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Green Bean (*Phaseolus vulgaris*): A New Source of IgE-Binding Lipid Transfer Protein

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Green beans belong to the Fabaceae family, which includes widely consumed species, such as beans, peanuts, and soybeans. In the literature, few cases have described allergic reactions upon the exposure to green bean boiling steam or ingestion. Here, we describe five patients reporting documented adverse reactions upon the ingestion of cooked green beans, and we characterize the responsible allergen. Fresh and cooked green beans were tested by a prick + prick technique. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis and IgE immunoblotting were performed with boiled vegetable extract, and the N-terminal sequence of the immunoreactive protein was obtained by analyzing the excised band in a protein sequencer. Immunoblotting inhibition of cooked green bean with in-house-purified peach lipid transfer protein (LTP) Pru p 3 was performed. An interesting green bean protein was chromatographically purified, tested with a pool serum, and inhibited with Pru p 3. Moreover, its molecular mass was determined by mass spectrometry. Prick + prick tests with raw and cooked green beans were positive for all of the patients. IgE immunoblotting showed that all of the patients reacted toward a unique IgE-binding protein at about 9 kDa. The obtained N-terminal sequence revealed the following amino acids: Ala-Ile-Ser-X-Gly-Qln-Val-Thr-Ser-Ser-Leu-Ala, corresponding to an LTP. A complete inhibition of the IgE binding to this protein, in both raw and purified extract, was obtained by purified peach Pru p 3, confirming previous IgE immunoblotting results.

KEYWORDS: Food allergy; green bean allergy; legume allergy; lipid transfer protein

INTRODUCTION

Legumes (*Fabaceae* family) are widely consumed around the world due to their high biological value proteins, variable amounts of lipids, and wide range of vitamins (*1*). Studies regarding legume allergy have focused on those particularly consumed vegetables with regard to dietary habits. In Spain, where sensitization to legumes represents the fifth most common cause of food allergy in children younger than 5 years old, these vegetables are widely investigated (*2*). In India, where most of the population is vegetarian and legumes are a staple food, chickpea is one of the major food allergens (*3*).

In contrast, few reports have described allergic symptoms upon exposure to boiled green bean (GB) by vapors or ingestion. Igea et al. (*4*) described a single case of a homemaker who experienced rhinoconjunctivitis, asthma, and contact urticaria after handling and boiling GB. Daroca et al. (*5*) reported three cases of asthma and rhinitis after exposure to raw GB, but all of the patients tolerated cooked GB. The IgE immunoblotting revealed two IgE-binding proteins at about 41 and 70 kDa in both raw and cooked GB extracts, while an IgE-binding protein at 47 kDa, responsible for the clinical symptoms, was found only in raw extract. Asero et al. (*6*) described a case of anaphylaxis after the ingestion of boiled GB, and IgE immunoblotting showed only a heat-resistant allergen at 35 kDa. However, no GB allergen has been characterized until now. Here, we describe five cases that present documented mild to severe adverse reactions upon the ingestion of cooked GB, and we investigate the responsible allergen.

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MATERIALS AND METHODS

Patients. For the present study, we selected five patients that reported documented adverse reactions to cooked GB. Their clinical data are presented in **Table 1**. These patients were previously enrolled in a larger study registered at the clinical trial Gov register (FDA #NCT00715156), aimed at evaluating either the diagnostic role of recombinant allergens or the other most frequently allergenic foods in peach-allergic patients. This study was approved by the Niguarda Ca' Granda Ethical Committee, and all of the patients signed an informed consent to participate.

