

ORIGINAL ARTICLE

Eosinophilic airway inflammation is increased in children with asthma and food allergies

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Abstract

Background: Asthma is associated with food allergies in a significant number of children, with evidence linking allergies to asthma severity and morbidity. In this study, we tested our hypothesis that the eosinophilic lower airway inflammation is higher in asthmatic children with food allergies.

Aims: The aims of the study were to compare the eosinophilic inflammatory markers in asthmatic children with and without food allergies.

Materials and Methods: Children with asthma, with ($n = 22$) and ($n = 53$) without food allergies were included. All subjects were classified according to the GINA guidelines (2009) and had received at least 3 months of anti-inflammatory therapy prior to testing. Fractional exhaled nitric oxide and sputum differential counts were performed using standard techniques.

Results: Children with asthma and food allergies had significantly higher fractional exhaled nitric oxide median (range) [(22.4 (6.1–86.9) vs. 10.3 (2.7–38.7) ($p = 0.01$)] and sputum eosinophil percentage [15.5 (5.0–53.0) vs. 2.0 (0–20) ($p < 0.001$)] compared with asthmatic children without allergies.

Conclusion: These results suggest that the children with asthma and food allergies have increased eosinophilic inflammation of the airways.

Asthma is associated with food allergies in a significant number of children (1, 2) and is a risk factor for fatal anaphylactic reactions (3, 4). A recent National Health and Nutrition Examination Survey in United States indicates higher prevalence of food sensitization in those with current asthma and emergency department visits for asthma in previous year (5). The presence of food allergies especially to multiple allergens increases the risk of hospitalizations and corticosteroid use in asthma (6, 7). Even asymptomatic children with food allergies have been shown to have higher bronchial hyperreactivity (8).

Eosinophilic inflammation, which is monitored by measuring induced sputum differential count and exhaled nitric oxide, is a hallmark of asthma. In children with asthma, eosinophilic airway inflammation is associated with worsening symptoms (9). In addition, it has been shown that successful weaning of corticosteroids can be achieved in children with normal sputum eosinophil counts (10). More recently, treatment strategies aimed at normalizing the sputum eosino-

phil count in asthma in adults have been shown to reduce exacerbations without an increase in overall corticosteroid therapy (11). Although the link between asthma severity and food allergies is becoming more evident (1), it is not known whether eosinophilic airway inflammation is also increased in these subjects. We therefore hypothesized that the eosinophilic airway inflammation is higher in asthmatic children with food allergies. To test our hypothesis, we compared induced sputum eosinophil differential count and exhaled nitric oxide in asthmatic children with and without food allergies.

Methods

Subjects

Data were collected retrospectively from asthmatic children with ($n = 22$) and without ($n = 53$) food allergies, attending Istituto Pio XII, Misurina, Belluno (High Altitude Paediatric

Asthma Centre) between May 2009 and April 2010. These children had performed spirometry, FeNO measurements satisfactorily and produced adequate sputum sample for cell counts. Children with chronic respiratory conditions like bronchopulmonary dysplasia, bronchiectasis, and congenital cardiac defects were excluded. Clinical symptoms and reversibility testing ($>12\%$ increase in FEV₁ after short acting bronchodilator treatment) were used to diagnose asthma. All children were on inhaled corticosteroid therapy. Spirometry was performed according to the American Thoracic Society Guidelines (12). The institute in Misurina is located at 1756 m above sea level; therefore, children were evaluated within 2 days of admission to reduce any confounding effects of the reduction in allergens seen at high altitude (13). The doses of inhaled steroids, number of exacerbations, exacerbations requiring oral corticosteroids in previous 3 months were recorded. Children's demographic data along with exposure to tobacco smoke were recorded. Children were grouped into low-, medium-, and high-dose range of inhaled corticosteroid doses as described in the GINA 2009 guidelines. Ten children with allergic rhinitis and food allergies but without a history of wheezing or asthma were evaluated. Diagnosis was based on a history of a typical hypersensitivity reaction to definite ingestion of the allergen. Subjects had urticaria, angioedema, and/or gastrointestinal symptoms (abdominal pain and/or vomiting) during their worst ever reaction. Their lung function tested by spirometry was in the normal range, and change in FEV₁ after the bronchodilator inhalation (400 μ g salbutamol inhaled using the non-electrostatic spacer, Vortex; Pari GmbH, Stamborg, Germany) was also in the normal range ($<8\%$). The ethical approval was obtained for the analysis by local ethical committee for clinical research.

