

Asthma and rhinitis induced by exposure to raw green beans and chards

Pilar Daroca, MD*; Jesus F Crespo, MD*; Mar Reaño, MD*; John M James, MD†; Aranzazu Lopez-Rubio, MD*; and Julia Rodriguez, MD*

Background: Although the vast majority of IgE-mediated allergic reactions to foods occurs through ingestion, a few cases of unexpected allergic reactions to foods may occur through the exposure to airborne food allergen particles.

Methods: Case reports. Skin prick tests and serum-specific IgE (CAP-FEIA) were used to identify specific IgE antibodies. Bronchial provocation tests were performed to determine the clinical relevance of inhaled exposure to raw and cooked green beans and raw chards. After demonstrating specific reactivity to them, SDS-PAGE and immunoblotting of raw and cooked green beans were carried out to identify relevant antigens.

Results: Three women developed bronchial asthma and rhinitis after exposure to raw green beans, and one of them also when exposed to raw chards. All women tolerated ingestion of green beans. Patients reported multiple episodes while handling these vegetables for cooking activities. Allergy to green beans and chards was demonstrated by skin testing and serum-specific IgE. Bronchial challenge test with these allergens showed positive responses to raw, but not cooked, green beans and chards. Oral food challenges with green beans (raw and cooked) and chards were negative in all patients. In order to further characterize the allergenic components of these extracts, SDS-PAGE and electroblotting studies were also performed. Immunoblots of raw and cooked green beans extract showed two IgE-binding bands with apparent molecular weights of 41.1 and 70.6 kD. Interestingly, a 47-kD IgE-binding protein was detected only in raw green bean extracts.

Conclusions: We report three patients who developed asthma and rhinitis caused by exposure to raw, but not to cooked, green beans and chards in a non-occupational environment. Only minor differences of IgE reactivity between nitrocellulose-blotted raw and boiled green bean extract were found.

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INTRODUCTION

Although the vast majority of IgE-mediated allergic reactions to foods occur following ingestion of the allergen, a few cases of unexpected allergic reactions to foods can occur through the exposure to airborne food particles.¹ Most of these cases have been reported in patients exposed to food allergens in the workplace, which result in occupational asthma.^{2–7} Exposures to potential airborne food allergens frequently

occur through the handling and processing while cooking in home, rather than in the food-processing industry. Unfortunately, minimal information is available on asthma induced by inhalation of vegetable food allergens in the domestic setting (ie, outside the workplace).

Quirce et al⁸ reported two housewives with raw potato-induced bronchial asthma. De la Hoz et al⁹ studied a patient in whom the inhalation of vapor from boiling Swiss chard caused rhinoconjunctivitis and asthma. Martin et al¹⁰ reported the case of a young man who experienced asthmatic attacks when exposed to the steam from cooking either chick pea or lentil. Specific bronchial challenges with these food

extracts elicited isolated immediate responses. Parra et al¹¹ reported an atopic housewife, in whom rhinitis and acute asthma were associated with Swiss chard and green bean. Bronchial responses after specific bronchial challenge indicated that these vegetables might be the causative factor for asthma. Igea et al¹² described a case of asthma in a homemaker, whose symptoms occurred during preparation and cooking of raw green beans.

In this investigation, we report the diagnosis of an IgE-mediated bronchial asthma induced by inhalation of raw green beans (*Phaseolus* spp.) and chards (*Beta vulgaris*) exposure. This diagnosis was accomplished by means of immediate skin reactivity, IgE determination in serum, and specific bronchial challenges with the food extracts in three patients. In addition, several allergens were identified in green bean extracts.

CASE REPORTS

Patient 1

During the past 2 years, a 31-year-old woman, who tolerated ingestion of cooked green beans without any symptoms, developed rhinoconjunctivitis, dyspnea, and wheezing each time she handled (cutting and processing) raw green beans in preparation for cooking. She required treatment in the emergency department several times for acute asthma after handling green beans. Of note, there was no previous history of respiratory illness until the previous year, when she began with respiratory symptoms being diagnosed with seasonal allergic rhinitis and asthma from sensitization to grass pollens.

Patient 2

A 37-year-old woman developed rhinoconjunctivitis, dry cough, dyspnea,

* Servicio de Alergia, Hospital Universitario 12 de Octubre, Madrid, Spain.