GB Open Food Challenge (OFC). Patients with allergic symptoms strictly localized to the oral mucosa underwent an oral provocation test, with doses administered at 15 min intervals. The test was performed using tap water-boiled GB for about 15 min. The total GB protein content was 1.8 g/100 g vegetable, determined by the Kjeldhal method. A minimum starting dose of 250 mg was given; the following doses were then doubled up to the maximum dose corresponding to 32 g of raw material or until

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Table 1. Demographic Data, Allergic Symptoms to Cooked GB, P+P Tests with Raw and Cooked GB, Skin Prick Test (SPT) with Histamine Dihydrochloride (Hist), Other Sensitizations, Peach Symptoms, and Serum Levels of Anti-recombinant Pru p 3 Determined by ImmunoCAP System (kUA/L)

			GB P+P (mm)					
patient	age/sex	GB symptoms[a]	raw	cooked	SPT *Hist*	other sensitivities[b]	peach symptoms[a]	anti-rPru p 3 (kUA/L)
1	38/f	OAS	8.4	6.0	6	PE, S, B	OAS, GI	1.90
2	22/f	A	10.2	4.3	8	PE, GP, BP, P, B	A, U	9.41
3	18/f	OAS	6.9	3.6	7	PE, CP, B, P, L, N	OAS, AE, GI	21.30
4	36/m	OAS, GE, A	9.0	8.1	8	PE, A, N, B, P, S, C, L, LU	OAS, GI	2.78
5	33/f	OAS	11	5.4	10	PE	OAS, GI	2.09

[a] A, asthma; AE, angioedema; GE, glottis edema; GI, gastrointestinal symptoms; and OAS, 1st grade oral allergy syndrome. [b] PE, peach and other *Prunoideae* fruits; S, soybean; B, bean; GP, grass pollen; BP, birch pollen; P, pea; CP, *Compositae* pollens; L, lentil; N, tree nuts; A, apple; C, chickpea; and LU, lupin.

symptoms arose. The test was considered positive when objective symptoms appeared. In the case of subjective symptoms, the challenge was considered positive when the same symptoms occurred in two separate tests (*7*). The patients that documented more severe symptoms were not challenged because of the severity of their reaction to GB.

Skin Tests. All patients underwent prick + prick (P+P) tests according to the EAACI recommendations (*8*) with fresh and boiled GB, obtained after about 15 min of cooking (until an acceptable softening was reached). Histamine dihydrochloride (10 mg/mL) and saline solution were also tested as positive and negative controls, respectively. To be considered positive, a skin test had to induce a wheal and flare reaction of at least 3 mm diameter.

Pru p 3 IgE Determination. Antirecombinant Pru p 3 serum levels were determined by ImmunoCAP System (Phadia Srl, Milan, Italy), according to the manufacturer's instructions.

Protein Extracts. Proteins from boiled GB were extracted according to Björksten et al. (*9*). Briefly, we boiled 300 g of GB in tap water for about 15 min and then diluted it 5:1 (w/v) in 10 mM phosphate-buffered saline (PBS) (pH 7) with 2% solid polyvinyl-polypyrrolidone, 2 mM ethylenediaminetetraacetate (EDTA) disodium salt, 10 mM sodium diethyldithiocarbamate, and 3 mM sodium azide (NaN_3). After the mixture was homogenized and centrifuged at 16000 rpm at 4 °C for 30 min, the supernatant was dialyzed (membrane cut off 3000 Da) against 10 mM PBS (pH 7) with 3 mM NaN_3 for 48 h at 4 °C, changing the buffer at 16−18 h intervals. The protein concentration of GB extract was determined by the method of Lowry et al. (*10*).

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE) and IgE Immunoblotting. Protein band separation was carried out as described by Pastorello et al. (*11*), using a stacking gel of 6% and a gradient separation gel of 7.5−20%. Separated proteins were electroblotted onto a nitrocellulose membrane, which was cut into strips and incubated overnight with individual or pooled serum. IgE binding was detected by incubation with ^{125}I-labeled antihuman IgE antibodies and exposure on X-ray film at −70 °C for 4 days (*11*).

IgE Immunoblotting Inhibition. Whole cooked GB extract and purified 9 kDa GB protein underwent immunoblotting inhibition (*11*). Briefly, 500 μL of a pooled serum of all of the patients was preincubated for 1 h with 500 μL containing 4 and 2 μg of in-house-purified peach Pru p 3 (*12*). Then, the analysis was performed as the immunoblotting protocol described above.

