

Bronchial asthma caused by two unrelated vegetables

Fernando M. Parra, MD; Milagros Lázaro, MD; Manuela Cuevas, MD*; M. Carmen Ferrando, MD†; Jose A. Martín, MD; Apolinar Lezaun, MD; M. Dolores Alonso, MD; and Moises Sanchez-Cano, MD

31297

Vegetable-induced asthma is an uncommon hazard. We report an atopic housewife, in whom rhinoconjunctivitis and acute asthma were associated with two vegetables of taxonomically unrelated botanical families (Swiss chard/Chenopodiaceae family and green beans/legume family). Type I hypersensitivity to the antigens in Swiss chard and green beans was demonstrated by means of skin tests, histamine release test, and specific IgE determination by RAST. Bronchial responses after specific bronchial challenges indicated that Swiss chard and green beans might be the causative factors for the acute asthma.

INTRODUCTION

Allergic symptoms caused by foods are more often produced after their ingestion. Food allergens acting as inhalants, however, can induce respiratory symptoms in sensitized subjects, and even primary sensitization might develop by this route.

Fruits and vegetables are common dietary items worldwide. They have important nutritive properties but can also be harmful in sensitized individuals. In fact, vegetables are among the more important sources of organic allergens known.¹ Despite frequent food sensitization, cases of allergic reactions due to inhalation of vegetables have rarely been reported.²⁻⁶

Recently, we studied a housewife who suffered from acute asthma associated with handling Swiss chard and green beans and inhaling vapors of boiling Swiss chard and green beans. Swiss chard (*Beta vulgaris L. cycla*) is a vegetable belonging to the Chenopodiaceae family, which also includes spinach and beetroot. Green beans (*Phaseolus multiflorus*) belong to the legume family, which includes vegetables widely

consumed throughout the world. A possible cross-reactivity between Swiss chard, green beans and botanically related vegetables and weed pollens was investigated. To our knowledge, there is only one previous report published (by our group) about allergic complaints due to Swiss chard.⁶

CASE REPORT

The patient was a 46-year-old housewife, a nonsmoker, with a 10-year history of seasonal rhinoconjunctivitis and mild asthma.

During the last 8 years she had presented with nasal and ocular itching, sneezing, and rhinorrhea within a few minutes of handling or inhaling the vapor of Swiss chard or green beans cooked at home. These symptoms were followed in 30 minutes by cough, wheezing, and shortness of breath which persisted for a few hours after exposure. The same symptoms developed when she visited a grocery. She was able to eat and to handle cooked Swiss chard and green beans without any reaction and had no reactions to any other foods.

Clinical and immunologic studies were performed out of pollen season. Physical examination, full blood count, chest and sinus radiographs all were normal. Total IgE was 115 IU/mL (4, 8 to 160 IU/mL). Spirometry revealed an FEV₁

of 120% and an FVC of 128% of predicted normal.

MATERIALS AND METHODS

Swiss Chard, Green Bean and Spinach Extracts for in Vivo and in Vitro Tests

One hundred grams of raw Swiss chard, green beans, and spinach were ground and passed through filter paper. The solutions were dialyzed by a Spectrapor (standard cellulose dialysis tubing, molecular weight cutoff 10 kD) (Spectrum Medical Industries Inc, US) in phosphate buffered saline (PBS) (pH 7.3) for 24 hours at 4 °C. Afterwards, the solutions were centrifuged at 2500 g for 15 minutes and passed through a 0.22-μ millipore filter (Millipore Corp., Bedford, MA) for sterilization. These extracts were considered 1:1 vol/vol concentration, and had a final protein concentrations of 2.8 mg/mL (Swiss chard), 0.25 mg/mL (green bean), and 0.9 mg/mL (spinach) as measured by the Bradford method (Bio Rad, München, Germany). Nondialyzed and heated (100 °C × 30 min) Swiss chard and green bean extracts were also obtained.

Legume Extracts for in Vivo and in Vitro Tests

Two grams of lentil, chickpea, and *Lathyrus sativus* respectively was ground and dissolved in 10 mL of

Servicios de Alergia, *Inmunología y †Neumología. Hospital Ramón y Cajal, Madrid, Spain.

