodium may be a valid therapeutic option, effective since from the first administration, made shortly before exercise, but with short duration of action both alone and with other drugs are indicated in case of EIB (4, 60). Inhaled anticholinergic agent before exercise may be considered as an alternative and suggested treatment. However, in the clinical practice, a daily inhaled ICS or daily LTRA appears at the moment as the first option, chosen case by case depending on patient's lung function and characteristics. There is an intrapatient and interpatient variability in the treatment efficacy. In allergic patients, with poor improvements and control with only inhaled SABA before exercise, ATS suggests administration of an antihistamine. For all patients with EIB, interval or combination warm-up exercise before planned exercise is recommend and in subjects with EIB who exercise in cold weather, routine use of a device (i.e., mask) that warms and humidifies the air during exercise is recommended. In conclusion, any intervention that reduces the amount of water lost or increases the water content of the inspired air will reduce the severity of EIB. EIB is often the first sign of asthma to come and the last to go with treatment so that control of EIB is an indicator of asthma control (15).

IMPORTANCE OF SPORT IN CHILDREN WITH EIB

The impact of EIB significantly reduces quality of life and participation in sports. When participating in systematic physical training, the asthmatic adolescent or child improves fitness and quality of life as confirmed by a Cochrane-based meta-analysis of eight training studies, including 226 asthmatics from 6 years of age (30). Physical activity is generally accepted to be an advantage to young children in terms of bone development, motor skills, improved cardiovascular fitness, and self-esteem (61). There is evidence that asthmatic children with well-controlled disease, even those with documented bronchial hyperresponsiveness, can achieve levels of exercise performance similar to those of non-asthmatics (62). Several studies have identified significant improvements in aerobic fitness (63, 64) and asthma-related benefits such as reduced hospital admissions, reduced absences from school, reduced medication use, and fewer doctor's visits after exercise performance (65). Moreira et al. also demonstrated, in children with persistent allergic asthma, that a physical training program did not increase airways inflammation but decreased their total and allergen-specific IgE levels (29, 66). Finally, preliminary data show that regular exercise reduces IL-2 production, meaning that lymphocytes are probably less responsive to exogenous stimuli, and IL-4 producing lymphocytes are also reduced, suggesting a better clinical condition for allergic people that exercise regularly (67). Overall, children with asthma should be medicated appropriately and encouraged to participate in regular physical activity. Successful management allows for participation in the chosen sport for the pediatric recreational athlete as well as with elite Olympic athletes with asthma. Olympic athletes with asthma have won gold and silver medals proportionately with their non-asthmatic competitors (18). But which sports? Subjects who participate in endurance and winter sports as well as swimming are at higher risk for EIA/EIB. Long-duration exercise and very low air temperature easily expose these patients to the osmolar and vascular changes in the airway, fundamental in the EIA/EIB pathophysiology. Types of training and atopy are independent risk factors for EIA/EIB but combining the two factors in a logistic regression model and atopic speed, EIA/EIB is significantly more common in athletes compared with control subjects, especially in swimmers but also in long-distance runners and track and field athletes. Furthermore atopic disposition appears strongly associated with increased bronchial responsiveness, being the most important risk factor for EIB and asthma (68). However, the apparent highest prevalence of bronchial hyperresponsiveness in swimmers is due to the fact that athletes selected swimming as their primary event but is known that swimming does not cause EIA/EIB as much as other activities like running, for example, even by avoiding outdoor allergens (68). In addition, for the asthmatic athlete it is also important to avoid strenuous exercise during temporarily increased exposure to "biological stress." This can be increased aeroallergen load, extreme cold air environment, or strenuous exercise too close to a recent viral respiratory tract infection. With an early and precise diagnosis, insightful precaution protecting the airways from extreme biological stress and an early start of anti-inflammatory treatment, the progression of bronchial hyperresponsiveness and asthma in these children having sport may usually be well controlled. It should be useful warm up with gentle exercises for about 15 min before to start more intense physical activity. Cover the mouth and nose with a scarf or face mask when subjects exercise in cold weather and try to breathe through the nose during the exercise. Sports that require only short bursts of activity are preferred, including volleyball, gymnastics, baseball, wrestling, golf, swimming, football and short-term track and field events. Some swimming events can demand constant activity, but the warmth and humidity from the water make it easier for people with EIB to breathe, activities such as walking, hiking, and recreational biking. Sports and activities most likely to cause EIB symptoms are those requiring constant activity or done in cold weather, such as soccer, basketball, long-distance running, ice hockey, ice skating, and cross-country skiing (47, 48). Sports with low risk for the development of asthma and bronchial hyperresponsiveness are the ones in which the physical effort is of short duration and in which high ventilatory levels are not reached. Medium-risk sports are team sports in general, in which the alternation of aerobic and anaerobic phases, as well as the relatively brief periods of continuous high-intensity exercise (in any case usually lower than 5–8 min), results in a lower risk of bronchial hyperreactivity. High-risk sports, as already stated, are endurance and winter sports in general (26). It is important that children's physical activities are adapted to their situation, teachers should know what to do in emergency and that parents help their children to take their medication properly but the most important thing is to choose a sport that each children enjoys (69).

AUTHOR CONTRIBUTIONS

AT and RC conceptualized and designed the review. SC contributed to data collection, carried out the initial analyses and

interpretation of data in literature, and wrote the manuscript. AT coordinated and supervised analysis and interpretation of data, critically reviewed, and revised the manuscript. AM contributed to data collection and interpretation of literature. All the authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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MINI REVIEW
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Asthma, Food Allergy, and How They Relate to Each Other

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The association between atopic diseases is well known, and previous research has shown that having one atopic disease can predispose to having another. The link between asthma and food allergy has been well researched, but the exact relationship between the two atopic conditions is not fully understood. Food allergic infants are at increased risk for the development of asthma and are at risk of food-induced asthmatic episodes and also anaphylaxis. Having a diagnosis of both food allergy and asthma has also been shown to have an effect on the severity of a patient's disease including being at greater risk of severe asthmatic episodes. Therefore, understanding the relationship between these two conditions in order to treat and manage these children safely is crucial to clinicians.

Keywords: asthma, food allergy, children, wheeze, anaphylaxis

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BACKGROUND

Diseases including asthma, eczema, allergic rhinitis, and food allergy are typically considered as allergic diseases, although the exact association with atopy is frequently debated for eczema and asthma. Nonetheless, such diseases commonly coexist and are common in pediatric populations worldwide. Children affected with one allergic disease frequently develop other allergic diseases. The sequence of disease progression is often referred to as the "atopic march" (1). For example, infants with eczema are at higher risk of developing food allergy, children with egg allergy are at increased risk of developing allergic respiratory diseases, and children with allergic rhinoconjunctivitis are at increased risk of developing asthma. Furthermore, children with a single food allergy frequently develop additional food allergies. The causal relationship between these atopic diseases remains unclear as it is not absolute in all patients and the sequence may vary (2–5). The dual-allergen hypothesis provides a plausible explanation as to how allergic disease may progress. It describes how early allergic sensitization occurs through breakdown of skin barrier integrity that allows for exposure to food and environmental allergen, an effect that can be moderated be early-life ingestion for foods such as peanut and is some studies hens egg (6–10). Research has shown that the development of eczema can be associated with mutations of the filaggrin gene that is responsible for a major structural protein in the epidermis (11). Thus, children with eczema are at greater risk of developing food allergies due to a weakened skin barrier; for example, a study reported that 50% of children with eczema developed food allergy by 1 year (12). Similarly, Martin et al. (13) showed that in infants with eczema, the earlier onset and greater severity of their eczema symptoms increased their risk of food allergy. This emphasizes the hypothesis that allergen exposure through the cutaneous route contributes to allergic sensitization and highlights the key role of skin barrier integrity in protecting the infant immune system, which has been seen in children sensitized to peanut allergy (14, 15). A review showed that approximately 70% of patients with severe eczema developed asthma or allergic rhinitis later in life, and asthmatic patients who had filaggrin mutations had a difficult disease course with more asthma exacerbations (11). Other work has shown that patients who have filaggrin

loss-of-function mutations have a significant association with food challenge confirmed peanut allergy (16).

Asthma

Asthma is one of the most common long-term childhood conditions of which approximately 9% of children are affected by it (17). Asthma is defined as a chronic respiratory disease characterized by recurrent attacks of wheeze and breathlessness. These symptoms occur due to irritation that occurs in the airways causing inflammation and swelling resulting in reduced lung airflow (18). Over time with advances in asthma medicine, management continues to change but treatment primarily focuses on assessment of asthma severity, the use of acute and chronic medications including bronchodilators, anti-inflammatory medication (i.e., steroids), and treatment of comorbidities (19). In the management of acute asthma, the goals are to reverse airflow obstruction, correct significant hypoxia, and prevent future relapses (20). In order to achieve this, management for acute exacerbations includes the use of oxygen, short-acting inhaled beta-agonists, ipratropium bromide, systemic corticosteroids, and magnesium sulfate. With regards to long-term management of asthma, stepwise escalation strategies following regular symptom assessment and lung function tests include using medications such as inhaled long-acting beta-agonists, inhaled and systemic corticosteroids, and leukotriene-receptor antagonists (21).

Food Allergy

In the last three decades, there has been a worldwide increase in the prevalence of food allergy with 3.5–8% of children having food allergies (17, 22, 23). Food allergy is defined as an adverse immunological reaction that occurs on exposure to a food that re-occurs on repeat exposure (22). It is usually classified into immunoglobulin-E (IgE)-mediated food allergy, non-IgE-mediated food allergy, or mixed IgE- and non-IgE-mediated allergy. IgE-mediated allergy has an acute onset (within 2 h of exposure), and presenting symptoms are often respiratory, skin, and gastrointestinal in nature, whereas non-IgE-mediated food allergy has a delayed onset of symptoms (from 1 to 24 h), and the symptoms tend to be skin and/or gastrointestinal (24). The key investigations for diagnosing food allergy include taking a thorough clinical history, skin prick testing, serum-specific IgE, and the double-blinded oral food challenge, which considered the gold standard investigation (25). For food allergies proven by positive oral food challenges, recommendation is for strict avoidance of the causative allergen. For those patients who experience life-threatening symptoms of IgE-mediated food allergy, an emergency self-injectable adrenaline device is often prescribed as well. However, over the last 10 years, increasing research has been performed looking at the use of oral, sublingual, and epicutaneous immunotherapies to desensitize patients through tolerance induction (26).

