

Cabbage lipid transfer protein Bra o 3 is a major allergen responsible for cross-reactivity between plant foods and pollens

Aránzazu Palacín, PhD,^{a,b} Jose Cumplido, MD,^b Javier Figueroa, MD,^b Oussama Ahrazem, PhD,^a Rosa Sánchez-Monge, PhD,^a Teresa Carrillo, MD, PhD,^b Gabriel Salcedo, PhD,^a and Carlos Blanco, MD, PhD^b Madrid and Las Palmas de Gran Canaria, Spain

Background: Food IgE-mediated allergy to members of the Brassicaceae family has been increasingly reported.

Objective: To characterize cabbage—*Brassica oleracea* var *capitata*—allergy and its major allergens.

Methods: A prospective study was performed, recruiting 17 patients allergic to cabbage, and control subjects. Skin prick tests and double-blind placebo-controlled food challenges were performed. A major allergen was isolated from cabbage by RP-HPLC and characterized by N-terminal amino acid sequencing and matrix-assisted laser desorption/ionization mass spectrometry analysis. Specific IgE determinations, IgE immunoblots, and CAP-inhibition assays were also performed. **Results:** Skin prick test and specific IgE were positive to cabbage in all patients. Five of them referred anaphylactic reactions when eating cabbage, and in another 5 patients, cabbage allergy was further confirmed by double-blind placebo-controlled food challenge. Most of them showed associated sensitizations to mugwort pollen, mustard, and peach. A 9-kd cabbage IgE-binding protein, Bra o 3, was identified as a lipid transfer protein (LTP) with 50% of identity to peach LTP Pru p 3. Skin prick test with Bra o 3 showed positive results in 12 of 14 cases (86%). On CAP inhibition assays, Bra o 3 managed to inhibit significantly the IgE binding to cabbage, mugwort pollen, and peach. Both Bra o 3 and Pru p 3 were recognized by IgE from the patients' sera. **Conclusion:** Bra o 3, a cabbage LTP, is a major allergen in this food, cross-reacting with mugwort pollen and with other plant foods, such as peach.

Clinical implications: Cabbage IgE-mediated allergy is a potentially severe condition that can present with other plant food and pollen allergies. (J Allergy Clin Immunol 2006;117:1016-1026.)

Key words: Allergen, anaphylaxis, *Brassica oleracea*, cabbage, cross-reactivity, food allergy, lipid transfer protein, mugwort pollen, mustard, peach

IgE-mediated allergy to foods belonging to the Brassicaceae family has been increasingly reported in recent years, especially to mustard.¹⁻³ Cabbage—*Brassica oleracea* var *capitata*—is another member of the same plant food family that is consumed worldwide both cooked and raw. Despite its wide use, only anecdotal cases of cabbage immediate hypersensitivity have been described so far.^{4,5}

Regarding mustard allergy, possible cross-reactivity with other members of the Brassicaceae family has been suggested, but some authors could not confirm this hypothesis.⁶⁻⁸ However, in a previous study describing 38 patients allergic to mustard, we have found that near 40% of them were also allergic to cabbage.⁹ Moreover, most of these patients showed also associated sensitization to mugwort—*Artemisia vulgaris*—pollen, as well as to other plant foods, such as Rosaceae fruits, nuts, legumes, and corn, suggesting a new mugwort pollen plant food cross-reacting syndrome.⁹ The panallergens responsible for this syndrome remain to be identified.

Major allergens of white and oriental mustard seeds, Sin a 1 and Bra j 1, respectively, have been previously characterized as seed storage proteins, belonging to the 2S albumin family, with an approximate molecular weight of 14 to 16 kd.^{10,11} More recently, an 11S globulin storage protein of 51 kd has been isolated and identified as a novel major allergen of mustard seeds.¹² The low levels, or even absence, of both groups (2S and 11S) of seed storage proteins in pollens and other plant organs, such as leaves and fruits, lead to the suspicion that these allergen families are probably not responsible for the cross-reactivity among cabbage, mustard, and other plant foods and pollens.

