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Asthma and Allergies Through the Lifespan

Abstract

Asthma is a common, chronic inflammatory airways disease characterized by a clinical syndrome of bronchial hyperresponsiveness, inflammation, and reversible airflow obstruction. Individuals with asthma can vary widely in clinical presentation, severity, and pathobiology. The incident factors, pathogenesis, prognosis, and treatment of asthma remain incompletely understood. Utilizing measurable characteristics of asthmatic patients, including demographic, physiologic, and biologic markers, can however identify meaningful phenotypic categories in asthma. Identification of these phenotypes may help improve precision therapeutics targeted toward an individual's' disease, and may identify strategies for preventing progression of disease severity.

Keywords: Severe asthma, Phenotype, Heterogeneity

Background

Asthma is a chronic inflammatory disease of the airways. Individuals with asthma may experience recurrent wheezing, dyspnea, chest tightness, and cough. These symptoms reflect episodes of reversible airflow obstruction, which may remit spontaneously or with treatment. Over time, many asthmatics experience progressive airway remodeling, leading to an incompletely reversible, or fixed, airflow obstruction. Further, inflammation in the asthmatic airway induces airway bronchial hyper-responsiveness to a variety of allergic, infectious, or irritant stimuli.

Public health impact of asthma

Asthma is a very common chronic disorder. Asthma severity can range from intermittent to severe; more severe asthma is associated with significant morbidity and mortality. Further, asthma prevalence is increasing with time [1], perhaps due to better recognition and phenotyping. It is estimated that, in the United States in 2013, asthma affected 16.5 million adults and 6.1 million children, reflecting 8.3% and 7.0% of the population, respectively [2]. Approximately half of those individuals experienced an asthma attack, which is defined as sudden worsening of asthma symptoms due to bronchoconstriction, and when severe, hyperinflation and "air trapping" [3]. Asthma is the leading cause of absenteeism in children in the United States, causing approximately 50% of children to miss at least one school day each year, and one in three adults to miss at least 1 day of work. Three out of five asthmatics are forced to limit their usual activities because of this disease.

Asthma remains a prevalent disease worldwide. Estimates from worldwide analyses such as the Global Burden of Disease Study from the Forum of International Respiratory Societies suggest that asthma affects at least 235–334 million individuals [4, 5]. Using data from the International Study of Asthma and Allergies in Childhood surveys, approximately 14% of the world's children suffer from asthma in any given year. Latin American and English-speaking countries of Australiasia, Europe, North America, and South America have the highest prevalence of childhood asthma, estimated at over 20% [6]. Reported asthma symptoms in children increased from 1993 to 2003 in low- and middle-income countries. Estimates of asthma prevalence in adults are more difficult to obtain. Approximately 8.6% of adults worldwide between the ages of 18–45 have asthma symptoms. The morbidity and mortality burden of disease, however, disproportionately affects older adults [5].

Global measures of disability rank asthma 14th in number of years lost to asthma-associated morbidity and mortality [7]. This most significantly affects individuals in some countries of Europe, Central and South America, Africa, and Austrailasia. Annually in the United States, asthma accounts for approximately 15.5 million outpatient health care visits, 1.8 million emergency department visits,

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and 439,000 hospitalizations, costing the US $56 billion each year, or roughly $3259 per person [8]. In a European study from 2011, the estimated total cost of asthma in adolescents and adults was €19.3 billion [9]. In the Asia-Pacific region, the estimated direct and indirect cost of asthma per person range from $184 to 1189. In the United States in 2013, 3630 individuals died from asthma, or nine people per day [1, 8]. These data suggest asthma is often poorly controlled, despite the availability of pharmacologic therapies that are recommended in National and International Asthma Guidelines [10–12].

Development of asthma

An individual's susceptibility to the development of asthma, or to severity of asthma, are likely determined by an interaction of host or genetic characteristics that interact with environmental exposures. For example, specific genotypes can confer susceptibility to developing wheezing with rhinovirus exposure [13], atopy, or responsiveness to bronchodilator therapy [14, 15]. Currently, there are a number of genes that are associated with asthma susceptibility [16]. An important question in whether these or different genes influence asthma progression and severity. Environmental exposures, including prenatal influences [17], allergens [18, 19], respiratory infections [20–22], cigarette smoke [23], and air pollution [24] are implicated in the development of asthma. Cumulative environmental exposures may lead to persistent, progressive disease with potentially irreversible changes in lung structure and function. These concepts are illustrated in Fig. 1 which describes the interaction between genetics and environment in the development and progression of asthma. Because of differences in the influence of genes and environment, there is a wide range of disease heterogeneity and severity in asthma.

Assessment of asthma

All that wheezes is not asthma, and all asthma does not wheeze. Accurate diagnosis of asthma is important, as treatment will benefit both morbidity and mortality from this disorder. As many non-asthmatic diseases have overlapping clinical findings with asthma, accuracy of clinical diagnosis is critical for planning appropriate treatment strategies. The Global Strategy for Asthma Management and Prevention 2015 report update [12] and the National Institutes of Health Guidelines for the Diagnosis and Management of Asthma Expert Panel Report-3 [10] provide recommendations for the diagnosis of asthma. In addition to obtaining a detailed history of symptoms and physical exam, these guidelines suggest obtaining studies of lung function such as spirometry to measure forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) measurement, as the FEV1/FVC ratio objectively measures airflow obstruction. Additional pulmonary function testing such as diffusing capacity, lung volumes, or bronchoprovocation studies to support or refute the asthma diagnosis. Comorbidities and alternate diagnoses should be evaluated when symptoms are atypical or not responding to therapy.

Treatment of non-severe asthma

Goals of asthma treatment are multifaceted. A combination of controller and rescue therapy for asthma usually allows an individual to achieve and maintain control of asthma symptoms. Control of asthma should confer a normal day-to-day activity level, including exercise capacity. Treatment of asthma may prevent the development of irreversible airflow limitation and allow maintenance of best possible pulmonary function. Adequate control of asthma, by definition, should prevent exacerbations and limit mortality due to asthma [10, 12]. Importantly, treatment should also identify and minimize medication side effects.

The Global Strategy for Asthma Management and Prevention 2015 report update [12] and the National Institutes

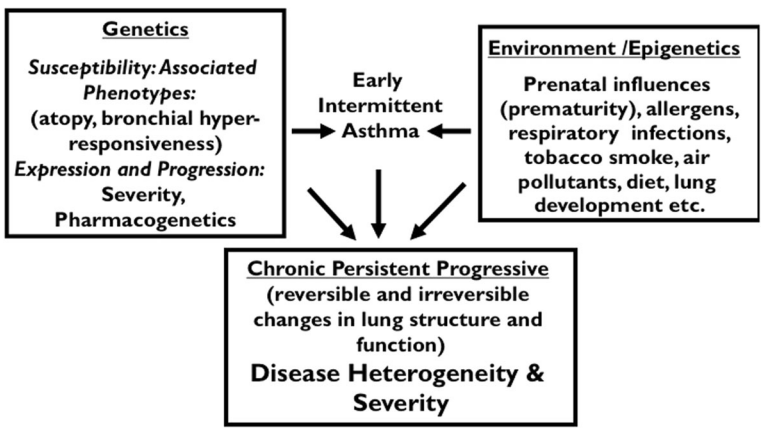

Fig. 1 Gene-environment interactions in susceptibility and severity of asthma

of Health Guidelines for the Diagnosis and Management of Asthma Expert Panel Report-3 [10] also provide a framework for the treatment of asthma. These guidelines emphasize evaluation of impairment and risk, with ongoing assessment of control. The domains of impairment and control focus on assessment of symptom frequency, frequency of use of rescue medications, impact on activity levels, and lung function. The risk domain identifies risk of exacerbations and adverse outcomes utilizing an individuals' history of exacerbations and lung function, with a goal of prevention of future exacerbations or fixed airflow limitation. The severity of asthma as measured through these domains is then used to guide treatment.

A stepwise approach to therapy is recommended, which highlights use of controller medications, particularly inhaled corticosteroids, then titrating doses or adding additional therapies as needed to achieve the necessary level of symptom control. At every level, assessment of proper inhaler device techniques, adherence to therapy, environmental control, and use of rescue inhalers for quick relief of sudden symptoms are recommended. Well recognized, however, is the inter-individual variability in response to each treatment [25, 26], reflecting the heterogeneity of disease which exists across severity groups.

Severe asthma

Task force definitions of severe asthma

The American Thoracic Society and European Respiratory Society released a Task Force document in 2014 entitled "International ERS/ATS Guidelines on Definition, Evaluation and Treatment of Severe Asthma [11]." The purposes of this document include defining severe asthma and treatment-resistant asthma; discussing phenotypes of severe asthma with respect to genetics, natural history, pathobiology, and physiology; outlining evaluation of a patient with severe asthma; and providing recommendations for treatment of severe asthma in children and adults. Assuming asthma diagnosis is accurate and comorbidities are being addressed, severe asthma is defined as asthma that requires treatment with guidelines-suggested medications such as high dose inhaled corticosteroids and a second controller for the previous year, and/or systemic corticosteroids for at least half of the previous year, to prevent it from becoming 'uncontrolled' or which remains 'uncontrolled' despite this therapy. Uncontrolled asthma is defined as the presence at least one of the following characteristics: persistently poor symptom control, two or more exacerbations requiring bursts of systemic corticosteroids in the preceding year, at least one serious exacerbation requiring hospitalization in the previous year, or chronic airflow limitation of FEV1 < 80% predicted with FEV1/FVC ratio less than the lower limit of normal [11].

Evaluation of patients with severe asthma

Individuals with severe asthma should undergo a careful systematic assessment to confirm this diagnosis. Lung function testing is utilized to confirm airflow obstruction and to measure reversibility or variability of airflow obstruction. Bronchoprovocation testing, such as with methacholine inhalation or exercise, may also be utilized. Medication noncompliance or poor inhaler technique can be identified in many severe asthmatics [27, 28]. Atopy and unregulated allergic exposures, such as ongoing house dust mite or cockroach exposure in an individual with sensitization to these antigens, may contribute to severe asthma, particularly in children [29]. Chronic rhinosinusitis is a very common comorbidity of asthma and contributes to disease severity [30, 31]. Obesity, obstructive sleep apnea, and psychological factors may contribute to asthma severity or perception [11, 32]. Symptomatic gastro-esophageal reflux disease is common in asthmatics, but the effect of treatment on asthma control or severity is currently unclear. The contribution of tobacco smoke exposure, hormones, and medication use should be carefully considered, as avoidance of the offending agent can confer major benefits on asthma control [11].

Treatment of patients with severe asthma

Inhaled corticosteroids remain the mainstay of asthma treatment, particularly in mild to moderate disease. By definition, those individuals with severe asthma require high doses of corticosteroid to control disease, and often remain symptomatic despite this therapy. Further, a subset of severe asthmatics is relatively corticosteroid insensitive, with relative or complete lack of clinical improvement from treatment with inhaled or systemic corticosteroids. While corticosteroid insensitivity seems more common in those with vitamin D deficiency or obesity, eosinophilic or type-2 inflammation-high asthma may have a relative benefit from steroids when compared to those with non-eosinophilic, non-type-2 inflammation [33].

Other controller therapies may benefit some individuals with severe asthma. Beta-agonists provide smooth muscle relaxation and bronchodilation through beta-adrenergic receptors. While short acting and long acting beta-agonists are used in asthma, concern that these drugs may contribute to asthma treatment failure, particularly in individuals with genetic differences in the beta-adrenergic receptor, may impact use [15]. However, recent results of United States Food and Drug Administration-mandated safety studies with inhaled corticosteroid-long acting beta agonist combination therapy do not show evidence of adverse effects [34, 35]. Leukotriene modifiers may benefit severe asthmatics with aspirin exacerbated respiratory disease. Anticholinergics block smooth muscle contraction through inhibition of the muscarinic receptor-3. The long-acting muscarinic antagonist Tiotriopum bromide has shown

benefit some individuals with severe asthma [36, 37]. These treatments, as well as potential future approaches, are highlighted in Fig. 2.

Biological therapeutics, those with a specific pathobiological target, have been and continue to be developed for use in severe asthma with particular phenotypes. Three are available currently in the United States for clinical use. Omalizumab, a monoclonal anti-Immunoglobulin E antibody, may be beneficial for some allergic asthmatics uncontrolled on therapy [38]. Mepolizumab and Reslizumab, both monoclonal anti-IL5 antibodies, reduce asthma exacerbations in those with severe eosinophilic asthma [39, 40], Different treatments, particularly for those with both-type 2 and non-Th2 inflammatory asthma, are under active development [41–43].

Asthma heterogeneity

With a developing understanding of the marked heterogeneity within the disease of asthma, we hypothesize, and expect to confirm, that the heterogeneity of asthma is attributable largely to individuals' genetic and epigenetic variability, mediated by certain environmental exposures. Environmental exposures are highly dependent on regional characteristics with varying climatic conditions, geography and population distributions. This variability in turn drives the immunologic mechanisms, or endotype, that confer the pathobiological and physiologic characteristics of asthma, the phenotype, as measured in the clinical setting. Importantly, our understanding of this variability and the mechanisms causing this disease may facilitate the development of interventions for primary prevention, disease modification, and precision therapeutics.

Hypothesis-driven univariate approaches to phenotyping have been utilized to clarify differences among groups of asthmatics. This type of approach defines groups based on the presence or quality of one variable, which is chosen to support testing a specific hypothesis. Disease severity may be the most straightforward, if not oversimplified, way of delineating disease phenotype. As anticipated, and likely as a result of the definitions of severe asthma, groups with severe asthma can be distinguished from non-severe asthmatics in terms of disease duration, symptomatology, health care utilization, lung function, and comorbidities [44–47]. However, it is well recognized that disease heterogeneity is present and vitally important among these severity classes, particularly among the more severe asthmatics [48] wherein cellular characteristics and airway remodeling have been long shown to confer different physiologic subtypes. Phenotypic characterization solely by disease severity therefore lacks the granularity to understand and delineate subtypes of asthma.

Other clinical characteristics have been assessed using hypothesis-driven univariate approaches. Reduction in mid forced expiratory flow rates (FEF25-75), as well as in FEV1, have been shown to be independently associated with markers of asthma severity, including ICU admissions, persistent or nocturnal symptoms, peripheral blood eosinophilia, and bronchial hyperreactivity [49]. A striking relationship between age and the probability of severe asthma was identified, particularly in men, increasing with duration of disease and from ages 18 to 45 [47, 50]. Airway mast cell phenotype and activation may contribute to phenotype and clinical characteristics. Indeed, mast cells containing both tryptase and chymase have been identified as the predominant phenotype in patients with severe asthma, whereas mast cells containing only tryptase are identified in biopsies from individuals with mild disease [51].

Inflammatory mediators within the airway may also be used for disease phenotyping. These inflammatory markers, present in sputum supernatant or bronchoalveolar lavage, may be related to cellular patterns that then relate to clinical phenotypes [52], or to disease characteristics such as eosinophilia, neutrophilia, airway bronchial

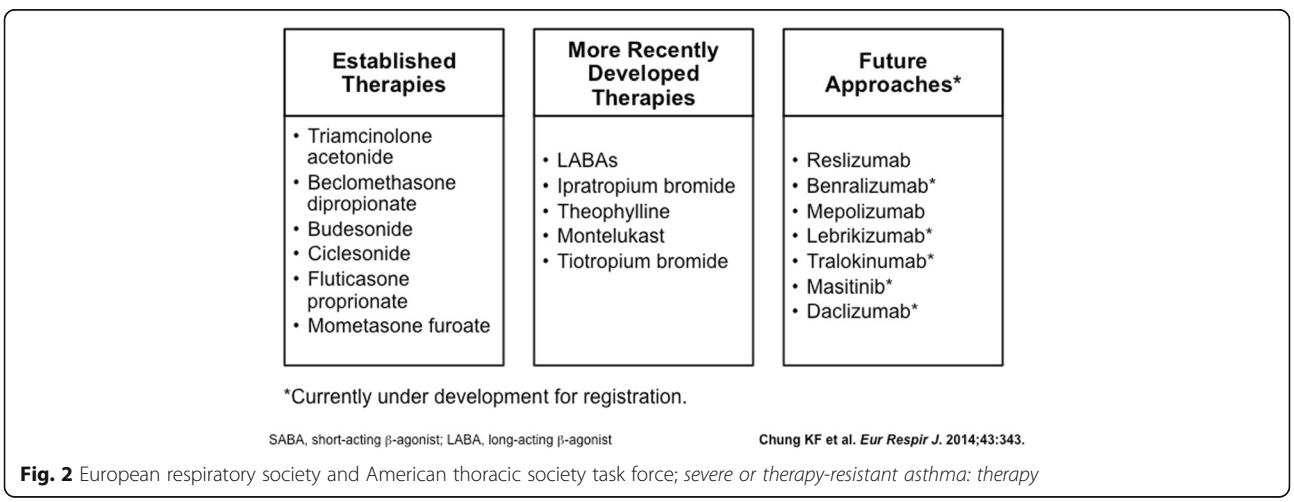

Fig. 2 European respiratory society and American thoracic society task force; *severe or therapy-resistant asthma: therapy*

hyperresponsiveness, and bronchodilator response [53]. Interestingly, when examining broncoalveolar lavage of children with asthma, while markers such as IL-13 and IL-6 can differentiate asthmatics from controls, and other cytokines can distinguish moderate from severe asthma, severe asthma itself does not have a clearly TH1 or TH2 inflammatory pattern [54]. This further underscores the heterogeneity of severe asthma.

Finally, technology to measure gene expression such as microarray and RNA-seq can provide insight into abnormally expressed pathways. Bronchial airway epithelial gene expression patterns were assessed in relationship to the clinical biomarker fractional exhaled nitric oxide (FeNO). Using a subset of genes that correlated with FeNO, subject clusters can be identified as having distinct clinical and molecular characteristics [55].

Model-free multivariate (Unbiased Cluster) approaches

Unbiased approaches to phenotyping utilize computer algorithms to evaluate hypothesis-free relationships among many clinical and biological characteristics. The resultant clusters, because they were created in an unbiased manner, can provide novel insights into asthma phenotypes.

The National Institutes of Health-sponsored Severe Asthma Research Program (SARP) enrolled and carefully assessed large cross-sectional cohorts of mild, moderate, and severe asthmatic adults and children. Unsupervised hierarchical cluster analysis performed on clinical and physiologic data from ~700 adult asthmatics in the SARP cohort identified five clusters of asthmatic subjects (Fig. 3) [47, 56]. Clinical clusters 1, 2, and 4 contain early onset, atopic asthmatics of increasing disease severity and worsening lung function. Cluster 3 is characterized by older, obese women with late-onset non-atopic asthma, with moderate lung function deficits and frequent exacerbations. Cluster 5 is characterized by later onset non-atopic asthma with more severe, irreversible airflow obstruction and high health care utilization. The most influential variables in forming these clusters include gender, age of asthma onset, asthma duration, use of inhaled beta-agonists and corticosteroids, and lung function pre- and post-bronchodilator administration [57].

With an unrelated cohort, investigators from Leicester likewise examined adult asthmatics through cluster analysis, revealing similar phenotypes of benign (mild) asthma, early onset atopic asthma, early onset symptom predominant asthma, obese non-eosinophilic asthma, and late onset inflammation predominant asthma [58]. The reproducible findings of these and other unrelated cohorts support these phenotypes as relevant [44, 59, 60].

Unsupervised cluster analyses were also performed on 161 subjects in the pediatric asthmatic cohort from SARP [61]. Four clusters were identified. Cluster 1 consists mainly of mild, later onset, less atopic asthma with normal lung function. Clusters 2, and 3 represent the spectrum of early onset, atopic asthma with increasing severity and worsening lung function. Cluster 4 identified a subset with more severe, fixed airflow obstruction and the highest health care utilization. These clusters have similarities to those seen in the adult SARP analyses.

Unsupervised cluster analysis was similarly utilized by researchers from the Trousseau Asthma Program in Paris,

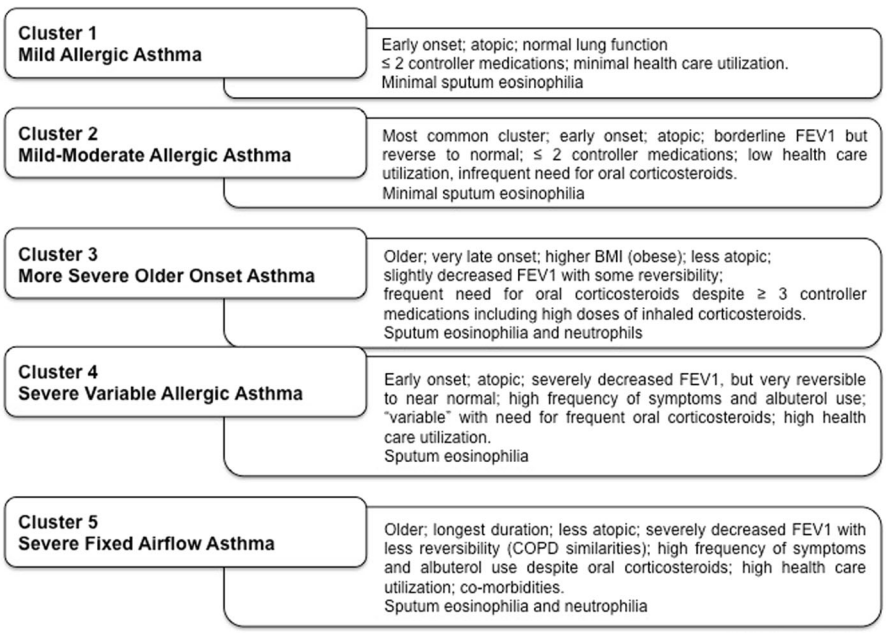

Fig. 3 Severe asthma research program clinical clusters

France [62] to identify phenotypic clusters in a pediatric severe asthma cohort of 315 subjects. Clinical and inflammatory markers were included in these analyses. Three clusters were identified: one of mild asthma, one of highly atopic asthma with eosinophilia and severe exacerbations, and one of higher body mass index, neutrophilia and more severe airflow obstruction. Despite the differences between the SARP and Trousseau cohorts, the clusters have features that generally overlap: SARP cluster 1 similar to the "mild" cluster, SARP cluster 3 to the "atopic severe" cluster and SARP cluster 4 to the "airflow obstruction" cluster.

Sputum cellular characteristics can identify patterns of airway inflammation and may have clinical utility. For example, individuals with sputum eosinophilia are likely to derive benefit from use of inhaled corticosteroids [63]. Phenotyping by cellular characteristics also can identify groups with differences in clinical and inflammatory markers. Airway neutrophilia has been associated with severe asthma defined by low lung function and use of high dose inhaled or oral corticosteroids [64]. Similarly in the SARP cohort, when using pre-defined normal and elevated cell counts, in the absence of cluster analysis, cellular asthmatics with elevated sputum eosinophilia (≥2%) and neutrophilia (≥ 40%) tended to have lower lung function, increased symptoms and health care utilization when compared with others [52].

A further examination of the adult SARP data integrated inflammatory cellular measures with the clinical variables in an unsupervised cluster analysis. Four phenotypic clusters were identified, which represented a severity spectrum from those with mild-to-moderate allergic disease (SARP clusters 1,2), having predominantly paucigranulocytic or eosinophilic sputum, to those with moderate-to-severe asthma or impaired lung function, most of whom had significant sputum neutrophilia with or without significant eosinophilia (SARP clinical clusters 3, 4, and 5) [65]. Importantly, the more inflammatory and severe clusters had markedly increased asthma medication use and health care utilization, including bursts of systemic corticosteroids and hospitalizations [57, 65].

Data collected from longitudinal cohorts can also be used for unsupervised cluster analyses, leveraging the power of the longitudinal design to provide insight into the variable patterns of disease over time. Analyses of pediatric birth cohorts have identified clusters of wheeze, atopy, or other characteristics that are associated with risk for asthma-related outcomes into the teenaged years. For example, the Avon Longitudinal Study of Parents And Children (ALSPAC) study collected data on wheezing at multiple time points from birth to age 7 years, for 6265 children in the United Kingdom [66]. The authors utilized wheeze data in latent class analysis to describe patterns of early wheeze, then examined clinical characteristics of individuals in these classes. Associations with atopy, airway hyper-responsiveness, and lung function abnormalities were seen in intermediate and late onset wheezing. These findings were similar to those from analyses of the Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, a multicenter birth cohort that enrolled 4146 pregnant women [67]. A latent class analysis of the PIAMA data identified 5 phenotypes of childhood wheeze, similar to those seen in ALSPAC [68]. The ALSPAC cohort was again assessed after age 16; latent class analysis identified early onset persistent wheeze to confer risk of lung function abnormalities.