Understanding the Relationship between Food Allergy and Asthma

Asthma and food allergy have been commonly shown to coexist with each other, especially as they often share risk factors (family history of allergy, atopic eczema, and asthma) but the way in which they interact and influence each other is yet to be fully understood. Studies have shown that food allergies can develop in the first year of life and precede the development of asthma (17, 27). There has also been increasing recognition that there is an allergic component to asthma as a disease with particular focus on the role of environmental allergens (i.e., house dust mite and cat allergens). Exacerbations of asthma can be caused by exposure to inhalant allergens, although avoidance of these allergens on asthma disease is not entirely understood. For example, a study that looked at the effectiveness of avoiding house dust mite allergen on asthma management found that using allergen-impermeable covers to avoid house dust mite allergen did not have a significant effect on clinical asthma (28). Schroeder et al. showed that there was a higher prevalence of asthma in children with food allergy as well as it occurring at a 7 earlier age compared to children without food allergy (29). Another study showed that compared to children who were not sensitized to common food and aeroallergens, those who were cosensitized had a higher risk of developing respiratory allergic disease (27). Studies have also looked at the timing of when food sensitization occurs and have shown that food sensitization early in life (within the first 2 years of life) is a strong predictor of allergy by school age and also children with food allergy have approximately double the chance of developing asthma and rhinitis (30, 31).

There also seems to be an association between asthma and non-IgE-mediated food allergy, although it is less prevalent than that seen in IgE-mediated allergy (32). In a study, approximately one-third of children with non-IgE-mediated food allergy had asthma and allergic rhinitis (33). Higher rates of asthma (26–66%) have also been reported in eosinophilic esophagitis, which is considered a food allergy disease (34–36).

ASTHMA TRIGGERED BY FOOD ALLERGENS

The way in which food allergens may trigger asthma symptoms is not fully understood. The respiratory symptoms that occur in food allergic reactions commonly include rhinitis, bronchospasm, cough, and laryngeal edema (17). One theory is that particles of ingested food are inhaled into the airway, and exposure of these allergenic proteins to mast cells in the lungs causes inflammation and therefore respiratory symptoms (17, 24). Commonly documented respiratory reactions from aerosolized food proteins have been well documented over the years, mostly in adults. One of the most commonly described examples is baker's asthma where exposure to inhaled flour proteins causes an IgE-mediated type reaction, which manifests as asthmatic symptoms. Diagnosis is based on a history of work-related asthma symptoms, skin prick tests, and inhalation challenges to bakery allergens, which is the gold standard test (37). Aerosolized fish protein allergens have also been detected in open-air fish markets and can cause respiratory related symptoms due to inhalation of fish proteins (38). Similarly, occupational asthma and allergy has been reported in snow crab-processing workers who on cumulative exposure to snow crab have developed symptoms of asthma and allergy

(39). With regards to children, Roberts et al. performed bronchial challenges in children with proven IgE-mediated food allergy and asthma using aerosolized foods (40). In this study, despite dietary avoidance of allergens (i.e., fish, milk, eggs, chickpeas, and buckwheat), the children had worse chronic asthma symptoms when there was environmental exposure to the foods (i.e., families cooked with the allergenic foods at home). However, when the families stopped cooking the allergenic food(s) at home, the child's symptoms improved, and they needed less inhaled corticosteroid treatment (40).

There has also been research performed on specific food allergens and their association with respiratory symptoms and the development of asthma. For example, in a large birth cohort study, having egg allergy during infancy was predictive of respiratory allergy later in childhood (2). In fact, they reported a positive predictive value of 80% if the child also had eczema (2). Rhodes et al. found that in a study of 100 infants who were deemed at high risk of developing asthma and atopy (i.e., had atopic parents), those who were sensitive to egg and milk in the first year of life was predictive of having asthma as an adult (5). Another study looking at peanut or tree nut allergies showed that patients with a severe history of asthma were at greater risk of life-threatening bronchospasm occurring after ingestion of nuts ($p < 0.0001$) (41).

Factors Contributing to the Development of Food Allergy and Asthma

In children, 4–8% of asthmatic patients have food allergies and approximately 50% of those with food allergies have allergic reactions that involve acute respiratory symptoms (24, 42). Various factors have been found to affect the risk of developing asthma in children with food allergy but some of the key factors include seasonal changes, the host immune response, and the use of anti-IgE treatments. Asthma exacerbations seem to coincide with seasonal changes, for example, increase aeroallergen levels such as grass pollen in the spring (43) and dust mite in the autumn (44). The specific-IgE antigens bind to mast cells and basophils causing an inflammatory response within the airways, which over time can cause airway modeling (45). The host immune response to allergens activates an inflammatory process causing allergic cytokines to be released and a subsequent rise in IgE levels, which have been shown to be associated with an increased risk of asthma (45, 46). In one study, they found that high IgE levels at 6 months old was associated with early-onset of asthma and also a strong relationship with the development of asthma in school-age years (47, 48). Milgrom et al. showed that 90% of children in their study with asthma had positive skin prick tests to common allergens (45). Also, the use of anti-IgE treatment (i.e., monoclonal anti-IgE antibodies such as omalizumab) in asthmatic patients has been successful, which suggests there is a role of IgE in the pathogenesis of asthma. There have been studies that have shown that omalizumab is effective in reducing the need for corticosteroids including complete withdrawal of corticosteroids compared to placebo, a reduction in asthma-related symptoms, and improvement in quality of life of these patients (45, 49).

Morbidity and Mortality in Asthma

In adults, there is evidence that being allergic to more than one food is associated with an increase frequency of oral steroid use and a higher risk of lifetime hospitalizations and emergency department attendances (50). In children, a study showed that having a milk or peanut allergy was associated with an increased number of hospitalizations ($p = 0.009, 0.016$) (51). More specifically, having a milk allergy was associated with an increased use of systemic steroids ($p = 0.001$) (51). Simpson et al. also showed that children with asthma who also had peanut allergy had a 1.59 times greater rate of systemic steroid use and 2.32 times greater rate of hospitalization (52).

There is also evidence that suggests that exposure to food allergens can be a risk factor for life-threatening asthma. For example, in a study of children with peanut allergy, 9% (4/46) of the children died from an exacerbation of asthma that represents a significantly higher fatality rate for an asthmatic population (53). Roberts et al. compared children aged 1–16 years with life-threatening asthma (defined as requiring admission to pediatric intensive care) to those without non-life-threatening asthma and showed that life-threatening asthma was significantly associated with having food allergy (OR 5.89, 1.06–32.61) and having multiple previous admissions for asthma (OR 9.85, 1.04–93.27) (54). Ernst et al. conducted a study in patients aged 5–54 years and 129 of the patients had "fatal" asthma. The main finding in this study was that over 10 prescriptions or more of bronchodilators was associated with an increased risk of near-fatal asthma, but they also found that food allergy was an independent risk factor for near-fatal asthma (odds ratio 5.1, 95% CI 2.4–11.1) (55). Similarly, a case–control study showed that patients with near-fatal asthma (defined as requiring ventilation on intensive care unit) were more likely to be food allergic (OR 3.6, 1.6–8.2) and/or have had anaphylaxis (OR 5.6, 2.7–10.6) (56). Vogel et al. compared children who had ward-based care or ambulatory care (i.e., no hospitalization required) with children with potentially fatal asthma (requiring pediatric intensive care admission) and also found food allergy to be a risk factor for life-threatening asthma (57).

Asthma has also been identified as a risk factor for anaphylaxis and is associated with poorer outcomes in children with food allergy (24). Boyano-Martínez et al. conducted a study where children with cow's milk allergy had a 10 times higher chance of a severe reaction if they also had asthma (58). Another study found that the majority of fatal reactions attributed to food allergy were asthmatic reactions that occurred in patients on daily asthma treatment (59). Furthermore, in a series looking at fatalities due to food-induced anaphylactic reactions, the majority of the children were asthmatic, and their respiratory symptoms were identified as the main cause for the severity of their reactions (60). With this evidence over time, guidelines have been produced by various organizations of which pediatric allergists are recommended to prescribe self-injectable adrenaline devices to patients who have both food allergy and asthma (24, 61, 62).

There have also been studies looking at preventative measures [i.e., house dust mite avoidance measures (i.e., protective mattress covers), allergen food avoidance] to prevent atopy in

children, of which there is some evidence showing a reduction in respiratory symptoms (i.e., nocturnal cough, severe wheeze) by 1–2 years of age (63–65). Some studies suggest that immunotherapy to respiratory allergens can prevent allergic sensitization to new allergens, but this has not been observed in all studies (66, 67).

Clinical Implications for Patients

When reviewing food allergic or asthmatic children, a detailed clinical history should be taken to identify potential triggers for both allergic disease and asthma. If a specific trigger can be identified, primary advice is for avoidance of the allergen. Asthmatic patients with food allergies require regular assessments, careful monitoring and dietary and emergency management plans, review of treatment, and medication adherence reviews. In cases of status asthmaticus, the use of intramuscular adrenaline should be considered if there is a history of food allergies. Equally, patients who have known food allergies with respiratory symptoms should be offered beta-agonist inhalers.

CONCLUSION

Children who have both food allergies and asthma are at an increased risk of severe asthmatic episodes and may be at greater risk of food-induced anaphylaxis and also food allergen-triggered asthmatic episodes. It is important that clinicians educate patients appropriately regarding the higher risk of life-threatening asthma and anaphylaxis and ensure they receive regular assessments regarding their treatment and management.

Summary of key points

- Food allergic infants are at increased risk of developing asthma.
- Children with both asthma and food allergies are at increased risk of severe asthmatic episodes.
- Children with both asthma and food allergies may be at greater risk of allergen-triggered asthma episodes and food-induced anaphylaxis.
- Patients with known IgE-mediated food allergies and asthma should have immediate access to self-injectable adrenaline and inhaled beta-agonists.

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REVIEW
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The Nose and the Lung: United Airway Disease?

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Epidemiologic, pathophysiologic, and clinical evidences recently revealed the link between upper and lower airways, changing the global pathogenic view of respiratory allergy. The aim of this review is to highlight the strong interaction between the upper and lower respiratory tract diseases, in particular allergic rhinitis and asthma.