We have studied a group of patients allergic to cabbage, and have searched for major allergens that could cross-react among cabbage, mugwort pollen, and other plant foods.

METHODS

Selection of patients and control subjects

A prospective study was designed in our Allergy Section, composed of a detailed clinical history, *in vivo* tests, and blood

From ^athe Departamento de Biotecnología, Escuela Técnica Superior Ingenieros Agrónomos, Universidad Politécnica de Madrid, Madrid, and ^bthe Sección de Alergia, Hospital Universitario de Gran Canaria Dr Negrín, Las Palmas de Gran Canaria.

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Reprint requests: Carlos Blanco, MD, PhD, Servicio de Alergia, Hospital de Gran Canaria Dr Negrín, C/Bco de la Ballena s/n, 35012 Las Palmas de GC, Spain. E-mail: cblague@gobiernodecanarias.org.

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Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge
 LTP: Lipid transfer protein
 MALDI: Matrix-assisted laser desorption/ionization mass spectrometry
 OAS: Oral allergy syndrome
 rPru p 3: Recombinant Pru p 3
 SPT: Skin prick test

drawing for *in vitro* assays. During a 2-year running period, every patient reporting immediate adverse reactions related to cabbage ingestion, suggestive of being IgE-mediated, and showing positive skin prick test (SPT) to fresh cabbage, was included. A control group of 17 subjects allergic to dust mite, nonsensitized to either foods or pollens, matched for age and sex with our patients, was also recruited. The same *in vivo* tests were performed in both patients and controls. An additional group of 8 patients allergic to mugwort pollen with no associated food sensitization was also used as control for SPT. Meanwhile, a pool of sera from those control subjects allergic to dust mites who showed specific IgE to *Dermatophagoides pteronyssinus* higher than 3.5 kU/L ($n = 5$) was used as control for *in vitro* tests. Written informed consent was obtained from all participants, and the protocol of this study was approved by the Research and Ethics Committees of the Hospital.

Crude food extract preparations

Cabbage (*Brassica oleracea* var *capitata*) leaves, mustard (*Sinapis alba*) seeds, and peach (*Prunus persica*) peels were defatted with cold acetone ($2 \times 1:5$ [wt/vol] for 1 hour at 4°C) and ethanol: ether ($1:3$ [vol/vol], $3 \times 1:5$ [wt/vol] for 1 hour at 4°C). After drying, mustard and cabbage were extracted with 0.5 mol/L NaCl ($2 \times 1:10$ [wt/vol] for 1 hour at 4°C). Peach peels were extracted with 0.1 mol/L TRIS-HCl buffer, pH 7.5, 10 mmol/L EDTA ($1:5$ [wt/vol] for 1 hour at 4°C), then washed with H₂O and finally re-extracted with 1.5 mol/L LiCl ($1:5$ [wt/vol] for 1 hour at 4°C). These extracts were dialyzed (cutoff point, 3.5 kd) against H₂O and freeze-dried. Protein concentration was quantified according to the method of Bradford.¹³

Allergen purification and characterization

The cabbage extract was separated by RP-HPLC on a Nucleosil 300 C4 column (8×250 mm; particle size 5 μ m; Scharlau Science, Barcelona, Spain). Elution was performed with a linear gradient of acetonitrile in 0.1% (vol/vol) trifluoroacetic acid (0% to 10% for 15 minutes and 10% to 100% for 150 minutes, at a flow rate of 1 mL/min). Lipid transfer protein (LTP) containing fractions were immunodetected with both anti-Pru p 3 antibodies¹⁴ and a serum pool from patients allergic to cabbage, and then pooled, freeze-dried, and dissolved in H₂O. The isolated protein was quantified by a commercial bicinchoninic acid test (BCA; Pierce, Cheshire, United Kingdom).