Food and aeroallergen sensitization

Allergy to food was diagnosed by history, skin prick test (performed in all children), and specific IgE (ImmunoCAP ISAC, Phadia Multiplexing Diagnostics GmbH, Vienna, Austria). The reactions to food substances included anaphylactic shock ($n = 2$), angioedema ($n = 8$), urticaria ($n = 5$), bronchospasm worsening of dermatitis ($n = 7$), and oral symptoms ($n = 4$). Skin prick tests (SPT) were performed for common aeroallergens and food substances (Lofarma SpA, Milan, Italy) as suggested by history, according to international guidelines and manufacturer's instructions. The aeroallergens tested include house dust mite (*Dermatophagoides farinae* and *pteronyssinus*), fungi (*Alternaria*, *Aspergillus*, and *Cladosporium*), grass pollen (*Gramineae*), trees (*Cupressus arizonica*, *Corylaceae*, *Betula*, *Fagaceae*, and *Oleaceae*), weed (*Parietaria*, *Ambrosia*, and *Compositae*), and animal dander (dog, cat). For all children with food allergy, in addition to the allergens that induced a typical hypersensitivity reaction, a standard panel of allergen was tested that included cow's milk, egg, soya, wheat, peanut, hazelnut, apple, kiwi, peach, pear, strawberry, and anisakis. Positive (histamine hydrochloride 10 mg/ml) and negative (saline solution) controls were also included in each test. A positive result was considered as

the appearance of a wheal with a diameter >3 mm over that produced by the saline control at 15 min.

Food allergen microarray analysis

In the food allergy group, the positivity for specific serum IgE for food allergens was analyzed using ISAC technology (Phadia Multiplexing Diagnostics GmbH, Vienna, Austria). Microarray immunoassays were performed according to the manufacturer's recommendations as described by Harwanegg and Hiller (14). This technology allows the detection of specific IgE to microarrayed single allergenic molecules, either natural or recombinant, spotted in triplicate on a solid surface. A microarray carrying 103 different allergens was used (ImmunoCAP ISAC Assay Kit). Briefly, 20 μ l of serum was incubated on the reaction site of the microarray in a humid chamber for 2 h. Slides were washed, and fluorescein-labeled anti-IgE antibody was added and incubated for 1 h. Fluorescence was detected by a laser scanner (Capitalbio Corporation, Beijing, China). Images were processed and raw fluorescence values were analyzed by the ISAC software (Phadia Multiplexing Diagnostics). Values are expressed as kU_A/l. Values above 0.1 kU_A/l were counted positive.

Sputum induction and processing

Sputum was induced and processed as described previously (15). Air-dried cytopins were stained with Diff-Quik stain (Medion Diagnostics GmbH, Duding, Switzerland) (Romanowsky stain) as described by the manufacturer. In brief, the air-dried slides were dipped five times in fixative, eosin (red), and thiazine dye (in that order) and then rinsed with distilled water. The eosinophil differential count was obtained by counting 400 non-squamous cells (macrophages, eosinophils, neutrophils, and lymphocytes) and expressed as percentage.

Exhaled nitric oxide measurement

FeNO was measured by a standard technique complying with the ATS/ERS recommendations (16) using a chemiluminescence analyzer (Logan LR 2149; Rochester, Kent, UK) and expressed as parts per billion (ppb).

Statistics

Statistical analysis was performed using GRAPHPAD PRISM 5 (GraphPad, San Diego, CA, USA) and SPSS (PASW 18; SPSS Inc., Chicago, IL, USA). Subject characteristics are presented as mean (standard deviation), median (range), and number (percentages). The non-parametric data were compared using Kruskal-Wallis test, Mann-Whitney test, and proportions using Fisher's exact test. Differences were considered significant when $p < 0.05$. Multiple regression analysis (stepwise model) was performed to identify which of the variables [food allergy, exposure to tobacco smoke, aeroallergen sensitization, forced vital capacity (FVC), FEV₁, inhaled corticosteroid dose, and course of oral corticosteroid dose] were significant determinants of sputum eosinophilia.