† Colorado Allergy and Asthma Centers, Fort Collins, Colorado, USA.

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and wheezing 5 to 10 minutes after handling (cutting and cleaning) either raw green beans or chards. These symptoms developed over the previous 2 years and the patient reported eating cooked legumes and other vegetables without any symptoms. She had a positive history of seasonal allergic rhinitis and asthma with sensitization to olive and grass pollens since age 30 years.

Patient 3

Over the past 2 to 3 years, a 47-year-old woman developed rhinoconjunctivitis and asthma attacks, including wheezing, and chest tightness, immediately after she started to clean and handle raw green beans. The symptoms lasted approximately three to four hours. She tolerated the ingestion of cooked green beans. She had been diagnosed as having seasonal allergic rhinitis and asthma with sensitization to grass pollens 6 years before.

METHODS

Allergenic Extracts

Extracts from raw and cooked green beans and chards were prepared separately by blending 100 g of fresh vegetable in 500 ml 0.01 M PBS (pH 7.4) in a commercial Waring blender (New Hartford, CT) for 4 minutes at room temperature. Mixtures were extracted overnight at 4°C under constant stirring. Extracts were centrifuged (12,000 g) for 20 minutes at 4°C. The supernatant was subsequently dialyzed (Spectra/por, Spectrum Medical Industries, LA, USA) (cutoff ≥ 3.5 kD) at 4°C against 100 vol of 10 mM of glycine for 24 hours, centrifuged at 30,000 g, and filtered through a 0.45-mm Millipore filter (Millipore Corp., Bedford, CT). The material was stored at -20°C . For preparation of skin and specific bronchoprovocation tests, extracts were sterile filtered, and the filtrates were assessed for sterility. For skin prick testing, extracts were diluted 1:1 with sterile glycerol.

Immunologic Studies

Skin prick tests (SPT) were performed with cooked and raw green bean and

chard extracts at 1:10 wt/vol. A positive SPT response was defined as a mean wheal diameter > 3 mm in subjects who react to the positive control (10 mg/mL histamine hydrochloride), and who are negative to the diluent control (50% glycerol in PBS). Green bean-specific serum IgE levels were determined by CAP-FEIA (Pharmacia Diagnostic, Sweden). Results were expressed by $\text{kU}_\text{A}/\text{L}$, according to the manufacturer's instructions.

SDS-PAGE was performed with a 12% polyacrylamide separating gel and an stacking gel of 4%. Twenty milliliters at a final concentration of 1 mg/mL of each allergenic extract was applied to each well. Electrophoresis was performed for 1 hour at 130 V per gel for the 8 cm by 7.5 cm gels (Mini-Protean II System, Bio-Rad Laboratories, Richmond, USA). Proteins were electrophoretically transferred from the separating gel to a nitrocellulose membrane in a transfer buffer. After transferred from the transblot apparatus, the nitrocellulose was placed in blocking solutions for 1 hour. The nitrocellulose blot was then washed 4 times with TTBS and incubated with the patient's serum (1:2 vol/vol) overnight with rocking. The nitrocellulose blot was again washed with TTBS 4 $\times$, and Alkaline phosphatase-conjugated goat anti-human IgE (1:2000 vol/vol) was added and incubated for 6 hours. After an additional wash with TBS three times, the blot was developed with 250 mL of 30 mg of nitroblue tetrazolium in 70% dimethylformamide and 250 mL of 15 mg of 5-bromo-4-chloro-3-indolyl-phosphate in 70% dimethylformamide solution in 25 mL of carbonate buffer (0.2 mol/L, pH 9.8) for 15 minutes. The immunoblots were scanned in the DeskTop Densitometer (pdi Inc., New York, USA) and analyzed by using the Diversity One software (pdi Inc., New York, USA).

