

Characterization of asparagus allergens: A relevant role of lipid transfer proteins

Araceli Díaz-Perales, PhD,^a Ana I. Tabar, MD, PhD,^b Rosa Sánchez-Monge, PhD,^a Blanca E. García, MD, PhD,^b Belén Gómez, MD,^b Domingo Barber, PhD,^c and Gabriel Salcedo, PhD^a Madrid and Pamplona, Spain

Background: No asparagus allergen has been characterized to date. Lipid transfer proteins (LTPs) have an ubiquitous distribution in plant foods and have been identified as relevant allergens in some fruits, seeds, and pollens.

Objective: We sought to identify asparagus allergens and to evaluate the potential involvement of the panallergen LTP family in asparagus allergy.

Methods: Eighteen patients with asthma, anaphylaxis, and/or contact urticaria after asparagus ingestion or exposure and positive skin prick test (SPT) responses and serum-specific IgE levels to asparagus were selected. Two LTPs were isolated from crude asparagus extract by using chromatographic methods and characterized by means of N-terminal amino acid sequencing. Both isolated proteins were tested by means of immunodetection, CAP inhibition assays, and SPTs. Additional asparagus allergens were located by using immunodetection with a pool of sera from patients allergic to asparagus and with rabbit polyclonal antibodies to sunflower pollen profilin and anti-complex asparagine-linked glycans antibodies.

Results: The purified LTPs showed an N-terminal amino acid sequence similar to that of Pru p 3 and a strong reaction to anti-Pru p 3 antibodies. Each isolated protein reached inhibition values of up to 60% in CAP inhibition assays against asparagus extracts and elicited positive SPT responses in 9 of 18 patients with asparagus allergy. Immunodetection assays allowed us to identify profilin and cross-reacting carbohydrate determinants as asparagus IgE-binding components.

Conclusion: Asparagus LTPs are relevant allergens. In addition, profilin and glycoproteins harboring complex asparagine-linked glycans can also be involved in asparagus allergy. (J Allergy Clin Immunol 2002;110:790-6.)

Key words: Asparagus allergy, lipid transfer protein, profilin, cross-reacting carbohydrate determinant, Pru p 3

Abbreviations used

CCD: Cross-reacting carbohydrate determinant

LTP: Lipid transfer protein

SPT: Skin prick test

Asparagus (*Asparagus officinalis*), onion (*Allium cepa*), and garlic (*Allium sativum*) are the most widely consumed vegetables belonging to the Liliaceae family. Several cases of IgE-mediated adverse reactions to asparagus, most of them related to occupational asthma or contact urticaria, have been reported.¹⁻⁵ All of these studies included a single patient or a very limited number of patients, usually with associated allergies to other foods, pollens, or both. Different and exclusive routes of sensitization (inhalation or ingestion),⁵ as well as heat inactivation of some asparagus allergens,¹⁻³ have been suggested in some cases. Cross-reactivity between asparagus and other Liliaceae foods, such as onion and garlic, has been shown by using RAST inhibition assays.^{1,5} However, no allergen from these foods has been isolated or even identified to date. Only a putative lipid transfer protein (LTP) has recently been located as an IgE-binding component of onion extract by using serum from a single patient.⁶

Plant LTPs constitute a family of 9-kd polypeptides involved in defense mechanisms against phytopathogens.⁷ This protein family has an ubiquitous distribution throughout the plant kingdom, being present in both foods and pollens. LTPs have been identified as the major peach (Pru p 3), apple (Mal d 3), and apricot (Pru ar 3) allergens in the Mediterranean population.^{8,9} Furthermore, allergenic members of the LTP family are also present in other plant foods, such as maize (Zea m 14),¹⁰ chestnut (Cas s 8),¹¹ and carrot (Dau c NLTP)¹² and in *Artemisia vulgaris* (Art v 3), *Olea europaea* (Ole e 7), and *Parietaria judaica* (Par j 1) pollen.^{11,13,14} Cross-reactions between Pru p 3 and other allergenic LTPs have been demonstrated in vitro and in vivo.^{11,12,15} LTPs are highly resistant to heat treatment and pepsin digestion.^{12,16} These properties, together with the cross-reactions described among reactive LTPs, point to them as potentially severe food panallergens.