Chromatographic Purification of the GB 9 kDa Protein. The 9 kDa protein was purified from boiled GB extract by subsequent chromatographic steps (AKTA Purifier, Amersham Biosciences, Uppsala, Sweden). First, proteins were separated by a cation-exchange Resource-S column (Amersham Biosciences, volume = 6 mL), in 30 mM sodium citrate, pH 6, and a linear gradient of 0−1 M NaCl. The concentrated fractions were then separated on a gel filtration Superdex 75 HR 10/30 column (Amersham Biosciences), in a 30 mM sodium citrate, pH 6, and 150 mM NaCl buffer. The eluted peaks were analyzed by SDS-PAGE and immunoblotting.

N-Terminal Sequencing. The 9 kDa protein band was excised from the SDS-PAGE gel, dried in a Speed Vac, and then reswelled in 100 μL of 200 mM Tris-HCl, pH 8.5, and 2% SDS. After the addition of Milli-Q water and a polyvinylidene fluoride (PVDF) membrane prewetted in methanol, the solution was maintained in agitation at room temperature. After 24 h, 50 μL of methanol was added as a transfer catalyzer and was incubated for 4−5 days at room temperature until the solution cleared.

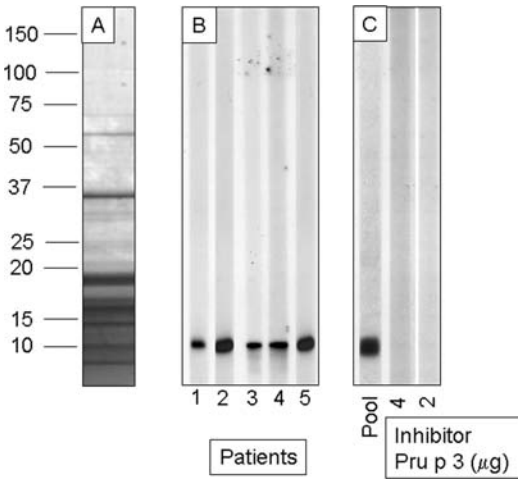

Figure 1. Electrophoretical analyses of cooked GB extract: (**A**) SDS-PAGE, (**B**) IgE immunoblotting results using individual serum (patients 1−5), and (**C**) immunoblotting inhibition with purified Pru p 3 at different concentrations using a pooled serum of all the patients.

After it was washed with 10% methanol and Milli-Q water, the air-dried membrane was inserted in a Procise 492 protein sequencer (Applied Biosystems, CA), as described elsewhere (*13*).

Mass Spectrometry. An aliquot of the purified protein was dialyzed against 0.1% trifluoroacetic acid (TFA) three times using a Microcon Ultracel YM-3 device (Millipore, Billerica, MA) at 14000*g* and concentrated to one-third. Then, 2 μL of sample was added to 2 μL of 2,5-dihydroxybenzoic acid (DHB) solution [10 mg/mL in 0.1% TFA/33% acetonitrile (ACN)], and 1 μL of the mixture was spotted on a MALDI plate and analyzed by a matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) mass spectrometer (Bruker, Bremen) in linear modality. The instrument was calibrated by using the PI standard from Bruker, obtaining a mass accuracy of ±1 Da.

RESULTS

Patients. Five patients (four females and one male, mean age = 29.4) were admitted to the study. Patients 1, 2, and 5 were submitted to provocation tests and responded to the maximum dose (32 g, corresponding to 0.58 g of total GB proteins), showing a first grade oral allergy syndrome (OAS), as previously described (*14*). Patient 2 complained of asthma after GB exposure, and her symptoms worsened after ingestion of the cooked vegetable. Patient 4 experienced OAS plus glottis edema and asthma after ingesting cooked GB. With respect to other allergic sensitizations, all of the patients suffered from severe allergic symptoms after peach ingestion (strongly positive serum levels of anti-rPru p 3), and four patients (1−4) reported adverse reactions to other legumes. Patients 2 and 3 presented with pollen sensitization: in particular, patient 2 was sensitive to grass and birch pollen, and patient 3 was sensitive to *Compositae* pollen. Indeed,