SDS-PAGE was performed by the method of Laemmli¹⁵ on XCell SureLock Mini-Cell (Invitrogen, Carlsbad, Calif). N-terminal amino acid sequencing was performed with an Applied Biosystems 477A gas-phase sequencer (Foster City, Calif) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry analysis with a Bi-flex III Spectrometer (Bruker-Franzen Analytik, Bremen, Germany), according to standard methods.¹⁶

SPTs

Skin prick tests were performed in all patients and controls according to standard procedure by trained personnel who were unaware of the subjects' clinical histories, with panels of aeroallergen

(including dust mites, fungi, pollens, animal epithelia, and natural rubber latex) and food (milk, egg, fish, nuts, fruits, mustard, kiwi, cereals, seafood, and legumes) commercial extracts, as detailed elsewhere.⁹ In the same way, SPTs were also performed with the crude extract from cabbage (2 mg/mL protein in a 1:1 PBS buffer/glycerol solution), with purified LTP from cabbage (named as Bra o 3 by the International Union of Immunological Societies Allergen Nomenclature Sub-Committee), as well as with previously described purified LTP from peach (Pru p 3) and mugwort pollen (Art v 3, isolated as previously reported),¹⁷ using these purified allergens at a protein concentration of 20 μ g/mL in a 1:1 PBS buffer/glycerol solution.

Meanwhile, skin tests with fresh cabbage was made by the prick-prick method, which was also applied for testing patients who underwent double-blind, placebo-controlled food challenge (DBPCFC) with the cabbage preparation used for that purpose. Results of SPT are expressed as the mean wheal diameter (in mm) after 15 minutes of puncture. Histamine dihydrochloride (10 mg/mL) and physiologic saline solutions served as positive and negative controls, respectively. A mean wheal diameter greater than 3 mm compared with the saline control was considered a positive response.

Food challenges

Patients were challenged in a double-blind, placebo-controlled fashion, following a standardized protocol.¹⁸ Food challenge was not performed when a patient had a convincing history of cabbage severe anaphylaxis. A fresh cabbage was liquified with an electric mixer and then freeze-dried (7% wt recovery rate). The lyophilized cabbage was masked in a natural-yoghurt based vehicle, containing a mix of vanilla and anisette juices, sugar, and crushed wheat biscuits. This mix was free of sulfites, and all ingredients of the vehicle were known to be tolerated by all patients. Normal intake in a serving of cabbage salad was estimated to be around 10 g for an adult, equivalent to 700 mg of our lyophilized cabbage. A previous study in volunteers without cabbage allergy was performed to demonstrate that such a dose could be masked by this method.

Preparation of challenges was performed in the same morning, and subjects were then randomly assigned to either food or placebo (vehicle), with a 2-hour interval between first and second part of the challenge. Increasing doses (60, 180, 460 mg) were administered with a 30-minute interval until symptoms appeared or a cumulative dose of 700 mg of lyophilized cabbage was reached. Cabbage allergy was accepted if the subject had symptoms after challenge with active substance and not with placebo.

Total and specific IgE determinations

Total IgE was determined by the UniCAP System (CAP; Pharmacia Diagnostics, Uppsala, Sweden), following the manufacturer's instructions. Specific IgE to cabbage (code f216), to common aeroallergens, and to other foods implicated in adverse reactions for each patient were also determined with the CAP assay, and values greater than 0.35 kU/L were considered positive.

Specific IgE binding to Bra o 3 was performed as previously described for recombinant LTP from peach (rPru p 3)¹⁴ by using 1:9 dilutions in PBS buffer (0.1 mol/L sodium phosphate, pH 7.0, 0.15 mol/L NaCl) of individual sera from patients allergic to cabbage, or of a pool of sera from control subjects allergic to dust mites. Microtiter plates were coated with 50 μ L purified proteins—Bra o 3 or rPru p 3—at 3 μ g/mL, or cabbage extract at 15 μ g/mL, in PBS buffer for 1 hour at 37°C. PBS buffer with 1% BSA (wt/vol) was used as negative control for solid phase. These tests were performed in triplicate.