The Manchester Asthma and Allergy Study is an unselected birth cohort of over 1000 children with periodic lung function and assessments of atopy and other clinical characteristics. Principal component analysis was performed using twenty one variables available at up to 5 years of age; patterns of wheeze and cough components were significant contributing components to the groups [69]. With the availability of 8-year old data for this cohort, a latent class analysis was performed, which identified differences in lung function trajectories over time among the classes, as well as more severe asthmatics with exacerbation risk in the persistent troublesome wheezing group [70].

In a population-based longitudinal cohort that enrolled 1,650 preschool children in Leicestershire, United Kingdom, early life wheeze and atopy data were used for latent class analysis [71]. The three wheeze and two cough phenotypes identified from early life data were assessed for associations with school age respiratory outcomes. The atopic persistent wheezers from early life had highest rates of current or frequent wheeze at ages 8-13. These authors identified a validation cohort of 6970 children born in a different county of the United Kingdom, for whom atopy and respiratory assessments were available at ages 8–13 in approximately 900. Latent class analyses revealed five groups with very similar characteristics to the groups seen in the original cohort [72].

Unbiased analyses from longitudinal cohorts indeed complement those of the cross-sectional cohorts. Despite slight differences among the clusters in each cohort, these unsupervised analyses ultimately identify clearly that asthma phenotypes vary by atopy, age of wheeze onset, clinical and physiologic characteristics. The stability of these clusters into adulthood is not well known, however, and the potential for progression from milder asthma to more severe disease, or vice versa, needs further elucidation.

Conclusion

We can easily recognize the clinical syndrome of asthma, presenting as symptoms of reversible airflow obstruction with airway hyper-reactivity and inflammation. More

severe asthma is associated with exacerbations that cause a significant degree of morbidity and even mortality. However, the incident factors, pathogenesis, prognosis, and treatment of asthma remain incompletely understood. Utilizing measurable characteristics of asthmatic patients, including demographic, physiologic, and biologic markers, can identify meaningful phenotypic categories of asthma. These phenotypes, while providing a helpful albeit partial understanding of disease state, can be further leveraged toward endotypic characterization, with the ultimate goals of identifying preventative strategies and improving precision therapeutics targeted toward an individual's disease.

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Abstract

The human microbiome can be defined as the microorganisms that reside within and on our bodies and how they interact with the environment. Recent research suggests that numerous mutually beneficial interactions occur between a human and their microbiome, including those that are essential for good health. Modern microbiological detection techniques have contributed to new knowledge about microorganisms in their human environment. These findings reveal that the microbiomes of the lung and gut contribute to the pathogenesis of asthma and allergy. For example, evidence indicates that the microbiome of the gut regulates the activities of helper T cell subsets (Th1 and Th2) that affect the development of immune tolerance. Moreover, recent studies demonstrate differences between the lung microbiomes of healthy and asthmatic subjects. The hygiene and biodiversity hypotheses explain how exposure to microorganisms is associated with asthma and allergy. Although those living in developed countries are exposed to fewer and less diverse microorganisms compared with the inhabitants of developing countries, they are experiencing an increase in the incidence of asthma and allergies. Detailed analyses of the human microbiome, as are being conducted under the auspices of the Human Microbiome Project initiated in 2007, promise to contribute insights into the mechanisms and factors that cause asthma and allergy that may lead to the development of strategies to prevent and treat these diseases.

Keywords: Asthma, Allergy, Microbiome

Background

Microbiomes exist in numerous diverse environments such as soil, freshwater, and seawater. The human microbiome was studied for the first time in the 1600s when Antonie van Leeuwenhoek scraped the coating of his teeth and studied the contents using his self-fabricated microscope [1]. Just over 300 years later, the Nobel Laureate Joshua Lederberg coined the term "the human microbiome" to describe the ecological community of symbiotic and pathogenic microorganisms that inhabit the body [2]. Lederberg believed that microorganisms in the human body are significant for health and disease [2]. Scholars distinguish between the concepts of microbiome [3, 4] and microbiota [5] to separate the collective genomes of microorganisms from the microorganisms themselves, although the original definitions do not distinguish between them [3–5].

An adult human harbors approximately 100 billion bacteria in the intestine alone, and the microbiome accounts for 90 % of the cells in the human body [3]. The human genome comprises about 21,000 genes that encode proteins [6]. In contrast, the microbiome may comprise approximately three million genes [4]. The microbiome may be considered a "new organ system," because its existence and contributions to human health and disease was uncovered by researchers between 15 and 20 years ago [7]. The composition and function of the microbiome of the human gut evolves during the first years of life and stabilize within the first 3 years of life [8–10]. The development of the gut microbiome is influenced by interactions between diet, environment, and host- and microbe-associated factors [10–18]. The gut microbiome plays a fundamental role in shaping host immunity by balancing the activities of Th-1 cells and Th-2 cells [19–23]. In contrast to the extensive knowledge of the

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gut microbiome [11, 24], information on the lung microbiome is limited [25, 26]. However evidence suggests a distinct microbiome of the lungs of healthy subjects [27] and a difference between the microbiomes of healthy people and those with obstructive lung diseases such as asthma [28].

The aim of the present review was to provide a general account of current research on the gut and lung microbiomes of humans and their association with the pathogenesis of allergy and asthma.

Review

The human microbiome

Over the last 30 years, the development of techniques to sequence microbial ribosomal RNA genes has led to the reconstruction of the evolutionary history of microorganisms [29, 30] and the illumination of their ecology. The United States National Institutes of Health initiated the Human Microbiome Project (HMP), which was allocated a budget of 150 million USD to investigate the microbiota of the human nose, mouth, gut, skin, and genitourinary tract with the aim of documenting the changes in the microbiome associated with human health [4]. Contributions of the HMP include the discovery that the microbiomes of the anatomic sites described above are similar among individuals but vary between the hair, nose, mouth, gut, skin, and genitourinary tract [24]. However, there may be large individual variations in microbiomes at the same sites on the body among individual [31].

The gut microbiome

The most thoroughly studied microbiome is that of the human gut [26]. Swift adaptation to modern lifestyles and environmental transitions likely changed the human gut microbiome with clear effects on immunological and physiological processes that affect human health [11]. The stability and diversity of the gut microbiome increase over the first 3 years of life [8–10]. Perinatal exposure [14], gestational age [16], mode of delivery [12], host genetics [15], and breastfeeding [10, 13] influence the development of the microbiomes of infants while antibiotics [18] and diet may also influence the microbiomes of older people [17]. *Firmicutes* and *Bacteroidetes* dominate the gut microbiome of adults; however, the representation and diversity of different classes of bacteria vary enormously [4].

A recent study of fecal samples of 124 European subjects identified between 1000 and 1150 bacterial species. Each individual in the cohort harbored at least 160 bacterial species, many of which were shared [32]. Knowledge gained from research on the gut microbiome provides insight into the development of allergic diseases and why the far less studied lung microbiome may contribute to the development of asthma. Factors such as birth by cesarean section [12, 33], breastfeeding [33], introduction of solid foods [10], and use of antibiotics by the mother or infant [34] affect the gut microbiome and are associated with increased incidences of asthma and allergies [35]. For example in a study including 2733 1-month-old infants, colonization of with *Clostridium difficile* is associated with birth by cesarean section and an increase in the risk of asthma (aOR = 2.06) at 6 years of age [36]. However, there are great inconsistencies in the findings of studies on the association between delivery by caesarian section and proven food allergy [37].

However, there is persuasive evidence indicating that children born by cesarean section have twice the risk of being sensitized to egg and milk allergens [38]. A recent study [40] of the association between gut microbiota and food sensitization in the first year of life among 166 infants found that 7.2 % were sensitized to one or more food allergens at 1 year of age and had lower gut microbiota richness and an elevated *Enterobacteriaceae*:*Bacterioi daceae* ratio. These findings suggest that early gut colonization may contribute to the development of atopic diseases such as food allergy [39]. Interaction between the host and the gut microbiome may explain the relationship between the microbiome and asthma and allergies.

Studies conducted using mice show that the gut microbiome is important in shaping the host's immune system [23]. Mice with different genetic predispositions to polarize responses to different antigens in opposite directions, toward either Th1 cells (possibly suppressed in asthma and allergy [40]) or Th2 cells (enhanced in asthma and allergy [40]) depending on the stimulus, both have airway response when the microbiome is disrupted [41]. By controlling the activation of antigen-presenting dendritic cells in the gut, single bacterial species may regulate the differentiation of naïve T cells into regulatory T cells [20]. The microbiome may affect the balance between Th1 cells and Th2 cells and thus the outcomes of subsequent infections with pathogens [20]. This is just one of many examples of immune regulation in symbiosis with bacteria [17, 19]. Further, specific bacterial species directly influence the development of regulatory T cells in mice [21] and humans [22]. Thus, symbiotic bacteria may regulate the immune system.

The lung microbiome

Researchers believed mistakenly for many years that the lungs are sterile [42]. For example, in a study of 28 healthy adults, the most common phyla detected in the nose and oropharynx are *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* and that 2000 bacterial genomes per cm^2 are present in the upper left lobe of the

lung [26]. *Proteobacteria*, particularly *Haemophilus* spp., are more common in the lungs of asthmatic adults than in those of healthy controls who harbor a higher proportion of *Bacteroidetes*. Further, the abundance of *Proteobacteria* in asthmatic children is higher compared with healthy controls [28].

In children with asthma, the bacterial load is significantly higher in the lung compared with healthy controls [44]. Further, Airway microbiological diversity in the lungs was significantly greater in asthmatic children who demonstrated significant reduction in bronchial responsiveness after antibiotic treatment [43]. Six weeks of treatment of patients with asthma with azithromycin reduces the relative abundance of *Prevotella* from 4.54 to 3.43 %, *Staphylococcus* from 10.49 to 4.59 %, and *Haemophilus* from 10.74 to 3.28 %. In some patients treatment with azithromycin reduces the bacterial richness in the airways and *Anaerococcus becomes* the most abundant bacteria. [44]. Further research is required to understand the role of the lung microbiome in the pathogenesis of asthma.

The microbiome in allergy and asthma

Increasing evidence suggests that the compositions of the lung and gut microbiomes determine the risk of asthma and allergies. It is interesting to consider how these findings support or contradict the "hygiene" and "biodiversity" hypotheses (see below) that were proposed to account for the influence of the human microbiota on allergies and immune tolerance. The hygiene hypothesis argues that the lack of early exposure to infectious agents, symbiotic microorganisms (such as intestinal flora and probiotics), and parasites increases susceptibility to allergic diseases through insufficient stimulation of Th1 cells [45], which cannot counterbalance the effects of Th2 cells, leading to predisposition to allergic diseases [20]. The biodiversity hypothesis suggests that disturbances in the composition of the gut microbiome of citizens of western countries induced by antibiotics, diet, and lifestyle disrupt the mechanisms of mucosal immunological tolerance [46]. The biodiversity hypothesis extends the hygiene hypothesis by stating that the gut microbiome interacts with the immune system to maintain the efficiency of the immune system [47].

The hygiene hypothesis

The hygiene hypothesis was proposed to explain the invers association between the risk of hay fever and the number of older siblings [46]. Thus, infections in early childhood transmitted through unhygienic contact with older siblings, or through the mother who is infected by her older children, prevent the development of allergic disease [45]. This hypothesis is supported by the findings of a study on school children in areas of Germany that were formerly East and West Germany that showed a decreased risk of allergic sensitization with an increasing number of older siblings, which was indicated by a rate of allergic sensitization this was three times higher in the former West compared with East Germany [48]. Numerous subsequent cross-sectional studies support the hygiene hypothesis, because they show that increased exposure to bacteria, fungi, airway infections, dogs, cats, and other animals in childhood is associated with decreased risk of asthma and allergies [49, 50]. Other studies show a lower incidence of allergic diseases in children raised on farms [51].

The hygiene hypothesis has been "updated" based on cross-sectional data together with experimental data and can be reconciled by a "new hygienic hypothesis" or a "Western lifestyle hypothesis," [52] which proposes that Th1 cells are not stimulated to balance the Th2 cells. Imbalance in the Th2 cell population leads to the production of interleukins (IL-4 and IL-5), which then induce the production IgE and eosinophils that cause atopic disease [53]. The relationship between exposure to different types of bacteria or fungi and asthma in children was assessed in a review of the characteristics of more than 15,000 children included in two different studies [50]. This analysis found that children who lived on a farm are exposed to diverse microflora compared to children not living on farms. The two studies analyzed show a reduced prevalence of asthma in children who live on a farm compared children not living on farms and an inverse association between exposure to microbes and the probability of asthma. However, a correlation between exposure to the microbiota and antigen-specific IgE was not detected [49].

Because cross-sectional studies simultaneously measure exposure and effect, they cannot establish a causal relationship. For example, the findings of cross-sectional studies may be explained if the parents of children with atopic disease have fewer children compared with other parents. Thus, the lower incidence of asthma and allergies in people who live on farms may be explained by families with asthma who discontinue farming and move; in contrast, healthy families continue to farm. Such an effect is called the healthy worker effect [54]. Occupational epidemiology studies suggest that the healthy worker effect reduces the association between exposure and outcome by 20–30 % [55] and that the effect of "cat avoidance" could correspond to a protective effect with an odds ratio of 0.83 [56]. Several longitudinal studies did not detect similar effects that support the hygiene hypothesis [57, 58], and several studies of birth cohorts show an positive association between exposure to cats and cat allergen-specific IgE [59, 60]. Other observations

such as increased atopy in poor and densely populated urban areas and among children in kindergarten argue against the hygiene hypothesis [61].

The biodiversity and the microflora hypotheses

Biodiversity is important for human livelihood and development and plays a paramount role in the quality of life of populations worldwide [62]. Moreover, the loss of biodiversity may have serious implications for human health [63]. Evidence indicates that the diversity of the body's microbiota is negatively associated with the risk of developing asthma and allergy [46, 64]. The biodiversity and disappearing microbiota hypotheses argue that changes in how people interact with the environment reduces exposure to microorganisms and that this may affect the mechanisms of development of immunologic tolerance [46, 65]. For example, consumption by mothers and infants of untreated cow's milk is negatively associated with asthma and allergies in children regardless of whether they live on a farm [66]. Further, a diverse intestinal flora in early life is associated with reduced risk of allergy at 5 years of age [67], and the diversity of gram-negative *Gammaproteobacteria*, common in soil but particularly dominant in aboveground vegetation, is reduced on the skin the atopic adolescents [68]. The association of gram-negative *Gammaproteobacteria* such as *Acinetobacter* with atopic disease is supported by the positive correlation of the levels of a regulator of immune tolerance, IL-10, with the abundance of *Acinetobacter* on the skin of healthy but not atopic adolescents [68].

The microflora hypothesis extends the hygiene and biodiversity hypotheses by proposing that the gut microbiome interacts with the immune system to maintain immune function. An imbalance in the gut microbiome caused by changes in the use of antibiotics and in the diet over the last three decades may cause dysfunction of the immune system [47]. This may explain why diseases such as asthma and allergy develop at any age. The evidence supporting the microflora hypothesis includes the increased incidences of asthma and allergies in industrialized countries during the last 50 years [69, 70] and includes the major relationships as follows: (1) correlation between allergic diseases and antibiotic use [71, 72], (2) correlation between allergic diseases and altered fecal microbiota [73, 74], and (3) correlation between allergic diseases and dietary changes [75]. Studies of mice demonstrate that antibiotic treatment that disrupts the microbiome may inhibit airway tolerance to airborne allergens such as fungal spores (e.g. *Aspergillus fumigatus*) [76] or aerosolized ovalbumin [41].

Probiotics

Probiotics are defined as viable microorganisms that enhance the host's health [77]. There is great uncertainty about the efficacy of probiotics for preventing and treating asthma and allergies. For example, when *Lactobacillus* was administered prenatally to mothers with at least one relative or partner with allergic rhinitis, atopic eczema, or asthma, and postnatally for 6 months to their infants, the outcome indicated a promising effect on the prevention of atopic disease [78]; however, the results of more recent studies (mainly of *Lactobacillus* and *Bifidobacterium*) are inconsistent [79]. A meta-analysis of 25 studies that assessed the effects of probiotic administration in children on atopy and asthma found that administration of probiotics reduces IgE levels and the risk of atopic sensitization but not the risk of asthma or wheezing [80]. The timing of ingestion, prenatally to the mother and postnatally to the infant versus postnatally to only the infant, did not influence IgE levels; however, the risk of allergic sensitization was significantly reduced only when the administration of probiotics commenced prenatally and continued after birth. The decrease in total IgE was more pronounced when probiotics were administered for longer, and the reduced risk of allergic sensitization may therefore depend on the specific bacterial strains. These findings indicate that administration of probiotics during pregnancy and to infants may contribute to the prevention of atopic diseases in high-risk infants [80].

Bacterial products may have great potential for preventing and treating allergies, although the bacterial species, their numbers, and the duration and timing of treatment that are safe and effective are unknown [46]. The results of a controlled clinical trial of newborns indicate that 6 months of treatment with oral probiotics protects against IgE-associated dermatitis at 2 years, but only for those at high risk of eczema. The effect disappears after 5 years of age [81]. Future probiotic supplements are likely to contain a wide range of microbes that can have long-term beneficial effects on the immune system [82]. Therefore, immunological, epidemiological, microbiological, and clinical studies are required to establish whether supplementation with bacterial products contribute to effective treatment and prevention of asthma and allergy. Future trials investigating the effects of probiotics should include analyses of specific strains of probiotic bacteria and longer follow-up.

Areas for future exploration

Allergy and asthma are heterogeneous diseases but are categorized according to an increased tendency of inflammation driven by changes in the environment, nutrition, the gut microbiome, and possibly the lung microbiome. The outcome of these changes may depend on genotype [83, 84], overall biodiversity [46], and other risk factors such as physical activity [85] and air pollution [86]. The findings may contribute a common explanation

of why the incidence of numerous inflammatory conditions such as asthma and allergy has increased in parallel with changes in the environment [11]. A broad interdisciplinary approach will be required to understand the complex pathogenesis of asthma and allergy and to understand the roles of the lung and gut microbiomes. The use of standardized outcomes and methodology may help address the gaps in our knowledge [11, 87]. For example, researchers should continue to focus on the functional and ecologic characteristics of the gut and lung microbiomes of healthy people as well as the features of the lung and gut microbiomes of patients with specific diseases. The development of strategies to improve microbiome colonization patterns will likely enhance health throughout an individual's life. It will be necessary to determine if variations in the microbiome are the cause or effect of allergies and asthma, and longitudinal studies are essential to control for different confounding factors. Further research on the microbiome should include organisms other than bacteria as some viruses [88] and fungi [89] interact with their hosts cells similar to what's observed with bacteria and their host. The results of such research can be ultimately translated to the clinic to improve diagnostics as well as treatment and prevention strategies that might include probiotics as well as dietary and lifestyle interventions.

Conclusions

The microbiome can be defined as all microorganisms that inhabit humans and their interactions with the environment. The findings of studies employing recently developed techniques such as metagenomics as well as those of epidemiological studies indicate that humans are exposed to fewer microorganisms because of changes in factors such as the use of antibiotics and diet, which are accompanied by increasing susceptibility to asthma and allergies. Moreover, these studies illuminate the differences in the microbiomes of healthy people and those with asthma and allergies. Moreover, they show that early exposure to bacteria may protect against these diseases. Further basic studies are required to characterize the lung and the gut microbiomes of healthy people was well as those with asthma and allergies. The ultimate goal is to understand whether aspects of the microbiome are linked to disease and whether manipulation of the microbiome will be useful to preserve lung function, prevent, and treat allergies.

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Abstract

Every fifth pregnant woman is affected by allergies, especially rhinitis and asthma. Allergic symptoms existing before pregnancy may be either attenuated, or equally often promoted through pregnancy. Optimal allergy and asthma diagnosis and management during pregnancy is vital to ensure the welfare of mother and baby.
For allergy diagnosis in pregnancy, preferentially anamnestic investigation as well as in vitro testing should be applied, whereas skin testing or provocation tests should be postponed until after birth. Pregnant women with confirmed allergy should avoid exposure to, or consumption of the offending allergen. Allergen immunotherapy should not be initiated during pregnancy. In patients on immunotherapy since before pregnancy, maintenance treatment may be continued, but the allergen dose should not be increased further. Applicable medications for asthma, rhinitis or skin symptoms in pregnancy are discussed and listed.
In conclusion, i) allergies in pregnancy should preferentially be diagnosed in vitro; ii) AIT may be continued, but not started, and symptomatic medications must be carefully selected; iii) management of asthma and allergic diseases is important during pregnancy for welfare of mother and child.

Keywords: Allergy, Atopy, Newborn, Pregnancy, Prevention

Background

In the USA, about 18–30% of women in the childbearing age suffer from allergic diseases, and around 20% of pregnant women are affected by allergies, especially rhinitis and asthma. These two conditions often are present in the same patient (reviewed in [1, 2]). Other medical conditions that often complicate pregnancy include allergic conjunctivitis, acute urticaria, anaphylaxis, food allergy and drug allergy. Optimal management of these disorders during pregnancy is vital to ensure the welfare of the mother and the baby (Fig. 1). In this review, we focus on the current recommendations for diagnosis, management and therapy for allergic diseases and asthma in women during pregnancy and/or lactation, as well as on risk factors and preventive measures for mothers and their children.

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A secondary publication. An earlier version of this review was posted on the World Allergy Organization website at http://www.worldallergy.org/professional/allergic_diseases_center/allergy_in_pregnancy/ [1].
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Diagnosis of allergy during pregnancy

The diagnosis of allergy in pregnant women should focus on a detailed medical history and symptom analysis. For diagnosis, (i) a diary of allergy symptoms and (ii) avoidance of suspected allergens accompanied by monitoring of changes of allergic symptoms may be helpful. It has to be emphasized that it is important not to put the mother on a rigid elimination diet for diagnosis of food allergy, as this could negatively influence the nutritional status of both the mother and the growing infant.

In vitro diagnostic tools such as serologic tests for allergen-specific IgE, e.g. ImmunoCAP or radioallergosorbent test (RAST), or the lymphocyte transformation test for type IV allergy diagnosis are preferred to skin and provocation tests, which should be postponed until after birth because of possible, though rare, anaphylactic reactions [3]. The same applies to food and other challenge tests. Despite the fact that there are no harmful effects of patch testing during pregnancy or lactation known, most physicians deter testing as general precaution, and furthermore because test results can interfere with immunological changes due to pregnancy [4].

Fig. 1 Management of asthma and allergic diseases are decisive during pregnancy for welfare of mother and child. (Fotolia.com©Reicher)

Management of allergic diseases during pregnancy

Mothers with allergy should avoid exposure to, consumption of and contact with diagnosed specific allergens. Patients should also especially avoid the inhalation of any potent triggers for asthma, such as animal dander, house dust, tobacco smoke and irritating pollutants.

Allergen immunotherapy (AIT, SIT, SLIT should ideally not be initiated during pregnancy because of the risk of systemic reactions. However, the initiation of immunotherapy can be considered in pregnant patients for clinical high-risk indication like anaphylaxis caused by *Hymenoptera* (insect venom) hypersensitivity. For patients who were already on immunotherapy prior to the pregnancy, maintenance treatment may be continued safely during pregnancy [5]. The allergen dose should not be increased during pregnancy. If pregnancy occurs while the patient is in the build-up phase of immunotherapy and on a low dose, which probably is not therapeutic, immunotherapy could also be discontinued [6].

Newer studies indicate that allergen immunotherapy is not only improving the disease in the pregnant patient, but that this treatment might also prevent allergic sensitization in the child. However, more studies are needed to confirm the effect of allergen immunotherapy during pregnancy on the development of sensitization in the child [7].