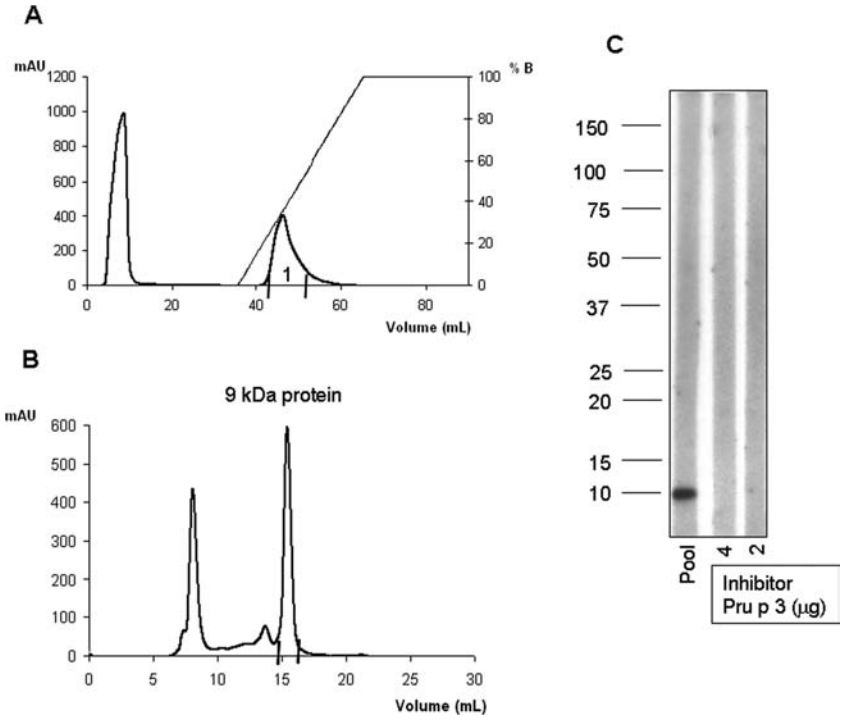

Figure 2. HPLC purification of the GB 9 kDa protein. (**A**) Cationic exchange profile, (**B**) gel filtration profile of the gradient peak obtained with the cationic exchange column, and (**C**) immunoblotting and immunoblotting inhibition results (with purified Pru p 3 and a pool serum) of the third peak obtained in gel filtration.