Results

Subject characteristics

The clinical characteristics of subjects are as shown in Table 1. There was no difference in age, gender, FEV₁ percent predicted, and Forced Expiratory Flow (FEF)_{25–75} percent predicted between the two groups. The mean (SD) age of children with rhinitis and food allergy was 10.8 (1.97) and 6/10 were boys; this was not significantly different to other two groups. The FVC percent predicted was higher in children without food allergies. A greater number of children were exposed to environmental tobacco smoke in the group without food allergies ($n = 29$, $p = 0.04$). Children had a maximum of one exacerbation or had one course of oral corticosteroids in previous 3 months. There was no statistically significant difference in inhaled corticosteroid dose, Long Acting Beta Agonist (LABA) use, exacerbations, and oral corticosteroid use between the two groups.

Food and aeroallergen sensitization

The common food allergens and number of children with allergies are summarized in Table 2. All subjects with food allergies were also sensitized to one or more aeroallergens, with Dermatophagoides (90.9%) being highest followed by pollens (81.8%), dog or cat (68.2%), and fungi (50%). Two of 22 children with food allergies were positive to Dermato-

phagoides alone. In children with asthma and without food allergies, Dermatophagoides sensitization was also higher (86.8%), followed by pollens (77.3%), dog or cat epithelium (70%), and fungi (28.3%). There was no statistically significant difference in sensitization to aeroallergens in children with or without food allergies. Children with food allergies and rhinitis were allergic to one or more aeroallergens: Dermatophagoides (70%) being highest followed by pollens (80%), dog or cat (50%), and fungi (20%).

Markers of eosinophilic inflammation

Children with asthma and food allergies had significantly higher FeNO median (range) [(22.4 (6.1–86.9) vs. 10.3 (2.7–38.7) ($p = 0.01$)] (Fig. 1B) as compared to children without food allergies. The sputum eosinophil differential count in both groups is shown in Table 3. The sputum eosinophil count was higher in children with food allergies and asthma ($p < 0.0001$) (Fig. 1A). There was no significant difference in sputum eosinophilia for children with or without milk, egg, and nut allergy within the food allergy group. A small number of children ($n = 4$) within the food allergy group were evaluated in between the months of March and June; there was no statistically significant difference in FeNO ($p = 0.44$) or sputum eosinophil differential count ($p = 0.3$) to those evaluated during winter and autumn months. The multiple linear regression analysis demonstrated that allergy to food

Table 1 Demographic characteristics, lung function, treatment, and aeroallergen sensitization in asthmatic children with and without food allergies

	With food allergies $n = 22$	Without food allergies $n = 53$	p-Value
Age (yr) mean (SD)	11.8 (2.9)	11.4 (3.3)	0.55
Gender (boys) – n (%)	15 (68.2)	32 (60.4)	0.6
Ethnic origin (Caucasians) – n (%)	22 (100)	49 (92.4)	0.3
Weight kgs – mean (SD)	40.6 (13.2)	48.4 (21.3)	0.11
Height cms – mean (SD)	149.6 (18.1)	147.4 (18.4)	0.63
Family history of allergies positive – n (%)	21 (95.5)	48 (90.5)	0.66
Exposure to smoke – n (%)	6 (27.2)	29 (54.7)	0.04
FVC % predicted – mean (SD)	95.7 (9.7)	101.0 (8.9)	0.02
FEV ₁ % predicted – mean (SD)	101.1 (9.3)	104.2 (10.4)	0.33
FEF _{25–75} % predicted – mean (SD)	98.9 (27.1)	96.79 (21.9)	0.93
Equipotent dose* of inhaled corticosteroids – n (%)			
Low	20 (90.9)	45 (85)	0.71
Medium	2 (9.1)	7 (13.2)	
High	0	1 (1.8)	
LABA use – n (%)	7 (31.8)	14 (26.4)	0.77
One course of oral steroids last 3 months n (%)	1 (4.5)	6 (11.3)	0.66
One exacerbation in last 3 months n (%)	6 (27.2)	27 (50.9)	0.07
Aeroallergen sensitization – n (%)			
Dermatophagoides	20 (90.9)	46 (86.8)	1.0
Pollens (grasses and trees)	18 (81.8)	41 (77.3)	0.76
Animals (cat, dog)	15 (68.2)	37 (70)	0.12
Fungi (Alternaria, candida)	11 (50)	15 (28.3)	0.10

FVC, forced vital capacity, FEV, forced expiratory volume.