Medication for asthma and allergy in pregnancy

The ideal situation during pregnancy is "no pharmacologic therapy", especially during the first trimester. However, in practice, medications must be considered for pregnant patients with medical disorders, based on a thorough appreciation of the potential deleterious effects of untreated disease in the mother, and also potential harm for the unborn [8]. For instance, women suffering from asthma require drug therapy during pregnancy to prevent life threatening episodes to the mother, as asthma exacerbations during pregnancy have been linked to a higher risk of pre-eclampsia, gestational diabetes, placental abruption and placenta praevia [9]..

Most of the existing data regarding asthma and allergy medications during pregnancy have not demonstrated adverse effects (Table 1), even though in infants of corticosteroid-treated mothers an increased risk of oral clefts, preeclampsia, preterm birth, and lower birth weight have been reported. Many of the case controls, which showed the association between oral corticosteroid and oral clefts did not provide information on dose, duration or indication. Other studies, which have demonstrated association with OCS and preterm delivery and low birth weight, have been linked with higher doses for longer periods. For example in Bracken's study, which showed an association with OCS use and pre-eclampsia, the subjects were on OCS for the duration of pregnancy [10]. However, the potential side effects of any drug must be balanced against the risks to the mother or the infant of suffering from inadequately treated disease.

Treatment of asthma

Certain physiological changes occur normally during pregnancy, like increased tidal volume and minute ventilation, and decreased residual volume, functional residual capacity and diffusion capacity. These alterations are primarily the result of hormonal effects. The physiologically elevated position of the diaphragm and hyperventilation occurring in pregnancy further increase the risk of hypoxia. Preexisting asthma symptoms may worsen, improve, or remain unchanged during pregnancy. Each of these three possibilities is observed in about one third of cases. Optimal asthma treatment is crucial [8], as the risk of pre-eclampsia, premature birth, low birth weight, and maternal and neonatal hypoxia and morbidity posed by undertreated asthma may be greater than that from the use of oral steroids for the treatment of asthma.

Table 1 Recommendations for treatment of asthma and allergies in pregnancy

Drug	Safety Data
Common asthma medications and safety data	
Inhaled bronchodilators (e.g. Albuterol, Formoterol and Salmeterol)	Human data generally reassuring for short acting and long-acting bronchodilators
Theophylline	Reassuring human data; serum levels must be monitored very closely to avoid toxicity
Systemic corticosteroids	Human data from smaller case control studies show increase in oral clefts. Larger prospective studies show increase in low birth weight, preterm birth, preeclampsia and intrauterine growth retardation.
Inhaled corticosteroids	Human data mainly reassuring. There may be an increased risk of malformations seen with higher doses.
Leukotriene Receptor Antagonist (e.g. Montelukast, Zafirlukast)	Human data are generally reassuring
5-Lipoxygenase-Inhibitor	Generally avoided during pregnancy due to the available less reassuring animal data.
Omalizumab	Increased risk of low birth weight and preterm birth; likely severity of asthma may confound to these observations.
Common allergic rhinitis medications and safety data	
Oral antihistamines (e.g. Azelastine, Cetirizine, Chlorpheniramine, Dexchlorpheniramine, Fexofenadine, Diphenhydramine, Hydroxyzine, Loratadine)	Human data are generally reassuring. Hydroxyzine should be used cautiously during first trimester based on animal data. Fexofenadine (an active metabolite of Terfenedine): no reports of increased congenital malformations, however, no epidemiologic studies in human pregnancy available.
Oral and Nasal Decongestants (e.g. Oxymetazoline, Phenylephrine, Phenylpropanolamine, Pseudoephedrine)	Should be avoided during pregnancy: Oxymetazoline has been associated with possible uteroplacental insufficiency at higher doses. Phenylephrine has been associated with clubfoot and eye/ear malformations. Phenylpropanolamine associated with congenital malformations, gastroschisis and ventricular septal defect. Pseudoephedrine associated with gastroschisis, hemifacial microsomia and small intestinal atresia in some case–control studies.
Intranasal Antihistamines (e.g. Azelastine, Olapatadine)	Animal studies are reassuring.
Intranasal Corticosteroids (e.g. Budesonide, Fluticasone, Triamcinolone, Mometasone)	Substantial reassuring data for inhaled corticosteroids. Risk of increased malformations at high dose, but severity of allergic rhinitis may be a confounding factor for these outcomes.

The recommendations from Table 1 have been reviewed in detail in [11, 12] (table modified from [13]). FDA categorization by letters has been removed for labeling of drugs used during pregnancy and lactation. New FDA regulations for labeling of mediations have been published in 2014 [14]

Treatment of *acute* asthma is similar to that recommended for non-pregnant patients (reviewed in detail in [11, 12]), including inhaled beta2 agonists, oxygen (essential), and corticosteroids (oral or parenteral). It is also wise to add nebulized ipratropium bromide in patients who do not respond to beta2 agonists. Intravenous aminophylline is not generally recommended in the emergency management of acute asthma (because of its potentially harmful effects) but may be used in pregnant patients hospitalized for acute asthma (theophylline levels should be monitored). Intravenous magnesium sulfate may be beneficial in acute severe asthma as an adjunct to inhaled beta2 agonists and corticosteroids.

The goals of management of *chronic* asthma are the same as those for asthma in general, including prevention of severe exacerbations, improvement of quality of life (no interference with sleep or daily activities) and maintenance of normal lung function. The recommendations for medical treatment have been summarized by the Global Initiative for Asthma (GINA) working group including management of asthma during pregnancy [15]. A step-wise approach is suggested for medical treatment. Inhaled salbutamol is the preferred short-acting beta-agonist, with an outstanding safety profile, and among inhaled corticosteroids budesonide is preferred based on the available data. Salmeterol is the preferred agent when long-acting beta2 agonists are indicated in a pregnant woman as add-on treatment for persistent asthma. Leukotriene modifiers may be used as alternative add-on treatment: montelukast and zafirlukast are the preferred anti-leukotriene drugs. Zileuton in contrast, being the only leukotriene synthesis inhibitor, is not recommended in pregnancy due to its potential to cause abnormal liver function (FDA pregnancy category C).

Patients whose asthma is not controlled with maximal doses of bronchodilators and anti-inflammatory agents may need systemic corticosteroids. The lowest possible effective dose should be used. Patients must be monitored closely for potential adverse effects of corticosteroids, especially gestational diabetes, preeclampsia, and

intrauterine growth retardation. Based on the available data, control of maternal asthma is essential to reduce the risk of perinatal complications. As pregnant women are hesitant about continuing asthma medications during pregnancy, asthma education is a critical component in the management of the pregnant asthmatic patient.

One of the treatment options for moderate to severe persistent allergic asthma is the recombinant DNA-derived humanized IgG1k monoclonal antibody omalizumab (Xolair®), which specifically binds to free human immunoglobulin E (IgE) in the blood. It currently has an FDA Category B classification based on reassuring animal studies and the expected limited placental passage in the first trimester due to the size of the molecule. We have established an ongoing registry with a target goal of enrolling 250 asthmatic women treated with omalizumab during pregnancy [16].

Treatment of rhinitis

Significant nasal symptoms occur in approximately 30% of pregnant women. Pregnancy-associated hormones have direct and indirect effects on nasal blood flow and mucous glands. The most common causes of nasal symptoms necessitating treatment during pregnancy are allergic rhinitis, *rhinitis medicamentosa*, sinusitis, and (non-allergic) vasomotor rhinitis. "Vasomotor rhinitis of pregnancy" or pregnancy rhinitis is a syndrome of nasal congestion and vasomotor instability, limited to the gestational period. Allergic rhinitis commonly co-exists with asthma. As with asthma, pre-existing allergic rhinitis can worsen, improve, or remain unchanged during pregnancy.

The general principles of treatment for pregnant women with allergic rhinitis [17, 18] –as with asthma- and do not differ from the step-wise approach recommended for treatment of non-pregnant women. The initial treatment steps are non-pharmacological and shall include avoidance of allergens and irritants, furthermore, nasal lavages with salty water solutions. The mainstays of pharmacological therapy for allergic rhinitis in non-pregnant as well as pregnant patients are antihistamines and intranasal glucocorticoids. No important differences in efficacy or safety appear to exist between the various intranasal glucocorticoid preparations. Most pregnant women who require antihistamines for allergic rhinitis are appropriately treated with a second generation agent, because these drugs are less sedating and have fewer cholinergic side effects compared with first generation agents. Among second generation antihistamines, loratadine (10 mg once daily) and cetirizine (10 mg once daily) may be considered the second generation antihistamines of choice in pregnancy.

For decongestant treatment, there are insufficient safety data. The narrowing of blood vessel due to this medication could have negative effects on the fetus, and furthermore, decongestant nasal sprays can cause addiction. These medications should therefore be avoided during pregnancy.

Treatment of anaphylaxis

The management of anaphylaxis during pregnancy [3] is similar to treatment of non-pregnant patients. The first step is to avoid the trigger of the anaphylactic reaction. For the treatment of anaphylaxis, epinephrine (adrenaline) should be promptly injected i.m. Adequate intravascular volume repletion and oxygenation are particularly important in the management of anaphylaxis during pregnancy to prevent both maternal and fetal complications. The pregnant hypotensive patient should be placed on her left side to prevent additional positional hypotension resulting from compression of the *vena cava inferior* by the gravid uterus, with her lower extremities elevated. Intravenous epinephrine may be required, despite its potential to cause decreased uteroplacental blood flow. Glucocorticoids should be administered early to patients with severe anaphylaxis. For laryngeal spasm, intubation and in rare cases tracheotomy may be necessary.

Treatment of atopic eczema/dermatitis

Gestational itchy dermatoses are relatively common, with eczema being diagnosed in 36 to 49% of all pregnancy dermatoses. Treatment of atopic dermatitis during pregnancy [19] should emphasize avoidance of triggering factors and reliance on topical treatment with emollients to nourish and re-establish the skin barrier. Topical corticosteroids are prescription-dependent first-line treatment, however, they should only be initiated when clinically indicated with the least potent effective preparations. Oral antihistamines (AH) may be required as systemic treatment. Short-term use of (sedating) first-generation antihistamines may be beneficial in the setting of sleep loss secondary to itch [20]. Chlorpheniramine and diphenhydramine are considered safe during the first trimester. However, also second-generation drugs are generally safe, and loratidine is the preferred second-generation antihistamine in pregnancy (reviewed in [19]). In general, AH should be used cautiously in the last month of pregnancy, because of possible withdrawal symptoms in the child, like poor feeding, diarrhea, irritability, or tremulousness, which can last up to 4 weeks after birth [21, 22]. Atopic dermatitis can additionally be managed with UV phototherapy (UVA, broadband UVA and UVB, or narrowband UVB).

Treatment of urticaria and angioedema

The pattern and causes of urticaria and angioedema in pregnancy are similar to those in non-pregnant patients.

A unique form of urticaria associated with pregnancy ("pregnancy urticaria", Pruritic urticarial papules and plaques of pregnancy PUPPP) mainly occurs in primigravida mothers in the last trimester [23, 24]. The first step in treatment of urticaria and angioedema [25] in pregnancy is identification and avoidance of causative factors. Antihistamines should be avoided if possible, but if required, the lowest dose of chlorpheniramine, loratadine, or cetirizine may be used.

Risk factors for atopy

The causes of allergy in general and of specific sensitization in newborns in particular have not been completely determined yet. Besides the role of genetic predisposition, some factors have been identified that may either contribute to sensitization of the mother and to the subsequent transfer of a predisposition for allergy to the offspring, or that directly induce sensitization in the offspring, that manifests shortly after birth or at a young age (reviewed in [26]).

Family history of atopy/allergy

The degree of risk for atopy/allergy appears to be directly related to the family history of allergy and especially to maternal atopy. If neither parent is allergic, the chance for allergies in the child is about 5–16%. If one parent is allergic, the risk increases to 20–40% (father: 33%, mother: 45%), and if both are allergic, the risk is greater than 40–60% (if patients have the same allergy: 50–80%), especially for developing the same organ-specific symptoms [27].

Exposure to tobacco smoke

In a recent human study performed by parental questionnaires, exposure to smoke *in utero* or during infancy enhanced the risk for asthma and rhinitis primarily in early childhood, and the risk for eczema at later ages of the children [28]. In human blood samples, Th2 cytokines responsible for a predisposition toward allergy were elevated in the neonates only of mothers who had smoked during pregnancy. In addition, total and specific IgE levels, total eosinophil counts, incidence of airway disease and positive results on skin prick tests were also increased in children who were exposed to smoke either during pregnancy or in early childhood [29].

Alcohol consumption

Alcohol consumption by the mother during pregnancy is associated with higher total IgE levels in cord blood [30] and furthermore with an increased risk of atopic dermatitis in the child [31]. Apart from these atopy-associated negative effects, alcohol consumption should be avoided during pregnancy due to general health concerns (e.g. fetal alcohol syndrome).

Maternal diet

Recent research has focused on the role of several essential nutrients in the diet of the mother, like Vitamin D, zinc, folate and n-3 polyunsaturated fatty acids (PUFAs) [32]. Contrasting data exist on the effects of n-3 PUFA. On the one hand, a diet higher in n-6 polyunsaturated fatty acids (PUFAs) -as present, for example, in margarine and vegetable oils- seems to be more likely to induce eczema than n-3 PUFAs, which are found in fish. Accordingly, several observational studies show that a high intake of fish and oily fish during pregnancy results in a reduced incidence of allergy in the children (reviewed in [33]). On the other hand, a recent Cochrane systematic review revealed no evidence for supplementation of the mother with n-3 PUFA during pregnancy and lactation for prevention of allergy in the child [34].

Current evidence suggests a protective effect of maternal intake of vitamin D, vitamin E, or zinc for wheezing in childhood, but the data are not conclusive for an effect on asthma or other atopic conditions [35].

The effect of folate and folic acid supplementation is intensively discussed. Higher levels in maternal blood seem to be positively associated with atopic dermatitis in the offspring [36]. Controversially, recent studies and a systematic review found no association of prenatal folic acid supplementation and atopic diseases in children [37, 38]. Importantly, apart from the effects on atopy and allergy, sufficient levels of folic acid uptake by the mother before and during pregnancy have been shown to reduce the risk for neural tube defects [39].

Regarding allergenic food consumption during pregnancy and lactation, there has been extensive reviewing of data. According to the updated directive (No. 1169/2011, entered into application on 13 December 2014) of the Commission of European Communities, the 14 most allergenic foods have to be labeled on pre-packed food, and this declaration/information has also to be provided for non-pre-packed food [40]. These allergen sources are crustaceans, mollusks, fish, nuts, milk, egg, cereals containing gluten, peanuts, soybeans, sesame, mustard, celery, lupines, and the products of all these, as well as sulphur dioxide and sulphites. Some studies suggest that allergen exposure during pregnancy, lactation and early childhood may be necessary to induce tolerance [41]. Accordingly, avoidance of allergenic food by the mother, e.g. milk, egg and nuts during pregnancy, did not appear to lower the risk of sensitization in the child [42]. Moreover, a balanced diet prevents malnutrition of both mother and child.

Use of anti-acid medication

Changes of hormone levels during pregnancy and the growing volume of the fetus often lead to heartburn, reflux and abdominal pain in the mother. About 70% of

pregnant women are affected by these symptoms during their last trimester and 50% of them are likely to take acid-suppressing medication. However, animal and human studies indicate that acid suppression and the resulting elevated pH in the stomach may lead to an increased risk of sensitization to food ([43, 44], reviewed in [45]) and drugs [46, 47]. This mechanism was recently also shown to be true for children aged 0–18 years with gastro-esophageal reflux disease, who were treated with gastric acid suppression medication [48]. Importantly, a sensitization of the mother induced by acid-suppression was shown to lead to an increased risk of food allergy in the newborn in a BALB/c mouse model [49]. Also in a database-link study of human patients, the positive correlation between acid suppression during pregnancy and increased risk for asthma in children was shown [50]. Although more studies are needed, pregnancy-associated reflux should probably be treated by non-pharmacological measures first (avoidance of large meals, sleeping with elevated upper body, not lying down after a meal, avoiding sweet and fatty food as well as alcohol and smoking). In general, during pregnancy and lactation, patients should avoid intake of any medication including non-prescription over-the-counter substances, unless recommended and closely monitored by a physician.

Insufficient exposure to environmental bacteria

The "hygiene hypothesis" states that low exposure of the mother during pregnancy and of the newborn in early life to environmental bacteria contributes to a Th2-biased immune response. This hypothesis has been confirmed by several experimental animal and epidemiological human studies, whereas details about the mechanism are still under investigation [51].

Cohabitation with pets

In a recent longitudinal study the effects of ownership of a wide range of pets from pregnancy to 7 years of age were investigated [52]. Whereas cat ownership was associated with lower, rabbit and rodent ownership was associated with a higher risk of wheezing. In that study, dog ownership in pregnancy was associated with wheezing in the newborn at the age of 6 months. However, in studies on urban children, especially dog exposure was a clear protective factor against asthma and allergic diseases, at least in children without family predisposition for allergies [53]. Dogs also seem to protect from atopic eczema [54, 55]. The discussion is, however, ongoing. For instance, recent recommendations for the prevention of food allergy and atopic eczema again contained the recommendation to avoid pets during gestation [56]. It is anticipated that an exchange of immunomodulatory allergens such as lipocalins takes place between pets and humans [57]. Reptiles and exotic pets were so far not investigated in any birth cohort studies, but potent allergens may be expected from their feeding animals [58].

Conclusion

Diagnosis, preferably by in vitro testing and avoidance of skin and provocation testing, as well as management of asthma and allergic diseases are decisive during pregnancy for welfare of mother and child. The most important initial steps for allergic pregnant women are the avoidance of the offending allergen and the symptomatic asthma and rhinitis treatment to guarantee optimal oxygen supply, also of the unborn. Allergen-specific immunotherapy should not be initiated, but can without further dose increase be maintained during pregnancy and lactation.

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Abstract

Asthma is a chronic inflammatory immune disorder of the airways affecting one in ten children in westernized countries. The geographical disparity combined with a generational rise in prevalence, emphasizes that changing environmental exposures play a significant role in the etiology of this disease. The microflora hypothesis suggests that early life exposures are disrupting the composition of the microbiota and consequently, promoting immune dysregulation in the form of hypersensitivity disorders. Animal model research supports a role of the microbiota in asthma and atopic disease development. Further, these model systems have identified an early life critical window, during which gut microbial dysbiosis is most influential in promoting hypersensitivity disorders. Until recently this critical window had not been characterized in humans, but now studies suggest that the ideal time to use microbes as preventative treatments or diagnostics for asthma in humans is within the first 100 days of life. This review outlines the major mouse-model and human studies leading to characterization of the early life critical window, emphasizing studies analyzing the intestinal and airway microbiotas in asthma and atopic disease. This research has promising future implications regarding childhood immune health, as ultimately it may be possible to therapeutically administer specific microbes in early life to prevent the development of asthma in children.

Keywords: Microbiota, Asthma, Early life, Critical window, Hygiene hypothesis, Microflora hypothesis

Background

Recent evidence supports a role of the intestinal microbiome in the development of childhood asthma and atopic disease. Animal model studies have made significant advancements in the quest to understand the gut-lung axis; identifying large-scale shifts in gut microbial compositions in asthma and allergy-induced mice and manipulating the intestinal microbiome with antibiotics, which enhanced the severity of these diseases [1–3]. However, with the advancement of DNA and RNA sequencing technologies and the establishment of large longitudinal human birth cohorts, it is becoming clearer that gut microbial dysbiosis in human atopic diseases is characterized not by global changes to the composition of the intestinal microbiota, but by taxa-specific shifts in abundance at the family, genus, and even species' levels [4–6]. Perhaps unsurprisingly, these taxa-specific changes are most prominent within the first 100 days of life, during which the human immune system is most plastic in its development [4–6]. Given these scientific developments, this review aims to provide an overview of the recent advances in human microbiome research in asthma and atopic disease.

The global burden of asthma and atopic disease

Allergic asthma is an immunoglobulin E (IgE)-mediated chronic inflammatory disease of the airways [7]. Other manifestations of IgE-mediated or "atopic" diseases include: atopic dermatitis (also referred to as eczema), allergic rhinitis, and food allergy [8]. These diseases typically manifest in early childhood and can be chronic lifelong burdens for many people. However asthma is often viewed as the most burdensome atopic disorder, due to the prevalence (235 million people worldwide) and associated mortality (an estimated nine asthma-related deaths per day in the United States) [9, 10]. Asthma has become the most prevalent childhood disease in recent decades, affecting approximately one in ten children

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worldwide [8]. Aside from the obvious danger associated with asthma, this disease is very disruptive to a normal daily lifestyle for children, and is the leading cause of emergency room visits and absenteeism from school [11].

Some of the most striking data related to asthma prevalence comes from the United States, where it was reported from 1999 to 2009, that the proportion of people diagnosed with asthma increased by 15% [10]. In other Westernized countries (e.g. Canada, Australia, and the UK) the prevalence of this disease was reported to be even higher (up to 30% in some countries), while many countries in Eastern Europe and Asia report a much lower prevalence of this disease (~5%) [8, 12, 13]. This rapid increase in prevalence of asthma (and other atopic diseases) as well as the apparent geographical disparity suggests an etiology that is more complex than population genetic variation.

'The post-industrial epidemic'

The underlying cause of asthma is a complex product of genetic and environmental factors resulting in significant heterogeneity of the disease. Parental history of asthma increases the likelihood of developing this disease, however assessment of this factor alone is not enough to confirm a person's risk of asthma development [14–16]. There is also evidence of a strong link between sex and increased risk of asthma development in children, as boys are more likely to develop childhood asthma than girls [17, 18]. Further, genome-wide-association-studies have identified candidate genes that play a role in asthma susceptibility (*ORMDL3* and *SMAD3*) [19]. Thus it is clear that human genetics contribute to asthma pathogenesis, however the rapid rise in asthma prevalence suggests changing environmental factors are biasing the developing human immune system toward these hypersensitivity diseases [20].

In addition to the within-generation rise in the prevalence of asthma, there is also an inverse relationship between the incidence of infectious diseases and hypersensitivity diseases, where a high incidence of infectious diseases appears to protect against allergic and autoimmune diseases [21]. Further, the geographical disparity of asthma and atopic diseases is shifting, as developing countries become industrialized and their living conditions become more like the Western world [22]. Thus it appears that there may be a link between the development of hypersensitivity diseases and the urbanization or modernization of society [23]. Many urban environments have similar characteristics (lower air quality, higher population density, lower economic status) that predispose populations to asthma; and similar to the geographical disparity of this disease, rural areas with comparable environments do report greater incidences of hypersensitivity diseases [24, 25]. There is also the possibility that urbanization does not support optimal immune development due to a decrease in exposure to environmental microbes as humans shift from an outdoor lifestyle to a more indoor lifestyle that is characteristic of urban societies [26].

This concept of decreased microbial exposure in modern or more urban societies has become a booming research area in the etiology of immune dysregulation. One particular arm of asthma etiology in particular, focuses on factors associated with improved health and hygiene; for example, increased antibiotic exposure, and household size [23, 27–35]. In particular, David Strachan extensively studied the relationship between household size and atopic disease in the late 1980s, and his initial findings led him to propose the hygiene hypothesis of allergic disease in 1989 [36]. This hypothesis sets the stage for the current analyses assessing the role of microbial exposure in the development of asthma and other hypersensitivity disorders.

External and internal microbes as protectors against asthma

The hygiene hypothesis

The hygiene hypothesis proposes that a lack of early life exposure to microbes alters early life immune system priming and, consequently, increases susceptibility to atopic diseases [20]. Strachan theorized that older siblings promote increased exposure to environmental microbes through inevitable unhygienic contact, which results in decreased likelihood of atopic disease development in younger siblings [36]. He supported his hypothesis by showing that household size was inversely correlated with the development of hay fever (i.e. allergic rhinitis) in a cohort of 11,765 children [37]. Since Strachan's original proposal, the hygiene hypothesis has expanded to include additional environmental factors (such as mode of birth, antibiotic exposure, household pets, etc.), which also alter the microbial exposure of infants [33–35]. Further, with substantial improvements in genetic sequencing technology, the role of indigenous microbes has also been added to the mix.