We report the involvement of LTPs in asparagus allergy demonstrated by the in vitro and in vivo reactions exerted by Pru p 3 and its purified homologues from

From ^aUnidad de Bioquímica, Departamento de Biotecnología, E.T.S. Ingenieros Agrónomos, Madrid; ^bSección de Alergología, Servicio Navarro de Salud, Pamplona; and ^cDepartamento de I+D, ALK-Abelló, Madrid.

Supported by Dirección General de Enseñanza Superior e Investigación Científica, MEC (grant PB 98-0735), and Dirección General de Investigación, MCYT (project BMC2002-00196).

Received for publication April 26, 2002; revised July 10, 2002; accepted for publication July 16, 2002.

Reprint requests: Gabriel Salcedo, PhD, Unidad de Bioquímica, Departamento de Biotecnología, E.T.S. Ingenieros Agrónomos, Ciudad Universitaria, 28040 Madrid, Spain.

© 2002 Mosby, Inc. All rights reserved.

0091-6749/2002 \$35.00 + 0 1/87/128242

doi:10.1067/mai.2002.128242

TABLE I. Clinical and demographic data of selected patients allergic to asparagus

Patient no.	Age/sex	Profession	Symptoms	Associated pollinosis	Asparagus-specific IgE (kU/L)
1	23/M	Cannery operator	BA	Yes	12.70
2	31/F	Housewife	BA	No	9.17
3	35/M	Cook	CU	Yes	1.98
4	55/F	Cannery operator	BA	Yes	0.45
5	45/F	Housewife	BA	No	1.58
6	42/F	Cook	BA	Yes	4.77
7	26/F	Grocery worker	BA/CU	Yes	9.70
8	34/F	Cannery operator	BA/A	No	4.47
9	28/M	Grocery worker	RC/A	Yes	12.00
10	18/M	Student	A	Yes	0.43
11	32/M	Carpenter	OAS	No	1.17
12	21/F	Cannery operator	CU	Yes	1.18
13	9/M	Student	CU	No	9.25
14	19/F	Cannery operator	CU	No	0.41
15	36/F	Housewife	CU	No	1.24
16	68/F	Housewife	CU	No	0.68
17	33/M	Cannery operator	CD	No	1.57
18	23/M	Cannery operator	CD	No	0.52

BA, Bronchial asthma; CU, contact urticaria; A, anaphylaxis; RC, rhinoconjunctivitis; OAS, oral allergy syndrome; CD, contact dermatitis.

asparagus in a group of 18 patients with allergy to this food. Additionally, evidence indicating that profilin and complex asparagine-linked glycans are IgE-binding components of asparagus is also presented.

METHODS

Patients and sera

Patients were selected from a population with allergy to asparagus during a prospective study performed by the Sección de Alergología of Servicio Navarro de Salud (Pamplona, Spain). The hospital institutional review board approved the study, and informed consent was obtained from all participants. Clinical and demographic data of the selected patients are summarized in Table I. Selection of patients for the present study was based on the following criteria: (1) a clinical history of immediate reactions to asparagus; (2) a specific IgE level of greater than 0.35 kU/L to this food (mean, 4.12 kU/L), as determined by using the Pharmacia CAP System FEIA; and (3) a positive prick-by-prick test response to asparagus. Diagnosis of patients with bronchial asthma or contact dermatitis and urticaria was confirmed by means of specific bronchial challenge or epicutaneous testing, respectively (A. Tabar, et al, unpublished data). A pool of sera from 6 of the selected patients (patients 1, 6, 7, 8, 9, and 13 in Table I) with specific IgE levels of greater than 3.5 kU/L (mean, 8.81 kU/L) was used for immunodetection and CAP inhibition assays. Five volunteers allergic to mites (*Dermatophagoides pteronyssinus*) but not to plant foods or pollens were included as the control group.

Crude extract and LTP-enriched preparations

Freeze-dried asparagus was ground and extracted with PBS buffer (0.1 mol/L sodium phosphate [pH 7.0] and 0.15 mol/L NaCl; 1× 1:5 [wt/vol] for 1 hour at 4°C). After centrifugation (10,000 rpm for 30 minutes at 4°C), the supernatant was dialyzed (cut-off point, 3.5 kD) against H₂O and freeze-dried. This crude extract (15 mg of protein) was fractionated by means of cation-exchange chromatog-

raphy on an Acell Pluss CM Waters SepPak cartridge (Waters Corp). MOPS buffer (50 mmol/L 3-[N-morpholino]propanesulfonic acid [pH 7.0]) was used for elution, and the retained material (enriched in LTPs) was then eluted with 0.75 mol/L NaCl in the same buffer (1 mL/min). Fractions were dialyzed against H₂O and freeze-dried.