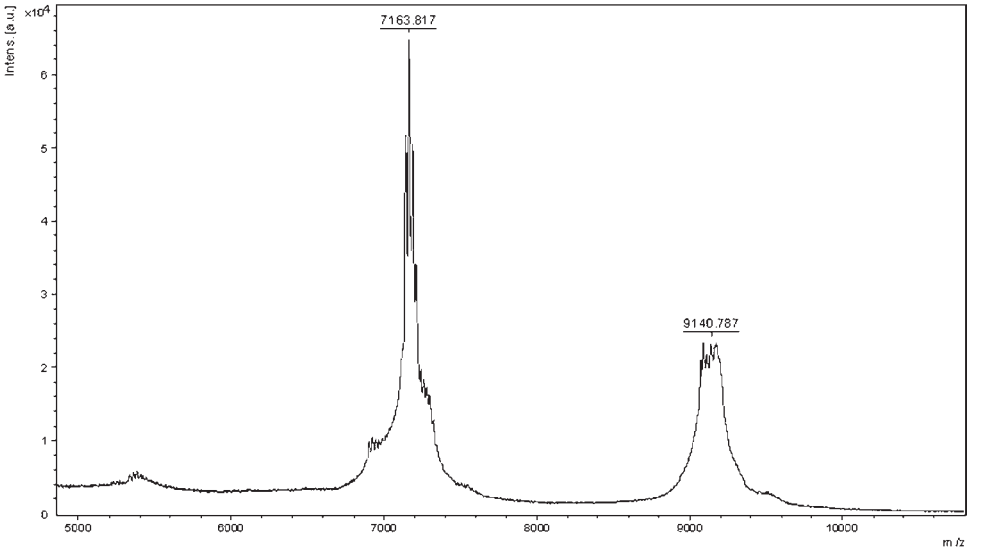

Figure 3. MALDI-TOF/TOF spectrum of the GB purified proteins.

all patients exhibited strongly positive skin reactions with raw and cooked GB.

SDS-PAGE, IgE Immunoblotting and Immunoblotting Inhibition of Cooked GB Extract. In spite of the many protein bands present in cooked GB extract after electrophoresis (**Figure 1A**), the IgE immunoblotting performed with individual serum showed that all of the patients reacted to a unique IgE-binding protein at about 9 kDa (**Figure 1B**). The major GB allergen at about 9 kDa was completely inhibited by Pru p 3 at different dilutions (**Figure 1C**). Because all of the patients reacted to this protein band, further investigations were undertaken to identify this protein.

Purification of GB 9 kDa Protein. The chromatogram in **Figure 2A** shows the elution profile of cationic separation of the whole cooked GB extract with a protein concentration of 4.4 mg/mL. Besides the exclusion peak, there was only one peak during the gradient phase, whose good resolution suggested satisfactory protein purification. **Figure 2B** shows the size exclusion profile of the gradient peak. The purified GB 9 kDa protein was able to bind IgE of a pooled serum of all patients and was completely inhibited by raw peach extract (**Figure 2C**).

Characterization of the GB 9 kDa Protein. The N-terminal sequence of this protein was A-I-S-X-G-Q-V-T-S-S-L-A, corresponding to a lipid transfer protein (LTP) with 100% sequence identity to French bean LTP1 (accession #O24440) and higher than 90% to apple and peach LTPs. Surprisingly, mass spectrometry analysis revealed the presence of two components in the purified sample with molecular masses of 9068.8 ± 1 and 7141.24 ± 1 Da

(**Figure 3**). Both of these proteins belonged to the LTP family: The first corresponds to the LTP previously described, while the second protein presented 80% sequence identity with a maize LTP (accession #B6U7P4). Further analyses will be necessary to better characterize this component, which seemed to have no IgE-binding ability for GB-allergic patients' sera.

DISCUSSION

Despite the large number of studies concerning adverse reactions to some selected legumes, such as peanut and soybean, few reports described GB sensitization. The present study aims to describe the IgE-binding profile of cooked GB. We carefully selected five patients with documented mild to severe symptoms to GB, also presenting with peach allergy, with clinical severe manifestations and Pru p 3 positivity.

While previous studies did not identify LTP in GB, our results demonstrated the presence of at least one allergenic LTP in cooked GB, sharing a high homology with peach Pru p 3. Asero et al. (*15*) suggested that LTP-allergic patients could usually tolerate legumes. The authors explained the lack of reactivity by an epitopic difference between legumes and peach LTP or the lack of LTP expression in legumes. Our results confirmed the rarity of legume allergy and in particular GB; in fact, only 5 out of 104 selected Pru p 3-allergic patients reported adverse symptoms for GB.

In conclusion, for the first time, we describe five patients with an IgE positivity toward a 9 kDa allergen in GB, corresponding to an LTP. This reactivity is quite rare (4.8% in our Pru p 3-positive patients) but possible, so GB can be added to the list of foods causing the "LTP syndrome". Further studies will be performed to better characterize the GB LTP.