The microflora hypothesis

The microflora hypothesis extends Strachan's hygiene hypothesis by emphasizing the role of microbes residing in and on the human body (collectively known as the human microbiota) [38]. Originally, these microbes were considered to be commensal, having little effect on human physiology [38]. However they have since been implicated extensively in human health and development, and it is clear that there is a microbial-immune cell interface, in which cross-talk between microbes and

immune cells aids in the development of immune tolerance [39–42]. Notably, the focus of this hypothesis is the gastrointestinal tract, one of the most populated zones of the human body [38]. It proposes that perturbations to the colonization and composition of the intestinal microbiota (dysbiosis), disrupts this natural microbe-immune cell interface, biasing the developing infant immune system toward a hyper-sensitive (allergic) state [38, 43]. In support of the microflora hypothesis, a recent study found that uncontacted Amerindians (indigenous peoples of the Americas) exhibited higher levels of bacterial and functional diversity in their skin and fecal microbiota than any other human population previously reported, suggesting that modern societal practices (perturbations) have strong implications in the development of the microbiota [44]. Regarding the role of the intestinal microbiome in asthma, the gut-lung axis attempts to explain the mechanisms guiding gut microbe-lung immune cell cross talk.

The gut-lung axis

Innate immunity microbial crosstalk

The gut-lung axis attempts to mechanistically define how microbes in the gut might influence immune function in the lung [45]. One potential connection is through interactions of the gut microbiota with pattern recognition receptors of the innate immune system [46]. It is well established that pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), CpG, and peptidoglycan can stimulate Toll-like receptor (TLR) signaling, which confers downstream activation of many genes that regulate inflammation and innate immune responses [47]. Similar to the antigen-recognition and IgE-mediated hypersensitivity pathways, dendritic cells (DCs) are also the intermediaries of gut microbiota-immune cell cross talk, as they regularly sample gut microbes in the intestinal lumen or lymphoid tissues [46]. DC sensing of gut microbiota PAMPs promotes immune tolerance in the intestine, but also results in phenotypic changes to DCs and migration to the mesenteric lymph node (MLN) to promote T cell priming [48]. In the MLN, T cells also acquire homing molecules (e.g. CCR4, CCR6), which initiate migration to other parts of the body, including the respiratory mucosa [49].

Thus it is possible that interactions with specific gut microbes, via their corresponding PAMPs, could result in varying phenotypic changes in DCs, with downstream effects on lymphocyte priming/homing and ultimately, shifts in anti-inflammatory responses in the airways [49]. In a house dust mite (HDM) model of allergic inflammation, chronic intranasal exposure to endotoxin (bacterial LPS) has been shown to protect mice from HDM-induced asthma [50]. The proposed mechanism of this protection is through A20 (ubiquitin modifying enzyme)-mediated inhibition of HDM-induced recruitment of conventional DCs to the lungs and mediastinal lymph nodes [50]. Further, prior 2-week treatment of mice with LPS suppressed proliferation and differentiation of adoptively transferred CD4+ HDM-specific 1-DER T cells in the mediastinal lymph nodes into IL-5 and IL-13—secreting T-helper (Th)-2 cells, highlighting the T cell priming effects of these DCs [50]. Though this is not a gut microbiota mediated pathway, it does highlight the ability of bacterial PAMPs (specifically LPS) to alter DC recruitment to the lungs and protect mice against asthma symptoms.

Role of microbial-derived metabolites—short chain fatty acids

Another area of gut-lung axis research involves microbial-derived metabolites, such as short chain fatty acids (SCFAs). SCFAs are direct by-products of bacterial fermentation of carbohydrates and are key energy sources for many host tissues and gut bacterial species [51]. There are three major SCFAs, acetate, propionate, and butyrate, which are present in a molar ratio of 60:20:20, respectively [51]. These metabolites are known to modify gene expression through inhibition of histone deacetylases (HDACs), cytokine and chemokine production, and cell differentiation, proliferation, and apoptosis [52]. With regard to immune tolerance and inflammatory mechanisms, butyrate and propionate induce extrathymic T-regulatory (T-reg) generation through direct interactions with T cells and indirect interactions through DCs, potentially through the inhibition of HDACs [53]. Clostridial species are prominent SCFA producers, and butyrate production by these particular bacteria was associated with the generation of peripheral T-reg cells in the colon [54]. In a HDM-mouse model of experimental asthma, both acetate and propionate were capable of reducing cellular infiltration into the airways after HDM exposure [55]. Systemic propionate treatment modified bone marrow hematopoiesis and enhanced the generation of DC and macrophage precursors and subsequent recruitment of DCs less effective in promoting Th-2 cell polarization in the lungs [55]. In a later study using the same asthma mouse model, maternal intake of acetate was shown to reduce allergic airways disease in the adult offspring of mice [56]. Notably, both these studies initially assessed the role of a high fiber diet on the production of SCFAs and colonization of intestinal bacteria—highlighting the influence of diet, mediated by gut microbial changes, on the development of the immune system [55, 56]. The latter study, however, emphasizes intrauterine effects on the infant immune system, mediated by maternal diet, suggesting the need to consider prenatal prevention strategies using these gut microbial metabolites [56].

Microbial influences on epigenetics

It is also possible that the intestinal microbiota is linked to lung immunity through microbe-mediated epigenetic modification. Distinct whole blood DNA methylation patterns were associated with two major bacterial phyla, either Firmicutes or Bacteroidetes and pathway analysis revealed differential methylation (associated with a high or low Firmicute/Bacteroidetes ratio) among genes enriched in functional networks such as cardiovascular disease, inflammatory responses, obesity, and lipid metabolism [57]. Further, production of bacterial methyl groups, cofactors (e.g. folate), and enzymes (e.g. methyltransferases) can both directly and indirectly affect host DNA methylation, and consequently may bias cell differentiation toward or against an immune profile that confers tolerance [58, 59]. There is also evidence that early life farm microbial exposures may influence the methylation of genes related to asthma and allergies [60, 61]. Lastly, reinforcing the age-sensitive role of the intestinal microbiota in hypersensitivity diseases, the presence of a conventional gut microbiota in previously germ-free (GF) neonatal (but not adult) mice decreased hypermethylation of *CXCL16*, which in turn decreased accumulation of invariant natural killer T (iNKT) cells (prominent in the pathogenesis of asthma) in the colon [41]. Thus ultimately, there is much more to learn regarding the mechanisms of the gut-lung axis in asthma and other lung disorders. Current research in asthma and atopic disease centers around how, mechanistically, the intestinal microbiota is linked to these disorders and whether early life changes to the intestinal microbiome can be therapeutically manipulated to promote immune tolerance.

The intestinal microbiota in asthma and atopic disease

Mechanistic studies analyzing how the intestinal microbiome is involved in asthma and atopic disease are typically conducted in mouse models of allergic inflammation. GF mice lacking a microbiota show increased allergic responses, including increased lymphocyte and eosinophil inflammation in the airways, accompanied by increased Th-2 cytokines and elevated IgE production [62]. However many animal studies also focus on roles of distinct bacterial taxa in atopic disease development. In an ovalbumin (OVA)-model of asthma, oral supplementation of mice with two types of *Lactobacillus* showed that protection from allergic responses is mediated by specific bacterial species [63]. Supplementation with live *Lactobacillus reuteri* resulted in decreased airway hyper responsiveness, while treatment with *Lactobacillus salivarius* had no effect on the allergic symptoms of the mice [63]. This species-specific effect was also shown using three bacterial species, *Bifidobacterium longum*, *Bifidobacterium breve* and *L. salivarius* [1]. *L. salivarius* had no effect, and both *Bifidobacterium* species increased Peyer's patch and splenic Foxp3+ T-reg cells in infant mice [1]. However, only *B. longum* introduced in the perinatal period resulted in T-reg cell induction in adult mice and protected against allergic airway inflammation in OVA-sensitized mice [1]. Notably, the age-sensitive induction of T-regs in adult mice by *B. longum* suggests the presence of an early life window in which microbial-driven immune changes are most effective.

Antibiotics (which disturb the intestinal microbiota composition) have also been shown to increase airway inflammation in mouse models of experimental asthma [2, 64, 65]. One study showed that combined oral antibiotic treatment of mice resulted in increased allergic inflammation, characterized by increases in serum IgE and circulating basophils [65]. Conventionally raised mice showed decreased proliferation of bone-marrow resident basophil precursors compared to the antibiotic treated mice, suggesting that these shifts in immune cells were mediated by alterations to the microbiota [65]. Thus collectively, these mouse model studies show that gut microbial alterations can result in changes in lung function, but it is becoming clearer through improved mouse-models and longitudinal human cohort research, that these microbe-mediated changes in immune development are most effective in early infancy.

Mouse studies suggest an early life critical window

Age is the main driver of compositional and functional differences in the intestinal microbiota [4, 66]. Thus it is perhaps unsurprising that many mouse studies assessing the role of the gut microbiota in atopic disease, find the results to be time sensitive (Fig. 1a). Cahenzli et al. demonstrate that global shifts in the composition of the intestinal microbiota (increased microbial diversity) in early life is required to regulate IgE production and decrease disease severity in a mouse-model of antigen-induced oral anaphylaxis [67]. In OVA- and HDM-models of allergic airway inflammation, oral infection with CagA-positive *Helicobacter pylori* resulted in protection against OVA and HDM-induced airway hyper responsiveness [68]. However, this bacterium-mediated protection against asthma was more apparent in mice infected neonatally compared to mice infected as adults [68]. As noted in the previous section (microbial influences on epigenetics), neonatal (but not adult-life) exposure to a conventional microbiota in GF OVA-challenged mice abrogated iNKT cells in the lungs and reduced serum IgE, proinflammatory cytokine levels, and eosinophilia in the bronchoalveolar lavage fluid, protecting mice from developing allergic asthma symptoms [41].

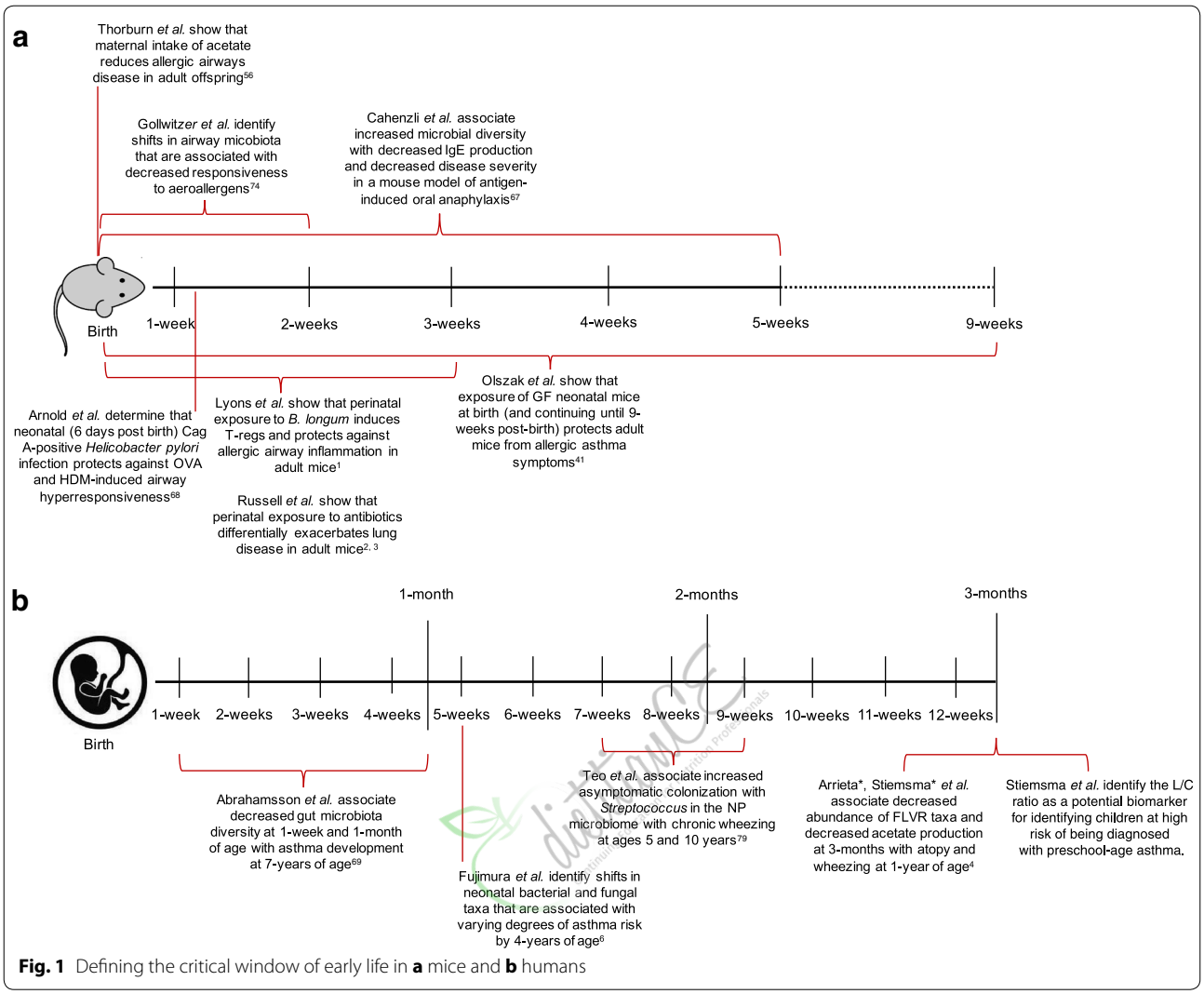

Fig. 1 Defining the critical window of early life in **a** mice and **b** humans

Russell et al. demonstrate the effects of early life antibiotic exposure in mice [2, 3]. This group showed that perinatal (in utero and up to 21 days after birth until weaning) versus strictly prenatal (in utero) vancomycin treatment of OVA-challenged mice exacerbates asthma-related immune responses [2]. Further, perinatal treatment of mice with another antibiotic, streptomycin, exaggerated lung inflammation in a Th-1/Th-17-driven model of hypersensitivity pneumonitis [3]. Notably, each antibiotic promoted expansion of specific bacterial phyla; streptomycin promoted expansion of Bacteroidetes, while vancomycin promoted expansion of Firmicutes [3]. This highlights both the selective effects of antibiotics on gut microbial taxa and the ability of an antibiotic-altered microbiota to differentially enhance disease susceptibility to specific lung diseases [3, 64]. Notably, all of these studies highlight a critical window (from birth to weaning in mice) in which microbial alterations can promote or protect against asthma and atopic diseases. However until recently, this critical window was not characterized in humans.

Longitudinal human studies define the early life critical window

Mouse studies have made substantial mechanistic strides in microbiome-atopic disease research. In parallel, improvements in DNA sequencing technology over the past decade have made analyzing the microbiomes of humans much more feasible. With these sequencing improvements, a similar 'critical window' in which microbial alterations can be associated with the development of asthma and atopic disease is also becoming more apparent in humans (Fig. 1b). Using 454-pyrosequencing, one study associated changes in gut microbial diversity at 1-week and 1-month of age with asthma development at school age [69].

However more recent studies have identified shifts in specific bacterial taxa in early life, rather than global compositional changes, that are associated with increased risk of asthma later in life. In fact, our group identified decreases in the abundances of four bacterial genera, *Faecalibacterium, Lachnospira, Rothia, and Veillonella* (FLVR), in the 3-month fecal microbiota, which were associated with atopy and wheezing at 1-year of age among 319 infants enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) Study [4]. Atopy and wheezing are clinically used to predict asthma development in children, and subjects positive for both atopy and wheezing were most likely (compared to wheeze only, atopy only, and control subjects) to develop asthma by 3-years of age—suggesting that these early life genera shifts are associated with increased risk of asthma development [4]. Further, these four bacterial taxa ameliorated asthma in an OVA-challenged mouse model, supporting their immune-modulatory roles in protecting against asthma development [4].

Since the CHILD Study is a longitudinal cohort, our group was able to conduct a follow-up study on this same cohort when they reached 4-years of age and could be diagnosed with preschool-age asthma [5]. We found that *Lachnospira* remained decreased in the 3-month fecal microbiota while one particular bacterial species, *Clostridium neonatale*, was increased in asthmatics at this time-point [5]. Demonstrating the diagnostic potential of these particular microbes, we calculated a ratio of *Lachnospira* to *C. neonatale* (L/C) and using quartile analysis, showed that children with the lowest L/C ratio (quartile 1) were 15 times more likely to be diagnosed with preschool-age asthma than children in the other L/C quartiles [5]. Most interestingly, however, both of these studies identified these gut microbial changes in the first 3 months of life only, highlighting this time frame as the early life critical window during which gut microbial dysbiosis is most influential in promoting asthma and atopic disease in humans [4, 5].

Notably however, additional bacterial taxa as well as other microbes (e.g. fungi) have been associated with asthma and atopic disease development in children [6, 70]. In fact, a recent study published in *Nature Medicine* was able to distinguish asthmatic and atopic children by their neonatal (35 days post birth) intestinal microbiome compositions [6]. Children in the highest risk group showed shifts in specific bacterial and fungal taxa, highlighting roles of various gut microbes in human immune development, which are identifiable even earlier than 3-months of age [6]. Thus, even now it is becoming more evident that; (i) there are likely many other gut microbes associated with asthma and atopic disease development in humans; and (ii) that the 'critical window' for identifying these gut microbial shifts in humans could be even smaller than 100 days post birth. Further, in an effort not to overlook a potentially obvious link between the microbiome and airway inflammation, recent studies have identified associations and mechanistic links between airway microbes and asthma and atopic disease development.

Role of the airway microbiota

Though the intestinal microbiome is one of the most populated regions of the human body, recent research supports a role of the airway microbiome in asthma and atopic disease pathogenesis. In an OVA-induced mouse model of asthma, administration of a common gut pathogen, *E. coli*, to the lung was shown in a TLR4-dependent manner to induce γδ-T cells, decrease activation of lung DCs, and abrogate Th-2 cytokine production to confer protection of mice from allergic airway inflammation [71].

In humans, airway microbial dysbiosis has been associated with increased risk of asthma [72, 73]. 16S rRNA analysis of sputum samples showed higher bacterial diversity and increased abundance of Proteobacteria in asthmatic adults compared to non-asthmatic adults [73]. Another adult study analyzing the bronchial microbiota was able to identify differences in microbial composition associated with asthma severity [72]. When compared to healthy controls, severe asthmatics were enriched in Actinobacteria and *Klebsiella* species [72]. However compared to patients with moderate asthma, patients with severe asthma were enriched in many Actinobacterial taxa and showed decreased abundances of Proteobacteria [72].

Continuing the early life theme, Gollwitzer et al. provide evidence of a 2-week window in which shifts in the airway microbiota are associated with decreased responsiveness to aeroallergens and the induction of Helios^{-} T-regs in a programmed death ligand 1 (PD-L1)-mediated manner [74]. If PD-L1 is blocked only in the first two weeks of life and allergic airway inflammation is induced after 4-weeks of age, the exaggerated allergic airway inflammation in neonatal mice is maintained to adulthood [74]. Further, in a study of 234 human children, researchers associated early (7–9 weeks post-birth) asymptomatic colonization with *Streptococcus* in the nasopharyngeal (NP) microbiome with chronic wheezing at ages 5 and 10 years [75]. Interestingly, they also suggest the NP microbiome as a determinate for the spread of respiratory infections to the lower airways, which are also significant risk factors for asthma development [75]. Thus there may be specific early life non-pathogenic airway microbes associated with asthma, but it is also possible that dysbiosis in the airway microbiota is the mediator

between respiratory infections and subsequent development of asthma.

Conclusions and future directions

In conclusion, the current literature suggests a role of the microbiome in asthma and atopic disease development, with particular emphasis on early life dysbiosis. Notably, recent studies have identified shifts in specific bacterial genera and species, which could ultimately be applied as probiotic interventions prior to the development of asthma. These probiotic interventions could be given directly to the baby in early infancy once all safety concerns have been addressed. Another option for colonizing the infant is through maternal exposure to these microbes either before or after delivery. Prior to establishing these probiotic regimens however, shifts in early life gut and airway microbes could also be applied as microbe-based diagnostics to identify children at the highest risk of developing asthma and related allergic diseases.

Before any of these preventative or diagnostic techniques can be applied, future research should focus on validating the current findings in additional longitudinal human cohorts and improving humanized microbiome mouse models of airway and lung inflammation to mechanistically characterize the microbe-immune cell interactions promoting or protecting against asthma and atopic disease. Additionally, targeted-metabolomic and shotgun metagenomic sequencing strategies using stool, urine, and potentially breast-milk samples in human cohorts will better characterize the functional roles of these specific taxa in infant immune development.

Further, to better elucidate this early life critical window in humans, additional longitudinal cohorts should begin stool sample collection beginning at birth and continuing up to age 1 year (with at least bi-weekly collection points within the first 3-montths of life). Additionally, the collection of additional biological samples (namely blood and urine) during the first 3-months of life (though this is not often feasible) would be ideal to determine whether these gut microbial alterations occur prior to immune-dysregulation or vice versa.

Moreover, although this review focuses on the bacterial microbiome in asthma, there are many other microbial organisms (fungi and other eukarya, and viruses) that also play key roles in host physiology and immune development [6, 76–79]. Also, with the characterization of other microbiomes within the human body (i.e. placental, blood, breast-milk), it is likely that we will identify even more microbial taxa that are associated with airway diseases. As discussed in the previous sections, there is evidence in mice that asthma is a developmental origin disease, mediated by maternal gut microbial alterations in utero [56]. Thus it will be important to incorporate multi-biome analyses to potentially identify: (i) how children are being colonized with specific asthma related microbes; and (ii) roles of other microbial taxa in the pathogenesis of asthma and atopic disease. Ultimately however, this literature review presents research with promising future directions, offering an exciting outlook for future microbe-based preventative treatments and diagnostic strategies for asthma and atopic disease in children.

NutritionCE Continuing Education for Nutrition Professionals

Abstract

Background: Asthma is an increasingly common chronic disease among children, and data point toward a complex mechanism involving genetic, environmental and epigenetic factors. Epigenetic modifications such as DNA hypo- or hyper-methylation have been shown to occur in response to environmental exposures including dietary nutrients.

Methods: Within the context of the asthma randomized trial of indoor wood smoke (ARTIS) study, we investigated relationships between diet, asthma health measures, and DNA methylation. Asthma health measures included a quality of life instrument, diurnal peak flow variability (dPFV) and forced expiratory volume in the first second (FEV_1). Dietary intake was assessed with a food frequency questionnaire. Methylation levels of LINE-1 repetitive element and two promoter CpG sites for interferon gamma (IFNγ, -186 and -54) from buccal cell DNA were measured using pyrosequencing assays.

Results: Data were collected on 32 children with asthma living in western Montana who were recruited to the ARTIS study. Selenium and several methyl donor dietary nutrients were positively associated with the asthma quality of life measure. Intake of methyl donating nutrients including folate was positively associated LINE-1 methylation and negatively associated with IFNγ CpG-186. Higher levels of LINE-1 methylation were associated with greater dPFV.

Conclusion: We identified several nutrients that were associated with improved quality of life measures among children with asthma. The IFNγ promoter CpG site -186 but not -54 was associated with the intake of selected dietary nutrients. However, in this small population of children with asthma, the IFNγ promoter CpG sites were not associated with respiratory health measures so it remains unclear through which epigenetic mechanism these nutrients are impacting the quality of life measure. These findings add to the evidence that dietary nutrients, particularly foods containing methyl donors, may be important for epigenetic regulation as it pertains to the control of asthma.

Trial registration ClincialTrials.gov NCT00807183. Registered 10 December 2008

Keywords: Asthma, Methylation, Spirometry, Diet, Nutrition, Children, Epigenetics, Quality of life

Background

Asthma is an environmentally triggered disease that affects nearly 26 million people in the United States [1]. Dietary intake represents a modifiable environmental exposure that could partially explain the current burden of chronic disease, including asthma, in industrialized countries [2]. Epidemiological studies suggest that dietary patterns are linked to the risk of developing asthma, however, the evidence from longitudinal birth cohorts has not clearly defined the importance of specific nutrients or fully elucidated the mechanistic pathways linking diet to chronic respiratory disease. Further, there have been few studies aimed at determining if nutrient intake contributes to asthma control in children. One potential mechanism whereby dietary intake affects respiratory health in children is through epigenetic modulation of immunoregulatory cytokines.