The protein concentrations in these crude and enriched preparations were determined by using the method of Bradford.¹⁷

Isolation and characterization of asparagus allergens (LTPs)

The LTP-enriched preparation from the cation-exchange chromatography was further separated (2 mg of protein) by means of reverse-phase HPLC on a Nucleosil 300 C₄ column (4.6 × 250 mm; particle size, 5 µm; Scharlau). Elution was performed with a 2-step linear gradient of acetonitrile in 0.1% trifluoroacetic acid (0%-60% in 120 minutes and 60%-85% in 15 minutes, 0.5 mL/minute). Peaks containing LTPs were identified by using SDS-PAGE and immunodetection with anti-Pru p 3 antibodies (see below). Protein concentration was quantified by using the bicinchoninic acid method.¹⁸

Natural Pru p 3 was obtained as previously described.¹⁹ Recombinant profilin from sunflower pollen (rHel 2)²⁰ was obtained from Bial-Aristegui.

SDS-PAGE was carried out on Bio-RAD Miniprotein II system gels (15% polyacrylamide), according to the method of Laemmli.²¹

N-terminal amino acid sequences were determined by using standard methods with an Applied Biosystems 477A gas-phase sequencer.

Immunodetection

Samples fractionated by means of SDS-PAGE were electrotransferred to polyvinylidene difluoride membranes, as previously reported.²² After washing and blocking, membranes were incubated with a serum pool from patients with asparagus allergy or with control sera (1:3 dilutions) and then with alkaline phosphatase-conjugated monoclonal anti-human IgE (1:500 dilution, Sigma) and developed by adding 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium solution.

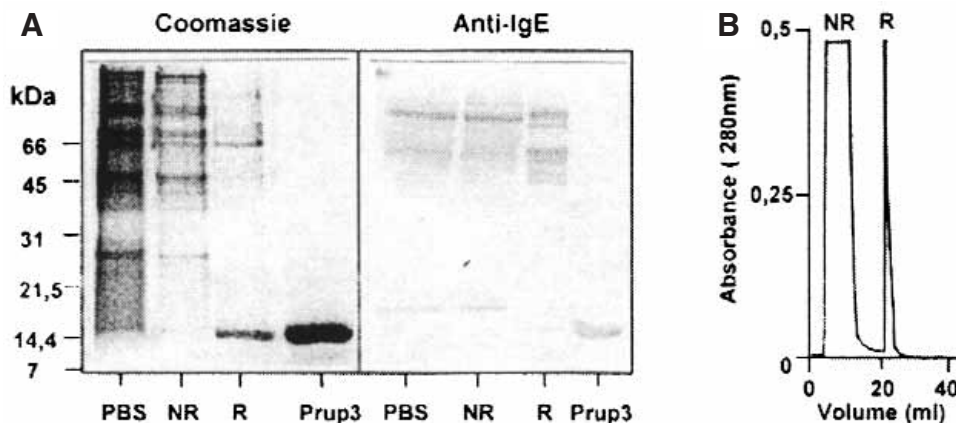


FIG 1. A, Protein staining and IgE immunodetection of the crude PBS asparagus extract, its nonretained (NR) and retained (R) fractions after cation-exchange chromatography, and purified Pru p 3. B, Cation-exchange chromatography fractionation of the PBS extract from asparagus.

Alternatively, replica-blocked membranes were immunodetected with rabbit polyclonal antibodies to natural Pru p 3 (1:200 dilution),¹⁹ to sunflower pollen profilin Hel a 2 (1:3000 dilution; kindly provided by Dr A. Martinez, Aristegui, Bilbao, Spain),²⁰ or to complex asparagine-linked glycans of plant glycoproteins (1:80,000 dilution; kindly provided by Dr MJ Chrispeels, University of California, San Diego, Calif).²³ Membranes were then incubated with an alkaline phosphatase-conjugated anti-rabbit IgG (1:1000 dilution, Sigma) and a 5-bromo-4-chloro-3-indolylphosphate/nitro blue tetrazolium solution.