CAP inhibition assays

A pool of sera from 12 patients allergic to cabbage, each showing specific IgE values to cabbage higher than 3.5 kU/L (CAP class III and up), was used for CAP inhibition assays. Duplicated samples of

TABLE I. Clinical characteristics of 17 patients with cabbage IgE-mediated hypersensitivity

Patient no.	Age (y)/sex	Atopy	Total IgE (kU/L)	Symptoms reported	Cabbage			Other clinically relevant food allergies
					Prick-prick (mm)	Specific IgE (kU/L)	DBPCFC symptoms	
1	31/F	EIA	39	EIA	5	4.7	ND	Mustard, kiwi
2	32/F	R, BA	12	U/AE	14	3.0	OAS/AE	Mustard, peach, nuts, legumes
3	27/F	R	361	OAS	10	10.3	Negative	Mustard, peach, nuts, banana
4	29/F	R	50	A	9	12.1	ND	Mustard, peach
5	28/M	R	49	A	7	5.8	ND	Peach, nuts
6	32/F	R, BA	221	U/AE	4	0.9	OAS/U	Mustard, peach, nuts
7	22/M	R, BA	1516	OAS	10	26.4	OAS	Mustard, tomato
8	39/F	R	322	U/AE	6	3.4	Negative	Peach, avocado, nuts
9	31/M	R	710	OAS	20	5.9	ND	Peach, nuts, corn
10	43/F	R, BA	345	OAS	13	4.9	OAS	Mustard, peach
11	30/F	R, BA	681	OAS	9	17.9	OAS	Mustard, nuts
12	17/M	R, BA	130	OAS	9	1.9	ND	Peach
13	37/F	R, BA	113	OAS	10	4.0	Negative	Mustard, nuts
14	31/F	R	36	A	7	0.9	ND	Mustard, peach, corn
15	30/M	R	294	OAS	6	8.8	ND	Mustard, kiwi
16	31/M	R	314	A	4	15.0	ND	Peach
17	32/F	R	596	OAS	7	11.4	Negative	Mustard, nuts

A, Anaphylaxis; AE, angioedema; BA, bronchial asthma; EIA, exercise-induced anaphylaxis; ND, not done; R, rhinitis; U, urticaria.

the undiluted pool of sera were premixed 1:1 (vol/vol) with 5 progressive 1:10 dilutions of the inhibitor solution in PBS buffer, pH 7.4, containing 0.03% (wt/vol) of serum albumin. After 2-hour incubation at room temperature, specific IgE levels to cabbage, mustard, peach, broccoli, Brussels sprouts, and cauliflower, as well as to mugwort pollen, were determined, following the CAP manual. Mustard, peach, and milk (as negative control) commercial extracts (ALK-Abelló, Spain); crude cabbage extract starting at 2 mg/mL protein; and purified proteins Bra o 3, Art v 3, and rPru p 3, starting at 20 µg/mL, were used as inhibitors.

Immunodetection assays

Samples (15 µg of protein of crude extracts from cabbage, mustard, and peach, and 3 µg of purified allergens) were fractionated by SDS-PAGE using Novex tricine gradient 10% to 20% acrylamide gels (Invitrogen), and electrotransferred onto nitrocellulose membranes as described by Towbin et al.¹⁹ After blocking with 5% BSA (wt/vol) in PBS buffer, membranes were incubated overnight with the pool of sera from patients allergic to cabbage (1:5 dilution in 0.5% BSA, 0.05% Tween-20 [vol/vol] in PBS buffer), washed with 0.05% Tween-20 in PBS buffer, and then treated with mouse anti-human IgE mAb HE-2 ascitic fluid (1:3000 dilution for 1 hour).²⁰ After washing, a 1:5000 dilution of rabbit antimouse IgE peroxidase-conjugated antibody (DAKO A/S, Glostrup, Denmark) was added, and the membranes were incubated for 1 hour. Detection of IgE-binding components was performed by enhanced chemiluminescence, following the manufacturer's instructions (Amersham Biosciences, Little Chalfont, United Kingdom). A pool of sera from subjects allergic to dust mite was tested as negative control.