Keywords: allergic rhinitis, asthma, non-allergic rhinitis, local allergic rhinitis, airway disease

INTRODUCTION

During the second century, Claudius Galenus identified the effect of the upper airway on the lower airway and defined the nose as a "respiratory instrument" in his work "De usu partium." Nevertheless, the concept of the upper and lower respiratory passages being a continuum and forming a single unified airway has been highlighted only over the last 10–15 years, starting from the Allergic Rhinitis and its Impact on Asthma (ARIA) World Health Organization workshop (1–3).

The mechanisms of nose and lung interaction are complex, not entirely understood, and they have been long investigated (4). Dating back to 1919, Sluder hypothesized the existence of a nasal-bronchial reflex (5), supported by the evidence of a similar innervation of both upper and lower airways (6). More recent studies have demonstrated the role of localized inflammatory changes in the upper and lower airways, which lead to a systemic response (7–9).

The aim of this review is to underline the strong anatomical, epidemiologic, pathophysiologic, clinical, and therapeutic evidences (summarized in **Table 1**) supporting the connection existing between the so-called United Airway Disease (UAD). We focus our attention on rhinitis and asthma, the most frequent and chronic inflammatory diseases of the upper and lower airways, presenting with several phenotypes.

RHINITIS AND ASTHMA

The term rhinitis includes several different phenotypes and the diagnosis of each of these subtypes is often an interesting challenge (10). It is usual to divide rhinitis in allergic rhinitis (AR) and non-allergic rhinitis (NAR), based on allergological evaluation (10, 11). AR is a disease of the nasal mucous membranes, induced by an IgE-dependent inflammation after the exposure to allergens (12). Symptoms of AR include rhinorrhea, nasal obstruction or blockage, nasal itching, sneezing, and postnasal drip, which reverse spontaneously or after treatment. AR is classified based on the frequency of symptoms in intermittent (symptoms occurring on less than 4 days per week or for less than 4 weeks per year) or persistent (symptoms occurring on at least 4 days per week or for more than 4 weeks per year). In addition, considering the severity of symptoms, AR can be divided into mild (normal sleep; no impairment of daily activities, no troublesome symptoms) or moderate to severe (one or more of: abnormal sleep; impairment of daily activities, and severe symptoms) (12). On the other hand, NAR includes a group of diseases characterized by the presence of at least two nasal symptoms, such as pruritus, rhinorrea, obstruction, sneezing in patients who are

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TABLE 1 | The evidence and the mechanisms of nose and lung interaction.

Anatomical and histological evidence	Epidemiologic evidence	Pathophysiologic evidence	Emerging biomarkers	Clinical and treatment evidence
The nasal and bronchial mucosae consist of ciliary epithelium resting on a basement membrane. Beneath the basement membrane is the lamina propria, glands, and goblet cells (17, 18)	19–38% of patients with allergic rhinitis (AR) have concomitant asthma and 30–80% of asthmatics have AR (15, 19)	The communication between the upper and lower airways is suggested to be *via* a bone marrow- derived systemic inflammatory response (17)	The role of microbiome: children being raised on traditional farms have a much lower prevalence of allergic disease as children grown up in urban settings (15, 18)	The treatment of AR can improve asthma symptoms (15, 18)
Both act as a transport system moving air in and out of the lungs (16)		The presence of epithelial basement membrane thickening, the typical hallmark of lower airway remodeling, not only in asthmatic patients but also in atopic patients without asthma and patients with AR (24)	The role of microRNA (miRNA): presence of the same particular miRNAs in different pathogenetic mechanisms of both AR and asthma, such as IL-13 pathway, GATA binding protein 3, and mucin secretion (42)	Decrease in asthma symptoms and AR after intranasal corticosteroid treatment of rhinitis (41)
Both provide defense against inhaled foreign substances, with most particles of 5–10 μm diameter filtered out by the nose, and irritant and soluble gases being extensively removed by dissolution in nasal secretions. The lower airway functions similarly, with smaller inhaled particles that reach the lower airway being trapped and cleared by the mucociliary escalator (19)		In non-allergic asthma it has been highlighted the importance of the presence of IgE in the bronchial mucosa, as in the nasal mucosa in local allergic rhinitis (10)		Leukotriene receptor antagonists are known to be useful for long-term management of asthma patients complicated by AR (42)
				The recombinant, humanized, monoclonal anti-IgE antibody Omalizumab improved nasal and bronchial symptoms and reduced unscheduled visits due to asthma (45)
				Allergen immunotherapy is effective for treating both rhinitis and asthma (38)

not sensitized to any allergen. The diagnosis is mainly based on exclusion (11). In the last few years, a new phenotype called local allergic rhinitis (LAR) has been defined (13). It is characterized by symptoms suggestive of AR due to a localized allergic response in the nasal mucosa, in the absence of atopy assessed by conventional diagnostic tests. The immunological characteristics of LAR are nasal Th2 allergic inflammation, with positive response to nasal allergen provocation test, and nasal production of IgE and inflammatory mediators (14). However, a better understanding of the underlying immune mechanisms of this disease is essential for developing diagnostic methods and targeted therapies.

According to the recent GINA guidelines, asthma is a chronic disease, potentially serious, characterized by a reversible airway obstruction, chronic airway inflammation, and bronchial hyperreactivity (15). It is diagnosed by the pattern of respiratory symptoms, such as wheeze, chest tightness, dry cough, and shortness of breath, that vary over time and in intensity, together with variable expiratory airflow limitation, induced by the bronchoconstriction, and morphological changes in the bronchial wall ("remodeling"). Symptoms are usually worse during the night or upon awakening, and the principal triggers are viral infections, allergens, tobacco smoke, exercise, and stress (15).

ANATOMICAL EVIDENCE

Anatomically, the respiratory tree is divided into upper (nose, pharynx, and larynx) and lower respiratory tract (trachea, bronchi, bronchioles, alveolar duct, and alveoli), separated by the larynx. Functionally, it is divided between conductive airways and gas-exchange region of the lungs, consisting of the respiratory bronchioles with the air cells (16). Although in daily practice, the nose and the lungs are considered as separate entities and treated by two different specialists, the upper and the lower respiratory tracts have anatomical and histological similarities, including the basement membrane, lamina propria, ciliary epithelium, glands, and goblet cells (17, 18). Moreover, both allow the passage of air into and out of the lungs and during inspiration the air is humidified, tempered, filtered, and supplied with nitric oxide before entering the lower airways. Nasal airconditioning capacities and filter function protect the lower airways from potentially harmful agents and it also participates to innate and adaptive immune defense (16). Patients with AR have partial or complete loss of function of the nose due to mucosal congestion and in this case the inhalation of cold or dry air can favor bronchoconstriction (19). Moreover, the nose protects the lower tract from inhaled

foreign substances by filtering out the particles of 5–10 μm diameter, while the irritant and soluble gases are extensively removed by dissolution in nasal secretions (19). The lower airway acts similarly: the smaller inhaled particles are trapped and cleared by the mucociliary escalator (19). Furthermore, in case of disease, they share macroscopic pathological characteristics (**Figure 1**) and also the histological appearance is similar, with a comparable allergic response in rhinitis and asthma (**Figure 2**). The main difference between the nose and the lungs is that upper airway obstruction is mainly caused by vasodilatation and edema, whereas the lower airway patency is influenced by smooth muscle function.

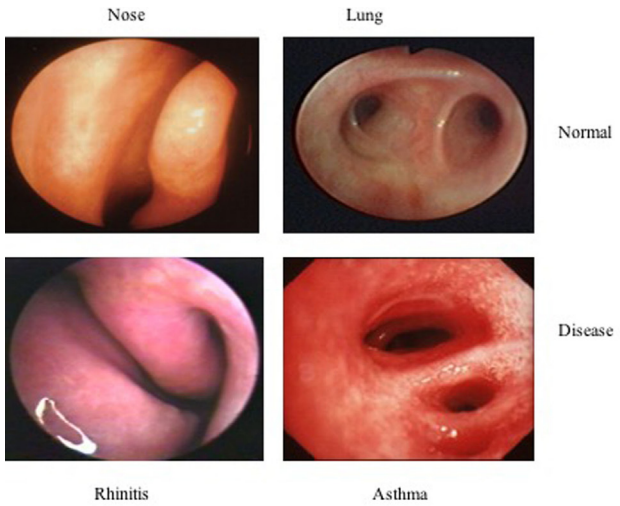

FIGURE 1 | **Macroscopic pathological characteristics.**

EPIDEMIOLOGIC EVIDENCE

Asthma and rhinitis are a major public health problem because of their frequency, their impact on quality of life, school performance, and economic burden (1). The influence on patients' quality of life has also been highlighted by a recent study on 2,896 children enrolled in the Taiwan Children Health Study, which demonstrated an association between allergic diseases, such as asthma and AR, and deficit hyperactivity disorder and oppositional defiant disorder (20). According to the International Study on Asthma and Allergy in Childhood, the prevalence of asthma worldwide was found to be 20%, and the prevalence of AR in Europe was observed to be 25% (1). Moreover, the prevalence of asthma and rhinitis varies all over the world: in countries with a more rural tradition, the numbers are usually lower than in countries with a higher level of urbanization (15, 19). Interestingly, the prevalence of asthma in subjects without rhinitis is usually less than 2% (17). The prevalence of AR appears to be at least triple the prevalence of asthma, and it has also been demonstrated that 19–38% of patients with AR have concomitant asthma and 30–80% of asthmatics have AR (15, 19).