CAP inhibition assays

The assay was carried out in triplicate samples. The serum pool (1:3 dilution) was premixed with the same volume of 4 serial 1:10 dilutions of each inhibitor solution in PBS buffer with 1% (wt/vol) BSA and incubated for 1 hour at room temperature. After incubation, commercial asparagus Immuno-CAPs were then added, and specific IgE levels were assessed according to the CAP System procedure. Crude asparagus extract (25 mg/mL), its nonretained protein preparation obtained after cation-exchange chromatography (25 mg/mL), and purified asparagus LTPs (30 µg/mL) were used as inhibitors.

Skin tests

Skin prick tests (SPTs) with the crude extract (2 mg/mL), the nonretained protein preparation (2 mg/mL), and the purified LTPs (20 µg/mL) were performed according to standard procedures²⁴ on the volar side of the forearm by using 0.9% (wt/vol) NaCl with 0.03% (wt/vol) human serum albumin as a diluent. Histamine phosphate (10 mg/mL) and NaCl-human serum albumin were used as positive and negative controls, respectively. Duplicates of each sample were assayed. A mean wheal area of 7 mm² or greater (15 minutes after puncture) compared with that produced by using the negative control was considered a positive response.

RESULTS

Separation by means of SDS-PAGE and protein staining of the asparagus PBS extract showed a complex pattern of bands of approximately 10 to 100 kd (Fig 1, A).

IgE immunodetection with a serum pool from patients allergic to this food revealed reactive components with apparent molecular sizes of approximately 15 kd and 45 to 70 kd. Interestingly, the serum pool also strongly reacted to isolated Pru p 3, the major allergen and LTP from peach fruit (Fig 1, A). No band equivalent to that of Pru p 3 was detected in the asparagus PBS extract. However, when this extract was subjected to cation-exchange chromatography (Fig 1, B), the fraction retained on the cartridge included such a band, which was recognized by both the serum pool (Fig 1, A) and the anti-Pru p 3 antibodies (Fig 2, B). In contrast, the 15-kd IgE-binding component appeared in the fraction not retained on the chromatographic cartridge (Fig 1, A). No band was detected in the asparagus extract when using negative control sera (not shown).

The retained fraction from the cation-exchange chromatography was further separated by means of reverse-phase HPLC to isolate and characterize putative asparagus LTPs (Fig 2, A). Immunodetection of the resulting fractions with anti-Pru p 3 antibodies allowed us to identify 2 peaks of the chromatographic profile that contained reactive proteins. Both components behaved as a single band when analyzed by means of SDS-PAGE, showed molecular sizes similar to that of Pru p 3, and bound IgE from patients with asparagus allergy (Fig 2, B). Furthermore, both isolated proteins presented N-terminal amino acid sequences similar to that of Pru p 3 (11/22 identical residues in the case of LTP-1 and 10/19 identical residues for LTP-2; Fig 2, C). Joint consideration of the above-mentioned data clearly allowed the inclusion of both purified asparagus proteins in the LTP allergen family.

In vitro and in vivo tests were carried out to evaluate the relevance of the isolated LTPs in asparagus allergy. CAP inhibition assays (Fig 3) showed a progressive inhi-

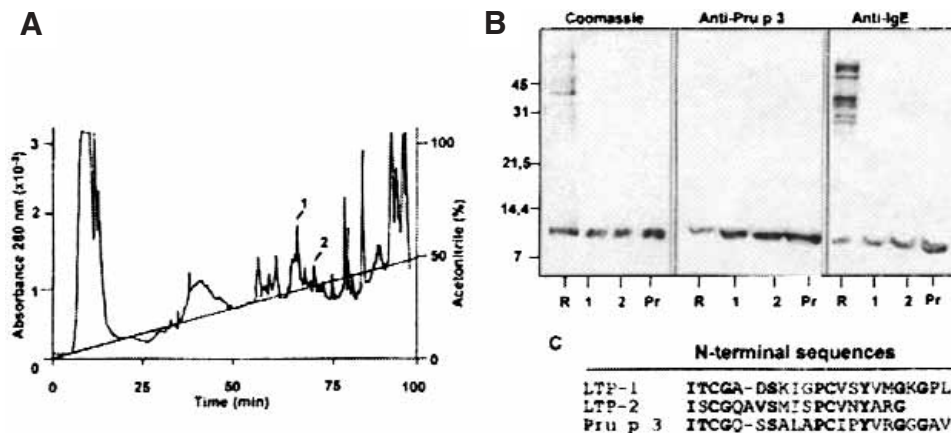


FIG 2. A, HPLC separation of the retained fraction from the cation-exchange chromatography indicated in Fig 1. B, Peaks corresponding to LTP-1 and LTP-2 are indicated. C, Protein staining and immunodetection with anti-Pru p 3 and anti-IgE antibodies of the retained fraction (R) of Fig 1, B, and purified LTP-1 (1), LTP-2 (2), and Pru p 3 (Pr). D, N-terminal amino acid sequences of asparagus LTP-1 and LTP-2 aligned with that of Pru p 3. Gaps (-) to obtain the proper sequence alignment are indicated.