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PBS at laboratory temperature. After stirring for 30 minutes, the same procedure described above was followed. The three extracts obtained had final concentrations of 1:5 wt/vol and protein concentrations of 11.6 mg/mL (lentil), 9.6 mg/mL (chickpea), and 12.6 mg/mL (*Lathyrus sativus*).

Skin Prick Tests (SPT)

Skin prick tests were done with the dialyzed, nondialyzed, and heated Swiss chard and green bean extracts. Three normal persons and seven atopic individuals served as controls. We also tested the spinach and the legume extracts, as well as raw Swiss chard, green beans, and other vegetables of *Chenopodiaceae* family (spinach and beetroot) by the prick method. A battery of commercially available common inhalants and food allergens (Bencard, Worthing, UK) were also tested. Finally we performed SPT with other pollens (birch, mugwort, ragweed, *Chenopodium album*, and *Salsola kali*), and commercial vegetable extracts (Swiss chard, spinach, green beans, chickpea, lentil, green peas, and haricot bean) (Leti, Barcelona, Spain). All skin tests were examined at 15 minutes, 6 hours, and 24 hours. We measured the largest wheal diameter and considered a reaction positive if the wheal size induced by the allergen was 3 mm or more larger than the negative control (PBS). Histamine phosphate, 10 mg/mL, was used as positive control according to the recommendations of the European Allergy Subcommittee on skin tests.⁷

Leukocyte Histamine Release Tests

This was performed using an automated fluorometric method according to Siraganian⁸ with the modifications previously described,⁹ with four different concentrations (1:5, 1:25, 1:125, and 1:625) of the following extracts: Swiss chard, spinach, and green bean extracts; legume extracts; commercial vegetable extracts; and pollen extracts (*Chen-*

opodium album, *Salsola kali*, grass pollen, European olive, birch, mugwort, and ragweed). A result of more than 10% release was considered positive.¹⁰ Three pollen-sensitive subjects were also tested with our own extracts.

Specific IgE Determinations

We used the CAP assay for commercially available allergens, following manufacturer's instructions (Pharmacia, Uppsala, Sweden). Specific IgE to Swiss chard, green beans, and spinach was measured by modification of the RAST. Swiss chard, green bean, and spinach extracts were coupled to nitrocellulose paper discs (Hoeffer Scientific Instrument, UK), by a 48-hour incubation period at 4 °C. Then the patient's serum was added to the discs and incubated for 48 hours at room temperature. Afterwards, ¹²⁵I labeled anti-IgE was added and results were expressed in PRU/mL as derived from the Phadebas RAST reference system. For present purposes we have arbitrarily defined results equal to or above 0.35 PRU/mL as positive. The RAST was performed in duplicate for each extract. The control group consisted of a pool of sera from seven pollen-sensitive subjects and a pool of sera from three nonatopic subjects.

RAST Inhibition Assays

RAST inhibition assays were performed as follows: 25 µL of three different dilutions (1:1, 1:10, 1:100 vol/vol) of the inhibitor allergen and 25 µL of PBS as negative control were incubated with 50 µL of serum for one hour at 24 °C as liquid phase. Then, our own discs mentioned above were added as solid phase. After incubating for three hours, the standard RAST technique (Pharmacia, Uppsala, Sweden) was followed. The percent RAST inhibition was expressed as:

$$1 - \left(\frac{\text{cpm test sample/cpm PBS control}}{\text{PBS control}} \right) \times 100\%$$

Swiss chard and green bean discs as solid phase and Swiss chard, green bean and *Chenopodium al-*

bum, *Lolium perenne*, and *Olea europaea* pollens as inhibitor antigens were used.

Bronchial Provocation Tests (BPT)

Methacholine inhalation was performed according to the method of Cockcroft¹¹ with some modifications. The aerosolized particles were generated by a continuous pressurized nebulizer model De Villbis 646, with a fitted output of 0, 28 mL/min.