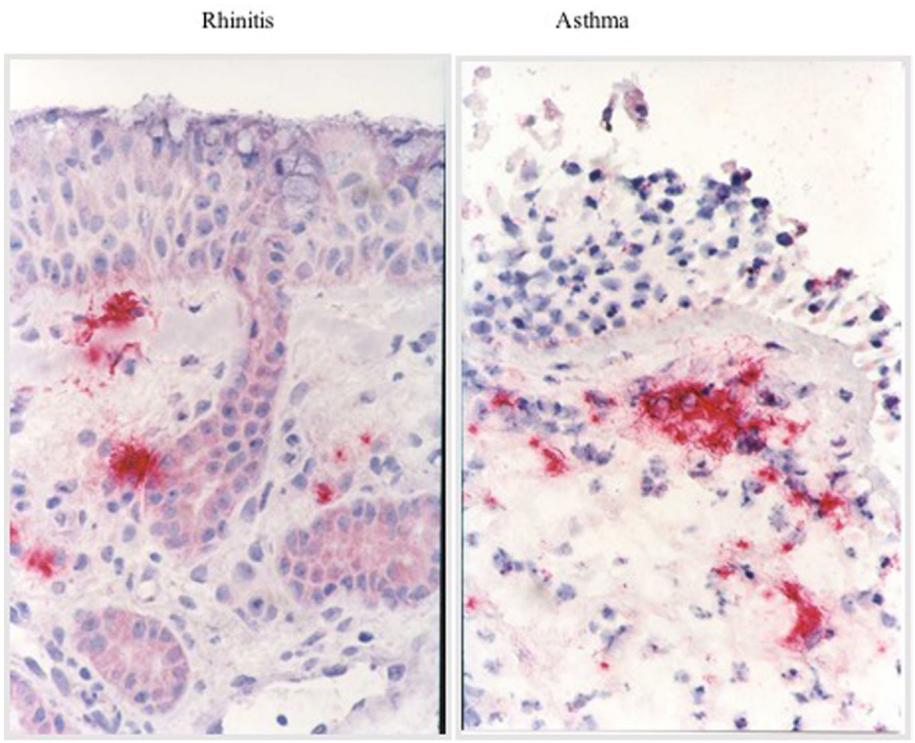

FIGURE 2 | **Histological appearance.**

PATHOPHYSIOLOGIC EVIDENCE

Allergic UAD

An immune response to external antigens induces the production of antibodies that are typically, but not exclusively, IgE antibodies. Allergic airway disease is caused by hypersensitivity or IgE-mediated reactions when inhaled allergen reacts with mast cells and basophils, the major effector cells, bearing IgE antibodies. The following cross-linking of allergen-specific IgE molecules bound to cells by allergen particles, results in the release of granule-associated mediators (i.e., histamine, tryptase), membrane lipid-derived mediators (i.e., leukotrienes), and cytokines (15, 20). Over time, the role of lipids in the pathogenesis of allergic disease has continued to expand thanks to sophisticated techniques capable of identifying and quantifying diverse lipid mediators (21). The early allergic response (maximal at 10–20 min) is usually characterized by edema, itching in the skin, rhinorrhea, sneezing, and erythema in the upper airways and bronchospasm, edema, mucous secretion, and cough in the lower respiratory tract. Instead, the late allergic response (within 2–6 h) in both the upper and lower airways is associated with an eosinophil activation and CD4 T cell tissue infiltrate, essential to maintain the chronic inflammatory process and tissue damage (20, 22).

To explain the interaction between the upper and lower airways, several mechanisms have been suggested (1, 16). The most likely mechanism is that localized inflammatory changes in the upper and lower airways leads to a systemic response, with bone marrow involvement, resulting in the release of progenitor cells that are then recruited to tissue sites. Inflammatory secretions may propagate from the upper airways to the lower *via* postnasal drip and systemic circulation. Although the nose is usually the first site of exposure to allergens or other noxious substances, despite a minimal nasal epithelial damage, a marked bronchial epithelial disruption may be present. Hence, researches hypothesized that the nasal mucosa has developed protective mechanisms that minimize remodeling and enhance epithelial regeneration (23). An important proof of the UAD interplay is the presence of epithelial basement membrane thickening, the typical hallmark of lower airway remodeling, not only in asthmatic patients but also in atopic patients without asthma and patients with AR. However, remodeling appears to be less extensive in the nasal mucosa than in the bronchial mucosa. The reason of this difference could be explained by the specific cytokine production of smooth muscle cells and by the presence of the genes of the embryologic differentiation in the nose and lungs or their different re-expression in asthma and rhinitis (24).

Non-Allergic UAD

In contrast to allergic UAD, the etiology and mechanisms involved in non-allergic UAD is still unknown. In non-allergic asthma, it has been highlighted the importance of the presence of IgE in the bronchial mucosa, as in the nasal mucosa in LAR (10). However, it is still not clear what is the role of allergens in the asthmatic symptoms in patients with LAR. Some of the possibilities include allergy triggered by unknown antigens (fungi), persistent infection (caused by *Chlamydia trachomatis*, *Mycoplasma* spp., or viruses), and autoimmunity (10).

RISK FACTORS

Allergen Exposure

The allergen exposure has been considered the major risk factor for the development of UAD (15, 18). Rhinitis and asthma are characterized by a high prevalence of sensitization to those allergens that are common in the community, i.e., aeroallergens. When considering different aero/inhaled allergens, the most important distinction is that between outdoor allergens (i.e., pollen and molds), indoor allergens (i.e., cat, dog, mite), and occupational agents. Nevertheless, there are still important questions about the relevance of current allergen exposure to these diseases and to their management. Different factors can influence the IgE antibody response including genetics, allergen dose, and early life exposures that may inhibit or enhance the response (25).

Genetic Factors

It is well known that allergic diseases run in families, implying a role for genetic factors in determining individual susceptibility: hereditability varies from 35 to 95% for asthma and from 33 to 91% for AR (26). Studies on the prevalence of allergic traits in relation to family history demonstrated incremental increases in risk of developing asthma or AR with the presence of one or both parents with allergic disease, and greater than three times the risk if allergic disease occurred in more than one first degree relative (26). To date, a positive family history remains one of the most reliable tools for prognosis of allergic disease (27). Moreover, the Multicentre Allergy Study has demonstrated that having parents with allergies is not only a strong predictor to develop any allergy but also strongly increases the risk of developing allergic multi-morbidity (28). A link of asthma and AR with different chromosomal regions was recently found thanks to genome-wide association studies. Actually, there are about 161 different potential biomarkers involved in respiratory inflammation (26). For example, Liu et al. have recently shown that the single-nucleotide polymorphisms in the TNFSF4 and FAM167A-BLK genes may be involved in asthma and AR gene (29) and Zhao et al. associated the PBX2 gene in the 6p21.3 asthma susceptibility locus with an increased risk for both AR and asthma (30). The genetic studies of allergic disease pave the way for tailored treatments to specific genotypes to improve therapeutic outcomes and minimize side effects.

Other Risk Factors

Environmental exposures during pregnancy including diet, nutrient intake (especially vitamin D) (31), toxins (smoking, air pollution, microbes, infection) can alter the epigenome and interact with inherited genetic and epigenetic risk factors to directly and indirectly influence organ development and immune programming (32). Considering these data, the primary prevention of allergic disease should begin very early in life, even *in utero* (25).

EMERGING BIOMARKERS

The Role of Microbiome

Microbiome is the totality of microbes, their genes, and their interactions in a given environment. It is increasingly accepted that human microbiome may play an important role by promoting the maturation of the host immune system. Thanks to advances in sequencing technologies, such as real-time quantitative PCR, it is now known that the microbes that inhabit healthy and diseased nose and lungs are different (33). According to the "hygiene hypothesis," microbial exposures in early childhood may prevent allergies and asthma by modulating the Th1/Th2 and Treg imbalance (33). As matter of fact, children being raised on traditional farms have a much lower prevalence of allergic disease as children grown up in urban settings. The diversity of the microbial exposure has been shown to account for the asthma-protective farm effect. Nevertheless, in urban areas high exposure to environmental microbes also relates to a lower prevalence of allergic disease (15, 18). So, the microbiome itself could be considered as a potential biomarker source. A recent study demonstrated an association of asthma with reduced α- and β-diversity of the nasal microbiota and the relative abundance of a bacteria belonging to the *genus Moraxella*. The linking of asthma and *Moraxella*, however, was restricted to children not living on farms. In contrast to the nasal samples, the throat microbiota characteristics were not related to asthma (34).

The Role of microRNA (miRNA)

microRNAs, a recently discovered regulators of gene expression, might be another non-invasive biomarkers to diagnose and characterize asthma and allergic. Circulating miRNAs have been considered to be involved in many inflammatory diseases, although gene regulation in the common inflammatory processes in UAD remains unclear (35). Panganiban et al. identified 30 miRNAs that were differentially expressed among healthy, allergic, and asthmatic subjects. These miRNAs fit into five different expression pattern groups. Among asthmatic patients, miRNA expression profiles identified two subtypes that differed by high or low peripheral eosinophil levels. Circulating miR-125b, miR-16, miR-299-5p, miR-126, miR-206, and miR-133b levels were most predictive of allergic and asthmatic status (35). These findings have shown the presence of the same particular miRNAs in different pathogenetic mechanisms of both AR and asthma, such as IL-13 pathway, GATA-binding protein 3, and mucin secretion. Interestingly, recent studies have shown that miRNAs could be used as potential pharmaceutical targets for anti-inflammatory treatment (36).

CLINICAL AND TREATMENT EVIDENCE

The interaction between nose and lung in allergic airways disease is a bidirectional process, indeed it has been proved that the treatment of AR can improve asthma symptoms (15, 18). Subsequent ARIA updates and other reviews have made an attempt to summarize the diagnostic and therapeutic implications of this link based on these published evidence, but the evidence is still far from conclusive, due to limited number of randomized controlled trials available on subjects with concomitant AR and asthma (15, 18). Therapy for UAD is based on avoidance of the main allergens and irritants and pharmacotherapy [nasal and inhaled steroids, antihistamines, leukotriene receptor antagonists (LTRA), anti-IgE therapy, and allergen immunotherapy (AIT)] (11).

Allergen Avoidance

Once allergy testing is complete, the physician may devise a comprehensive program of allergen avoidance. The lack of hay fever outside the pollen season indicates that complete allergen avoidance can be effective. Unfortunately, complete avoidance is rarely possible, especially for outdoor allergens. The effects of environmental control strategies have been most heavily studied with regard to dust mites and furry pets (37). Compliance with these measures may be difficult but will certainly be helpful in many patients with hypersensitivity to these allergens. Avoidance of other rhinitis and asthma triggers, such as cigarette smoke, outdoor pollutants, fumes, and irritants, is sensible in clinical practice (38).

Pharmacologic Therapy

Oral and/or intranasal antihistamines and nasal corticosteroids are both appropriate for first-line AR treatment although the latter are more effective (39, 40).