bition when incubating the serum pool with increasing amounts of the purified allergens. Similar inhibition profiles were obtained with both proteins, reaching maximum inhibition values of 60%. Inhibition levels of close to 90% were reached by using the crude PBS extract and the nonretained fraction from the cation-exchange chromatography but with approximately 10³ times more protein than for the purified allergens. Furthermore, SPT responses to asparagus LTP-1 and LTP-2, as well as to Pru p 3, were positive in 9 (50%) of 18 allergic patients tested (Table II). Seven patients reacted with all 3 purified LTPs, whereas 3 of them had positive responses to the pairs LTP-1/Pru p 3, LTP-1/LTP-2, and LTP-2/Pru p 3, respectively. The PBS asparagus extract and its nonretained fraction induced skin reactions in 83% of the same patients. In all cases except 2, the same patient had positive SPT responses with both protein preparations.

The crude PBS extract was immunodetected with specific polyclonal antibodies against sunflower profilin (Hel a 2) and against complex asparagine-linked glycans (cross-reacting carbohydrate determinants [CCDs]) of plant glycoproteins (Fig 4) to further identify other putative allergens. Comparison with the immunoblots obtained when using the pool of sera from patients allergic to asparagus indicated that the 15-kd IgE-binding component corresponded to asparagus profilin, and most of the reactive bands from 45 to 70 kd corresponded to glycoproteins harboring CCDs.

DISCUSSION

The lack of identification and characterization of the main allergens from many plant foods has strongly restrained the standardization of commercial diagnostic tools based on crude food extracts, thus resulting in low

INHIBITION (%)

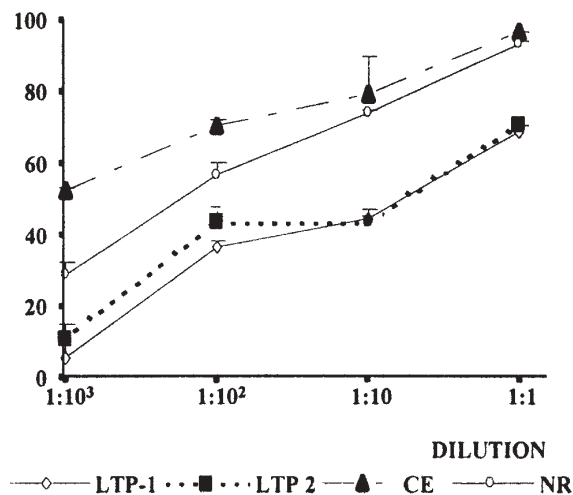


FIG 3. CAP inhibition assays of asparagus with crude PBS asparagus extract (C), its nonretained fraction on cation-exchange chromatography (N), and purified asparagus allergens LTP-1 and LTP-2. A serum pool from patients with allergy to this food was preincubated with 1:10 dilutions of each inhibitor (initial concentrations: 25,000 µg/mL for crude extract and nonretained fraction and 30 µg/mL for the purified LTPs).

diagnostic accuracy.²⁵ Moreover, the rationale to explain the observed clinical cross-reactivity among plant foods and among plant foods and pollens is mainly supported by the identification of homologous allergens in the cross-reacting sources.^{26,27} Foods belonging to the Liliaceae family are a paradigmatic example of such diag-

