Bronchial Hyperactivity, Sputum Analysis and Skin Prick Test to Inhalant Allergens in Patients with Symptomatic Food Hypersensitivity

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Abstract

Background: Dyspnea may be a presenting symptom of type I food hypersensitivity; bronchial hyperactivity, without known asthma, can coexist in patients with food allergy.

Objective: To evaluate airway involvement in young adult patients with food allergy and no asthma and compare the findings to those of patients with food allergy and asthma, with food allergy and allergic rhinitis, with asthma and no food allergy, and of apparently healthy controls.

Methods: The evaluation involved prick skin test to food (65 allergens) and inhalants (24 allergens), spirometry, methacholine inhalation challenge, and induced sputum for cell analysis. The five groups consisted of 18 patients with food allergy alone, 11 with food allergy and asthma, 13 with food allergy and allergic rhinitis, 10 with asthma alone, and 10 controls.

Results: Patients with food allergy alone were mainly (86%) skin sensitive to pollens. Those with either asthma or allergic rhinitis were mainly (95%) sensitive to mites. BHR was detected in 40% of the patients with food allergy alone, 55% of the patients with allergic rhinitis, and 100% of the patients with asthma. Cell counts in the sputum of patients with asthma and in those with food allergy and asthma showed higher eosinophil counts compared to those with food allergy and allergic rhinitis. Patients with food allergy and no asthma, regardless of BHR status, had mainly neutrophils in the sputum.

Conclusions: Patients with food allergy are highly likely to have concomitant asymptomatic BHR. Mite sensitivity in patients with food allergy predicts respiratory allergy (either asthma or allergic rhinitis). High eosinophil levels in the sputum of food allergy patients predict respiratory involvement.

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Food hypersensitivity, defined as immunologically mediated adverse reaction to food, is becoming more common in western society [1,2]. The sites of reaction to the ingested food are the skin and the gastrointestinal, respiratory or (rarely) cardiovascular systems in most patients with type I food hypersensitivity. Bronchial involvement can occasionally occur during symptomatic food hypersensitivity reaction, causing dyspnea and oxygen desaturation [3]. In a study by James et al. [4], a food challenge in food-allergic asthmatic subjects temporarily increased bronchial hyperactivity and even caused an asthmatic attack. It was recently reported by Wallaert and co-workers [5] that patients with food allergy without known asthma might have asymptomatic airway inflammation. Moreover, asymptomatic BHR can occur in non-asthmatic subjects, such as those with allergic rhinitis [6], in patients with inflammatory bowel disease (e.g., Crohn's or ulcerative colitis) [7], as well as in healthy subjects [8]. It is not well understood why patients with food allergy and no history of asthma develop bronchoconstriction during an allergic reaction to food. It is also unclear what differentiates those who have food allergy but neither asthma nor allergic rhinitis, from patients with food allergy and concomitant respiratory involvement.

We hypothesized that findings in sputum analyses or perhaps the specific types of aeroallergens might hold the answers to these questions. We designed this comparative study to evaluate airway involvement of young adult patients with food allergy alone and patients with food allergy and known asthma or allergic rhinitis by performing both sputum induction and methacholine inhalation challenge. We also compared the results of aeroallergen skin tests in the various patient groups and in allegedly healthy controls.

Patients and Methods

Patients

The study group comprised 42 non-smoking patients with food allergy: 18 had food allergy alone, 11 had food allergy and asthma with or without allergic rhinitis, and 13 had food allergy and allergic rhinitis. Ten patients with asthma and no food allergy and 10 healthy subjects (controls) were also included. They all signed informed consent to participate and the study was approved by the institutional ethics committee.

The diagnosis of food allergy was based on positive prick skin test results or positive serum-specific immunoglobulin E test to food allergens, a well-documented history of reaction to certain foods and, in certain cases, immediate symptoms following open challenge or double-blind placebo-controlled challenge with suspected food [9]. The food to which the patients were sensitive were fruits (n=7), peanuts (n=4), tree nuts (n=4), wheat (n=2), sesame (n=2), sunflower seeds (n=2), soy (n=2), eggs (n=1) and milk (n=1). Dyspnea during the allergic reaction was reported by seven of the patients with food allergy alone and nine of those with food allergy and asthma. It was not clear whether the dyspnea was due to anxiety or to respiratory involvement.