RESULTS

General characteristics

Seventeen cabbage-hypersensitive patients were included in the study, whose general characteristics are

summarized in Table I. Mean age ($\pm$ SD) was 30.7 ± 5.9 years, ranging from 17 to 43, and with a predominance of female subjects (11:6). Sixteen patients (94.1%) were atopic, as defined by a personal history of respiratory allergy, together with positive SPT to several common aeroallergens, including mugwort pollen in all but 1 case. Out of the atopic group, 7 patients (41.2%) had rhinitis and bronchial asthma, and 9 (52.9%) showed only rhinitis.

Clinical manifestations of cabbage allergy reported by patients consisted of oral allergy syndrome (OAS) in 9 cases (52.9%), OAS with urticaria/angioedema in 3 (17.6%), and systemic anaphylaxis in 5 (29.4%), 1 of them with cabbage-dependent exercise-induced anaphylaxis. SPT with fresh cabbage were positive in all of them, with mean wheals ($\pm$ SD) of 8.8 ± 4.0 mm. Total IgE ranged from 12 to 1516 kU/L, with a geometric mean of 180.4 kU/L. Specific IgE to cabbage showed also a positive result in all patients, with a geometric mean of 12.7 kU/L, ranging from 0.88 to 26.4 kU/L, and negative in control subjects. Most of our patients showed several associated clinically relevant food allergies, including mustard in 12 (70.6%) and peach in 10 cases (58.8%; Table I). Regarding other vegetables belonging to the Brassicaceae family, 10 patients were also allergic to cauliflower, manifested as OAS in 7 and urticaria/angioedema in 3 cases, with all of them showing positive SPT (9.5 ± 3.0 mm) and specific IgE (mean, 6.6; range, 1.8–34.3 kU/L) to this food.

Double-blind food challenges

Eight patients did not undergo DBPCFC because of severe symptoms (n = 5), pregnancy (1), or denial of

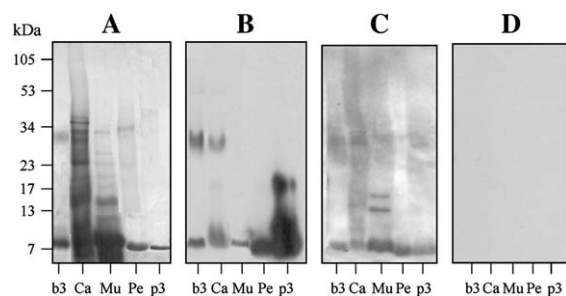


FIG 1. Protein staining with Coomassie blue (**A**) and immunodetection with anti-Pru p 3 rabbit polyclonal antibodies (**B**) or IgE of a pool of sera from patients with cabbage allergy (**C**) or from control subjects with dust mite allergy (**D**) of the following SDS-PAGE separated samples: cabbage leaves (Ca), mustard seeds (Mu), peach peels (Pe), purified nBra o 3 (b3), and rPru p 3 (p3).

consent (2). The remaining 9 patients underwent DBPCFC, with all of them showing positive SPT to the cabbage preparation used for oral challenge. In 5 cases (55.5%), cabbage challenge was positive, with isolated OAS the most frequently symptom observed (in 3 patients). Nevertheless, 1 patient showed angioedema and another patient urticaria, in both cases accompanied by OAS. All patients improved after symptomatic treatment in less than 90 minutes. Mean cumulative reactive dose of lyophilized cabbage ($\pm$ SD) was 145 ± 70 mg, ranging from 58 mg to 233 mg, equivalent to 2.1 ± 1.0 g of fresh cabbage.