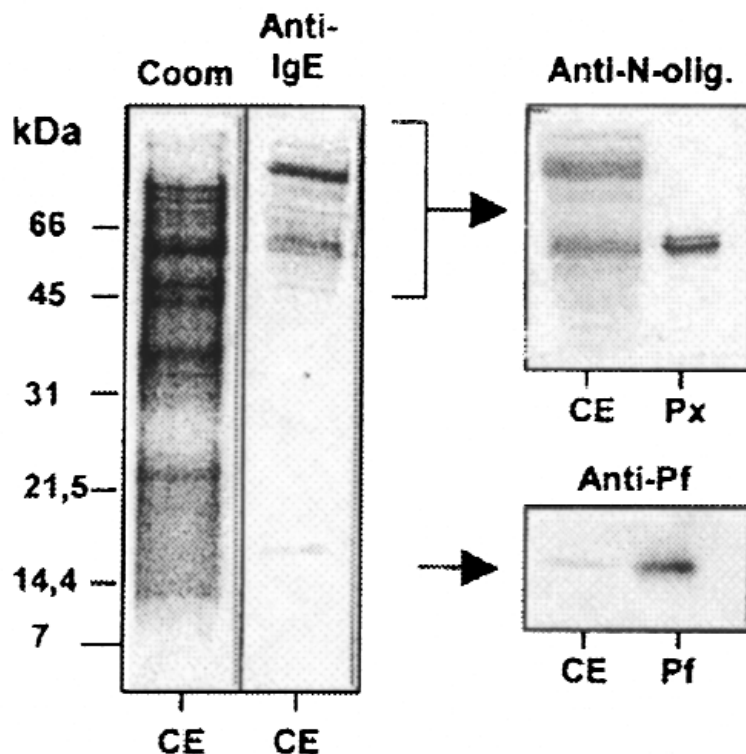


FIG 4. SDS-PAGE separation of the crude PBS extract from asparagus (CE; 20 µg of protein). Replica gels were stained with Coomassie Blue (Coom) or electrotransferred to polyvinylidene difluoride membranes and immunoblotted with a serum pool from patients with asparagus allergy (Anti-IgE), with anti-complex glycans antibodies (Anti-N-olig), or with anti-r Hel a 2 antibodies (Anti-Pf). Only the appropriate membrane section is shown for the anti-complex glycans antibodies (horseradish peroxidase [Px] is included as a positive control) and the anti-r Hel a 2 antibodies (rHel a 2 [Pf] is included as a positive control) immunoblots.

nostic constraints derived from the lack of knowledge of their main allergenic components. This study was designed to initiate the characterization of such allergens in asparagus, focusing particularly on the potential involvement of the panallergen LTP family on the adverse IgE-mediated reactions to this food.

Profilin and 40- to 70-kd glycoproteins harboring asparagine-linked glycans with xylosyl residues, fucosyl residues, or both (CCDs) were identified as the main IgE-binding proteins of the crude asparagus PBS extract (Figs 1 and 4). Both allergen types have been previously defined as cross-reacting IgE-antigenic determinants partially responsible for the cosensitization among plant foods and pollens.²⁷⁻³⁰ Profilin and glycoproteins are the major IgE-reactive components of the nonretained fraction obtained after subjecting the asparagus PBS extract to cation-exchange chromatography, which is virtually depleted of LTPs (Fig 1). The potential relevance of both profilin and CCDs as asparagus allergens is suggested by the high in vitro (up to 90% inhibition values in CAP inhibition assays) and in vivo (83% of positive SPT responses) responses reached by this nonretained protein fraction (Fig 3 and Table II).

The strong reaction of Pru p 3 with sera from patients with asparagus allergy led us to search for its homologous allergens present in this food. After a 2-step chromatographic fractionation of the crude asparagus extract, 2 LTP variants were isolated (Figs 1 and 2). Their recognition by anti-Pru p 3 serum, apparent molecular sizes on SDS-PAGE, and N-terminal sequences support their identification as members of the plant LTP family. The purified proteins were clearly recognized by the serum pool from allergic patients. The high in vitro reactivity (reaching up to 60% inhibition of the IgE binding to the commercial asparagus CAP) and in vivo reactivity (inducing positive SPT responses in 50% of patients) showed by both LTPs indicate their relevance as asparagus allergens. In fact, their in vitro IgE-binding capacity, uncovered by means of immunoblotting and ELISA inhibition assays, was validated by the SPT data. Consequently, the designations of Aspa o 1.01 (for LTP-1) and Aspa o 1.02 (for LTP-2) have been proposed by the International Committee of Allergen Nomenclature.

The allergenic LTPs are not detected in the crude PBS extract from commercial asparagus, indicating the low amount present in this extract. However, their low

extractability by PBS buffers (D. Barber, et al, unpublished results) and their location mainly in the external tissues of plant fruits,³¹ which are peeled during the manufacturing process, can drastically mask their actual levels in asparagus.