The diagnosis of allergic rhinitis was based on a history of nasal discharge accompanied by sneezing and itching of at least 4

BHR = bronchial hyperactivity

weeks duration each year, as well as positive skin test results to at least one aeroallergen [10]. The diagnosis of asthma was based on the American Thoracic Society criteria [11], and included a reversible airway flow limitation with at least a 20% increase in forced expiratory volume in one second following inhaled salbutamol.

The study exclusion criteria were a baseline FEV1 of <70%, acute or chronic disease, respiratory or gastrointestinal infection during the 12 weeks preceding study entry, and no use of daily medication other than salbutamol when needed.

Skin prick testing

The skin prick test to food and inhalants was carried out in the standard manner [12]. The food part included 65 common allergens (fruits, vegetable, dairy products, meat, fish, spices), and the aeroallergen part included 24 allergens common to the local coastal area in Israel (dust, mites, trees, grasses, weeds, molds, epidermal). A positive control (1 mg/ml histamine dihydrochloride and saline) was also used. The wheal diameter was measured 20 minutes after pricking the skin, and a response of 5 mm larger than the negative control was considered positive.

Food challenge

Diagnosis for the 42 patients with food allergy was initially based on a well-documented clinical history of food allergy. We elected not to perform challenge in 32 of these patients because the reaction was expected to be severe. Four patients refused to perform the challenge. The remaining six patients underwent one open challenge with the suspected food. The challenge was performed mainly with apple compote as the vehicle and it took place in the morning with the nurse and physician observing the patient in a special unit. Each food was challenged separately and at one dose per day. The dose was increased according to the history of the patient and the severity of the symptom. Pulmonary function was recorded at baseline and at the time of reaction.

Pulmonary function

Spirometry was performed with a Fukuda Spiroanalyzer (CSA 800, Japan) and begun at 8 a.m.. The forced vital capacity maneuver was performed at least three times with the subject in a sitting position, until a deviation of the sum of FEV1 and FVC was less than 5% in three curves. Only the best curve was used for further analysis.

Methacholine challenge

The method of Chai was used to evaluate BHR to methacholine [13]. MC (Provocholine) was diluted in free calcium and magnesium phosphate-buffered saline and delivered by a dosimeter (Morgan, UK). Patients inhaled five increasing concentrations of aerosol (0.07–25 mg/ml) from functional reserve capacity to total lung capacity, and the FEV1 was recorded for 3 minutes after each concentration. The provocation was stopped when a drop in FEV1 of at least 20% was reached. The PC20FEV1MC was calculated by linear interpolation between the last points on the dose response curve. In our laboratory, an MC challenge with a PC20 of $\leq$ 25 mg/ml is considered a positive test, correlating with clinical asthma.

Sputum induction

Subjects inhaled nebulized 3.5% saline for up to 20 minutes by an ultrasonic nebulizer [14] (RR project Astrana, SPA, Italy). The mouths were lavaged with normal saline, after which the patients were encouraged to cough. Sputum was collected in a sterile container and examined within 2 hours. It was poured onto a Petri dish and all portions with little or no squamous epithelial cells considered to originate from the lower respiratory tract were selected under an inverted microscope and placed in an Eppendorf tube, whereupon the weight was recorded. Dithiothreitol (sputalysin, Calbiochem, San Diego, CA, USA), freshly prepared in a dilution of 1:10 with distilled water according to the manufacturer's instructions, was added in a volume (in µl) equal to twice the weight of the sputum portion (in mg), mixed mechanically with the sputum by aspiration in and out of a pipette about 20 times and further diluted with PBS to a volume equal to the sputum plus dithiothreitol. The cell suspension was filtered through a 52 µm nylon gauze (BNSH Thompson, Scarborough, Canada) to remove debris and mucus, and the volume of the filtrate was recorded. The total cell count was measured using a homocytometer (Neubauer chamber). The filtered cell suspension was diluted with PBS to achieve a concentration of 1000/µl; one drop was placed in each cytocentrifuge cup already set in a Shandon III centrifuge (Shandon Southern Instruments, Sewickly, PA, USA), and cytospins were prepared at 1000 RPM for 5 minutes. Separate cytospin slides were stained by Giemsa. The cell counts were performed by scanning the cytospin, starting at the top while moving across the slide using high power (x500) magnification. Fifteen hundred cells were counted to obtain a differential cell count of metachromatic cells and 500 for all other cells.