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Significant observational data suggests that dietary status and intake of particular nutrients can affect respiratory health outcomes. Several recent studies have suggested that some dietary nutrients may be protective for respiratory health [3–12]. A recent review concluded that dietary intake in utero and throughout the lifecourse can influence respiratory health status, however definitively assessing causal relationships in human studies is a major challenge [13]. A cross-sectional study by Berthon et al. showed that among asthmatics, a high fat diet was associated with increased airway eosinophilic inflammation, and low fiber intake was associated with poor lung function [14]. Supplementation of dietary folic acid has been successful in the prevention of neural tube defects in the United States. However, longitudinal cohort studies have produced mixed results regarding maternal folic acid supplementation and asthma development [15, 16]. Antioxidants like selenium may play a role in respiratory health through systemic reduction of oxidative stress [17]. In a mouse model of allergic airway disease, a combinatory therapeutic that included selenium attenuated the physiologic airway damage that is typical of this model [18].

The rapidly evolving field of epigenetics has emerged as an appealing potential mechanistic bridge that could link environmental exposures to the development of asthma or the exacerbation of asthma-related symptoms [19]. The exact toxicoepigenetic mechanisms are far from elucidated, but landmark studies using the agouti mouse model have provided solid evidence that environmental exposures can affect phenotype through alterations in DNA methylation patterns [20]. Understanding how and when these mechanisms can impact asthma pathogenesis is paramount. In a mouse model of allergic airway disease, in utero dietary intake of methyl donating nutrients was associated with an enhanced disease phenotype as well as aberrant hypermethylation of runt-related transcription factor 3 (Runx3), a gene known to suppress allergic airway disease [21]. Although the perinatal exposure window may be especially important, data also suggest that environmental exposures could impact health via epigenetic mechanisms throughout the lifecourse. In humans, the production of regulatory T cells (Tregs), which are known to suppress immune responses, is controlled by transcription factor forkhead box p3 (FOXP3) [22]. Nadeau et al. demonstrated that patients with asthma in a polluted environment had a hypermethylated FOXP3 locus profile which was associated with impaired Treg function relative to patients with asthma in a less polluted area [23].

The relationship between dietary intake and epigenetic modifications is complex and compounded by sensitivity to timing of exposure (e.g. prenatal, postnatal, adolescent, or adult). Nevertheless, human and mouse data indicate several dietary nutrients play a role in epigenetic mechanisms [24], thus it is possible that nutrient intake is related to asthma pathogenesis through the epigenetic regulation of key genes. Asthma is phenotypically characterized by a shift toward type 2 T helper (Th2) polarization and consequently type 1 T helper (Th1) cell cytokines such as interferon gamma (IFNγ) play a critical role as counter regulators in the allergic asthma pathway [25, 26]. For example, in a follow-up study of adults recruited as children with a history of wheeze, those with persistent asthma were compared to those with resolved asthma to characterize the Th1/Th2 response following exposure to house dust mite allergen [27]. Smart et al. found that those with persistent asthma had much weaker Th1 responses and concluded that a measured decrease in IFNγ production in this group could be a major factor underpinning the presence of severe and chronic asthma symptoms. Meng et al. investigated the effect of diet on IFNγ production in humans and showed that cells extracted and purified from non-asthmatic adults produced differential amounts of IFNγ [28]. Interestingly, Meng found that the amounts of IFNγ were associated with intake of specific dietary variables and predicted upper respiratory tract infection incidence. Finally, a series of studies using either a ragweed or dust mite-sensitized mouse model of asthma showed that pre-treatment with a DNA adjuvant known to result in Th1 biased immune status with marked overproduction of IFNγ resulted in an ameliorated lung inflammatory phenotype [29, 30]. Thus IFNγ is a relevant candidate gene that plausibly exists in the mechanistic pathway linking dietary intake to respiratory health via epigenetic regulation of the Th1/Th2 cytokine balance.

Poor asthma control is associated with school absences, higher health care costs and worse long-term health outcomes. An understanding of the association between a child's recent dietary history and respiratory health measures could lead to important intervention strategies to improve outcomes among children with asthma. In this study we aimed to evaluate the relationship between a priori selected nutrients and asthma health. Although the link between current dietary status and asthma health is not clear, evidence suggests a potential role for an epigenetic mechanism. In addition to a measure of global gene methylation, IFNγ was chosen as a candidate gene because of its well-established role in the Th1/Th2 balance.

Methods

Study overview

Participants were recruited from the asthma randomized trial of indoor wood smoke (ARTIS) study. The rationale

and methods for the ARTIS study have been described previously [31–33]. The ARTIS study included 114 children with asthma (ages 6–17) from 97 homes in Montana, Idaho, and Alaska. This parent study was designed to test an indoor air quality intervention, and homes were assigned to either a placebo arm or an air filter intervention. Two in-home data collection visits occurred in each of two consecutive winter periods with the intervention occurring between these winter periods. The subcohort recruited to participate in this diet and epigenetics study included 32 participants living in western Montana who had been recruited in the final 2 years of the 5-year ARTIS study. Additional file 1: Figure S1 indicates when spirometry measurements, buccal cells, and food frequency questionnaires (FFQ) were administered. For the purpose of the currently described study, only data that was collected in conjunction with a FFQ was considered. In Additional file 1: Figure S1, this would be visits B and D. Health outcomes measures included a quality of life instrument and self-monitoring of spirometry measures using a peak flow meter. Buccal cell samples were collected for evaluating epigenetic markers. Anthropometric measures determined by trained staff using a digital scale and stadiometer along with the participant's gender and date of birth were used to calculate body mass index (BMI) percentile using the U.S. Centers for Disease Control and Prevention (CDC) calculator [34]. The study was approved by the University of Montana Institutional Review Board. In addition to the informed consent procedures for the parent study, children were separately assented to participate in this diet and epigenetics study and parents signed a parental permission and informed consent form.

Dietary nutrient collection

Dietary data was collected using the 2004 Block Kids FFQ (NutritionQuest, Berkeley, CA, USA) to characterize dietary intake among participants. This instrument has been validated in children, ages 6–17 years old [35–38]. The questionnaire includes 77 food items. In addition to intake of standard nutrients, this instrument was used to estimate intake of micronutrients that participate in the one-carbon metabolism pathway (i.e. betaine, choline, folate, etc.). These nutrients are important for the generation of methyl groups and are therefore potentially relevant to DNA methylation markers. The questionnaire was administered to each participant by trained staff using serving size visual aides, and parents were asked to assist their child with portion size recognition and remembering foods they ate during the last week. Questionnaires were processed by NutritionQuest, and the resulting data were analyzed at the University of Montana.

Health outcome measures collected in parent study

The pediatric asthma quality of life questionnaire (PAQLQ) is a 23-item asthma-specific battery which provides domain scores for symptoms (10 items), activity limitation (5 items), and emotional function (8 items) [39]. The total PAQLQ score and each domain score are calculated as mean scores ranging from one to seven with seven as the optimal score. The PAQLQ has been validated as an evaluative tool to measure within participant changes over time due to treatment, and changes in this scale of 0.5 or more points are clinically significant [39].

Using the PiKo-1 m (Ferraris Respiratory, Ayer, MA, USA) participants performed a test twice daily, in the morning and in the evening, for a period of 2 weeks. These 2-week periods were initiated at the beginning of each air sampling event. For each test, the child's parent records the observation as it appears on the meter and these observations are later checked for accuracy against the digital log of the instrument. The instrument records the best result for both peak expiratory flow (PEF) and forced expiratory volume in one second (FEV_1). Outcomes from these measures include average morning PEF and FEV_1, average evening PEF and FEV_1, and diurnal PEF variability (dPFV).

Cell collection, DNA extraction, and pyrosequencing

Buccal cells were collected from the participant's cheek by trained staff using a cytology brush and stored in Cell Lysis Solution (Qiagen, Valencia, CA, USA) at room temperature until all samples were collected. In compliance with this protocol, all samples were processed within 24 months from the day of collection. DNA from the buccal cells was extracted using Gentra Puregene Buccal Cell DNA Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The quantity of the purified DNA was measured using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) and then stored at −20 °C. DNA bisulfite treatment was carried out using the EZ DNA Methylation-Direct Kit (Zymo Research, Irvine, CA) according to the manufacturer's instruction, and stored at −20 °C. Pyrosequencing assay was used to measure methylation levels of LINE-1 repetitive element and the promoter region of INFγ. Briefly, 50 μg of bisulfite-modified DNA was PCR amplified by polymerase chain reaction (PCR) using specific primers (Additional file 1: Table S1) and the PyroMark PCR kit (Qiagen, Valencia, CA, USA). After annealing, pyrosequencing was conducted using a Pyromark Q96 MD (Qiagen, Valencia, CA, USA). Samples were run in duplicate and only samples with a coefficient of variation less than 5% were used in the final analysis. Epitect (Qiagen, Valencia, CA, USA) bisulfite treated controls, which include a methylated and unmethylated human genome

sample, along with a no template control were used on each plate.

Statistical analysis

All analyses were conducted using SAS v9.4 (Cary, NC, USA). To evaluate if cross sectional measurements of IFN CpG sites are correlated with each other and/or correlated with LINE1 global methylation we estimated Pearson correlation coefficients using the first available observation for each participant (n = 32). A subset of 17 macro- and micronutrients from the total 73 nutrient variables generated by the FFQ were chosen after a literature review of diet as it relates to asthma. Relationships between a priori selected dietary nutrients and both epigenetic markers and asthma outcomes were considered in separate models using all available and complete data, which included multiple visits for some participants. Their associations with continuous epigenetic markers (i.e. global and gene-specific methylation) and asthma measures were evaluated using generalized estimating equations (GEE), which account for correlations between repeated measures on the same participant. Tertiles of dietary nutrients were included in analyses as three-level indicator variables to investigate potentially non-linear relationships with epigenetic and asthma outcomes. Analyses were adjusted for age (continuous) and gender. Although this diet and epigenetics repeated measures study was not directly related to the indoor air quality intervention study, we included in our models indicators for pre- versus post-intervention winter and home intervention assignment (i.e., placebo versus air filter). Inclusion of the following potential confounders: presence of cat or dog in home (yes or no), family income (above or below $50,000) and parent education (college degree or no college degree) appreciably impacted parameter estimates. Therefore, the final model included age, gender, winter and intervention group assignment, presence of cat or dog, income and education. We investigated relationships between epigenetic markers and asthma measures in a similar manner. Due to the number of comparisons (n = 264), a false discovery rate correction [40] was applied and adjusted p-values (q-values) were calculated for each relationship where the GEE model was used. A threshold for significance was set at $q < 0.20$ which means we accept that 20% of the observed significant relationships (i.e. 3.4 out of 17) could be false positives.

Results

A subset of 32 children from the ARTIS cohort participated in this study of diet, asthma health and epigenetics and was included in the analyses described here. Diet data was collected once per winter in conjunction with buccal cells, PAQLQ and spirometry and therefore, only these 'complete' visits were considered in the analysis. Approximately 63% of subcohort, or 20 participants, had both a year one and year two 'complete' visit, while 12 participants only had one 'complete' visit, which occurred in either year one or year two, for a total of 52 observations. Reasons for these 12 participants having one rather than 2 years of data included missing data, participant not available during scheduled visit, or the participant chose to only participate in one year of the study. Moreover, in the final GEE models, which were adjusted for several covariates, one participant (two observations) was excluded because income and education data was missing, therefore the results from these models include 50 observations from 31 participants. Ages ranged from 8 to 17 years and 47% were male (Table 1). The study population was 94% non-Hispanic white. The mean (sd) BMI percentile was 70.6 (20.1) and 34% (n = 11) were above the 85th percentile, which is considered overweight according to the CDC [34]. Baseline asthma-related respiratory health values can be found in Table 1. Mean values for both dPFV and FEV_1 were at the approximate threshold used to designate poor asthma control [41]. Mean (sd) LINE-1 methylation was 65.3% (3.4) with a range of 56.1–73.2%. Mean (sd) IFNγ CpG-54 was 79.6% (4.5) with a range of 68.6–92.4%. Mean (sd) IFNγ CpG-186 was 70.1% (6.6) with a range of 49.1–81.6%. IFNγ CpG-54 and IFNγ CpG-186 observations were moderately correlated with each other (r = 0.42; p = 0.02) as were IFNγ CpG-54 and LINE-1 methylation (r = 0.44; p = 0.01). IFNγ CpG-186 and LINE-1 methylation were not significantly correlated (r = 0.26; p = 0.15).

Table 1 Selected characteristics of subset of ARTIS participants included in epigenetic study

	N	Mean	SD	Range
Age	32	12.8	2.5	8.0–17.0
Gender				
Male	15 (46%)			
Female	17 (53%)			
Ethnicity				
Non-Hispanic	30 (94%)			
BMI percentile	32	70.6	24.1	5.8–99.0
dPFV	32	20.0	14.0	2.0–66.0
% Predicted morning FEV_1	32	81.7	19.7	26.9–112.9
% Predicted evening FEV_1	32	82.3	19.3	19.3–110.1
PAQLQ	32	5.6	1.1	3.1–7.0
LINE-1 (%)	32	65.3	3.4	56.1–73.2
IFNγ-54 (%)	32	79.6	4.5	68.6–92.4
IFNγ-186 (%)	32	70.1	6.6	49.1–81.6

SD standard deviation, *BMI* body mass index, *dPFV* evening to morning peak flow variability, *FEV_1* forced expiratory volume in 1 s, *PAQLQ* pediatric asthma quality of life questionnaire, *IFNγ* interferon gamma

Evaluating dietary nutrients with respect to respiratory health

When considered across categories of calculated intake, most dietary nutrients failed to show a consistent association with respiratory health measures, but several differences in PAQLQ scores were observed between participants in the highest third versus the lowest third of intake for some nutrients (Table 2). Phosphatidylcholine was the only selected nutrient that was associated with any of the three pulmonary function measure assessed. Children in the middle tertile relative to the lowest had 16.04% point (95% CI 3.31, 28.78; q = 0.16) higher % predicted evening FEV_1. Intake of selenium and folate was associated with better PAQLQ scores. Specifically, participants with the highest tertile of selenium and folate intake had 1.4 unit (95% CI 0.90, 1.91; q = 0. 01) and 0.92 unit (95% CI 0.31, 1.53; q = 0.11) higher PAQLQ scores, respectively. Additionally, nutrients in the one-carbon metabolism cycle, phosphocholine (1.11 unit higher PAQLQ score; 95% CI 0.23, 1.98; q = 0.16) and betaine (0.98 unit higher PAQLQ score; 95% CI 0.30, 1.66; q = 0. 13) were positively associated with PAQLQ.

Evaluating dietary nutrients with respect to methylation outcomes

Intake of several nutrients was associated with LINE-1 methylation and methylation at CpG promoter site IFNγ-186, but not for IFNγ-54 (Table 3). Children in the highest tertile of kilocalories (3.2% points higher methylation; 95% CI 0.82, 5.58; q = 0.16) or the middle tertile of protein (2.67% points higher methylation; 95% CI 0.62, 4.71; q = 0.16) had higher LINE-1 methylation. Similarly, those in the highest tertile of methyl donating nutrients free choline (2.18% points higher methylation; 95% CI 0.54, 3.82; q = 0.16), total choline (2.60% points higher methylation; 95% CI 0.60, 4.60; q = 0.16) and folate (4.29% points higher methylation; 95% CI 2.25, 6.34; q = 0.01) also had higher LINE-1 methylation. Intake within the middle tertile of kilocalories (4.56% points lower methylation; 95% CI −7.44, −1.69; q = 0.09) and folate (4.05% points lower methylation; 95% CI −6.18, −1.19; q = 0.02) compared to the lowest was associated with less IFNγ-186 methylation. However, intake within middle tertile of monosaturated fat intake (6.88% points higher methylation; 95% CI 3.11, 10.62; q = 0.02) was associated with more IFNγ-186 methylation. Children in the highest tertile of betaine intake (4.34% points lower methylation; 95% CI −7.25, −1.42; q = 0.12) and both the middle and highest tertile of vitamin B6 intake (6.57% points lower methylation; 95% CI −10.65, −2.48; q = 0.09 and 6.63% points lower methylation; 95% CI −11.13, −2.14; q = 0.12, respectively) had less IFNγ CpG-186 methylation.

Evaluating DNA methylation with respect to respiratory health

We investigated the relationships between methylation markers and asthma-related respiratory health measurements (Table 4). A one-percentage point increase of LINE-1 methylation was associated with a 1.24 percentage point (95% CI 0.31, 2.16; q = 0.16) increase in dPFV. Neither IFNγ CpG-54 nor-186 methylation was associated with the respiratory health measures evaluated in this study.

Discussion

In this study of dietary nutrients, DNA methylation and asthma-related respiratory health outcomes, we observed positive associations between several nutrients related to one-carbon metabolism (e.g. folate, phosphocholine and betaine) and the PAQLQ score. These nutrients were not similarly associated with better self-monitored spirometry outcomes, suggesting that the nutrients may positively influence asthma quality of life through another mechanism. The composite PAQLQ score is comprised of symptom, activity and emotion domains; however, a post hoc analysis, which substituted individual domains for the composite PAQLQ in a model with dietary nutrients (i.e. those nutrients that had significant relationships with composite PAQLQ), revealed no difference for the impact of dietary intake on individual domains. Furthermore, the individual domains were highly correlated with one another (data not shown) and therefore it is unclear which of these domains may be more affected by diet.

Folate (or folic acid) is one of the most prominently studied methyl donors and is known to play a role in allergic asthma [42]. Many studies have investigated the efficacy of methyl donating nutrient supplementation to reduce the risk of asthma development. To date the results have been inconclusive (see reviews [2, 43]). Many of these supplementation studies have been either limited by ethical concerns or underpowered. We observed that folate intake was associated with a higher PAQLQ score in children with asthma. Based on the most recent reviews, the evidence suggests that early life exposure to folate has no major effects on asthma outcomes later in life [44]. However, relevant to our findings, few studies have investigated the relationship between folate intake and asthma measures in adolescent children with established asthma [45]. Folate deficiency has been associated with asthma-related symptoms and exacerbations [46–48]. Folate and betaine are involved in DNA methylation through the formation of S-adenosylmethionine and homocysteine metabolism and therefore have the potential to affect gene expression, thereby influencing asthma pathogenesis [43]. In addition to methyl donors, selenium was also positively associated with PAQLQ score. Fabian

Table 2 The relationship between select nutrients and asthma health measures by dietary tertiles in ARTIS where the lowest tertile of intake (T1) is the reference group

Asthma health measure	dPFV		% Predicted morning FEV_1		% Predicted evening FEV_1		PAQLQ	
Dietary factor	Estimate (95% CI)	q-value	Estimate (95% CI)	q-value	Estimate (95% CI)	q-value	Estimate (95% CI)	q-value
Kilocalories								
T3:T1	−3.18 (−11.41, 5.06)	0.80	13.09 (−4.04, 30.22)	0.61	4.18 (−14.08, 22.44)	0.87	0.62 (−0.25, 1.48)	0.68
T2:T1	−0.50 (−8.78, 7.78)	0.98	0.83 (−13.94, 15.60)	0.98	7.04 (−8.31, 22.40)	0.76	−0.34 (−1.20, 0.51)	0.79
Protein								
T3:T1	−6.96 (−20.40, 6.48)	0.72	19.26 (0.48, 38.05)	0.33	10.96 (−2.58, 24.51)	0.55	0.70 (−0.16, 1.56)	0.55
T2:T1	−3.42 (−14.56, 7.73)	0.83	6.31 (−8.30, 20.92)	0.77	1.14 (−12.42, 14.70)	0.97	0.59 (−0.09, 1.27)	0.51
M-fats								
T3:T1	−2.87 (−10.62, 4.87)	0.81	11.64 (−6.82, 30.11)	0.71	8.55 (−10.72, 27.81)	0.77	0.39 (−0.31, 1.09)	0.71
T2:T1	−0.57 (−8.81, 7.67)	0.98	3.89 (−9.58, 17.36)	0.83	3.26 (−9.25, 15.77)	0.85	0.52 (−0.03, 1.07)	0.41
S-fats								
T3:T1	−3.99 (−15.87, 7.89)	0.83	16.85 (1.36, 32.35)	0.29	11.73 (0.65, 22.82)	0.33	−0.16 (−0.82, 0.50)	0.87
T2:T1	−6.56 (−16.72, 3.59)	0.70	16.67 (2.96, 30.37)	0.28	10.19 (−0.41, 20.80)	0.41	−0.05 (−0.77, 0.67)	0.98
Omega 3:6 ratio								
T3:T1	−1.25 (−10.54, 8.03)	0.92	2.42 (−11.59, 16.44)	0.90	−0.39 (−13.84, 13.07)	1.00	−0.13 (−1.18, 0.93)	0.92
T2:T1	−1.29 (−12.09, 9.51)	0.92	5.40 (−4.00, 14.80)	0.71	1.46 (−6.18, 9.10)	0.90	0.11 (−0.54, 0.75)	0.90
Selenium								
T3:T1	5.44 (−2.98, 13.86)	0.70	6.63 (−6.31, 19.56)	0.73	6.37 (−6.57, 19.30)	0.74	1.40 (0.90, 1.91)	0.01*
T2:T1	2.52 (−4.56, 9.59)	0.82	0.20 (−14.11, 14.51)	1.00	2.91 (−12.96, 18.77)	0.90	−0.56 (−1.59, 0.47)	0.71
Fiber								
T3:T1	−0.39 (−9.02, 8.25)	0.99	7.75 (−5.27, 20.78)	0.71	5.86 (−8.02, 19.74)	0.77	0.66 (−0.52, 1.84)	0.71
T2:T1	0.63 (−9.85, 11.11)	0.98	−5.36 (−17.98, 7.27)	0.77	−3.60 (−15.79, 8.59)	0.83	0.32 (−0.38, 1.02)	0.76
Folate								
T3:T1	−3.43 (−14.76, 7.89)	0.83	10.07 (−7.95, 28.09)	0.71	3.75 (−10.53, 18.02)	0.85	0.92 (0.31, 1.53)	0.11*
T2:T1	−3.03 (−14.23, 8.17)	0.85	−0.03 (−17.50, 17.44)	1.00	−0.24 (−13.99, 13.50)	1.00	−0.25 (−0.83, 0.35)	0.78
Methionine								
T3:T1	−4.83 (−13.90, 4.24)	0.72	14.81 (−0.25, 29.88)	0.36	11.07 (−4.02, 26.17)	0.65	0.52 (−0.37, 1.41)	0.71
T2:T1	1.87 (−7.53, 11.28)	0.90	5.22 (−12.12, 22.57)	0.83	7.29 (−10.16, 24.73)	0.77	−0.06 (−1.58, 1.46)	0.99
Free choline								
T3:T1	2.44 (−4.68, 9.56)	0.83	11.20 (−6.45, 28.86)	0.70	7.69 (−10.98, 26.36)	0.78	0.00 (−1.34, 1.34)	1.00
T2:T1	5.35 (−3.47, 14.17)	0.71	−2.64 (14.74, 9.47)	0.88	−0.21 (−13.39, 12.97)	1.00	−0.31 (−1.26, 0.64)	0.83
Glycpp-choline								
T3:T1	2.84 (−5.75, 11.42)	0.83	−3.05 (−18.17, 12.07)	0.89	−3.64 (−19.88, 12.61)	0.88	0.61 (−0.17, 1.39)	0.59
T2:T1	−1.81 (−9.22, 5.59)	0.87	2.88 (−9.04, 14.80)	0.87	3.70 (−8.10, 15.50)	0.83	0.12 (−0.41, 0.65)	0.87
Pp-choline								
T3:T1	4.54 (−3.07, 12.15)	0.71	−2.95 (−12.99, 7.08)	0.83	−5.21 (−15.57, 5.14)	0.73	1.11 (0.23, 1.98)	0.16*
T2:T1	−1.03 (−7.55, 5.50)	0.90	−10.81 (−27.27, 5.64)	0.69	−12.35 (−29.79, 5.09)	0.68	0.50 (−0.61, 1.60)	0.77
Ppt-choline								
T3:T1	0.36 (−6.86, 7.58)	0.98	11.60 (−1.45, 24.65)	0.48	−1.13 (−19.21, 16.94)	0.98	0.24 (−0.86, 1.35)	0.88
T2:T1	5.71 (−2.92, 14.33)	0.69	−3.17 (−15.51, 9.17)	0.85	16.04 (3.31, 28.78)	0.16*	−0.19 (−0.58, 0.21)	0.76
Total choline								
T3:T1	1.33 (−6.50, 9.16)	0.90	8.61 (−7.92, 25.14)	0.72	5.96 (−12.23, 24.15)	0.83	0.18 (−1.26, 1.61)	0.92
T2:T1	3.76 (−4.42, 11.94)	0.76	−6.68 (−20.25, 6.90)	0.74	−2.06 (−16.74, 12.62)	0.92	−0.38 (−1.35, 0.59)	0.80
Betaine								
T3:T1	−2.62 (−9.35, 4.10)	0.80	12.85 (0.98, 24.72)	0.29	9.13 (−1.59, 19.85)	0.51	0.98 (0.30, 1.66)	0.13*
T2:T1	4.20 (−6.18, 14.58)	0.79	−9.34 (−21.55, 2.86)	0.61	−9.10 (−19.78, 3.59)	0.68	0.21 (−0.56, 0.98)	0.85
Vitamin B-2								
T3:T1	−0.95 (−8.43, 6.54)	0.92	−4.74 (−18.10, 8.61)	0.82	3.81 (−10.97, 18.58)	0.85	0.40 (−0.66, 1.46)	0.80
T2:T1	1.66 (−8.13, 11.44)	0.90	8.98 (−7.57, 25.52)	0.71	−4.04 (−17.65, 9.56)	0.83	−0.12 (1.12, 0.88)	0.92