Bronchial Provocation Tests

Bronchial provocation testing (BPT) was performed according to the Report prepared by the EAACI Subcommittee on provocation tests with allergens.¹³ These tests were performed during an

asymptomatic period, after at least 7 days without exposure to the allergen. The substance was delivered by means of a De Vilbiss 646 nebulizer (De Vilbiss Co., Somerset, Pa, USA) with an output of 0.28 mL/min, and an intermittent aerosol generation. Each dose of aerosol consists of five breaths, taken slowly from residual volume to the total lung capacity with a 2 to 5 second breath-hold. The different allergen concentrations were administered at 20-minute intervals. The test was considered positive when a FEV_1 decrease of at least 20% was obtained. Bronchial provocation testing with allergenic extracts were performed as for the methacholine tests, with a previous endpoint titration beginning with a dilution of 1:1,000,000 wt/vol of the extract and progressively increased by 10-fold, to select a safe initial allergen dose for inhalation. For both methacholine and specific bronchial challenges, the delivery system was identical and pulmonary function was measured by assessment of FEV_1 (Sensor Medics, Anaheim, USA). Spirometric controls were performed at 10-minute intervals until a positive response was obtained and, from this moment, at 10-minute intervals up to the first hour. After, FEV_1 values were followed hourly for the next 12 hours. The next morning, a new spirometry was performed. Two unexposed patients with seasonal allergic asthma were also challenged with a 1:10 wt/vol dilution of raw green bean and chard extracts after informed consent was obtained.

Oral Food Challenges

Open food challenges were carried out in each patient by giving increasing amounts of cooked green beans and chards in patient 2. Oral tolerance to raw green beans was evaluated by double-blind, placebo-controlled food challenge,¹⁴ using freeze-dried raw green beans. Increasing doses from 100 mg to 17.5 g of the freeze-dried material were administered, at 30-minute intervals, in a mix of pineapple and tomato juice as a vehicle. The juice, without the active material, was

used as a placebo. The test was performed in 2 days, with serial FEV₁ measurements every 30 minutes up to the last dose, and hourly until the fifth hour. From this moment on, the patient was given a peak-flow meter, with hourly measurements, until bedtime. All provocation studies were performed after obtaining informed consent.

RESULTS

Results of skin prick tests were positive in response to raw green bean in all patients. Patient 2 reacted also to cooked green beans and raw and cooked chards. Results of the same test performed in five atopic subjects were negative. Specific IgE to green beans was positive in all patients (5.6 kU/L, 6.3 kU/L, and 1.33 kU/L, respectively). Bronchial provocation testing with raw green bean extract in patients 1 and 3 elicited a fall in FEV₁ of 24% and 25%, respectively, from baseline 20 minutes after challenge without late response. In these patients, BPT with cooked green bean and control extracts were negative. In patient 2, BPT with raw green bean extract induced an isolated immediate fall in FEV₁ of 34% and with raw chard extract elicited dual bronchospastic reactions. Specific BPT with cooked green bean and control extracts were negative (Fig 1). Results of BPT with raw green bean and chard extracts in two control patients were negative. The results of methacholine inhalation tests showed a moderate bronchial hyperresponsiveness (PD₂₀ 18 IU, 23 IU, and 4 IU, respectively). Oral food challenges with raw and cooked green beans and with cooked chards (in patient 2) were negative. Immunoblots of raw and cooked green beans extract showed two IgE-binding bands with apparent molecular weights of 41.1 and 70.6 kD. A 47-kD IgE-binding protein was detected only in raw green bean extract (Fig 2). No bands were observed in raw and cooked green bean immunoblots when serum from a nonatopic subject was used (not shown).

DISCUSSION

Our patients experienced respiratory symptoms immediately after handling green beans and chards in a domestic, non-occupational setting. The negative bronchial challenges to control extract in patients and the negative bronchial response to raw green bean in two control subjects with allergic asthma support a true, specific bronchospastic reaction to raw green beans and chards. These findings are also supported by the results of skin testing and in vitro studies. All of this data indicate that raw green beans and chards were antigens responsible for the asthma in our

patients. Our results also demonstrate that bronchial reactivity was limited to raw, but not to cooked extract. This observation is interesting because only minor differences of IgE reactivity between nitrocellulose-blotted raw and boiled green bean extract were found. As reported previously,^{9,12} our patient tolerated the ingestion of cooked green beans and chards. To further characterize the oral clinical reactivity, we challenged these patients with raw green beans which was tolerated in all the cases. It denotes the lability of green beans allergens to digestion and enzymatic processes, which could explain