Some authors reported a decrease in asthma symptoms and AR after intranasal corticosteroid treatment of rhinitis and a recent meta-analysis confirmed the beneficial effect of intranasal steroids in AR (41). LTRAs are known to be useful for long-term management of asthma patients complicated by AR (42). Leukotrienes are generated by the metabolism of arachidonic acid *via* the 5-lipoxygenase (5-LO) pathway, which is involved in the rapid initial inflammation response. LTRAs block the cysteinyl-leukotriene receptor, which are peptide-conjugated lipids produced by activated basophils, eosinophils, mast cells, and macrophages, to relieve the symptom of AR (42). Recent studies demonstrated that LTRAs have a significant influence in improving patients' nasal symptoms and quality of life and the use of LTRAs in combination with antihistamines has generally resulted in greater efficacy than when these agents were used alone (43). The recombinant, humanized, monoclonal anti-IgE antibody (Omalizumab) forms complexes with free IgE, blocking its interaction with mast cells and basophils and lowering free IgE levels in the circulation (44). Omalizumab has been tested in several clinical trials, and its beneficial effect has been established in patients with uncontrolled allergic asthma, leading to its approval by FDA (38). In patients with severe asthma and rhinitis, omalizumab improved nasal and bronchial symptoms and reduced unscheduled visits due to asthma. The clinical benefit of treatment with omalizumab is associated with an anti-inflammatory effect on cellular markers in blood and nasal tissue, as well as with a reduction in FcRI expression and function (45).

The only treatment potentially able to interfere with the natural history of respiratory allergy is AIT, specifically aimed at modifying the response to sensitizing allergens (37). Current data support the effectiveness of AIT in AR and a beneficial effect in allergic asthma (38). In particular, AIT may prevent

the onset of asthma by halting the progression from rhinitis, by preventing new sensitizations or by avoiding the primary development of allergy (46). In the context of the UAD, AIT consistently shows a clear benefit for both the upper and the lower airways.

CONCLUSION

The link between upper and lower airways, the so-called UAD, has been revealed by several epidemiologic, pathophysiologic, and clinical evidences, changing the global pathogenic view of respiratory allergy. AR and asthma are both manifestations of a single inflammatory process and require an integrated diagnostic and therapeutic approach in order to get global disease control.

AUTHOR CONTRIBUTIONS

All authors made substantial contribution to the conception of the work, reviewed the literature on the subject, and drafted the final version of the manuscript; AL and GM revised it critically for important intellectual content. All authors finally approved the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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REVIEW
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Subcutaneous and Sublingual Immunotherapy in Allergic Asthma in Children

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This review presents up-to-date understanding of immunotherapy in the treatment of children with allergic asthma. The principal types of allergen immunotherapy (AIT) are subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT). Both of them are indicated for patients with allergic rhinitis and/or asthma, who have evidence of clinically relevant allergen-specific IgE, and significant symptoms despite reasonable avoidance measures and/or maximal medical therapy. Studies have shown a significant decrease in asthma symptom scores and in the use of rescue medication, and a preventive effect on asthma onset. Although the safety profile of SLIT appears to be better than SCIT, the results of some studies and meta-analyses suggest that the efficacy of SCIT is better and that SCIT has an earlier onset than SLIT in children with allergic asthma. Severe, not controlled asthma, and medical error were the most frequent causes of SCIT-induced adverse events.

Keywords: subcutaneous immunotherapy, sublingual immunotherapy, children, allergy, asthma

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INTRODUCTION

Asthma is one of the most common chronic inflammatory disorders in children, and airway remodeling can cause it to persist into adulthood. It affects up to 300 million people worldwide, and it is believed that an additional 100 million people will be suffering with asthma by 2025 (1). It has been shown that there are numerous asthma phenotypes from infancy to adulthood. Although asthma is not exclusively associated with allergy/atopy, about 75% of all children with asthma are atopic (1).

It is known that asthma pharmacotherapy can effectively control symptoms and the ongoing inflammatory process. However, it can not affect the underlying immune response; when medication is discontinued, symptoms may recur. This is where allergen immunotherapy (AIT) comes into play, as the only management that can interfere with the underlying immune pathophysiology. AIT is recommended for patients with moderate to severe allergic rhinitis with/without mild to moderate asthma due to inhalant allergens (2, 3). AIT is the only therapeutic method that may alter the natural course of allergy affecting both the development of new sensitizations and the clinical disease development (including deterioration of symptoms and progression of rhinitis to asthma) (4). The predominant mechanism is dependent on the type of allergen-specific T_H cells (5). The efficacy of both subcutaneous subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) has been shown by systematic reviews and meta-analyses for both perennial and seasonal allergic respiratory disease (4, 6). However, the clinical evaluation of AIT must take into account

the high heterogeneity among studies. Nevertheless, the Global Initiative for Asthma Report has been updated in 2017 and stated that potential benefits of AIT, compared to pharmacological and avoidance options, must be weighed against the risk of adverse effects, and the inconvenience and cost of the prolonged course of therapy (7).

The objective of the current review is to summarize the evidence for the efficacy, safety, potential barriers to, and facilitators of the use of AIT in pediatric asthma.

DEFINITION

Subcutaneous immunotherapy is the term used to describe a process of repeated doses of a specific relevant allergen, for the treatment of IgE-mediated allergic disease (8). The conventional schedule for SCIT that employs unmodified allergen extracts consists of a weekly dose buildup by subcutaneous injections, followed by maintenance doses at 4 or 8 week intervals. Fewer buildup doses are possible with the use of modified allergenic extracts (such as allergoids), and/or adjuvants (9).

Sublingual immunotherapy is an alternative approach of allergen immunotherapy, whereby allergens are administered orally and—more specifically—by the sublingual route. In SLIT the allergen is given as either a dissolvable tablet or an aqueous/liquid extract (10), and the time interval between each maintenance dose varies from one product to another; generally, the once-a-day administration is preferred (11).

HISTORY

History of SCIT

In 1911, Dr. L. Noon and Dr. J Freeman published their findings on allergy desensitization through subcutaneous injections of pollen extract. By 1935, Cooke and colleagues identified a protective factor in serum, which was induced by AIT. This finding led to the concept of "blocking antibodies" (12). In 1953, Johnstone and Dutton randomized all children attending their clinic to receive either treatment (higher doses of SCIT) or placebo. The asthma symptoms of the treatment group resolved after 4 years (12).

After the second World War, aluminum hydroxide (Alum) was used as adjuvant in most allergen preparations. In the last decades, other modalities were tried, showing promising results (13). Other modifications also took place such as the use of inactivated allergoids in order to reduce their ability to bind to IgE, while retaining their ability to stimulate immune responses (12). In 1986, however, concerns were raised in the UK regarding the safety of desensitization, as several severe reactions had occurred in people with asthma. As a result, regulatory authorities prohibited SCIT in the UK outside of clinics that were familiar with its use and had appropriate resuscitation facilities. In the United States, serious adverse events (AEs) were reported in patients with relatively mild disease, and severe reactions kept being reported until the end of the century (14). These concerns about safety and a need to perhaps simplify administration led to various improvements, and also to the development of SLIT.

History of SLIT

Oral and sublingual route for the administration of allergen extracts was attempted in the 1900s, and the available vaccines were single allergen preparations (15); these efforts, however, failed to establish this method at the time. In the 1980s, several landmark studies kept demonstrating the safety and effectiveness of SLIT. Since 1986 there has been a revival of interest in SLIT.

Currently, there is no difference between the allergens used for SLIT and SCIT, although there are differences in the product quality requirements for each method (e.g., natural allergen extracts versus recombinant allergens) (16, 17).

Sublingual immunotherapy is now being used routinely in some parts of Europe (especially Italy and France) and is gradually spreading to Northern Europe and the United States. The introduction of SLIT could widen the scope of AIT and allow an increased number of patients to receive therapy (12).

MECHANISMS OF IMMUNOTHERAPY TO AEROALLERGENS

With AIT, allergen extracts are presented to the immune system either subcutaneously (SCIT) or sublingually (SLIT). As the patient is already sensitized to the allergens in question, they react with a localized immune response. The allergens arrive in local lymph nodes either unbound *via* free diffusion or are taken up by dendritic or B cells (18). Breg cells, which also play a key role in the induction of immune tolerance to allergens, can suppress allergen-mediated inflammation through secretion of IL-10 and TGF-β. Thereby, effector T-cell responses are suppressed, and Treg cells are induced (19). Likewise, Breg cells might promote allergen tolerance through preferential production of IgG4 antibodies on differentiation to plasma cells. Further, B-cells produce IgG4 antibodies, which bind to the allergens without initiating a reaction, thus acting as "blocking antibodies" (20). In a recent study, the authors showed that Bregs were less prevalent in lungs of mice after allergen exposure confirming that the development of asthma alters the homeostasis of IL-10+ regulatory B cells, emphasize the importance of B cells in asthma, not only as IgE producers but also as suppressive cells able to constrain the pathological process (21). Additionally, Tregs cells suppress allergic responses directly and indirectly. They migrate from the site of their development in the lymph nodes back to the area of inflammation and release IL-10 and TGF-β, thereby reducing local inflammation (22). IL-10 can decrease B cell antigen-specific IgE production and increase IgG4 levels; reduce proinflammatory cytokine release from mast cells, eosinophils, and T cells; and elicit tolerance of T cells. As a consequence, responses to allergens are reduced after induction of regulatory T cells (23). The data also support the concept of a later allergen-specific immune deviation from a TH2 to a TH1 cytokine profile (24). Furthermore, Tregs suppress effector Th1/Th2/Th17 cells, allergen-specific IgE, mast cells/basophils/eosinophils; inhibit migration of effector T cells to tissues; and facilitate release of IgG4 (25).

A schematic representation of the mechanisms involved in AIT is shown in **Figure 1**.