Table 2 continued

Asthma health measure	dPFV		% Predicted morning FEV_1		% Predicted evening FEV_1		PAQLQ	
Dietary factor	Estimate (95% CI)	q-value	Estimate (95% CI)	q-value	Estimate (95% CI)	q-value	Estimate (95% CI)	q-value
Vitamin B-6								
T3:T1	2.53 (−5.37, 10.43)	0.83	4.19 (−10.21, 18.59)	0.83	0.26 (−13.43, 13.94)	1.00	0.46 (−0.21, 1.13)	0.69
T2:T1	4.64 (−3.10, 12.38)	0.71	−4.96 (−20.50, 10.57)	0.83	0.81 (−14.85, 16.46)	0.98	−0.65 (−1.20, 0.00)	0.36
Vitamin B-12								
T3:T1	−0.02 (−8.26, 8.22)	1.00	6.42 (−8.93, 21.77)	0.77	1.70 (−11.74, 15.13)	0.92	0.79 (0.09, 1.50)	0.29
T2:T1	1.53 (−7.17, 10.23)	0.90	−2.40 (−16.46, 11.66)	0.90	−3.38 (−16.85, 10.10)	0.86	0.29 (−0.53, 1.11)	0.82

M-fats monosaturated fats, *S-fats* saturated fats, *Glycpp-choline* glycerophosphocholine, *Pp-choline* phosphocholine, *Ppt-choline* phosphotidylcholine, *dPFV* evening to morning peak flow variability, *FEV_1* forced expiratory volume in 1 s, *PAQLQ* pediatric asthma quality of life questionnaire

* q-value < 0.20

et al. found that children with asthma compared to healthy control children had lower plasma levels of selenium and higher exhaled nitric oxide, a marker of poor lung health [49]. However, a group of Swedish researchers found no impact of selenium intake on allergic disease in young children [50]. The inconclusive results regarding the impact of selenium intake on allergic asthma could be attributed to the fact that while selenium does have antioxidant properties it also has the ability to upregulate some immune responses [17, 51]. In our analysis selenium status is associated with better asthma quality of life measures, however, this nutrient was not associated with LINE-1 or IFNγ methylation profiles.

Among the dietary nutrients investigated in this study only phosphatidylcholine was modestly associated with self-administered spirometry measures, specifically higher evening FEV_1. Phosphatidylcholine is phospholipid and a major dietary source of choline, which is involved in one-carbon metabolism. Phospholipids can also impact T cell function in a number of ways including membrane fluidity and gene expression, which could have indirect immunomodulatory effects [52]. Therefore, our observation of a positive association between phosphatidylcholine and FEV_1 in these children could be reflective of reduced lung inflammation. However, this association was not consistent across tertiles of phosphatidylcholine intake nor was there a consistent response across the different FEV_1 measures, evening versus morning. These concerns together with the studies that have linked phosphatidylcholine to cardiovascular disease [53] and related inflammatory symptoms [54], suggest that the association between phosphatidylcholine intake and FEV_1 should be interpreted with caution.

In addition to evaluating the relationship between nutritional intake and asthma health, a potential epigenetic mechanism was examined by determining if global methylation or IFNγ promoter methylation was associated with nutritional intake. Global methylation, though informative, is difficult to interpret in the context of respiratory health and may be even more complex in this cohort of children with asthma. Few studies have looked at buccal DNA LINE-1 global methylation in healthy children. A study of 57 healthy girls aged from 6 to 15 investigated LINE-1 global methylation in saliva samples and the average (SD) was 75.2 (3.4) [55]. The cells collected from buccal and saliva should be similar, however the mean LINE-1 methylation in our study overall was considerably lower, which could be attributed to asthma status. It is clear from the literature that intake of methyl donors can result in measureable changes to the mammalian epigenome [20]. Our study shows that dietary intake of folate, free choline, and total choline is positively associated with LINE-1 methylation. By convention, an increase in global methylation is thought to be protective, while a shift toward genome-wide hypomethylation is often associated with a poor health outcome or disease [56, 57]. Nevertheless, our data suggested that global methylation was positively associated with dPFV, an indicator of airway hyper-reactivity. This finding could be a characteristic of the study population, which had relatively low average global methylation. Further, DNA methylation is dynamic and global methylation is a reflection of the epigenetic changes occurring at many gene locations.

Our study specifically focused on IFNγ as a candidate gene, hypothesizing that this gene would lie in the mechanistic pathway linking dietary intake to asthma health outcomes in children. Previous studies have established that IFNγ CpG-54 and -186 (-53 and -190 are the corresponding murine CpGs) are relevant to allergic outcomes in animal models [58, 59] and humans [60–62]. In the mouse, Jones et al. showed that these CpGs are functionally relevant (i.e., methylation status affects transcription of the IFNγ gene) and that de novo methylation of these sites plays a key role in Th2 polarization at least within the CD4+ T cells [63]. In an human asthma

Table 3 The relationship between select nutrients and DNA methylation markers by dietary tertiles in ARTIS, where the lowest tertile of intake (T1) is the reference group

Epigenetic measure	IFNγ CpG-54		IFNγ CpG-186		LINE-1	
Dietary factor	Estimate (95% CI)	q-value	Estimate (95% CI)	q-value	Estimate (95% CI)	q-value
Kilocalories						
T3:T1	0.46 (−2.46, 3.38)	0.90	−0.11 (−4.35, 4.13)	1.00	3.20 (0.82, 5.58)	0.16*
T2:T1	−0.40 (−2.87, 2.07)	0.90	−4.56 (−7.44, −1.69)	0.09*	0.00 (−2.45, 2.46)	1.00
Protein						
T3:T1	2.60 (−0.90, 6.10)	0.65	−0.04 (−3.41, 3.32)	1.00	1.62 (−0.79, 4.03)	0.69
T2:T1	2.17 (−0.46, 4.80)	0.55	2.02 (−1.24, 5.27)	0.71	2.67 (0.62, 4.71)	0.16*
M-fats						
T3:T1	−1.10 (−3.70, 1.51)	0.77	4.77 (−0.04, 9.57)	0.36	1.14 (−1.23, 3.51)	0.76
T2:T1	−2.42 (−4.74, −0.11)	0.33	6.86 (3.11, 10.62)	0.02*	1.12 (−1.00, 3.24)	0.72
S-fats						
T3:T1	−0.75 (−3.11, 1.61)	0.83	5.11 (−1.08, 11.31)	0.55	1.52 (−1.09, 4.13)	0.71
T2:T1	1.02 (−1.91, 3.96)	0.82	3.00 (−2.48, 8.48)	0.71	1.26 (−1.45, 3.97)	0.76
Omega 3:6 ratio						
T3:T1	1.44 (−1.23, 4.10)	0.71	2.29 (−1.85, 6.44)	0.71	−0.09 (−2.70, 2.52)	0.99
T2:T1	3.72 (0.44, 7.01)	0.29	0.24 (−4.72, 5.20)	0.98	0.96 (−1.03, 2.95)	0.74
Selenium						
T3:T1	0.34 (−2.45, 3.13)	0.92	−1.40 (−5.17, 2.38)	0.81	2.32 (−0.15, 4.79)	0.45
T2:T1	1.40 (−0.60, 3.40)	0.68	−2.38 (−5.01, 0.24)	0.45	−0.18 (−2.69, 2.33)	0.98
Fiber						
T3:T1	0.53 (−2.31, 3.37)	0.90	−4.67 (−9.09, −0.24)	0.33	1.89 (−1.02, 4.80)	0.69
T2:T1	0.19 (−2.29, 2.68)	0.98	−2.52 (−6.54, 1.49)	0.71	−0.27 (−2.70, 2.16)	0.94
Folate						
T3:T1	2.93 (0.06, 5.80)	0.36	2.46 (−2.25, 7.17)	0.72	4.29 (2.25, 6.34)	0.01*
T2:T1	1.95 (−0.03, 3.93)	0.36	−4.05 (−6.18, −1.91)	0.02*	0.46 (−1.24, 2.16)	0.85
Methionine						
T3:T1	0.63 (−2.42, 3.68)	0.89	−2.70 (−6.86, 1.45)	0.69	1.12 (−1.78, 4.03)	0.80
T2:T1	0.43 (−2.25, 3.11)	0.90	−1.38 (−5.68, 2.92)	0.83	1.58 (−1.04, 4.21)	0.71
Free choline						
T3:T1	1.33 (−1.08, 3.74)	0.71	−2.61 (−5.84, 0.63)	0.55	2.18 (0.54, 3.82)	0.16*
T2:T1	1.29 (−1.03, 3.60)	0.71	−3.28 (−6.33, −0.24)	0.29	0.01 (−1.82, 1.85)	1.00
Glycpp-choline						
T3:T1	0.44 (−2.23, 3.12)	0.90	−2.81 (−5.95, 0.33)	0.48	0.66 (−1.48, 2.80)	0.83
T2:T1	1.68 (−0.54, 3.89)	0.65	−0.31 (−3.84, 3.22)	0.97	0.24 (−1.74, 2.21)	0.92
Pp-choline						
T3:T1	0.50 (−2.65, 3.65)	0.90	−1.77 (−5.27, 1.73)	0.73	0.41 (−1.43, 2.25)	0.88
T2:T1	1.47 (−0.52, 3.46)	0.65	−1.54 (−5.83, 2.75)	0.82	−2.08 (−4.41, 0.24)	0.48
Ppt-choline						
T3:T1	1.49 (−1.08, 4.06)	0.71	−2.33 (−6.06, 1.39)	0.71	0.64 (−1.66, 2.94)	0.84
T2:T1	−0.78 (−3.40, 1.84)	0.83	−1.55 (−6.86, 3.76)	0.83	−1.66 (−4.03, 0.71)	0.68
Total choline						
T3:T1	1.29 (−1.06, 3.64)	0.71	−3.47 (−6.68, −0.26)	0.29	2.60 (0.60, 4.60)	0.16*
T2:T1	0.85 (−1.41, 3.11)	0.80	−3.58 (−6.59, −0.58)	0.28	0.42 (−1.88, 2.73)	0.90
Betaine						
T3:T1	−0.00 (−2.50, 2.49)	1.00	−4.34 (−7.25, −1.42)	0.12*	1.18 (−1.25, 3.62)	0.74
T2:T1	1.52 (−1.21, 4.26)	0.71	1.28 (−4.28, 1.73)	0.77	1.65 (−0.61, 3.91)	0.65
Vitamin B-2						
T3:T1	0.62 (−2.44, 3.68)	0.89	−4.19 (−7.91, −0.48)	0.29	2.47 (−0.37, 5.32)	0.51

Table 3 continued

Epigenetic measure	IFNγ CpG-54		IFNγ CpG-186		LINE-1	
Dietary factor	**Estimate (95% CI)**	**q-value**	**Estimate (95% CI)**	**q-value**	**Estimate (95% CI)**	**q-value**
T2:T1	1.41 (−0.74, 3.56)	0.69	−4.03 (−7.86, −0.20)	0.33	0.23 (−2.48, 2.94)	0.97
Vitamin B-6						
T3:T1	2.05 (−1.04, 5.14)	0.69	−6.63 (−11.13, −2.14)	0.12*	1.44 (−1.65, 4.52)	0.76
T2:T1	0.66 (−1.80, 3.12)	0.85	−6.57 (−10.65, −2.48)	0.09*	0.13 (−2.39, 2.65)	0.98
Vitamin B-12						
T3:T1	1.12 (−2.31, 4.55)	0.83	−2.31 (−7.00, 2.39)	0.74	0.59 (−2.43, 3.60)	0.90
T2:T1	2.21 (−0.48, 4.91)	0.55	1.84 (−2.37, 6.05)	0.77	0.87 (−2.09, 3.82)	0.83

M-fats monosaturated fats, *S-fats* saturated fats, *Glycpp-choline* glycerophosphocholine, *Pp-choline* phosphocholine, *Ppt-choline* phosphotidylcholine, *IFNγ* interferon gamma

* q-value <0.20

Table 4 The relationship between epigenetic measurements and asthma health outcomes in ARTIS

Epigenetic marker	dPFV		% Predicted morning FEV_1		% Predicted evening FEV_1		PAQLQ	
	Estimate (95% CI)	**q-value**	**Estimate (95% CI)**	**q-value**	**Estimate (95% CI)**	**q-value**	**Estimate (95% CI)**	**q-value**
LINE-1	1.24 (0.31, 2.16)	0.16*	−0.89 (−3.18, 1.41)	0.80	−1.19 (−3.41, 1.03)	0.72	0.04 (−0.11, 0.20)	0.84
IFNγ CpG-186	0.58 (0.06, 1.09)	0.29	−0.53 (−1.79, 0.72)	0.77	−0.57 (−1.62, 0.48)	0.72	0.03 (0.00, 0.07)	0.44
IFNγ CpG-54	0.22 (−0.74, 1.19)	0.87	−0.57 (−1.92, 0.77)	0.77	−0.84 (−2.10, 0.42)	0.69	0.02 (−0.07, 0.11)	0.85

dPFV evening to morning peak flow variability, FEV_1 forced expiratory volume in 1 s, *PAQLQ* pediatric asthma quality of life questionnaire, *IFNγ* interferon gamma

* q-value <0.20

cohort, Lovinsky-Desir et al. showed that there are differential methylation profiles for these CpGs relative to age, sex and tissue type [64]. For example, when methylation profiles of buccal cells and CD4+ lymphocytes isolated from whole blood were compared, IFNγ CpG-186 was correlated for males but not females. Further, methylation values for IFNγ CpG-54 and -186 were correlated for children and adults in CD4+ lymphocytes but only for adults in buccal cells. White et al. investigated IFNγ promoter methylation profiles by in vitro polyclonal expansion of CD4+ and CD8+ T cells sorted from peripheral blood mononuclear cells [62]. When samples collected from adolescent children were stratified by atopic status, the authors found that, for CD8+ T cells under Th1 polarizing conditions, IFNγ CpG sites -54 and -186 were less methylated in the non-atopic children.

When evaluating the impact of diet on IFNγ promoter methylation, we found that only IFNγ CpG-186 methylation patterns were affected by selected nutrients. We observed that intake of kilocalories and three methyl donating nutrients was associated with less IFNγ CpG-186 methylation, while children who had higher intake of monosaturated fats had more IFNγ CpG-186 methylation. Based on the functional data available for this CpG site, which we note does not come from buccal cells, we speculate that a negative association between a dietary nutrient and methylation at this site could impact the Th1/Th2 balance by increasing the expression of IFNγ. Overall nutrient intake has previously been linked to IFNγ production [65]. However, overnutrition is not likely a preferable or effective asthma intervention especially due to the potential links between obesity, inflammation, and asthma. Monosaturated fat was the only nutrient we found to be positively associated with IFNγ-186 methylation. In a study of approximately 1200 adolescent children conducted in Taiwan, intake of monosaturated fats was inversely associated with risk of asthma [66]. By contrast, a study of nearly 4000 adult European participants found that intake of monosaturated fats was positively associated with allergic sensitization [67].

IFNγ CpG promoter methylation at site -54 and -186 was not associated with respiratory health measures or PAQLQ. This suggests that the positive relationship revealed between PAQLQ composite score and intake of selenium, folate, phosphocholine, and betaine may not be working directly through epigenetic modification of these specific sites as we had hypothesized.

Limitations and cautions

We evaluated several dietary macro- and micronutrients in this study, but these factors likely include only a

portion of the exogenous factors that could influence DNA methylation in this population. While FFQs are an accepted and validated method for acquiring personal dietary information, we note that the portion sizes and specific foodstuffs were self-reported by the participants with assistance and input from parents. Though it is widely accepted, BMI may actually be a poor indicator for obesity in children and adolescents who have large, lean body mass from physical activity, high muscularity, or frame size. By focusing on select candidate DNA methylation markers, we recognize that numerous inflammatory pathways involving diet and asthma may not have been captured. We also are limited in our interpretation of the DNA methylation data because we did not measure IFNγ expression or protein levels in these samples. For example, we found some dietary factors to be negatively associated with DNA methylation, which could be informative for asthma intervention strategies, but such interpretations require further assessment as methylation changes do not necessarily translate to functional changes in the target tissue. Finally, although we accounted for false discoveries, we recognize that several statistical tests were performed and would expect some significant results due to chance alone. Thus these observations should be considered exploratory and requiring of further study in other populations.

Conclusions

Within this cohort of childhood asthmatics, we sought to identify dietary nutrients that may be beneficial for respiratory health. In addition we measured LINE-1 and IFNγ (CpG-54 and -186) methylation levels to identify pathways whereby diet influences the health among children with asthma. In this study, selenium and several nutrients involved in the one-carbon metabolism pathway were associated with improved asthma quality of life measures. Furthermore, these data showed that some dietary constituents were associated with both global and gene specific methylation in children with asthma. The two IFNγ CpG sites that were investigated appear to be uniquely affected by intake of micro- and macronutrients.

DietitianCE Continuing Education for Nutrition Professionals

Abstract

Background: Asthma in the elderly is poorly understood as very few studies have included these patients. DNA methylation can affect the expression of asthma susceptibility genes. Methyl groups can be produced through a choline dependent pathway. Asthmatics have decreased serum choline. We studied the effect of choline supplementation in elderly asthmatics and associations between different parameters at baseline.

Methods: This is a double-blind, placebo-controlled, cross-over study. Thirty asthmatics 65 years old and older were evaluated at baseline and 3, 6, 9, and 12 weeks later. They randomly received choline bitartrate 310 mg and placebo capsules twice daily for 6 weeks.

Results: Ninety percent of the study subjects were atopic and 97 % of them were using inhaled corticosteroids. Choline supplementation did not affect ACT (asthma control test), spirometric values, eosinophil counts or total serum IgE vs. placebo ($p > 0.86$ for all comparisons). In subjects with lower ACT (≤ 20), lower FEV1 % (<60 %), or higher eosinophil counts (≥ 0.6), there was similarly no difference between choline and placebo ($p > 0.63$). We found no significant association between eosinophil counts and IgE and the other parameters at baseline including in subjects with lower ACT or on higher inhaled steroid doses ($p > 0.09$). Asthmatic women had lower baseline ACT scores compared to men ($p = 0.02$).

Conclusions: In this study of elderly asthmatics, choline supplementation for 6 weeks did not affect ACT scores, spirometric values, peripheral blood eosinophils, or total serum IgE. These results will require confirmation in larger and longer studies.

Trial registration ClinicalTrials.gov NCT02371993

Keywords: Asthma, Choline, Elderly

Findings

Background

The U.S. population older than 65 years of age is growing rapidly and will likely increase to about 25 % of the total population by the year 2050 [1]. Among individuals 65 years old and older, about 7 % have asthma [1]. However, there is little information about asthma in these patients as most asthma studies have ignored this group. Older asthmatics have higher morbidity and mortality and are more likely to be underdiagnosed, undertreated, and hospitalized compared to their younger counterparts [1].

In elderly patients with asthma, we have previously studied the role of exhaled nitric oxide measurements and vitamin D [2, 3]. Indeed, there is growing interest in the possible role of dietary supplements (e.g., vitamins and methyl donors) in asthma [4, 5]. However, most of the studies of supplements in asthma have yielded conflicting or negative results [4, 5]. DNA methylation is a mechanism regulating gene-environment interactions in asthma, and its changes can affect asthma by increasing or decreasing the expression of asthma susceptibility genes [4, 5]. In humans, dietary methyl groups can be produced through folate and choline dependent pathways.

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Choline is a water soluble essential nutrient important in neurotransmission, lipid signalling and membrane structure besides being a methyl donor [6]. It forms methionine through the methylation of homocysteine. Choline is contained in foods such as meat, liver, eggs, poultry, fish, shellfish and peanuts. Its deficiency has been associated with neurological and cardiovascular diseases [7]. Mostly without strong scientific evidence, choline supplementation is used for liver disease, neurological diseases including depression, bodybuilding, in pregnant women to prevent neural tube defects and as a supplement in infant formula.

In mice, choline has been shown to decrease airway allergic inflammation and reduce bronchoalveolar lavage eosinophils [8]. Individuals with asthma were found to have decreased serum choline [9]. An open label study of Indian asthmatic adults showed a decrease of bronchial hyperreactivity and use of asthma drugs after choline supplementation [10]. In the same study, there was a reduction of cytokine levels (IL-4 and TNFα) and cysteinyl leukotrienes in the supernatants of the patients' blood mononuclear cells stimulated with phytohemoagglutinin [10]. In a recent, comprehensive review of vitamins and methyl donors in asthma, it was suggested that the current evidence strongly justifies clinical trials of choline supplementation as an adjuvant treatment in asthma [4].

The main purpose of this study was to investigate the effect of choline supplementation on asthma symptoms, assessed by the asthma control test (ACT), and spirometric values in elderly asthmatics. This is a double-blind, placebo controlled, cross-over study. Secondary objectives of our study included studying whether choline supplementation in elderly asthmatics affects peripheral blood eosinophil counts and total serum IgE levels. We also looked for associations between different parameters at baseline.

Methods

This study included five study visits (baseline, and 3, 6, 9, and 12 weeks later). Each study subject took one capsule of choline bitartrate (310 mg) (Vitamin Shoppe, North Bergen, NJ) twice daily (total daily dose = 620 mg daily) or one placebo capsule twice daily each for 6 weeks in a double-blind, cross-over design. Individuals with history of gastrointestinal cancers were excluded from this study. The bottles containing the unused capsules were collected at the end of the two study periods, the remaining capsules counted and their number recorded. Compliance with the study based on capsule count was very good (>90 %).

Thirty subjects 65 years old and older with asthma were included in the study. Twenty-nine were Caucasian and one was African-American. Current smokers or individuals with a 10 pack/year or longer history of smoking were excluded. Almost all of the study subjects were lifetime nonsmokers. The study subjects were recruited among interested and eligible patients with asthma followed in our practice. Allergic sensitization was verified by allergy skin tests for relevant perennial and seasonal allergens.

Spirometric values were obtained according to the ATS/ARS guidelines by a KoKo Spirometer (nSpire Health, Inc, Longmont, Colorado, USA). The ACT is a tool that allows patients to report asthmatic symptoms on a scale from 1 (severe) to 5 (no symptoms) by answering five questions about asthma control. Values lower than 20 are considered as indicative of suboptimal asthma control. Inhaled steroid doses are expressed as fluticasone equivalent. The inhaled steroids used by the study subjects were fluticasone (17), budesonide (7), mometasone (4), and beclomethasone (1). Long-acting bronchodilators were salmeterol (12) and formoterol (8). The only leukotriene antagonist used by the study subjects was montelukast. Drug treatment remained essentially unchanged throughout the study period. One subject was on prednisone 5 mg daily throughout the study. Peripheral blood eosinophils were enumerated and total serum IgE measured at the Main Line Health Laboratories. This study was approved by the Main Line Hospitals Institutional Review Board (F/N-R15-3427B).