Statistical analysis

Student's paired *t*-test was used for comparing the data. Results were considered significant when the level of significance was <0.05.

Results

The characteristics of the patients are shown in Table 1. The difference in the mean age of the five groups was insignificant. Their physiologic data are displayed in Table 2. The baseline FVC and the baseline FEV1 were all within normal limits, although there were significantly lower FEV1 values for the asthma group compared with those of the food allergy alone-group and the control group ($P < 0.05$).

BHR was demonstrated in 40% of the food allergy-alone patients (the mean PC20 was 1.3 ± 3 mg/ml MC). All of the patients with asthma either alone or with food allergy but only 55% of the patients with food allergy and allergic rhinitis and none of the controls had BHR.

FEV1 = forced expiratory volume in one second
FVC = forced vital capacity
MC = methacholine
PBS = phosphate-buffered saline

Table 1. Patients' characteristics

	No. of patients	Gender (M/F)	Age (yrs, mean ± SD)	Duration of illness (yrs, mean ± SD)
Food allergy alone	18	11/7	20 ± 4	13 ± 5
Food allergy and asthma with or without allergic rhinitis	11	7/4	22 ± 5	11 ± 4
Food allergy and allergic rhinitis	13	6/7	18 ± 3	9 ± 6
Asthma alone	10	4/6	25 ± 3	7 ± 4
Controls	10	7/3	23 ± 4	–

Table 2. Patients' physiologic data

	FVC baseline (% predicted, mean ± SD)	FEV1 baseline (% predicted, mean ± SD)	PC20 mg/ml* metacholine (mean ± SD)	Percent reactive
Food allergy alone	95 ± 7	91 ± 5	1.3 ± 3	40%
Food allergy and asthma with or without allergic rhinitis	93 ± 6	83 ± 4	0.4 ± 0.5	100%
Food allergy and allergic rhinitis	99 ± 6	89 ± 5	2.2 ± 2.0	55%
Asthma alone	93 ± 4	77 ± 6	0.2 ± 0.4	100%
Controls	102 ± 5	93 ± 4	–	None

* PC20 was calculated for the reactive patients only

Table 3. Skin prick test data

	Pollens	Mites	Molds
Food allergy alone	Grasses 13/18 Weeds 7/18 Trees 5/18 Combinations 8/18	2/18	2/18
Food allergy and asthma with or without allergic rhinitis	Grass 8/11 Weeds 5/11 Trees 3/11	10/11	2/11
Food allergy and allergic rhinitis	Grass 8/13 Weeds 6/13 Trees 7/13	10/13	1/13
Asthma alone	Grass 4/10 Weeds 2/10 Trees 2/10	10/10	3/10
Controls	Grass 2/10 Weeds 1/10 Trees 1/10	2/10	1/10

Table 4. Cell counts obtained from induced sputum samples

Metachromatic cells	Macrophages	Lymphocytes	Neutrophils	Eosinophils	Group
0	46 ± 4	13 ± 3	39 ± 5	0.8 ± 0.7	Food allergy
0.6 ± 0.2	52 ± 4	18 ± 2	20 ± 3	9 ± 11	Food allergy and asthma with or without allergic rhinitis
0.8 ± 0.3	53 ± 7	15 ± 2	29 ± 4	3 ± 4	Food allergy and allergic rhinitis
1.1 ± 0.5	49 ± 6	16 ± 1	22 ± 5	13 ± 7	Asthma
0	55 ± 8	17 ± 2	28 ± 3	0.3 ± 1	Controls

Values are given as mean ± SD%

Table 3 lists the results of the skin prick test for all 52 participants. The patients with food allergy alone were sensitive mainly to pollens, but only four of them were mildly sensitive to mites (n=2) and molds (n=2). The size of their wheals was smaller than those of the patients with respiratory symptoms (<5x5 mm). Patients with food allergy and asthma (11/11, 100%) and those with asthma alone (10/10, 100%) or food allergy and only allergic rhinitis (10/13, 77%) had significantly ($P < 0.001$) higher rates of mite sensitivity as well as a larger-sized wheal response. Two of the 10 control subjects had small-sized skin sensitivity to mites and pollens.