The patient was first skin tested with tenfold dilutions of the extracts to determine a safe starting dose for the BPT. At daily intervals, the patient inhaled each aerosolized allergen in progressive concentrations for two minutes at tidal volume. The initial concentration was a 1:1,000 vol/vol green bean extract and a 1:10,000 vol/vol Swiss chard extract. A control challenge with PBS was carried out before antigen challenges. Baseline pulmonary function measures of FEV₁, FVC, and PEFR were performed and repeated at 10, 20, 30, 40, 50 minutes, 1 hour, and 2 hours after each challenge. A positive immediate response was defined as $\geq 20\%$ fall in FEV₁. Peak flow measurements were performed every four hours during a 24-hour period following each challenge in order to evaluate the late response. This was considered to be present if a $\geq 25\%$ fall in PEFR from baseline was recorded.¹² Two nonexposed asthmatic patients with similar nonspecific reactivity were also challenged after obtaining their informed consent.

RESULTS

Immediate positive responses were obtained in the SPT with the Swiss chard and green bean extracts. All SPT were negative in the control subjects. Skin prick tests with lentil and *Lathyrus sativus* extracts and grass, European olive, and *Chenopodium a.* pollens were also positive in the patient but negative results were obtained with the other aeroallergens and food allergens tested. Skin prick test results are shown in Table 1.

Leukocyte histamine release test was positive in our patient with Swiss chard, green bean, lentil, and *Lathyrus sativus* extracts; with *Chenopodium a.*, *Salsola k.*, and European olive pollens; and negative with the other allergens tested (Fig 1). The histamine release test results with our own extracts were negative in control subjects.

Significant levels of specific IgE

Table 1. Skin Prick Test Results (size of wheal in mm)

Swiss chard	
Dialyzed extract	7
Nondialyzed extract	9
Heated extract	0
Commercial extract	0
Raw (prick)	5
Green bean	
Dialyzed extract	4
Nondialyzed extract	5
Heated extract	0
Commercial extract	0
Raw (prick)	4
Spinach extract	0
Lentil extract	3
<i>Lathyrus sativus</i> extract	4
Grass pollen	6
European olive pollen	7
<i>Chenopodium a.</i> pollen	4
Negative control (PBS)	0
Histamine, 10 mg/mL	4

antibodies were found in the patient's serum to Swiss chard (0.78 PRU/mL), green bean (0.68 PRU/mL), lentil (0.42 KU/L), and *Chenopodium a.* pollen (0.48 KU/L). Negative results with the noncommercial extracts were obtained in control subjects. Swiss chard RAST was inhibited up to 88% with the Swiss chard extract and up to 75% by the *Chenopodium album* pollen. Green bean RAST was inhibited up to 90% with the green bean extract. Only a 25% inhibition was observed between Swiss chard and green bean, while the inhibitions produced by *Olea europaea*, *Lolium perenne*, and between green bean and *Chenopodium album* pollen were below 15%.

Bronchial challenge with methacholine revealed bronchial hyper-reactivity (PC₂₀ 1 mg/mL). After bronchial provocation with Swiss chard extract 1:100 vol/vol, the patient had both immediate and late responses. FEV₁ decreased 21% at 40 minutes and returned to baseline two hours later. Eight hours after the challenge, a 35% fall in PEFR was recorded, which returned to baseline values 24 hours later. The patient also had a dual response with the 1:100 vol/vol green bean

extract dilution. FEV₁ decreased 25% at 20 minutes and returned to baseline one hour later. A 25% fall in PEFR was seen eight hours later and returned to normal values at 24 hours (Fig. 2). Control subjects showed no significant response after bronchial provocation with both extracts.

DISCUSSION

Reports published to date suggest that most adverse reactions to inhaled food allergens are the result of IgE-mediated reactions.¹³ In our case, the positive skin tests with Swiss chard and green beans as well as the positive histamine release tests and the demonstration of Swiss chard and green bean-specific IgE antibodies by RAST in patient's serum strongly suggest that the patient had developed type I hypersensitivity to the antigens in Swiss chard and green beans. Furthermore, the bronchial response after specific provocation tests confirmed the causative role of these two vegetables for the symptoms reported by the patient.

It's interesting to note the lack of cutaneous response to commercially available vegetable extracts, which is in accordance with previous observations.^{4,6,14} It should also be noted that loss of allergenicity was observed with the heated extracts, which might serve to explain why the patient was able to eat and handle cooked Swiss chard and green beans without ill effects. The positive skin prick test results obtained with the dialyzed as well as with the nondialyzed extracts suggest that antigens in Swiss chard and also green beans must be macromolecules (molecular weight > 10 kD).