The primary endpoint of this study was to evaluate the effect of choline supplementation on the ACT scores. The goal was set to see a 10 % improvement (effect size) of the ACT score following treatment with choline vs. placebo. In our recent study of asthma in the elderly (2), the mean ACT score was 22.2 and the standard deviation was 2.8 % (n = 30). The calculated standardized effect size is 10 % of 22.2 (2.2)/2.8 = 0.8. Therefore, β = 0.2 (1/0.8). For an α of 0.05 (two tailed t test) the number necessary to see a 10 % change is 25. Data were expressed as the mean ± standard deviation and analyzed by the two tailed t test and the correlation coefficient as indicated with significance accepted at <0.05.

Results

Table 1 summarizes the baseline characteristics of the study subjects. Most subjects were atopic (90 %), on inhaled corticosteroids (97 %), and had well controlled asthma. As shown in Table 2, choline supplementation for 3 or 6 weeks did not affect ACT or spirometric values when compared to placebo.

Similarly, peripheral blood eosinophil counts and total serum IgE were unaffected by choline supplementation vs. placebo (Table 3). In subjects with lower ACT (≤20, 16.7 ± 3.3, n = 6), lower FEV1 % (<60 %, 46.4 ± 9.2 %, n = 6), or higher eosinophil counts (≥0.6, 0.88 ± 035 K/

Table 1 Subjects' characteristics at baseline

Sex (F/M)	18/12
Age (years, range)	73.7 ± 5.9 (66–84)
BMI	25.6 ± 4.7
Atopy	27/30
Duration of asthma (years)	34.8 ± 21.2
Rhinitis	23/30
Gastroesophageal reflux disease	9/30
Inhaled steroids (dose, range)	29/30 (358 ± 255, 0–1000 mcg/day)
Long-acting bronchodilators	20/30
Leukotriene antagonists (montelukast)	11/30
Anticholinergic agents (tiotropium)	3/30
Theophylline	1/30
ACT score	22.1 ± 3.3
FEV1 %	75 ± 20.4
FEV1/FVC	0.73 ± 0.1
FEF25-75 %	71 ± 38.2
Peripheral blood eosinophils	0.38 ± 0.31 K/μL
Total serum IgE	198 ± 210 IU/ml

n = 30

Table 2 Effect of choline supplementation on ACT scores and spirometric values

	Baseline	3 weeks	6 weeks
ACT (Choline)	22.1 ± 3.3	22.9 ± 2.8	23.4 ± 2
ACT (Placebo)		23.7 ± 1.9	23.2 ± 2.5
FEV1 % (Choline)	75 ± 20.4		75.3 ± 19.3
FEV1 % (Placebo)			76 ± 20
FEV1/FVC (Choline)	0.73 ± 0.1		0.73 ± 0.1
FEV1/FVC (Placebo)			0.73 ± 0.1
FEF25-75 % (Choline)	71 ± 38.2		70 ± 34.2
FEF25-75 % (Placebo)			71.5 ± 34

n = 30

p > 0.86 for all comparisons

Table 3 Effect of choline supplementation on peripheral blood eosinophils counts (K/μL) and total serum IgE (IU/ml)

	Baseline	6 weeks
Eosinophils (Choline)	0.38 ± 0.31	0.34 ± 0.27
Eosinophils (Placebo)		0.33 ± 0.27
IgE (Choline)	198 ± 210	206 ± 262
IgE (Placebo)		220 ± 285

n = 30

p > 0.84 for all comparisons

μL, n = 6) there was also no difference between choline and placebo (Table 4). In subjects with lower serum IgE (≤151 IU/ml, 68.8 ± 46 IU/ml, n = 18), there was a trend for a decrease of the IgE by choline supplementation vs. placebo (56.1 ± 36.9 vs. 69. ± 55.4 IU/ml, respectively), but this did not reach statistical significance (p = 0.078).

We found no significant association between eosinophil counts and IgE (Table 5). Similarly, there was no association between eosinophils or IgE and ACT, age, body mass index (BMI), steroid dose, or duration of asthma (Table 5). In subjects with lower ACT scores (16.7 ± 3.3, n = 6), eosinophils and IgE were similar to their counterparts with controlled symptoms (p > 0.22). Eosinophils were similar in subjects treated with higher inhaled steroid dose (≥400 mcg/day, 583 ± 226, n = 13) and subjects on lower doses (p = 0.82). Women had lower baseline ACT scores compared to men (21.1 ± 3.7 vs. 23.6 ± 1.9, respectively, p = 0.02).

Table 4 Effect of choline supplementation in different patient subgroups

	Baseline	6 weeks
ACT ≤ 20 (Choline)	16.7 ± 3.3	21.5 ± 3
ACT ≤ 20 (Placebo)		22.3 ± 2.9
FEV1 % < 60 % (Choline)	46.4 ± 9.2	47 ± 10.3
FEV1 % < 60 % (Placebo)		46.6 ± 6.8
Eosinophils ≥ 0.6 K/μL (Choline)	0.88 ± 0.35	0.62 ± 0.4
Eosinophils ≥ 0.6 K/μL (Placebo)		0.62 ± 0.5

n = 6

p > 0.63 for all comparisons

Table 5 Associations between different parameters at baseline

	R
Eosinophils vs. IgE	0.180
Eosinophils vs. age	0.069
Eosinophils vs. ACT	0.071
Eosinophils vs. BMI	−0.003
Eosinophils vs. inhaled steroid dose	−0.034
Eosinophils vs. duration of asthma	−0.308
IgE vs. age	−0.243
IgE vs. ACT	0.019
IgE vs. BMI	0.154
IgE vs.inhaled steroid dose	−0.145
IgE vs. duration of asthma	0.008

n = 30

p > 0.09 for all associations

None of the subjects reported adverse effects during the course of the study.

Discussion

In this study, we investigated the effect of choline supplementation in elderly subjects with asthma. This group of patients has been mostly ignored in previous studies of this condition.

Choline supplementation for 6 weeks did not affect asthma symptoms or spirometric values, including in the subgroups of subjects with lower ACT scores and FEV1 values. Similarly, peripheral blood eosinophil counts and total serum IgE were unaffected by choline supplementation. These results suggest that supplementing this methyl donor has no effect on clinical and biologic parameters in elderly asthmatics. We cannot rule out that longer studies using higher doses of choline may have a different outcome. However, an open label study of Indian adults with asthma similarly showed that the same parameters were unaffected by choline chloride supplementation for 6 months [10]. Such study employed higher doses of choline than utilized in our study. In contrast, the same senior author of that study had previously reported that tricholine citrate supplementation for 16 weeks lead to an improvement in asthma symptom scores in small cohorts (10–12 subjects) of adolescent and young adults [11, 12]. One of these two studies was single blinded and the other was open label. It is unclear whether different formulations of choline may affect clinical response.

While blood eosinophil counts are similar in younger and older asthmatics, total serum IgE is significantly lower in elderly than in nonelderly asthmatic subjects [13]. In a study of adults with asthma, antigen specific serum IgE was associated with blood eosinophil counts [14]. We found no association between eosinophils and total serum IgE, or other parameters at baseline.

The results indicating that asthmatic women have lower symptom scores than men are in agreement with a recent study showing an association between female sex and poorer asthma symptom scores [15].

Although choline supplementation is considered fairly safe, and none of our study subjects experienced side effects during the study, one study found that a high dietary intake of choline was associated with an increased risk of colon adenomas in women [16].

Limitations of this study include the relatively small number of subjects, almost exclusively Caucasians and atopic with well controlled asthma. It is possible that including more subjects with uncontrolled symptoms and/or non atopic asthma could yield different results. In addition, it is also possible that choline supplementation may affect biologic markers that were not assessed in our study (e.g., cytokines, periostin).

Conclusions

In summary, in this pilot study of elderly asthmatics, choline supplementation for 6 weeks did not affect ACT scores, spirometric values, blood eosinophils or serum IgE. These results will require confirmation in subjects with uncontrolled symptoms, in other ethnic groups, and following a longer treatment period with higher dose of choline.

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Abstract

Background: Tropical forests cover less than 10 per cent of all land area (1.8×107 km^2) and over half of the tropical-forest area (1.1×107 Km^2) is represented by humid tropical forests (also called tropical rainforests). The Amazon basin contains the largest rainforest on Earth, almost 5.8 million km^2, and occupies about 40% of South America; more than 60% of the basin is located in Brazil and the rest in Bolivia, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname and Venezuela.
Over the past decade the positive role of tropical rainforests in capturing large amounts of atmospheric carbon dioxide (CO_2) has been demonstrated. In response to the increase in atmospheric CO_2 concentration, tropical forests act as a global carbon sink.

Main body: Accumulation of carbon in the tropical terrestrial biosphere strongly contributes to slowing the rate of increase of CO_2 into the atmosphere, thus resulting in the reduction of greenhouse gas effect. Tropical rainforests have been estimated to account for 32–36% of terrestrial Net Primary Productivity (NPP) that is the difference between total forest photosynthesis and plant respiration. Tropical rainforests have been acting as a strong carbon sink in this way for decades.
However, over the past years, increased concentrations of greenhouse gases, and especially CO_2, in the atmosphere have significantly affected the net carbon balance of tropical rainforests, and have warmed
the planet substantially driving climate changes through more severe and prolonged heat waves, variability in temperature, increased air pollution, forest fires, droughts, and floods. The role of tropical forests in mitigating climate change is therefore critical. Over the past 30 years almost 600,000 km^2 have been deforested in Brazil alone due to the rapid development of Amazonia, this is the reason why currently the region is one of the 'hotspots' of global environmental change on the planet.
(Continued on next page)

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Conclusion: Deforestation represents the second largest anthropogenic source of CO_2 to the atmosphere, after fossil fuel combustion. There are many causes of deforestation, including socioeconomic and natural factors, such as clear-cutting for agriculture, ranching and development, unsustainable logging for timber, as well as droughts, fires and degradation due to climate change. About natural causes of forest degradation, in the context of the Amazon, the major agent of change in the forest ecosystem would most likely be decreased dry-season precipitation. Of the 23 global climate models employed by the Intergovernmental Panel on Climate Change (IPCC) in their 2007 report, 50–70% predict a substantial (above 20%) reduction of dry-season rainfall in eastern Amazonia under mid-range greenhouse gas emissions scenarios, 40% in central Amazonia and 20% in the west. While annual carbon emissions from fossil-fuel combustion have been continually increasing since 1960s, historical trends of deforestation and associated carbon emissions have remained poorly understood.

Keywords: Respiratory allergy, Bronchial asthma, Climate change, Air pollution and respiratory diseases, Greenhouse gas emissions, Anthropogenic emissions of CO_2, Interaction between climate change and allergy, Deforestation and climate change

Background

Climate change represents a massive threat to global health, affecting local and national food supplies, air and water quality, weather, economics and many other critical health determinants [1, 2]. Air pollution is closely associated with climate change [1–4]. Over the last 50 years global earth's temperature has markedly risen [1]. Most of the observed increase in globally averaged temperatures is very likely due to the observed increase in anthropogenic greenhouse gas concentrations, as stated in the Working Group I Report of the Intergovernmental Panel on Climate Change [1]. The key determinants of greenhouse gas emissions are energy production, transportation, agriculture, food production and waste management, and attempts at mitigating climate change will need to address each of these. A huge increase in carbon dioxide (CO_2) concentrations during the last decades has been experienced [1]. CO_2 is the most important anthropogenic greenhouse gas, and its atmospheric concentration has increased from a pre-industrial value of about 280 ppm to 379 ppm in 2005 [1]. About 75% of the anthropogenic CO_2 emissions to the atmosphere during the past 20 years resulted from fossil fuel burning; most of the rest resulted from changes in land use, especially deforestation [1]. The same trend occurred for the other prevalent anthropogenic greenhouse gases: methane (CH_4), and nitrous oxide (N_2O) [1]. However, it is important to consider that after CO_2 emissions are reduced and atmospheric concentrations stabilize, surface air temperature continues to rise slowly for a century or more. Furthermore, rising temperatures contribute to the elevation of the concentrations of ozone (due to more sunlight and higher temperature) and particulate matter (due to wildfire, droughts, desertification, sandstorms and an increased use of coal-fired power to produce energy for cooling) at ground level [1, 2].

A growing body of evidence indicates that climate change has a strong impact on respiratory health, particularly on respiratory allergic diseases [1–6].

Many measures to reduce greenhouse gas emissions may have positive benefits for health. According to the intergovernmental panel on climate change (IPCC), it is necessary to reduce the anthropogenic emissions of CO_2; in this regard deforestation represents the second largest anthropogenic source of carbon dioxide to the atmosphere, after fossil fuel combustion [7]. The role of forest, particularly of rainforest of Amazon basin (the largest rainforest on Earth), in mitigating climate change is therefore critical.

This article aims to provide evidence to stimulate the debate on the impact of climate change on respiratory health and on the contribution of forests of Brazil in mitigating climate change.

The effect of climate changes on pollen allergy and respiratory allergic diseases

A body of evidence suggests that major changes involving the atmosphere and the climate, including global warming induced by human activity, have an impact on the biosphere and human environment [1, 2].

A summary of the potential health effects due to climate change is presented in Table 1.

Studies on the effects of climate changes on respiratory allergy are still lacking and current knowledge is provided by epidemiological and experimental studies on the relationship between asthma and environmental factors, eg, meteorological variables, airborne allergens and air pollution. Climate change is correlated with allergens for several reasons [8, 9]:

1) increase and faster plant growth;
2) increase in the amount of pollen produced by each plant;

Table 1 Potential health effects of climate change

Climate events	Agriculture, forestry	Human health impact
Heavy precipitation events: frequency increases over most areas	Damage to crops; soil erosion, inability to cultivate land, water logging of soils; Adverse effects on quality of surface and groundwater; contamination of water supply	Deaths, injuries, infectious diseases, allergies and dermatitis from floods and landslides
Area affected by drought	Land degradation, lower yields/crop damage and failure; livestock deaths; land degradation; More widespread water stress	Increased risk of food and water shortage; increased risk of water- and food-borne diseases; cardiovascular disorders
Number of intense tropical cyclones	Damage to crops; wind throw of trees; Power outages cause disruption of public water supply	Increased risk of water- and food-borne diseases; asthma
Incidence of extreme high sea level	Salinization of irrigation and well water; Decreased freshwater availability due to saltwater intrusion	Increase in stress-related disease; other allergic conditions

3) increase in the amount of allergenic proteins contained in pollen,
4) increase in the start time of plant growth and therefore the start of pollen production and
5) earlier and longer pollen seasons.

An earlier start and peak of the pollen season is more pronounced in species that start flowering early in the year. Moreover, plants flower earlier in urban areas than in the corresponding rural areas with earlier pollination of about 2–4 days. Pollen counts could rise due to multiple mechanisms such as increased ambient carbon dioxide levels [10], increased temperature or earlier spring seasons [11]. With warming over the longer term, changing patterns of plant habitat and species density are likely, with gradual movement northward in the Northern Hemisphere, and further south in the Southern Hemisphere [12]. The change in land use might also play a relevant role, especially for some important allergenic species, such as grasses. However, since most data come from the analysis of distribution of airborne pollen, these findings are potentially biased by the occurrence of long and medium distance transport episodes of allergenic pollen [13, 14].

Pollen allergy is frequently used to study the interrelationship between air pollution and allergic respiratory diseases (rhinitis and asthma).

Epidemiologic studies have demonstrated that urbanization, high levels of vehicle emissions and westernized lifestyle are correlated with an increase in the frequency of pollen-induced respiratory allergy in people who live in urban areas compared to those who live in rural areas [15]. Studies on plant responses to elevated CO_2 concentrations indicate that plants exhibit enhanced photosynthesis and reproductive effects and produce more pollen. Wayne et al. observed that a doubling of the atmospheric CO_2 concentration stimulated ragweed-pollen production by 61%. [11]. Furthermore, ragweed pollen collected along high-traffic roads showed a higher allergenicity than pollen sampled in vegetated areas, and it is probably due to traffic related pollution. Climate change may also affect the release and atmospheric dispersion of pollen [16]. The overall impact will be an altered pollen season timing and load, and hence change in exposure.

One of the effects of climate change is an increasing frequency and intensity of floods and cyclones. An example of how this effect can threaten respiratory health is "Thunderstorm related asthma" [17]. Actually, thunderstorms occurring during the pollen season have been observed to induce severe asthma attacks in pollen-allergic patients [15–17]. Associations between thunderstorms and asthma morbidity have been identified in multiple locations around the world [17–19]. The most prominent hypotheses for thunderstorm-related asthma are linked with bioaerosols, and involve the role of rainwater in promoting the release of respirable particulate matter [17, 20].

After hydratation and rupture by osmotic shock during the beginning of a thunderstorm, pollen grains release part of their cytoplasmic content into the atmosphere, including inhalable, allergen-carrying paucimicronic particles such as starch granules and other cytoplasmic components [17, 20].

In summary the occurrence of these epidemics is closely linked to thunderstorms; the thunderstorm-related epidemics are limited to late spring and summer when there are high levels of airborne pollen grains; there is a close temporal association between the arrival of a thunderstorm, a major rise in concentration of pollen grains and the onset of asthma epidemics. As a consequence, subjects affected by pollen allergy should be alert to the danger of being outdoors during a thunderstorm in the pollen season.

Interaction between climate change and urban air pollution

Climate change, coupled with air pollutant exposures, may have potentially serious adverse consequences for human health.

Some air pollution-related episodes of asthma exacerbations are due to climatic factors that favour the

accumulation of air pollutants at ground level, and some cities are continuously affected by pollution caused by motor vehicles [21, 22]. Furthermore, it is also important to consider that worldwide, hundreds of thousands of hectares of woods are destroyed each year by fire, thus millions of tons of CO_2 are producted, playing a role in the greenhouse effect [23–25].

Studies have demonstrated some effects of ozone over respiratory symptoms, acute decreases in lung function, increased airway responsiveness, airway injury and inflammation and systemic oxidative stress [26–29]. Gent et al. [26] examined the simultaneous effects of ozone and fine particulate matter ($PM_{2.5}$) at levels below EPA standards on daily respiratory symptoms and rescue medication use among children with asthma. Ozone level (but not $PM_{2.5}$) was significantly associated with respiratory symptoms and rescue medication use among children using maintenance medication. A 50 parts per billion (ppb) increase in 1-h ozone was associated with increased likelihood of wheeze (by 35%) and chest tightness (by 47%) [26]. The highest levels of ozone (1-h or 8-h averages) were associated with increased shortness of breath and rescue medication use [26].

One of the mechanisms whereby air pollutants can induce asthma is the interaction with allergen-carrying paucimicronic particles derived from plants [30]. The paucimicronic particles, pollen-originated or not, are able to reach peripheral airways with inhaled air, inducing asthma in sensitized subjects. Air pollution—in particular particulate matter (PM), and diesel exhaust particulate (DEP), ozone, nitrogen dioxide and sulfur dioxide — have been shown to have an inflammatory effect on the airways of susceptible subjects, causing increased permeability, easier penetration of allergens into the mucus membranes, and easier interaction with cells of the immune system [30]. There is also evidence that predisposed subjects have increased airway reactivity induced by air pollution and increased bronchial responsiveness to inhaled allergens [31]. By attaching to the surface of pollen grains and plant-derived particles of paucimicronic size, air pollutants could modify not only the morphology of these antigen-carrying agents but also their allergenic potential. In addition, by inducing airway inflammation, which increases airway permeability, pollutants overcome the mucosal barrier and could be responsible for "priming" the allergen-induced responses of pollinosis in allergic and atopic individuals.

The relationship between exposure to air pollution and the development of allergic respiratory diseases has been investigated in several studies, however there is still much to understand.

Nicolai and von Mutius carried out a study on this topic in reunified Germany. The prevalence of asthma and allergic disorders was assessed in East Germany and in West Germany [32]. In East Germany the main sources of air pollution were the industries and private coal burning for heating purposes, differently in in West Germany where traffic-related air pollutants and NO_2 exposure were prevalent [32]. The authors thus analyzed the impact of different environmental and social conditions on the development of allergies in two genetically homogeneous populations. The results showed that hay fever, skin test reactivity to common aeroallergens and asthma were considerably more prevalent in West Germany as compared to East Germany [32].

Recently a systematic review and a meta-analysis of birth cohort studies have shown that increased longitudinal childhood exposure to $PM_{2.5}$ and black carbon was associated with increasing risk of subsequent asthma in childhood [33]. Also, early childhood exposure to traffic-related air pollution was associated with development of asthma across childhood up to 12 years of age [33]. Increasing exposure to $PM_{2.5}$ was associated with sensitization to both aero- and food allergens [33].

How to reduce air pollution and global warming: the role of Brazilian forests and their message to the planet

Tropical forests cover less than 10% of all land area (1.8×107 Km^2) [34] and over half of the tropical-forest area (1.1×107 Km^2) is represented by humid tropical forests (also called tropical rainforests) [35]. The Amazon basin contains the largest rainforest on Earth, almost 5.8 million Km^2, and occupies about 40% of South America; more than 60% of the basin is located in Brazil and the rest in Bolivia, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname and Venezuela.

Over the past decade the positive role of tropical rainforests in capturing large amounts of atmospheric CO_2 has been demonstrated [36–39]. In response to the increase in atmospheric CO_2 concentration, tropical forests act as a global carbon sink. Accumulation of carbon in the tropical terrestrial biosphere strongly contributes to slowing the rate of increase of CO_2 into the atmosphere, thus resulting in the reduction of greenhouse gas effect [5]. Tropical rainforests have been estimated to account for 32–36% of terrestrial Net Primary Productivity (NPP) that is the difference between total forest photosynthesis and plant respiration [40, 41]. In this way tropical rainforests have been acting as a strong carbon sink for decades.

However, over the past years, increased concentrations of greenhouse gases, and especially CO_2, in the atmosphere have significantly affected the net carbon balance of tropical rainforests, and have warmed the planet substantially driving climate changes through more severe and prolonged heat waves, variability in temperature, increased air pollution, forest fires,

droughts, and floods [2, 42]. The role of tropical forests in mitigating climate change is therefore critical. Over the past 30 years almost 600,000 Km2 have been deforested in Brazil alone due to the rapid development of Amazonia, this is the reason why currently the region is one of the 'hotspots' of global environmental change on the planet [34]. Deforestation represents the second largest anthropogenic source of CO_2 to the atmosphere, after fossil fuel combustion [7]. There are many causes of deforestation, including socioeconomic and natural factors, such as clear-cutting for agriculture, ranching and development, unsustainable logging for timber, as well as droughts, fires and degradation due to climate change. About natural causes of forest degradation, in the context of the Amazon, the major agent of change in the forest ecosystem would most likely be decreased dry-season precipitation [43]. Of the 23 global climate models employed by the Intergovernmental Panel on Climate Change (IPCC) in their 2007 report, 50–70% predict a substantial (above 20%) reduction of dry-season rainfall in eastern amazonia under mid-range greenhouse gas emissions scenarios, 40% in central Amazonia and 20% in the west [44]. While annual carbon emissions from fossil-fuel combustion have been continually increasing since 1960s, historical trends of deforestation and associated carbon emissions have remained poorly understood [7, 45, 46]. Recently Song et al., using satellite-data of deforestation rates, derived from changes in tree cover density in the humid tropics, have estimated that between 2000 and 2010, a total of 15.9 ± 2.5 Mha (million ha) forests were lost, which represented 2.6% of the total basin area, or 2.9% of forests in year 2000 [47]. The Brazilian Amazon and the non-Brazilian Amazon lost a total of 12.5 ± 2.0 Mha and 3.4 ± 0.5 Mha forests respectively and over that decade. Brazil was the dominant country in terms of deforested area, which accounted for 79% of the total lost forests [47].