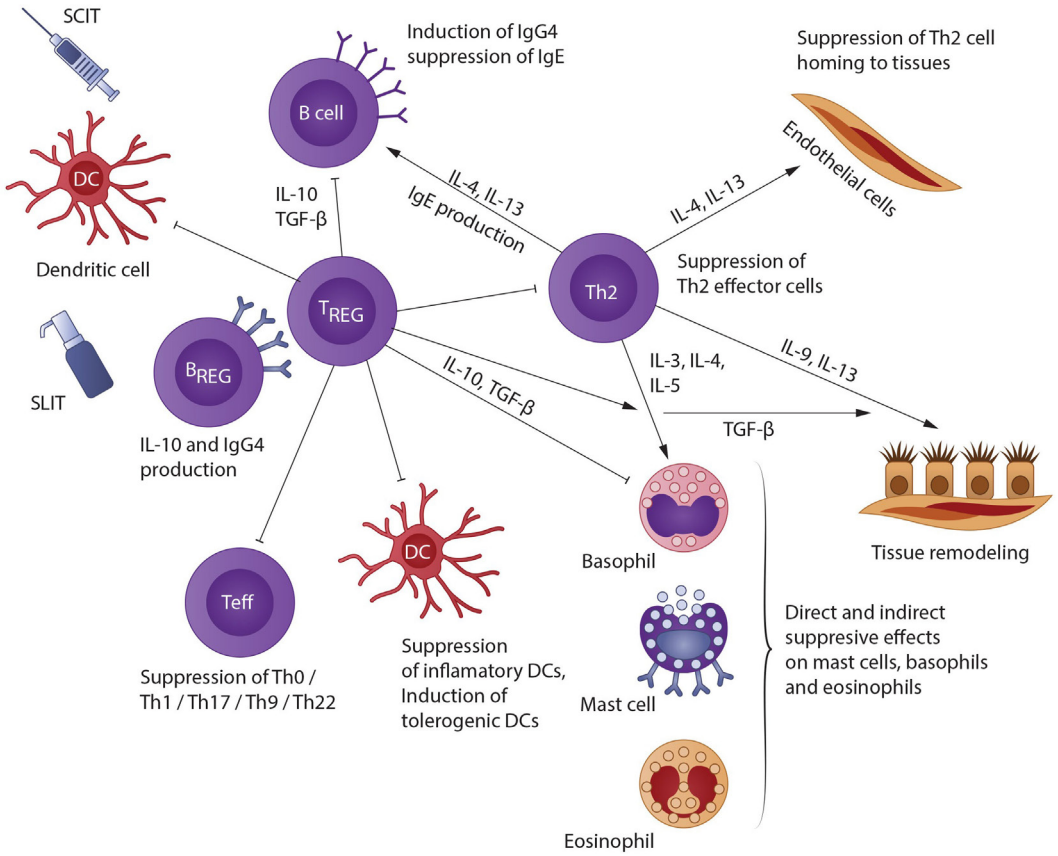

FIGURE 1 | A schematic representation of the mechanisms involved in AIT [modified from Akdis and Akdis (22)]. Allergen IT results in both a shift in allergen-specific T-cells from Th2 to Th0/Th1, and in generation of IL-10- and TGF-β-producing T regulatory (Treg) cells. Treg cells affect B cells directly or indirectly by facilitating IgG4 and IgA release and hindering IgE development; also, they impede Th2 cell homing to tissues; they suppress mast cells, basophils, and eosinophils *via* direct and indirect mechanisms; and they inhibit epithelial cell activation. In addition, Breg cells also suppress effector T cells and contribute to IgG4 synthesis.

INDICATIONS

Selection of patients for immunotherapy requires identification of the underlying antigenic trigger by combination of clinical history taking, and skin prick tests and/or blood tests for allergen-specific IgE (26). The current ARIA guidelines (27) give both SCIT and SLIT a conditional recommendation in allergic asthma, due to moderate/low quality of evidence. The majority of the guidelines agree that appropriate candidates for AIT are mainly children with allergic asthma that is difficult to control with conventional treatments. Asthma, nevertheless, must be well controlled by standard pharmacological treatment at the time the injection is administered, due to safety concerns (28). It is of particular interest in patients, who are sensitized to several pollens, to prescribe AIT only for major allergens (29), with the aim to increase the effectiveness of AIT and to better select patients who need a treatment. Hence, the use of molecular diagnosis techniques [component-resolved diagnostics (CRD)] (30) may allow physicians to better identify whether children with allergic respiratory symptoms are sensitized to major allergens or to cross-reactive molecules (31). In this context, an observational multicenter survey carried out by the Italian Pediatric Allergy Network suggest that a higher cutoff point of SPT-induced wheal reactions (e.g., 5 mm) should be used to take decisions when a confirmatory CRD assay cannot be implemented (32). In the UK, AIT is rarely used for asthma, partly because of the risk of adverse reaction with SCIT in uncontrolled asthma, and partly because of the lack of evidence for its cost-effectiveness versus the currently available routine treatments (33). Several factors may influence the decision for immunotherapy either way, such as poor adherence, clinically irrelevant allergens, poly-sensitizations, unavoidable adverse reactions of routine medication, etc. (34). Furthermore, the prescription of AIT depends also on the severity of the allergic asthma and duration of symptoms. Key parameters to evaluate the severity are the need of additional specialist visits; the response to pharmacotherapy and the recurrence of symptoms impairing school or sport activities or altering sleep quality (35). The decision between SCIT or SLIT hinges on several factors, including product availability, cost, patients ability to consistently attend the clinic, patient's characteristics, physician's/patient's preference, etc. (4). Also, SLIT could be tried if SCIT

causes systemic reactions (34). Indications for SCIT and SLIT are summarized in **Table 1**.

CONTRAINDICATIONS

The contraindications for SCIT or SLIT are either absolute contraindications (serious immunologic disease, major cardiovascular disease, cancer, chronic infections, lack of compliance, severe psychological disorders, etc.), or relative contraindications (pregnancy) (36). Severe asthma or uncontrolled asthma (regardless of its severity) is major risk factor for serious or even fatal adverse reactions and, therefore, represent important contraindications for SLIT/SCIT (37, 38). Interestingly, well-controlled asthma, regardless of its severity, was thought to *not* be a contraindication for AIT in a recent EAACI position paper (36). However, the strength of this recommendation was variable (36), and more evidence should become available before AIT can be safely considered for patients with severe asthma, even if well controlled. *Partially* controlled asthma is a *relative* contraindication for AIT in the same paper (36). Accordingly, German guidelines suggest that AIT may be performed in children with partially controlled asthma (39). Furthermore, well-controlled asthma, regardless of its severity, is not a contradiction for AIT (36). Any other condition that would reduce the patient's ability to survive a potential systemic allergic reaction could also be a relative contraindication (28). SLIT should not be administered in case of acute inflammation, injury and surgical interventions in the oral cavity, or acute gastroenteritis (39). Some contradictions are listed in **Table 1**.

DURATION OF TREATMENT

It is generally accepted that 3–5 years are required to achieve a clinical benefit and to maintain it after treatment cessation, for either SCIT or SLIT (39). Two studies showed no differences in the efficacy between a 3 and a 5 years course of house dust mite (HDM) in asthmatic children, or in the persistence of clinical benefit after discontinuation (40, 41). In another AIT study in asthmatic children sensitized to HDM, improvement has been shown from the first year of treatment (42, 43). The duration of the treatment may be prolonged (5 years or more), depending on the clinical response of subjects. Many patients experience a prolonged remission of symptoms after discontinuation of AIT (44, 45) whereas others may have a relapse of clinical manifestations. Currently, there are no specific laboratory tests or biomarker that can distinguish patients who will relapse from those who would have a prolonged clinical remission after discontinuing AIT (45). In keeping with that evidence, 3 years of SLIT in HDM sensitized children with asthma had a medication-sparing effect (46). The data are unclear, however, regarding the extend of the medication-sparing effect of AIT, with one study reporting no change in the asthma medication score after 1 year of treatment (43), whereas a pronounced effect was shown in a different work (42). Early treatment termination is a major problem (47) as only 35.4% of children were found to have completed at least 3 years of treatment (48). If AIT has been administrated for a number of years, current evidence suggests that it could induce long-term benefits, after its cessation (40, 46, 49). In any case, the duration of AIT should be individualized on the basis of the patient's clinical response, disease severity, AEs, and patient preference (28).

Position papers and practice parameters recommend well-standardized protocols for SCIT in asthma. Pajno et al. showed that during the first year of SLIT for children with rhinitis/asthma because of grass pollen, the continuous regimen performed better than the pre/co-seasonal; however, no significant difference was shown in the subsequent 2 years (50). Currently, there is no clear evidence of superiority for the pre/co-seasonal for pollen allergens (11). Nevertheless, due to improved adherence and better cost-effectiveness, pre/co-seasonal regimens are often preferred (38).

TABLE 1 | Indications and contraindications for subcutaneous immunotherapy and sublingual immunotherapy (SLIT) in asthmatic children.

Indications	Contraindications
• Mild–moderate allergic asthma, well or "partially" controlled by pharmacotherapy (53) • Clinically relevant sensitization (82) • Availability of a standardized product (28)	• Malignant/cardiovascular/autoimmune disease • Uncontrolled asthma • Pregnancy • Acute infections • < 5 years old (36, 66, 82) • Lack of compliance and severe psychological disorders (28) • Inflammation, injury, or surgical intervention in oral cavity SLIT • Acute gastroenteritis • Eosinophilic esophagitis (39)

EFFICACY

AIT is generally effective in asthmatic children who do not fully respond to asthma medication and environmental control. Nevertheless, it should be kept in mind that maintenance of asthma control *via* pharmacotherapy is vital both before and during AIT (51, 52). Several studies evaluating the efficacy of SCIT (53, 54) and SLIT (7, 55) have demonstrated effectiveness in controlling asthma symptom and reducing the medication use. A recent systematic review also concluded that SCIT and SLIT appear to be efficacious for the treatment of rhinitis and asthma in children (56).

Efficacy of SCIT

There is consensus that SCIT for asthma induced by the most common aeroallergens (grass, mite, and cat dander) is generally efficacious (57). The efficacy of SCIT for the treatment of asthma, including a steroid—sparing effect, was evaluated in a meta-analysis including 101 studies (3,792 patients) carried out both in adults and in children (53). In particular, 42 studies of AIT involved patients with mite allergy, 27 pollen allergy (mostly grasses), 10 animal dander allergy, 2 *Cladosporium* allergy, 2 latex allergy, and 6 patients with multiple aeroallergens allergy. A significant reduction of symptoms was found in patients treated with mite and pollen AIT, while no significant improvement was recorded for animal dander or allergenic mixtures. Despite the heterogeneity of the included studies, the overall reduction

of symptoms (for all allergens), the medication scores, and the bronchial hyperreactivity were significantly reduced, too. Saporta et al. evaluated 99 children and adults in regards to symptom score before and after either SCIT or SLIT. Coughing seemed to respond better to SCIT ($P = 0.037$), and wheezing to SLIT ($P = 0.024$), though both symptoms significantly improved regardless of regimen. For the remaining symptoms, there was no significant difference between SCIT and SLIT (58).