*GINA classification of low, medium, and high equipotent dose of inhaled corticosteroids.

Table 2 The common food allergens in children with food allergies and asthma confirmed by skin prick test (SPT) and ISAC (n = 22).

Allergen	SPT + ve n (%)	ISAC-specific IgE reactivity
Milk	10 (45.45)	Alpha-lactalbumin [nBos d 4] (n = 4), Casein [nBos d 8] (n = 5), Beta-lactoglobulin [nBos d 5] (n = 3) Trasferrin [nBos d lactoferrin] (n = 1), Bovine serum albumin [nBos d 6] (n = 2)
Egg	7 (31.82)	Ovomucoid [Gal d 1] (n = 5), Ovalbumin [Gal d 2] (n = 3), Lysozyme [LysoGal d 4] (n = 1)
Nuts	18 (81.82)	Peanut [nAra h 1] (n = 8) and [rAra h 2] (n = 10), Hazelnut lipid transfer protein [rCor a 8] (n = 7), Brazil nut [rBer e 1] (n = 2)
Fruit	6 (27.27)	Peach lipid transfer protein [Pru p 3] (n = 5), Kiwi [Act d 1] (n = 3) and [Act d 2] (n = 1), Apple [rMal d 1] (n = 3)
Others*	10 (45.45)	Parvalbumin (r Gad c 1) [n = 5], Anisakis (rAni s 3) [n = 3], Tropomyosin [rPen a 1] and [r Pen i 1] (n = 6)

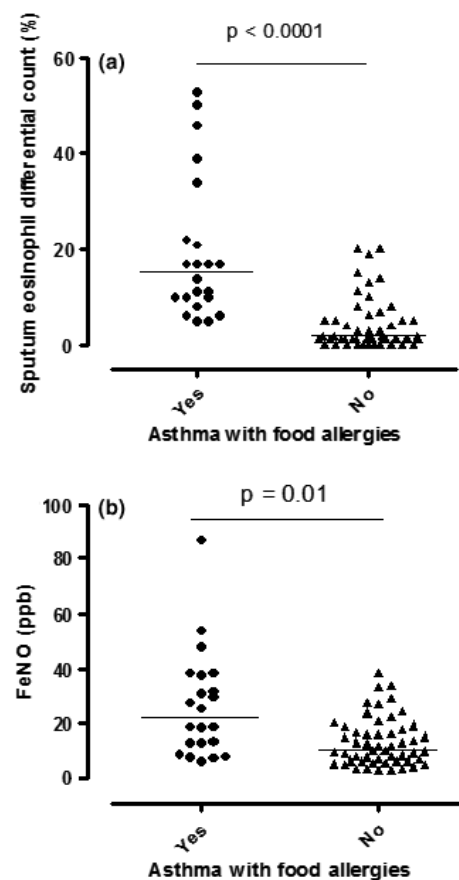
*Others, cod, anisakis, tuna, shrimp, mussel, and chestnut; Nuts, walnut, hazelnut, pine nuts, peanuts, and almond; Fruits, kiwi, banana, apple, and peach.

substances was the only factor that was significantly associated with sputum eosinophilia ($\beta = 0.6$, $p < 0.0001$). Children with food allergies and allergic rhinitis had lower eosinophil count compared with those with asthma with ($p < 0.0001$) and without food allergies ($p = 0.02$) (Table 3).

Discussion

This study compares the eosinophilic inflammation in asthmatic children with and without food allergies and children without asthma who had food allergy and rhinitis using well-established and non-invasive methods of measuring eosinophilic inflammation in the lower airways. The most important finding was that children with asthma and food allergies had a higher eosinophil differential count and FeNO than those without food allergies.

It is of concern that food allergy appears to be a risk factor for life-threatening asthma (17, 18) with children needing intensive care treatment 3.3 times more likely to report food allergy compared with those admitted to standard medical wards (19). Similarly, children with coexisting asthma are at higher risk of severe allergic reactions (20). Even in the less severe asthmatic children, food allergy, especially to milk and peanuts, was associated with increased hospitalizations and corticosteroid use. Although there is growing epidemiological evidence, little is known about the airway inflammatory sta-

**Figure 1** Scatter plots showing the comparison (a) sputum eosinophil differential count (b) FeNO in asthmatic children with and without food allergies. The bars represent medians.

tus in children with asthma and coexistent food sensitization. Kivity et al. (21) did not find higher eosinophil differential in eleven asthmatic young adults with food allergy compared with ten asthmatics without food allergy. A small number of patients included could be one of the possible reasons for no difference in eosinophil counts between these groups. However, similar to their study, we found children with allergic rhinitis had lower/normal sputum eosinophil differential count as compared to those with food allergy and asthma.