Recently reports by the Brazilian government, the FAO and other previous studies showed a declining trend in the Brazilian Amazon and the entire Amazon basin after 2005 [48–51]. The annual relative share of Brazil's deforestation changed dramatically over the study period – from the highest of 87% in the year 2004 to the lowest of 54% by the year 2010 [48–51].

Largely driven by Brazil's efforts to halt deforestation in recent years deforestation rates over the Brazilian Amazon and the entire basin declined significantly in the second half of the last decade, which resulted in greatly reduced carbon emissions [52].

Curbing deforestation in the Brazilian Amazon decreased the Brazilian Amazon's deforestation contribution to global land use carbon emissions from 17% in the 1990s and early 2000s to 9% by 2010 [53]. An opposite emission trend was observed in the non-Brazilian Amazon; this consisted of various inter-annual variabilities in the Bolivian Amazon, the Colombian Amazon and the Peruvian Amazon. Furthermore, forests of higher-biomass accounted for an increasing portion of the cleared area. According to the Intergovernmental Panel on Climate Change (IPCC), it is necessary to reduce the anthropogenic emissions of CO_2 to the atmosphere to avoid global warming beyond two degrees [54]. Although tropical deforestation was excluded from the Kyoto Protocol (KP), since 2005 there has been a common effort within the United Nations Framework Convention on Climate Change (UNFCCC) to develop a climate policy approach to deforestation that would compensate tropical nations which reduce carbon emissions from tropical deforestation and forest degradation [55, 56]. The result was a program, known as REDD ("Reducing Emissions from Deforestation and Degradation") that represents one of the most advanced components of the current round of climate treaty negotiations within the UNFCCC [57, 58].

Reducing fossil fuel emissions remains the key element for stabilizing atmospheric CO_2; however limiting the emissions from deforestation and degradation of forest represents one of the most cost-effective strategies that can help to stabilize atmospheric CO_2 levels [59, 60].

Conclusions

Climate changes affect many physical and biological systems including the immunologic and respiratory systems that are critical to human health, and it is foreseeable that environmental risk factors will have a stronger effect in the coming decades [59–62]. Climate changes interact with and affect air pollution and pollinosis, which in turn increases the frequency and severity of asthma, and affects the clinical expression of allergic disease [1–4]. Climate change affects the timing, dispersion, quantity, and quality of aeroallergens and the distribution and severity of allergic disease. Climate change alters local weather patterns including minimum and maximum temperature, rain precipitation, and storms, all of which affect the burden of allergic disease. A combined approach comprises primary prevention by greenhouse gas mitigation to stabilize the climate, and secondary prevention by clinical intervention to minimize climate change–related increases in asthma and allergic disease [61]. In the future climate changes may depend on how rapidly and successfully global mitigation and adaptation strategies are deployed. The effect of human intervention and efforts to minimize changes in vegetation and aeroallergen exposure remains to be seen.

Reducing air pollution might contribute to lessening the impact of climate change on pollen and thus directly

Table 2 What can we do to reduce the global warming?

• Decreasing use of fossil fuels and controlling vehicle emissions.
• Reducing the private traffic in towns.
• Increased use of public transport, cycling and walking.
• Planting in cities non-allergenic trees.
• Minimize outdoor activity on days with high pollution.
• Suggest patients live in remote areas from heavy traffic.
• Reduction in meat consumption.
• Two for the price of one: climate change mitigation measures also reduce air pollution.

on patients, while recognizing that ozone, the key pollutant associated with climate change, may be the major driver of pollutant/pollen interactions.

What can we do to decrease the effects of environmental factors affecting respiratory allergic diseases? Suggested measures are as follows: encouraging policies to promote access to non-polluting sources of energy; reducing the private traffic in towns and improving public transport; decreasing the use of fossil fuels and controlling vehicle emissions; planting non-allergenic trees in cities, and in this context the proposed implantation of new trees should be evaluated by allergy specialists in order to avoid high allergenic species. Although in this paper the direct impact of the increase in plant growth on allergy has not been dealt with, more studies are needed to assess its contribution to the increase in allergy prevalence.

Many measures to reduce greenhouse gas emissions may have positive benefits for health. These co-benefits will offset at least some of the costs of climate change mitigation and should be taken into account in international negotiations (Table 2). Strategies to reduce climate changes and air pollution are political in nature, but citizen and in particular health professionals and societies must raise their voices in the decision process to give strong support for clean policies on both national and international levels.

EuroDietitianCE Continuing Education for Nutrition Professionals

Abstract

Severe asthma is a major health concern. The allergic (IgE-mediated) form of asthma is well known from a pathogenic viewpoint. We searched the available literature to identify which allergens are most frequently associated with severe, refractory or life threatening asthma. According to the results, molds, pet dander, cockroach and ragweed were more frequently responsible for severe asthma. Thunderstorm asthma, in addition, represents a special association between allergic sensitization and an external climatic factor. A detailed knowledge of the most harmful allergens is mandatory for an appropriate diagnostic and preventive approach.

Keywords: Severe asthma, Atopy, Allergic sensitization, Allergens

Background

Bronchial asthma, which prevalence is around 5-10% worldwide [1, 2], remains a major health problem in all age groups for patients, their families and the community. The asthma-related respiratory symptoms cause a limitation of everyday activity with consequent absenteeism/presenteism. Exacerbations may require extra visits, emergency room admissions, and hospitalizations. Of note, fatalities continue to be reported [3]. Indeed, in the majority of patients, asthma can be adequately treated with the standard of care therapy [4], and most patients achieve a satisfactory control of the disease. Nonetheless, a not negligible subgroup of subjects remains "difficult-to-treat" or "uncontrolled" despite adequate therapy. "Severe" or "difficult to treat" or "refractory" asthma is a heterogeneous condition that encompasses different phenotypes/endotypes, such as eosinophilic, obesity-related, neutrophilic, late onset asthma, and remains a major unmet need [5, 6]. Severe asthma accounts for only 5–10% of all cases, but it is responsible for the majority of direct and indirect costs. Thus, severe asthma poses a significant health care burden accounting for up to 50% of the asthma budget in developed and developing Countries.

Several multicenter cohort studies of patients with severe asthma have been published [7–10]. These studies shed light upon some of the emerging clinical characteristics of this challenging group of patients and provide important insights into the strategic priorities for its management. Managing patients with severe asthma is complex, and requires a multidisciplinary approach and a standardized protocol, in addition to a uniform definition. Asthma is a rare cause of mortality, contributing to less than 1% of all deaths in most countries worldwide. Rates of death rise almost exponentially from mid-childhood to older ages, so the majority of asthma deaths occur after middle age [11]. Although asthma mortality trends declined in many high-income countries, studies have suggested that avoidable factors still play a major role in preventing severe asthma or asthma deaths. There is increasing evidence that severe asthma phenotypes are related to genetic factors, age of onset, disease duration, exacerbations, rhinosinusal disease and inflammatory characteristics [12–15]. For instance, in a study in a general population, as part of the European Community Respiratory Health Survey (ECRHS), there was no gender difference in asthma severity in the two surveys. However, those studies suggested that asthma severity might be less stable in women than in men [16]. Occupational exposures have also been associated with late-onset, and more severe asthma [17].

The evidence for a favorable clinical response to environmental control in severe asthma remains so far inconsistent. Atopy and allergy have long been

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associated with asthma and, to some degree, with severe asthma. (Table 1) [7–10]. It is known that early-life exposures and sensitization to various allergens, occur in children with severe asthma [18], and the association between allergy and asthma severity is stronger in children [19, 20]. In general, the association among specific IgE sensitization (skin prick test or serum IgE assay), exposure and symptoms usually help to identify the factors contributing to the severity of disease [21]. Some specific allergens, in particular cockroach [22] and *Alternaria* [23] have been associated with more severe forms of asthma, and asthma deaths have been associated with high fungal spore counts in the environment [24]. In parallel, sensitization to mold has been associated with increased asthma severity and intensive care admissions in adults [25]. Another relevant aspect of severe asthma with allergic sensitization is the relationship with thunderstorms ("thunderstorm asthma"). An increasing body of evidence confirmed the occurrence of asthma epidemics during thunderstorms in pollen seasons, in various geographical areas [26]. Patients without asthma symptoms, but affected by seasonal rhinitis can also experience severe asthma attacks, and there is a link between thunderstorm asthma and pollen sensitizations [27, 28]. Identifying the possibly related causal allergens is mandatory for an appropriate therapeutic strategy [29, 30], although the evidence supporting secondary prevention of asthma (allergen avoidance) is still weak [31, 32].

To assess the impact of allergenic sensitization on severe asthma, a search of current scientific literature was performed and the results were filtered to identify relevant articles or studies with data from well-designed clinical studies in human patients. A PubMed literature search was performed using the Boolean string severe asthma* [title/abstract] AND allergic sensitization [title/abstract] AND atopy [title/abstract].

Mold sensitization and severe asthma

Allergy is one of the major pathophysiological aspects of asthma requiring hospitalisation, and the concentration of IgE antibodies is relevant in severe allergic asthma, as happens for instance in exacerbations due to rhinovirus infections [33]. In principle, any allergen can cause severe asthma in sensitized patients. Among children admitted to hospital for acute exacerbations, more than 85% are allergic. Of note, allergen-carrying particles with a diameter ≤ 5 microns such as HDM, dog, cat dander and fungal spores (*Aspergillus, Penicillium, Alternaria)* can entry the lower airways, thus more likely to cause asthma. In this context, it is important to distinguish between sensitization to thermotolerant fungi which can colonise the airways (mainly Aspergillus, Penicillium and Candida) so providing a persistent allergenic stimulus in the absence of airborne exposure and non-thermotolerant allergens such as Cladosporium and Alternaria where allergenic effects are directly related to airborne content of the spores. The former are associated with lung damage, whereas the latter are more closely associated with epidemic thunderstorm episodes. Several studies have found that there is a correlation between the severity of allergic diseases and the proteolytic activity of *Alternaria* extracts [34]. Furthermore, a possible key role of sensitization to *Alternaria* species has been observed in thunderstorm related severe asthma [35, 36]. In the specific case *Alternaria*, the prerequisites for severe asthma epidemics associated with thunderstorms are: sensitization, current asthma, sudden large allergen exposure, thunderstorm with cold outflow occurring at a time and location during an allergen season, in which many asthmatics are outdoors, sudden release of large amounts of respirable allergenic fragments, particularly fungal spores such as *Alternaria* [35–38]. Mold sensitivity is usually associated with increased severity of

Table 1 Demography and atopic status data from three of most representative studies on severe asthma

	Enfumosa study	Tenor study	SARP Study
Study design/location	Cross-sectional European multicentre study (9 Countries)	U.S. Multicentre Observational study	Observational cross-sectional multicentre study (9 sites in U.S. and 1 site in U.K.)
Patients (N.) with severe asthma	163	2282 (48% OF TOTAL)	204
Age characteristics	Age range: 17-65 yrs	Mean age ± SD : 38.9 ± 20.92 yrs	Mean age ± SD: 41 ± 13 yrs
Sex	Sex ratio (F/M) : 4.4 / 1	Not reported[c]	Females: 64%
≥1 Positive skin prick test	58% (VS. 78% OF Controlled asthmatics)	-	71% (VS. 85% of mild and 87% of moderate asthma)
Mean total serum IgE	109 KU/L-^{1}, 95% CI: 85-139	106.6 IU/ml[a]	2.0 ± 7.6[b]
References	2	3	4

[a]Overall, the TENOR study patients with severe asthma had elevated geometric mean IgE values compared with patients with moderate and mild asthma. IgE values for children and adolescents increased with asthma severity
[b]Total serum IgE (log)
[c]Overall patients (mild/moderate/severe asthma): females : 2945 (62.2%); males : 1792 (37.8%)

asthma and hospital admission in adults, while in children is related to increased bronchial hyperactivity [39]. Asthma-related deaths are more frequent in young adults during spore peaking [26, 40]. Molds are an important causative agent of severe asthma as thus the term SAFS (Severe Asthma associated with Fugal Sensitization) has been coined to identify those patients with severe asthma, not-responsive to standard therapy [41]. The exact prevalence of mold sensitivity is not well defined, but it is believed to range between 13% and 78% in atopic patients [42, 43]. Several species of fungi are considered responsible for allergic sensitization, but the more relevant belong to the Ascomycetes family: *Alternaria, Aspergillus, Botrytis, Epicoccum, Fusarium* and *Penicillium* species [41]. Sensitization to *Alternaria* and *Cladosporium* species are more frequently associated with persistent and severe asthma [41].

Molds are both outdoor and indoor persistent allergens, although with seasonal variations, that depend on climatic factors (rain, humidity, temperature, air pollutants and wind). Indoor spores can derive from molds present outdoor, or from inside damp and moist walls in buildings. Rain is usually necessary for spores discharge in air, not also from outdoor, but even from house surfaces, especially during thunderstorms [44]. Each species of fungi presents several allergens, that can be structural components of the cell and/or derived from metabolic products [45]. Currently 30 allergens from *Aspergillus sp*, 16 from *Penicillium sp*, 10 from *Cladosporium sp* and 9 from *Alternaria* sp have been identified. Although there is some documented cross-reactivity among allergens [46, 47], also species-specific allergens, such as Asp 1 and Alt 1, have been characterized [46, 47]. Exposure to fungal allergens can cause severe asthma trough 3 different mechanisms: a) inhalation of spores or hyphae, that act as allergens in patients sensitized; b) fungal colonization of airway, i.e. allergic bronchopulmonary aspergillosis (ABPA), associated to sensitization to fungal antigens; c) fungal colonization of districts different from respiratory tract (generally skin) in patients sensitized to involved fungi [33]. This wide spectrum of clinical entities in patients with sensitization to fungi is due to their potent antigenicity and immunomodulatory activities [48]. Immunopathogenesis is mediated by β-glucans and theirs receptor, dectin-1. In fact, after the recognition of β-glucans, dectin 1 on macrophages surface triggers the release and the production of several inflammatory mediators [49]. Recent finding suggest that the immune response to fungi directs T cell towards Th17 cells, through dendritic cells action. In the same studies the Authors demonstrated that induction of Th17 cells strictly correlates with severe allergic asthma, with possible steroid resistance [46]. Fungal proteases act as allergens, and also as mediators of tissue injury, inducing production of IL-6 and IL-8, proinflammatory citokines involved in exacerbations of asthma. Moreover, it has been demonstrated that the receptor activated by protease (PAR) type 2 is overexpressed in bronchial tissue from asthmatic patients, suggesting a potential increased vulnerability to fungi in these subjects [42]. In case of colonization of the airways a Th-2 hypersensitivity mechanism is involved. Disease is characterized by increased severity of asthma, increased IgE serum level, transient infiltrates in lungs, presence of IgG and IgE against molds [33, 42]. The most common fungi implicated in pulmonary colonization are from *Aspergillum* species, less frequently *Candida*, *Curvularia* and *Penicillium* species can be involved [50]. Asthma therapy in this case is associated with antifungal systemic treatment [42]. The third mechanism was firstly proposed in 1930 by Wise et al., who observed that patients with skin and/or nails fungal infection presented an increased asthma severity [51], and a significant correlation with sinusitis, rhinitis and urticaria associated to fungal colonization of skin. Supporting evidences to this hypothesis are derived from several trials in which patients affected by severe asthma and fungal skin infection had a relevant improvement in asthma control after itraconozole and fluconazole [52, 53]. Sensitization to Asp f 1 and/or Asp f 3 may be more indicative of allergic asthma. [54]. It was also observed that pediatric severe asthma with fungal sensitization was associated with more oral steroid therapy and higher IL-33 levels [55].

Pollen sensitization and severe asthma

The relationship occurring between pollen sensitisation and asthma severity has been widely studied, and an abundant literature on this aspect is currently available [13, 33]. The IgE antibody profile for a broad spectrum of allergen molecules, exhaled nitric oxide (FEno) and bronchial responsiveness were assessed in asthma patients. Asthmatic patients showed more frequently sensitization to grass, tree, weed pollens, furry animals, mold, latex and foods of plant origin. Asthma prevalence was increased in patients with food-pollen-perennial sensitisation. In this group of patients also FEno and bronchial responsiveness were increased with respect to groups of patients with sensitization only to pollen , or food or perennial [56]. Some evidence suggested that air pollution may interact with airborne allergens enhancing the risk of atopic sensitization and exacerbation of symptoms in sensitized subjects. These phenomena are supported by current in vitro and animal studies showing that the combined exposure to air pollutants and allergens may have a synergistic or additive effect on asthma and allergies [57]. Ragweed pollen sensitization induces asthma much more frequently than other

pollens [58]. Concerning *Olea* sensitization, the association between the presence of asthma and sensitization to Ole e 7 resulted statistically significant [59]. To date, among pollens, only grass, *Parietaria (*Wall Pellitory) and *Olea* pollen have been suggested as possible triggers in thunderstorm-related asthma [60]. A thunderstorm-related asthma episode was observed in Naples (Italy) on June 2004, when six adults and one child received emergency treatment. All patients showed allergic respiratory symptoms upon exposure to *Parietaria* pollen but they were not sensitized to grasses [61]. Losappio et al. observed 20 patients with allergic sensitization to Olea pollen brought to an emergency department in Barletta, Italy, for sudden and severe asthmatic symptoms in May 2010 following a violent thunderstorm [36]. On the basis of these observations, all subjects affected by pollen allergy should be alerted to the danger of being outdoors during a thunderstorm in the pollen season, as such events may be an important cause of severe asthma exacerbations.

Animal dander sensitization and severe asthma

Pet allergy is considered one the most important causes of severe or uncontrolled asthma. A specific IgE response to more than three animal-derived components was observed to be more common among uncontrolled severe asthmatics children compared to those with controlled asthma [62]. Component Resolved Diagnosis (CRD) allows the identification of specific allergens associated with the severity of asthma and the identification of cross reactive molecules that are clinically significant as well as Can f 6 or Fel d 4. They are lipocalin allergens and could explain the role in cross reactivity of dog with cat and horse. The physician can advise on whether or not to keep a household pet or which species could be tolerated. This may have a huge impact on the child's well-being [63]. CRD allows to evaluate the pattern of sensitization to pet IgE components and its association with clinical symptoms and their severity. Specific IgE to Can f 2 was significantly associated with asthma diagnosis, Can f 3 with moderate/severe rhinitis and asthma diagnosis, Can f 5 with persistent and moderate-severe rhinitis, and Equ c 3 with persistent rhinitis and severe asthma [64]. Furthermore, the sensitization to Can f 2 and Equ c 1 was more common in severe asthma than in children with controlled asthma [65]. Asthmatic children with cat allergy have higher Fel d 1-specific IgE levels as compared to those with rhinitis alone, and this suggests that high IgE levels to Fel d 1 could be a marker of increased asthma risk [66]. Fel d 2 is a serum albumin, abundant in saliva and dander. It is a highly cross-reactive molecule, associated with cat – pork syndrome but only a small number (10-20%) of patients sensitised to Fel d 2 report immediate reactions to beef, whereas sensitization to Fel d 2 is associated with more severe respiratory symptoms [67].

Cockroach allergens sensitization and severe asthma

Cockroach allergy is recognized as an important cause of asthma and cockroach-induced asthma was described as a more severe disease, associated with perennial symptoms and high levels of total IgE. Cockroaches produce several allergens that induce sensitization, and exposure to high levels of cockroach allergens at home is a major risk factor for symptoms in sensitized individuals [68]. Recent data suggest that cockroach allergen may be a most relevant urban allergen exposure and may be related to asthma severity [69]. Indoor allergen exposure in inner-city areas has been of particular interest given that patients living in urban areas have increased asthma severity, decreased asthma control, and greater health care use [70]. Rosenstreich et al. found that cockroach allergens are easily detectable in inner-city homes [22]. Furthermore, this study demonstrated that asthmatic children sensitized and exposed to high levels of cockroach allergens had an increased asthma morbidity. Ahluwalia et al. observed that in an inner-city community with high exposure to cockroach allergens, there was a more strongly and consistent association with poor asthma outcomes [71]. Gelber et al. and Call et al. showed that cockroach sensitization was more common in asthmatics compared to non-asthmatics referred to an emergency room. When sensitization was associated with direct exposure, a very high association with asthma occurred. [72, 73]. In a study carried out in Thailand, the possible changes in disease severity and allergen sensitization of children with asthma in an interim period of 5 years was evaluated [74]. During the years 2004-2009, asthma severity increased with increasing sensitization to mite and mite plus cockroach. In another study conducted in a population of elderly urban patients with asthma, the presence of cockroach-specific serum IgE was associated with more severe asthma, as reflected by an increase in airway obstruction and hyperinflation [75]. In a Polish study it was observed that the concentration of Bla g 2 in houses was higher than previously reported in other European countries, and children with cockroach hypersensitivity had more often severe asthma than children with other allergies [76]. Previously identified allergens from Blatella germanica and Periplaneta americana (the most important domestic species), include Bla g 2 (inactive aspartic protease), Bla g 4 (calycin), Bla g 5 (glutathione-S-transferase), Bla g 6 (troponin), the Group 1 cross-reactive allergens Bla g 1 and Per a 1, Per a 3 (arylphorin), and Per a 7 (tropomyosin). On the basis of

this finding, we suggested that community-based asthma intervention strategies should prioritize reducing cockroach allergen exposure.

Sensitization to food allergens and severe asthma

Workers handling food products and derivatives are at increased risk of developing occupational asthma. Exposure to food allergens occurs primarily through inhalation of dust, steam, vapors and aerosolized proteins generated during the processing of foods. Most of the inhaled food allergies are IgE mediated, but objective evidence of asthma by monitoring peak expiratory flows during and off work or specific inhalation challenges usually provide a reliable diagnostic value [77]. Wheat may induce the well known baker's asthma: albumins and globulins (LTP, Tri a 14, alfa amylase inhibitor) are more involved than gluten (low – molecular – weight - glutenins, alfa, gamma omega 5 gliadin) [78]. Heat proteolytic resistant food allergens such as seafood (fish and shellfish), plants (LTP), spices, milk (casein), eggs (lysozyme, ovomucoid) and mushrooms can cause severe asthma attacks through inhalation [78, 79].

Conclusions

Severe asthma remains a major health concern, due to its relevant social costs. Despite these critical aspects, there are still many unmeet needs with regard in particular the interpretation and comparison of the various studies carried out; for example, the different definitions of "severe asthma" adopted in the most important studies published so far (ENFUMOSA , SARP, TENOR, etc.) [3–8]. Different clinical and biological phenotypes of asthma are currently identified. Allergic asthma, with the atopic sensitization and the TH2 driven inflammation is the most frequent and the better known form of asthma [80]. Among the allergens that may cause asthma, some results to be more frequently associated to severe forms: molds, cockroach, pet dander, inhaled food-derived allergens. Also, "thunderstorm asthma" is frequently associated with severe symptoms due to a massive and sudden exposure to inhalant allergens. Thus, a detailed knowledge about the relevant allergens and causative factors is essential to properly diagnose, prevent, and treat the most severe forms of asthma.

There is evidence from some randomized controlled studies that ABPA treatment with systemic antifungal therapy can offer a therapeutic benefit to about 60% of patients [81].

Allergen immunotherapy (AIT) represents a valuable therapeutic option. A study of sublingual immunotherapy to *Dermatophagoides* in patients with allergic rhinitis with or without mild intermittent asthma showed an improved bronchial threshold to allergen challenge [29]. Another recent double-blind, randomized, placebo-controlled trial, conducted in 109 European trial sites and including 834 adults with HDM allergy–related asthma not well controlled by ICS or combination products, and with HDM allergy–related rhinitis, demonstrated that the addition of HDM SLIT tablets increased the time to first exacerbation during ICS reduction, with an estimated absolute reduction at 6 months of 9 to 10 percentage points; the reduction was primarily due to an effect on moderate exacerbations [30]. Further studies of these approaches are required in severe asthma. An alternative and clinically proven approach for atopic severe asthma is the use of the monoclonal antibody to IgE (omalizumab), which reduces circulating IgE and leads to down-regulation of its high affinity receptor FcεR1 on mast cells and basophils [31]. Different promising therapeutic options are also currently in development and undergoing clinical trials for the treatment of severe asthma, including anti-interleukin agents (mepolizumab, benralizumab, reslizumab, dupilumab, brodalumab, lebrikizumab) [32].

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