Cells obtained from the induced sputum samples were counted and the results are presented in Table 4. Eosinophil counts were significantly higher in patients with asthma (13 ± 7%) and patients with food allergy and asthma (9 ± 11%) than in those with food allergy and allergic rhinitis (3 ± 4%) ($P < 0.05$), and even more significantly higher than in patients with food allergy alone (0.8 ± 0.7%) and in the controls (0.3 ± 1%) ($P < 0.001$). There were no significant differences in lymphocyte, macrophage or metachromatic cell counts. A significantly higher number of neutrophils ($P < 0.05$) was counted in the patients with food allergy alone (39 ± 5%). There was no significant difference between the cell counts of patients with food allergy with and without BHR.

Discussion

Food allergy is a common childhood manifestation of atopy [1]. The condition can be divided into IgE-mediated and non-IgE-mediated reactions. IgE-mediated reactions involve the release of mediators from mast cells and result in symptoms within 2 hours of exposure [1]. Another common manifestation of atopy is asthma, a very common disease in childhood [17]. Food allergy and asthma frequently coexist, and this relationship has recently raised much interest in the role of food in the development of asthma. There is now compelling evidence to indicate that food may be a risk factor for respiratory involvement and even for life-threatening asthma. One adult case-controlled study showed that a history of a previous asthma attack precipitated by food is the most significant risk factor for life-threatening asthma [15]. Sensitization to inhalants and foods has also been reported to be a risk factor for brittle asthma in adults [16].

The current study on young adult patients with food allergy investigated the degree of airways involvement by measuring BHR and induced sputum, and our results showed that 40% of the patients with food allergy without known asthma have a significant degree of BHR. This agrees with the finding of Wallaert et al. [5] who reported that 50% of their patients with food allergy also had BHR without having any symptoms of asthma. Increased BHR was found in all of their patients with active asthma whether or not they had food allergy, and in 55% of patients with food allergy and nasal symptoms only. BHR is the hallmark of patients with active asthma [17], but it is also known to exist in other conditions, such as inflammatory bowel disease [7], allergic rhinitis [6] and others [8,17]. BHR was found to be present in up to 50% of

Ig = immunoglobulin

patients with allergic rhinitis alone, whether seasonal or perennial [6]. This airways sensitivity can even increase following exposure to an allergen to which the patient is sensitive [18], a finding also described in patients with food allergy following food challenge [4]. Almost two decades ago, Brama and colleagues [19] proposed that BHR is a risk factor for the development of asthma in patients with allergic rhinitis, a finding that emphasizes the significance of BHR in patients with food allergy.

The number of sputum eosinophils is considered to be an inflammatory marker and correlates well with the severity of the disease in patients with asthma [20,21] [Table 4]. It is interesting that no sign of inflammation, as measured by sputum eosinophilia, could be found in our patients with food allergy alone, again similar to Wallaert's finding [5]. Since sputum eosinophilia is a known marker of active asthma, we can assume that our patients did not have an active asthma component but rather a non-symptomatic one. The level of sputum eosinophilia was also reported to be high in patients with allergic rhinitis alone, with a marked increase following nasal provocation with allergen [22]. Corren [23] suggested that those patients might become asthmatic in the future.

It is also interesting that the patients with food allergy without respiratory symptoms were mostly skin sensitive to pollens and not to mites, while mite allergy was detected in approximately 90% of our patients who had airway upper or lower symptoms [Table 3]. Mite sensitivity is known to be the most important factor for developing airway disease [24]. It is possible that chronic exposure to mites becomes a risk factor for the development of respiratory disease. We can therefore assume that the presence of BHR in the absence of eosinophils is indicative of a non-active state of asthma.

In summary, the increased BHR of patients with food allergy might indicate that they can also develop bronchoconstriction during a reaction to food to which they are allergic. Our findings on patients with food allergy point to the airways as a possible site for allergic reaction during food exposure in patients with type I sensitivity to a particular food. We therefore recommend that patients with concomitant food allergy and BHR, even in the absence of asthma, carry a beta 2 agonist inhalator in the event of an allergic reaction to food.

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