Inhalation of food allergens, a systemic reaction to food allergens, or coexistent aeroallergen sensitization could be possible explanations for airway inflammation in children with food allergies. The challenge studies have demonstrated that children inhaling food allergens developed clinical features of asthma (22). Krogulska et al. (23) have found higher levels of plasma interleukin-5 (IL-5) 24 h after oral food challenge. IL-5 increases differentiation and survival of eosinophils. Eotaxin (CC chemokines) increases recruitment of eosinophils, and Matsuura et al. (24) found plasma levels of eotaxin-1 (CCL11) higher in children with food allergy as compared to controls. The systemic effect of food allergens leading to blood eosinophilia may contribute to airway eosin-

Table 3 Induced sputum cell count in three groups

Induced sputum % median (range)	Allergic rhinitis and food allergy n = 10	Asthma with food allergies n = 22	Asthma without food allergies n = 53	p-Value
Macrophages	71.5 (53–87)	51.5 (8.0–80.0)	59.0 (5.0–83)	0.01
Neutrophils	26.5 (12–46)	28.0 (4.0–66.0)	36.0 (8.0–93.0)	0.07
Eosinophils	0.5 (0–3)	15.5 (5.0–53.0)	2.0 (0–20)	<0.0001
Lymphocytes	0.5 (0–1)	1.0 (0–2.0)	1.0 (0–8.0)	0.08

ophilia. Children with food allergies may be cosensitized to aeroallergens as shown in our study. However, multiple regression analysis suggested food allergy to be the only significant determinant (independent of other factors) of sputum eosinophilia. In addition, majority of children were sampled during non-pollen season with no difference among the small number of children sampled during spring excluding the seasonal variation.

As noted in other studies, milk, egg, and nuts were the common allergens in our group. We did not find an obvious difference in airway eosinophil differential count in children with or without milk, egg, or nut allergy. However, our subgroup numbers were too low to formally compare these groups.

There is growing evidence both from adult and from pediatric studies where the control of eosinophilic inflammation in airways is beneficial in reducing the dose of corticosteroids and exacerbations (10, 11, 25). Our results suggest that children with food allergies have higher eosinophilic inflammation of the airways, and therefore, evaluation of treatment regimes based on eosinophilic inflammatory markers in an attempt to reduce morbidity will be of interest. Bird and colleagues have suggested that aggressive asthma control is indeed required in this group. In our study, there was no statistical difference in severity or number of exacerbations between the two groups in the short duration, suggesting similar clinical control in last 3 months. But in spite of this apparent control, the baseline eosinophilic inflammation was higher.

Fractional exhaled nitric oxide is used as a surrogate marker of eosinophilic inflammation in children (26). It is a non-invasive marker, can be performed in children, but is not

without limitations. Although FeNO is not very specific for asthma, it has been shown to be responsive to corticosteroid therapy (27) and possibly helpful in evaluating subjects with discordant symptoms (28). Hughes et al. (29) found that children with peanut allergy who had outgrown asthma and children with wheeze but no treatment had higher levels of FeNO suggesting ongoing eosinophilic inflammation. Atopic status is a known confounder effecting FeNO levels, and high FeNO levels have been demonstrated previously in atopic children (30).

Our study has some limitations, particularly its short duration. However, both the groups were well matched for demographic and clinical severity criteria. Second, double-blind oral food challenge was not undertaken for diagnosis; however, all cases were confirmed by positive history of a typical hypersensitivity reaction to definite ingestion of allergen, skin prick test, and in some cases by specific IgE. Third, we had not performed skin prick tests or specific IgE testing for food allergens in children without history suggestive of food allergies, to determine whether they were sensitized.

A further longitudinal study in children with food allergies with long-term follow-up is required to confirm the usefulness of monitoring eosinophilic inflammation in helping to guide management.

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