Evidence of sensitization to other legumes was found in our patient although she tolerated their ingestion. Cross-reactivity among legumes without clinical relevance has been previously described.¹⁵ Although no cross-reactivity was observed among vegetables of the

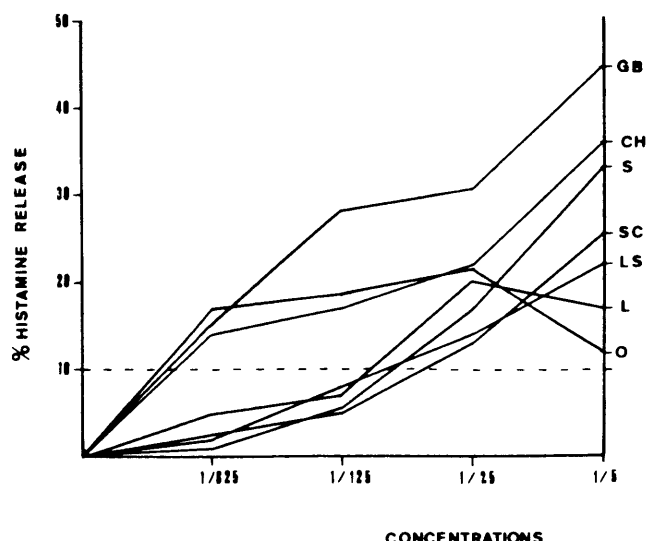


Figure 1. Histamine release test with four different concentrations of the following extracts: green bean (GB), Swiss chard (SC), lentil (L), and *Lathyrus sativus* (LS); and with the following pollens: *Chenopodium album* (CH), *Salsola kali* (S), and European olive (O).

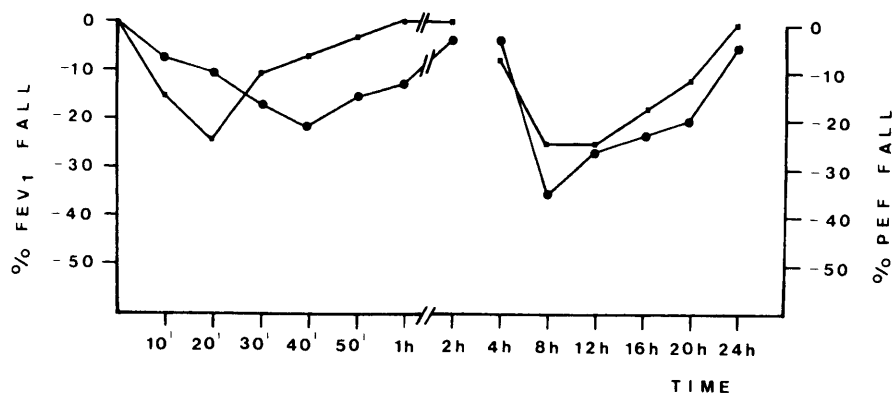


Figure 2. Bronchial provocation test with 1:100 vol/vol Swiss chard extract (●—●) and 1:100 vol/vol green bean extract (■—■). FEV₁ was measured during the first two hours and PEF from 4 to 24 hours.

Chenopodiaceae family, the patient was sensitized to *Chenopodium album* pollen, a weed belonging to this botanical family. Further, as RAST-inhibition assay results suggest, common allergenic determinants are present in Swiss chard and *Chenopodium album* pollen.

We were not able to detect significant cross-reactivity between Swiss chard and green beans by means of the RAST inhibition study. For this reason, we assume the patient developed independent type I hypersensitivity to the Swiss chard antigens as well as to the immunologically unrelated green bean antigens. These food allergens acting as inhalants are causative factors for occupational symptoms.

Housewives are occupationally exposed to numerous potential food allergens. Their causative role may easily remain undiagnosed. For these reasons, we consider this case of interest.

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Request for reprints should be addressed to:
Fernando-M. Parra Borreguero M.D.
Servicio de Alergia. Hospital Ramón y Cajal
Ctra. Colmenar Viejo, Km 9.1
Madrid 28034.
Spain