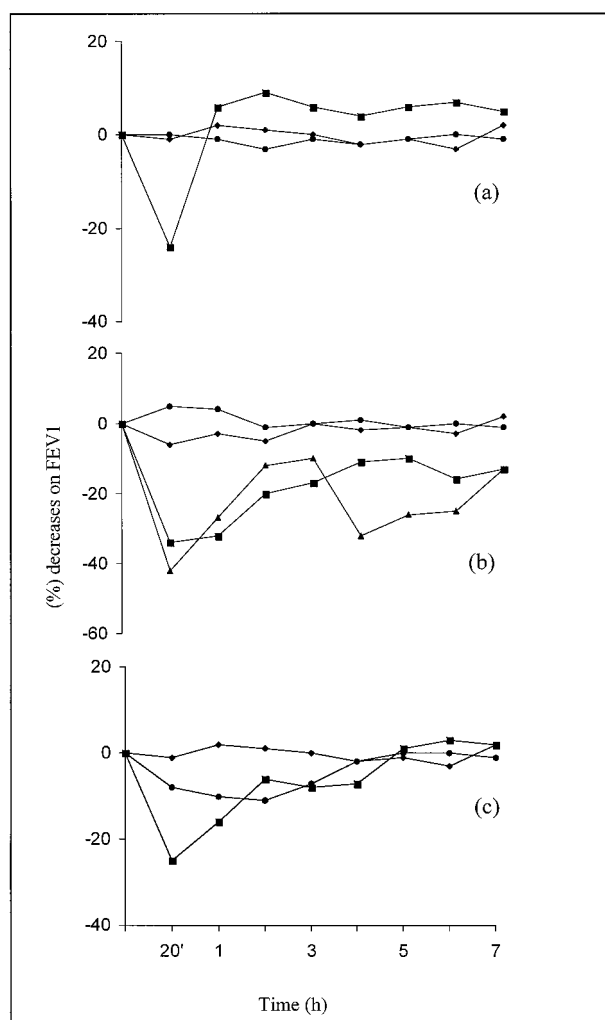


Figure 1. Percentage decreases on FEV₁ after inhalation of raw (squares) and cooked (circles) green bean and cat dander (control) (diamonds) extracts in patients 1 (a) and 2 (c) and raw (squares) and cooked (circles) green bean, raw chard (triangles), and cat dander (control) (diamonds) extracts in patient 2 (b).

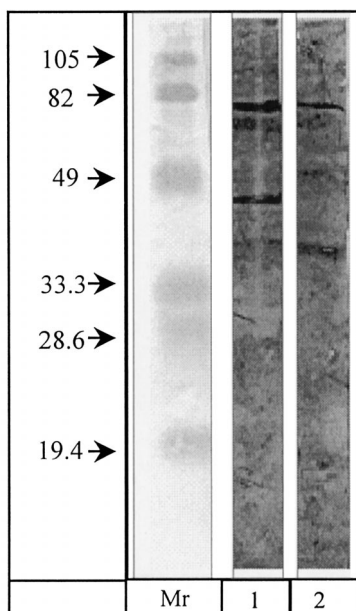


Figure 2. IgE antibody reactivity to green bean allergens by immunoblotting. Proteins in raw and cooked green bean were resolved by means of SDS-PAGE and transferred to nitrocellulose. Five millimeter strips from each blot were tested for reactivities to serum IgE from patient 1. Mr: molecular mass marker proteins; lane 1: raw green bean extract; and lane 2: cooked green bean extract.

the low rate of allergic reactions reported to these foods when ingested.

There was a period of 2 to 3 years from the onset of symptoms until the diagnosis was established in our patients. During this period, patients were evaluated by several physicians who were initially skeptical regarding the role of vegetables as precipitating factors in the respiratory symptoms,

although some cases of asthma caused by exposure to green beans and Swiss chard have been previously reported.^{11,12}

In summary, vegetable foods may induce respiratory symptoms by inhalation even when these foods rarely induce clinical allergic reactions following ingestion. Probably, most of these cases occurred outside an occupational environment, since most people are exposed to potential airborne food allergens by handling and processing while cooking in home. Therefore, respiratory symptoms occurring or worsening in the presence of foods, even in a non-occupational setting, should be considered when a patient is known to have or thought to have rhinitis and/or asthma is been assessed.

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Request for reprints should be addressed to:
 Dr P Daroca
 Servicio de Alergia
 Hospital Universitario 12 de Octubre
 CTRA Andalucía km 5,400
 28040 Madrid
 Spain