Interestingly, the only putative allergen identified in other Liliaceae foods seems to be an LTP from onion.⁶ Furthermore, the skin reactions induced by Pru p 3 in the group of patients with asparagus allergy suggest a substantial role of the panallergen LTP family in cross-reactivity between asparagus and other vegetables and fruits. The resistance of these panallergens to heat treatment¹⁶ could account for hypersensitivity in those patients who present symptoms after eating or manipulating heated asparagus. This treatment seems to inactivate most of the other allergenic components of this food.^{1,3}

We thank Dr A. Martínez (Aristegui, Bilbao, Spain) and Dr M. J. Chrispeels (University of California, San Diego, Calif) for kindly providing anti-Hel a 2 and anti-complex glycans serum, respectively; Mr J. Varela (CIB, CSIC) for protein sequencing; and Mr J. García-Guijarro (ETSIA, UPM) for technical assistance.

REFERENCES

- Eng PA, Yman L, Maaninen E, Wüthrich B. Inhalant allergy to fresh asparagus. *Clin Exp Allergy* 1996;26:330-4.
- Sanchez MC, Hernandez M, Morena V, Guardia P, Gonzalez J, Monteiserin J, et al. Immunologic contact urticaria caused by asparagus. *Contact Dermatitis* 1997;37:181-2.
- Lopez-Rubio A, Rodriguez J, Fernandez-Crespo J, Vives R, Daroca P, Reaño M. Occupational asthma caused by exposure to asparagus: detection of allergens by immunoblotting. *Allergy* 1998;53:1216-20.
- Escribano MM, Muñoz-Bellido FJ, Serrano P, De la Calle A, Conde J. Acute urticaria after ingestion of asparagus. *Allergy* 1998;53:622-3.
- Sanchez-Herandez MC, Hernandez M, Delgado J, Guardia P, Monteiserin J, Bartolome B, et al. Allergenic cross-reactivity in the Liliaceae family. *Allergy* 2000;55:297-8.
- Asero R, Mistrello G, Roncarolo D, Amato S. A case of onion allergy. *J Allergy Clin Immunol* 2001;108:309-10.
- Kader JC. Lipid-transfer proteins in plants. *Annu Rev Plant Physiol Plant Mol Biol* 1996;47:627-54.
- Sanchez-Monge R, Lombardero M, García-Sellés FJ, Barber D, Salcedo G. Lipid-transfer proteins are relevant allergens in fruit allergy. *J Allergy Clin Immunol* 1999;103:514-9.
- Pastorello EA, D'Ambrosio FP, Pravettoni V, Farioli L, Giuffrida G, Monza M, et al. Evidence for a lipid transfer protein as the major allergen of apricot. *J Allergy Clin Immunol* 2000;105:371-7.
- Pastorello EA, Farioli L, Pravettoni V, Ispano M, Scibola E, Trambaioli C, et al. The maize major allergen, which is responsible for food-induced allergic reactions, is a lipid transfer protein. *J Allergy Clin Immunol* 2000;106:744-51.
- Díaz-Perales A, Lombardero M, Sanchez-Monge R, García-Sellés FJ, Pernas M, Fernandez-Rivas M, et al. Lipid transfer proteins as potential plant panallergens: cross-reactivity among proteins of *Artemisia* pollen, *Castanea* nut and *Rosaceae* fruits, with different IgE-binding capacities. *Clin Exp Allergy* 2000;30:1403-10.
- Asero R, Mistrello G, Roncarolo D, de Vries SC, Gautier MF, Ciurana CLF, et al. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. *Int Arch Allergy Immunol* 2000;122:20-32.
- Bonura A, Amoroso A, Locorotondo G, Di Felice G, Tinghiano R, Geraci D, et al. Hypoallergenic variants of the *Parietaria judaica* major allergen Par j 1: a member of the non-specific lipid transfer protein plant family. *Int Arch Allergy Immunol* 2001;126:32-40.
- Rodríguez R, Villalba M, Monsalve RI, Batanero E. The spectrum of olive pollen allergens. *Int Arch Allergy Immunol* 2001;125:185-95.
- García-Sellés FJ, Díaz-Perales A, Sánchez-Monge R, Alcantara M, Lom-