ABBREVIATIONS USED

ACN, acetonitrile; DHB, 2,5-dihydroxybenzoic acid; EDTA, ethylenediaminetetraacetate; GB, green bean; HPLC, high-pressure liquid chromatography; LTP, lipid transfer protein; MALDI-TOF/TOF, matrix-assisted laser desorption/ionization time-of-flight/time-of-flight; OAS, oral allergy syndrome; OFC, open food challenge; PBS, phosphate-buffered saline; PVDF, polyvinylidene fluoride; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid.

LITERATURE CITED

(1) Duranti, M. Grain legume proteins and nutraceutical properties. *Fitoterapia* **2006**, *77*, 67–82.

(2) Martínez San Ireneo, M.; Ibáñez, M. D.; Fernández-Caldas, E.; Carnés, J. In vitro and in vivo cross-reactivity studies of legume allergy in a Mediterranean population. *Int. Arch. Allergy Immunol.* **2008**, *147*, 222–230.

(3) Patil, S. P.; Niphadkar, P. V.; Bapat, M. M. Chickpea: A major food allergen in the Indian subcontinent and its clinical and immunochemical correlation. *Ann. Allergy Asthma Immunol.* **2001**, *87*, 140–145.

(4) Igea, J. M.; Fernandez, M.; Quirce, S.; de la Hoz, B.; Diez Gomez, M. L. Green bean hypersensitivity: An occupational allergy in a homemaker. *J. Allergy Clin. Immunol.* **1994**, *94*, 33–35.

(5) Daroca, P.; Crespo, J. F.; Reaño, M.; James, J. M.; Lopez-Rubio, A.; Rodriguez, J. Asthma and rhinitis induced by exposure to raw green beans and chards. *Ann. Allergy Asthma Immunol.* **2000**, *85*, 215–218.

(6) Asero, R.; Mistrello, G.; Roncarolo, D.; Amato, S.; van Ree, R. String bean-induced anaphylaxis. *Allergy* **2001**, *56*, 259–260.

(7) Pastorello, E. A.; Farioli, L.; Pravettoni, V.; Robino, A. M.; Scibilia, J.; Fortunato, D.; Conti, A.; Borgonovo, L.; Bengtsson, A.; Ortolani, C. Lipid transfer protein and vicilin are important walnut allergens in patients not allergic to pollen. *J. Allergy Clin. Immunol.* **2004**, *114*, 908–914.

(8) Dreborg, S. Skin Tests used in type 1 allergy testing: Position paper of the European Academy and Clinical Immunology. *Allergy* **1989**, *44*, 22–59.

(9) Björksten, F.; Halmepuro, L.; Hannuksela, M.; Lahti, A. Extraction and properties of apple allergens. *Allergy* **1980**, *35*, 671–677.

(10) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

(11) Pastorello, E. A.; Pravettoni, V.; Farioli, L.; Ispano, M.; Fortunato, D.; Monza, M..; et al. Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. *J. Allergy Clin. Immunol.* **1999**, *104*, 1099–1106.

(12) Pastorello, E. A.; Farioli, L.; Pravettoni, V.; Ortolani, C.; Ispano, M.; Monza, M.; et al. The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *J. Allergy Clin. Immunol.* **1999**, *103*, 520–526.

(13) Pessione, E.; Giuffrida, M. G.; Prunotto, L.; et al. Membrane proteome of Acinetobacter radioresistens S13 during aromatic exposure. *Proteomics* **2003**, *3*, 1070–1076.

(14) Pastorello, E. A.; Ortolani, C.; Farioli, L.; Pravettoni, V.; Ispano, M.; Borga, A.; Bengtsson, A.; Incorvaia, C.; Berti, C.; Zanussi, C. Allergenic cross-reactivity among peach, apricot, plum and cherry in patients with oral allergy syndrome: An in vivo and in vitro study. *J. Allergy Clin. Immunol.* **1994**, *94*, 699–707.

(15) Asero, R.; Mistrello, G.; Roncarolo, D.; Amato, S.; Falagiani, P. Why do lipid transfer protein-hypersensitive patients tolerate bean (and other legumes)? *Int. Arch. Allergy Immunol.* **2005**, *137*, 236–240.

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