Efficacy of SLIT

The evidence for clinical efficacy of SLIT is not abundant, but good efficacy is generally reported for HDM, and grass pollen allergens. A recent review found a relative efficacy of SLIT (symptoms and/or medication score) in adults and children from 20 to 40% (7). With respect to appropriate doses, the 300 IR (index of reactivity) dose of SLIT is thought to offer optimal efficacy and tolerability for HDM-induced asthma (59). A meta-analysis that included 9 studies on 441 asthmatic children found a significant decrease in symptom and medication scores with SLIT, in comparison to placebo (60). In another meta-analysis that evaluated 9 studies in 452 HDM-allergic children aged 3–18 years with asthma treated with SLIT, marked improvement in asthma symptoms and medication scores, and a steroid-sparing effect was seen (61). Overall, the reviews of the literature on pediatric populations consistently support the efficacy and safety of SLIT compared to placebo.

SAFETY

In order to reduce the risk of adverse effects, AIT starts with very low doses that increase within the first few weeks to months of treatment (buildup/up-dosing phase), and until a maintenance dose is reached (28, 62). This does not, however, eliminate the risk of reactions, which is directly dependent on several factors such as allergen extract, injection schedule, dose, and patient factors (63). Such reactions could be local (in the immediate vicinity of the administration site) and systemic (SR), which can be further characterized as fatal, anaphylaxis, and systemic reactions not otherwise classified (wheezing and urticarial etch) (53). In most cases, symptoms can be managed if they are treated early.

The incidence of systemic reactions for AIT varies between 0.06 and 1.01% in those receiving SC dosing (64). In a recent prospective European survey, 762 children and 801 adolescents with AR (93.7%), AR and asthma (56.1%), and asthma alone (5.2%) had been included; they were sensitized to pollens (45%), mites (36.8%), dander (10.2%), or they were polysensitized (62.5%). A total of 29 reactions had been recorded, 23 by SCIT, and 6 by SLIT. The only three cases of anaphylaxis were related to SCIT, and they had a delayed onset (>2 h after administration) (65). Current recommendations suggest that children undergoing SCIT are observed for at least 30 min after injection (3, 66).

Typically, asthma is considered to be a risk factor for SRs, especially when it is uncontrolled (36). Other risk factors include polysensitization, grass pollen sensitization and—regarding SCIT—the use of natural extracts versus allergoids (65). All allergen preparations, such as standardized extracts (67), allergoids (68), or recombinant allergens (69), can cause side effects. Hence, research is being conducted to produce extracts using modified proteins or peptides that may increase safety and efficacy (33).

Sublingual immunotherapy appears to be quite safe for pediatric patients. In an observational study of 193 children receiving SLIT, who had a history of allergic rhinitis with or without asthma, there were nearly 500 mild/local adverse reactions but only 1 SR (severe asthma attack) (70). The main local AEs are oral/throat itching and mouth/tongue edema. In children, gastrointestinal complains have been mostly described during SLIT with HDM (7). Local symptoms can be, however, severe enough to warrant discontinuation of treatment. A grading system has been suggested with grade 1 corresponding to mild symptoms, grade 2 to moderate symptoms that require systemic treatment, and grade 3 to severe symptoms that could prompt termination of the SLIT regimen (7). The incidence of SRs with SLIT does not appear to be dose dependent, unlike SCIT where SRs are associated with higher allergen dose (71). A recent review summarized over 80 randomized double-blind placebo-controlled trials, and several reviews of both adult and children populations and concluded that, in most studies, the overall occurrence of systemic side effects is similar between placebo and active groups (7). To date, only few cases of anaphylaxis have been reported with SLIT (38), and some of these are probably due to overdose (72).

PREVENTIVE EFFECT OF AIT

Allergen-specific immunotherapy (AIT) is the only treatment capable of disease modification, as demonstrated by prevention of new sensitizations and inhibition of disease progression, especially in children monosensitized to HDM (73, 74). Due to its disease-modifying effects, AIT may be the closest that we currently have to a cure for allergic asthma (4). In the "Preventive allergy treatment (PAT) study," SCIT with birch and/or grass pollen reduced the risk of asthma development in children with allergic rhinoconjunctivitis (75). This effect was detectable 7 years following discontinuation of SCIT (76).

Sublingual immunotherapy was also shown to have a preventive effect in a study in which 113 children, aged 5–14 years with seasonal rhinitis due to grass pollen, were randomly allocated to pharmacotherapy plus SLIT, or pharmacotherapy only. After 3 years, only 8 of 45 SLIT patients had developed asthma as opposed to 18 of 44 controls (confidence interval: 1.5–10) (77). In another trial, 216 children aged 5–17 who had rhinitis with/without intermittent asthma received conventional medication plus SLIT, or medication only. After 3 years of observation, the prevalence of persistent asthma was 1.5 and 30% for SLIT and the control group, respectively (78). In a further study, the same authors prospectively evaluated the long-term effect of SLIT in 59 patients, compared with 12 control subjects. The total duration of the follow-up was 15 years. All the control subjects developed positive tests to allergens previously negative, while this occurred in less than a quarter of the patients receiving SLIT (44). Zolkipli et al. could recently demonstrate a significant reduction in sensitization to new allergens in children prophylactically treated with SLIT. This was a prospective, randomized DBPC, proof-of-concept study involving 111 infants <1 year of age at high risk of atopy (positive atopic family history) with no sensitization to

common allergens at randomization. After a year of treatment with a high-dose HDM SLIT, there was a 50% reduction in sensitization to any allergen in the active group (79).

SPECIAL CONSIDERATIONS

Age

Allergen immunotherapy for inhalant allergens is usually not considered for infants and toddlers. Although both SCIT and SLIT have been employed in children under 5 years and they appear to be effective (80), the evidence for the use of immunotherapy in this group is limited (81). For practical reasons, immunotherapy is not generally offered to patients below the age of 5, while for older ages there is no upper limit (82). In any case, each patient should be evaluated individually by considering the benefits and risks (83). SLIT drops are generally preferred for younger children over SCIT (48).

Polysensitized Patients

According to the review by Calderon et al., 50–80% of patients with allergies are polysensitized. This impedes appropriate selection of patients for immunotherapy (37) and renders the clinical history vital in the identification of the clinically relevant allergen(s) (84). The use of *in vitro* component-based IgE diagnostics can increase the likelihood of AIT being successful, by facilitating correct identification of the culprit allergen (39). Multiallergen immunotherapy is currently supported by little evidence, both regarding its efficacy and successful induction of immunological tolerance (37). Also, there are conflicting results for the efficacy of allergen mixes (85). Thus, large clinical trials are needed before SCIT and/or SLIT can be routinely carried out with an allergen mixture or concomitant use of several allergens in polysensitized patients.

Omalizumab and AIT

Omalizumab pretreatment has been shown to improve the safety and tolerability of cluster and rush immunotherapy schedules (86, 87). Additionally, omalizumab in combination with immunotherapy is more effective that AIT alone in managing symptoms (87). Treatment of >6 months with omalizumab was clinically effective in patients with severe uncontrolled asthma who could not tolerate immunotherapy (63, 88). This effect of omalizumab allowed the initiation of AIT in children with severe asthma. However, studies investigating AIT with omalizumab pretreatment and/or AIT-omalizumab combinations are lacking in children with severe asthma; further research is needed to evaluate the risk/benefit ratio of such regimens (63).

SCIT vs SLIT

There is conflicting evidence regarding which method is more effective. Chelladurai et al. showed little difference in treatment effectiveness when comparing SCIT with SLIT (89). From four dust mite studies, two studies favored SCIT in reducing medication use and two favored SLIT, while a birch study found SLIT to be more effective (89). A meta-analysis by Nelson found that SCIT was superior to SLIT (90). In general, although both SCIT and SLIT appear to be effective in allergic asthma, literature is more supportive of an SCIT predominance in clinical efficacy (91).

In regards to safety, SLIT appears to be better tolerated than SCIT. The majority of SLIT AEs are local reactions (e.g., oromucosal pruritus) that appear at the start of treatment and resolve within a few days or weeks, without any medical intervention. Only, a few cases of SLIT-related anaphylaxis have been reported (92). A novel approach for AIT in which SCIT is administered in the buildup phase and SLIT in the maintenance phase in a randomized, controlled, prospective manner in HDM–sensitive asthmatic children was conducted. The novel regimen proposed seems to successfully combine the advantages of both routes without loss of clinical benefit and might be a promising alternative in children undergoing AIT (93).

OTHER ISSUES

Compliance

It is important that AIT is carried out in accordance with prescriber's recommendations (2, 94). Adherence to therapy and the likelihood of treatment success are improved by thoroughly informing the patient about the way AIT works. Studies conducted on SCIT showed that the major cause of non-compliance was the inconvenience related to injections, and the cost of treatment (90). SLIT, on the other hand, had different compliance issues as it is administered at home by patients themselves. Although it was initially thought that SLIT would have a much better compliance than SCIT due to omitting the requirement to regularly attend clinics, it was soon shown that adherence to SLIT was not significantly better; this is probably because SLIT faces similar adherence problems with other conventional pharmacotherapy regimens (95).

Cost-effectiveness

Studies comparing cost-effectiveness between patients treated for 3 years with AIT versus those treated with pharmacotherapy alone have found that AIT might be associated with cost savings as high as 80% 3 years after completion of treatment (4). Nevertheless cost-effectiveness is difficult to review due to different national health systems, variable epidemiologic data, and different prescription habits and outcome measures used in studies (96). However, in general, AIT's cost-effectiveness appears to be good, as demonstrated by several pharmacoeconomics studies conducted within 6 years of treatment initiation (9).

CONCLUSION

AIT appears to be effective in children with IgE-mediated asthma who do not fully respond to the conventional antiasthmatic medications and environmental control and currently represents the only therapeutic approach capable to modify the natural evolution of a respiratory allergy. Its steroid-sparing effect is an important benefit for patients who have to use these drugs in high doses and in long-term regimens. Both SCIT and SLIT appear to be effective in allergic asthma, although

some reports suggest that the efficacy of SCIT may be better. Uncontrolled asthma remains a significant risk factor for side effects, and AIT should not be considered on safety grounds for patients who cannot get their symptoms reasonably under control with pharmacotherapy alone. As we are entering the era of personalized medicine, further research should be conducted with a view to individualize AIT using recombinant antigen technology: this way we could perhaps create allergen extracts against specific proteins to which the patient is allergic, or extracts with modified proteins or peptides that could increase safety/efficacy. Adjuvants that can stimulate the immune system are currently being developed. These approaches have the potential to transform AIT to a mainstream, first line therapy in the foreseeable future.