TABLE II. SPT results

Patient no.	SPT wheal area (mm ²)				
	CE	NR	LTP-1	LTP-2	Pru p 3
1	37	33	9	—	12
2	23	20	—	—	—
3	15	28	—	—	—
4	18	20	—	—	—
5	18	17	—	—	—
6	13	23	9	18	14
7	39	78	37	42	48
8	27	21	12	32	—
9	48	69	30	97	44
10	—	—	—	—	—
11	18	—	14	25	58
12	—	—	—	7	12
13	19	47	15	17	35
14	12	14	—	—	—
15	23	11	29	75	55
16	—	21	—	—	—
17	33	24	21	9	18
18	8	21	—	—	—
No. of positive responses	15	15	9	9	9

Testing agents used were asparagus crude extract (CE), its nonretained fraction on cation-exchange chromatography (NR), and the purified asparagus allergens LTP-1 and LTP-2 and peach Pru p 3. Samples were tested at 2000 µg/mL (crude extract and nonretained fraction) or 20 µg/mL (purified allergens). Values are given as means (n = 2).
—, Negative response.

- barbero M, Barber D, et al. Patterns of reactivity to lipid transfer proteins of plant foods and *Artemisia* pollen. An in vivo study. *Int Arch Allergy Immunol* 2002;128:115-22.
- Brenna O, Pompei C, Ortolani C, Pravettoni V, Farioli L, Pastorello EA. Technological processes to decrease the allergenicity of peach juice and nectar. *J Agric Food Chem* 2000;48:493-7.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al. Measurement of protein using bicinchoninic acid. *Anal Biochem* 1985;150:76-85.
- Díaz-Perales A, García-Casado G, Sanchez-Monge R, García-Selles FJ, Barber D, Salcedo G. cDNA cloning and heterologous expression of the major allergens from peach and apple belonging to the lipid-transfer protein family. *Clin Exp Allergy* 2002;32:87-92.
- Asturias JA, Arilla MC, Gomez-Bayon N, Aguirre M, Martínez A, Palacios R, et al. Cloning and immunological characterization of the allergen Hel a 2 (profilin) from sunflower pollen. *Mol Immunol* 1998;35:469-78.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
- Díaz-Perales A, Collada C, Blanco C, Sanchez-Monge R, Carrillo T, Aragoncillo C, et al. Cross-reactions in the latex-fruit syndrome: a relevant role of chitinases but not of complex asparagine-linked glycans. *J Allergy Clin Immunol* 1999;104:681-7.
- Lauriere M, Lauriere C, Chrispeels MJ, Johnson KD, Sturn A. Characterization of a xylose-specific antiserum that reacts with the complex asparagine-linked glycans of extracellular and vacuolar glycoproteins. *Plant Physiol* 1989;90:1182-8.
- Malling HJ. Methods of skin testing. In: Dreborg S, Frew A, editors. *Allergen standardization and skin test*. *Allergy* 1993;48(suppl):55-6.
- Sampson HA. Comparative study of commercial food antigen extracts for diagnosis of food hypersensitivity. *J Allergy Clin Immunol* 1988;82:718-26.

26. Sicherer SH. Clinical implications of cross-reactive food allergens. *J Allergy Clin Immunol* 2001;108:881-90.
27. Breiteneder H, Ebner C. Molecular and biochemical classification of plant-derived food allergens. *J Allergy Clin Immunol* 2000;106:27-36.
28. Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:77-85.
29. Garcia-Casado G, Sanchez-Monge R, Chrispeels MJ, Armentia A, Salcedo G, Gomez L. Role of complex asparagine-linked glycans in the allergenicity of plant glycoproteins. *Glycobiology* 1996;6:471-7.
30. Van Ree R, Cabanes-Macheteau M, Akerdaas J, Milazzo JP, Loutelier-Bourhis C, Rayon C, et al. β (1,2)-xylose and α (1,3)-fucose residues have a strong contribution in IgE-binding of plant glycoallergens. *J Biol Chem* 2000;275:11451-8.
31. Garcia-Olmedo F, Molina A, Segura A, Moreno M. The defensive role of nonspecific lipid-transfer proteins in plants. *Trends Microbiol* 1995;3:72-4.

Receive tables of contents by e-mail

To receive the tables of contents by e-mail, sign up through our Web site at

<http://www.mosby.com/jaci>

Choose *E-mail Notification*

Simply type your e-mail address in the box and click the *Subscribe* button

Alternatively, you may send an e-mail message to majordomo@mosby.com.

Leave the subject line blank and type the following as the body of your message:

subscribe jaci_toc

You will receive an e-mail message confirming that you have been added to the mailing list.

Note that TOC e-mails will be sent out when a new issue is posted to the Web site.