AUTHOR CONTRIBUTIONS

ST had the conception and designed the work. ST, AM, and GF collected the data. All the authors contributed to the data analysis and its interpretation. ST and GG made the critical revision of the article, and GG gave his final approval of the version to be published.

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OPINION
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How Much Asthma Is Atopic in Children?

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INTRODUCTION

Asthma is the most common chronic childhood disease worldwide and poses a significant health and socioeconomic burden. The prevalence of this disease differs geographically and seems on the rise in many parts of the world. (1).

Cross-sectional and longitudinal studies have identified several indicators associated with high risk of asthma (2). Personal history of atopy in early life seems to be one of the key factors of an individual's risk of persistent asthma. Indeed, numerous studies have demonstrated that early and multiple allergic sensitization (AS) specific to aeroallergens and some food antigens places a strong risk for the development of asthma in children (2–6).

Atopic asthma represents the most common form of asthma in the pediatric age and is characterized by eosinophilic airway inflammation associated with specific immunoglobulin E (IgE) antibodies sensitization to various allergens, as evidenced by serology or skin prick test (7). According to a large population survey, including data from people aged 6–59 years (the Third National Health and Nutrition Examination Survey), 56.3% of asthma cases in the United States were attributable to atopy (8). Similarly, the 14-year follow-up analysis of an Australian community-based birth cohort showed that the proportion of asthma associated with atopy was 52% overall (9). The results of a recent cohort study, which followed a large group of newborns over various periods, indicated that the prevalence of asthma from 4 to 16 years is markedly higher among ever-allergic sensitized children compared to never-sensitized ones (10). At a population level, a recently published longitudinal study that prospectively collected data over the first four decades of life showed that the occurrence of asthma before the age of 13 years was more strongly associated with atopy and greater airflow obstruction than later onset asthma (11).

Nevertheless, there is still controversy about the causal relationship between atopy and asthma, as other non-allergenic factors may trigger the specific IgE pathway and influence the occurrence of this disease (12). Over the last 20 years, accumulating evidence has shown that the interrelation between AS and subsequent development of asthma is more complex than a linear dose–response relationship, with gene–environment interactions and epigenetic modifications playing crucial pathophysiological roles (13).

One impediment to understand the relationship between atopy and asthma is the common use of atopy as a binary variable (i.e., sensitized or non-sensitized). Another major impediment is that asthma is a heterogeneous condition, which comprises several different disease endotypes sharing similar symptoms. Recent data indicate that atopy may also encompass distinct endotypes characterized by different patterns of association with asthma (13).

This opinion article outlines the most recent and debated findings about the interrelation between atopy and pediatric asthma.

Abbreviations: AS, allergic sensitization; IgE, immunoglobulin E.

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THE BURDEN OF ATOPY IN PEDIATRIC ASTHMA

Atopy is described as the personal tendency to produce IgE antibodies in response to exposure to common allergens, with an increased risk of developing typical diseases such as asthma, rhinoconjunctivitis, or atopic dermatitis. In clinical practice, as in most studies, atopy is often defined either as the presence of serum allergen-specific IgE antibodies or positive skin prick tests. However, a positive allergy test does not necessarily imply clinical reactivity upon allergen exposure. Indeed, a relevant proportion of such defined "atopic" children do not develop any allergy-related disease (10).

There is emerging evidence that both quantification of atopy and timing of onset of AS provide more valuable information to assess the risk of persistent asthma in children (6).

Indeed, different high-risk cohort studies showed that eczema may be a good predictor of childhood asthma when associated with AS; conversely, the risk of subsequent asthma seemed not to be increased with non-atopic eczema (14, 15).

The development of AS and lower respiratory tract viral infections in the first few years of life seem the most consistent environmental risk factors for subsequent asthma at school age, with sensitization to multiple aeroallergens (especially perennial allergens) and rhinovirus-induced wheezing conferring the greatest risk (16–18). There are several mechanisms by which respiratory viral infections are supposed to interplay with allergic inflammation to lead to airway dysfunction, wheezing, and asthma (19). Of interest, recent data showed that both rhinovirus infections and aeroallergens can induce airway epithelial cells to produce interleukin-33; this cytokine can promote allergic airway inflammation and remodeling (16). Nevertheless, the temporal relationship between atopy and viral wheezing during early life is not fully elucidated. The high-risk birth cohort "Childhood Origins of ASThma (COAST)" study recently showed a sequential relationship whereby AS seems to precede rhinovirus-induced wheezing (20).

Notably, it has become evident that early AS to aeroallergens and respiratory viral infections synergistically enhance both the risk of preschool wheezing and subsequent persistent asthma (17). In a recent extension of the COAST study, children with both inhalants sensitization and rhinovirus-induced wheezing by age 3 years had the highest odds ratios for asthma inception at school age and for the persistence of asthma out to adolescence (17). Interestingly, this study suggested that the timing of AS to aeroallergens in early life can significantly influence the risk of future asthma as children who were sensitized to one or more aeroallergens by age 1 year showed the highest rate of asthma at age 13 years; conversely, if sensitization occurred after age 5 years the risk of asthma at adolescence was not different from that of participants who were not sensitized through 13 years (17).

Increasing data also show that the level of allergen-specific IgE antibodies and the size of skin test wheal to aeroallergens can better help identifying children at risk of preschool wheezing and subsequent asthma than a simple positive allergy test (21–23). Similar quantitative relationship has also been reported in relation to asthma severity and severe asthma exacerbations in children (23, 24). Notably, a recent report suggested that in severe pediatric asthma both allergen-specific IgE antibodies and skin prick tests should be carried out and quantified, as these tests are not always concordant in this specific population of patients (25).

In the pediatric age, there is also evidence of a "quantitative synergism" between aeroallergen-specific IgE levels (e.g., to house dust mite) and respiratory viral infections in enhancing the odds of acute wheezing and asthma exacerbations, with higher levels of specific IgE conferring the greatest risk (26, 27). An indirect support to these findings comes from a recent clinical trial which showed that preseasonal treatment with anti-IgE antibody omalizumab decreased the rates of seasonal virus-induced exacerbations of asthma (28).

Although the association between early aeroallergen sensitization and allergic airway disease is acknowledged, it is less clear whether early life food sensitization influences the risk of subsequent asthma in children. Despite that several birth cohorts reported an association between early food sensitization and asthma, most of these studies neither have addressed this relationship beyond early childhood nor have considered important confounders (such as early life or concurrent eczema and wheezing) (29). Moreover, the number of tested food allergens varied across studies, with egg white and cow's milk being the most common (29). Recently, two independent prospective studies found that sensitization to food only in the first 2 years of life was associated with increased risk of asthma by age 10–12 years (30). However, both studies defined current asthma based on questionnaire data and were unable to address the relationship between specific food allergen sensitization and subsequent occurrence of asthma. Noteworthy, the greatest risk of asthma was observed in children who had sensitization to both food and aeroallergen by 2 years of life (30).

HETEROGENEITY OF ATOPY AND PEDIATRIC ASTHMA

Currently, the lack of agreement on the definition of asthma, both in clinical practice and research studies, strongly contributes to the complexity in understanding the relationship between atopy and asthma (31).

One major impediment to this process is the mounting recognition that asthma is not a single disease, but a collection of several disease entities, also referred to as endotypes. Although presenting with similar observable clinical characteristics (phenotypes), asthma endotypes seem to arise *via* distinct and unique pathogenic pathways and may also be associated with different genetic predisposition and environmental exposures (31, 32).

This constraint also applies to preschool wheezing illness, which is a heterogeneous condition with several phenotypic expressions and a complex relationship with development of asthma later in life (32). Even though recurrent wheezing is one of the major symptoms of asthma and that asthmatics are more likely than other children to wheeze in childhood, most children who wheeze during early life will have a resolution of the symptom by age 3 years or 6 years at the latest (33). Further impediment relevant to atopic asthma in preschool children is the difficulty in making a clear diagnosis; at this age, spirometry

cannot be reliably performed and the diagnosis of asthma is often based on the clinical pattern of wheezing episodes or parental reports of wheezing, which may overestimate the prevalence of this condition (34). To this purpose, during the past two decades, many attempts have been made to characterize childhood wheezing phenotypes and predict their trajectories of transition in asthma. Notably, distinct birth cohort studies showed that atopy is strongly associated with "intermediate and late-onset" wheezing phenotypes, which confer the higher risk of physician-diagnosed asthma at school age and up to adolescence (35–37).

There is growing evidence to show that atopy also comprises distinct endotypes that confer different risks of persistent asthma. Longitudinal follow-up of different birth cohorts across the globe showed that the combination of early and multiple AS represents a strong risk factor for persistent and severe asthma (18, 23, 38).

In a recent British unselected birth cohort study, Simpson and colleagues considered both the type and the time of onset of sensitization to specific allergens in relation to asthma occurrence and severity. Most participants in this cohort with "conventionally" defined atopy were clustered in a completely unsupervised manner into four different atopic classes, named "multiple early," "multiple late," "dust mite," and "non-dust mite" (18); interestingly, only those children sensitized to multiple aeroallergens at an early age reported a significant increase in risk for asthma inception, severe asthma exacerbations leading to admission and impaired lung function ascertained by age 8 years (18). Simpson and coworkers subsequently demonstrated that children belonging to different atopic classes had different environmental exposures, supporting the hypothesis that these classes may correspond to distinct endotypes of atopy (13). More recently, the same group of researchers corroborated all these findings by showing that strikingly similar atopy classes, with similar correlation with clinical outcomes (including asthma and lung function), could be identified in the independent population of children belonging to the Isle of Wight birth cohort (38).

CONCLUSION

From a clinical perspective, all these data suggest that AS should not simply be considered an all or nothing phenomenon; rather, the level of allergen-specific IgE and/or the size of skin prick test wheal diameter as well as the timing of occurrence should all be considered when defining the relationship between atopy and pediatric asthma.

Further studies should be designed to identify biological markers able to better characterize wheezing phenotypes in preschool children associated with elevated risk of persistent asthma. Classifying pediatric asthma into more specific clinical phenotypes and biological endotypes is mandatory to define personalized and effective treatment and to target the current elusive goal of primary prevention of asthma.

AUTHOR CONTRIBUTIONS

PC wrote the initial draft. All the authors participated in critical revision of the manuscript and provided important intellectual input and approved the final version. All the authors had full access to all of the data and participated in the interpretation of the findings.

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