Immunodetection assays

Extracts from cabbage leaves, mustard seeds, and peach peels were separated by SDS-PAGE, showing components from 5 kd to 40 kd, those corresponding to cabbage and mustard (Fig 1, A). Protein bands of approximately 9 kd were recognized by anti-Pru p 3 rabbit polyclonal antibodies in the 3 protein extracts (Fig 1, B). These 9-kd bands were also among the main IgE-binding proteins in all 3 extracts (Fig 1, C). Interestingly, the serum pool from patients allergic to cabbage clearly reacted also with purified Pru p 3.

Altogether, these results suggested that the 9-kd IgE-binding protein from cabbage could be a LTP.²¹ To support this notion, the protein was purified from the salt extract by RP-HPLC (not shown). The isolated allergen, Bra o 3, behaved as a single band of the expected molecular size in SDS-PAGE, which was recognized by anti-Pru p 3 antibodies and IgE of sera from patients allergic to cabbage (Fig 1).

Allergen characterization

Purity of Bra o 3 was confirmed by the single N-terminal amino acid sequence obtained that did not present heterogeneity at any determined residue, as well as by the single and sharp peak at 9026 d detected in MALDI analysis (not shown). The 20 N-terminal amino acids determined for Bra o 3 (AISC GTVTSNLAPCAVYLMK) displayed 50% identity with the corresponding region of Pru p 3 (identical residues are shown in *bold letters*),

TABLE II. Results of SPT in a group of 17 patients with cabbage immediate hypersensitivity expressed in millimeters of mean wheal diameter 15 minutes after puncture

Patient no.	Cabbage		Mustard	Peach		Mugwort pollen	
	Crude extract	Bra o 3		Commercial extract	Pru p 3	Commercial extract	Art v 3
1	6	—	—	5	3	—	—
2	11	5	8	13	7	8	5
3	7	6	5	7	7	8	7
4	9	6	6	6	7	8	5
5	9	7	5	6	3	6	5
6	ND	ND	6	4	ND	4	ND
7	11	10	15	6	5	4	3
8	8	6	5	14	8	5	5
9	ND	ND	10	—	ND	8	ND
10	12	7	9	8	7	5	11
11	11	5	8	10	8	8	5
12	ND	ND	12	10	ND	5	ND
13	7	6	4	7	4	7	5
14	7	5	6	11	9	7	6
15	11	3	7	6	5	4	3
16	10	6	9	7	6	7	15
17	5	—	6	—	5	5	—

—, Negative response; ND, not done.

and were identical to those deduced from a cDNA clone encoding a putative rape—*Brassica napus*—LTP (accession no. Q9ZSL7), included in the European Molecular Biology Laboratory database.

Skin tests with food extracts and purified proteins

Skin prick tests with the crude cabbage extract were positive in all patients, with mean wheals ($\pm$ SD) of 8.8 ± 2.2 mm (Table II). Most patients also showed positive SPT to mustard (16/17), peach (15/17), and mugwort pollen (16/17) commercial extracts. Fourteen patients were also skin tested with natural purified LTPs from cabbage, peach, and mugwort, showing positive results in 12 of 14 to Bra o 3 (85.7%), 14 of 14 to Pru p 3, and 12 of 14 to Art v 3, with mean wheals of 5.2 ± 2.5 , 5.4 ± 3.9 , and 6.0 ± 1.9 mm, respectively. Results of SPT were all negative in control subjects allergic to dust mite; meanwhile, 8 controls allergic to mugwort pollen showed positive SPT only to the mugwort pollen commercial extract (wheal, 7.5 ± 3.8 mm), whereas their SPT responses were negative to both plant food extracts and to the purified LTPs, including Art v 3.

ELISA and CAP assays

Specific IgE to Bra o 3 was found in 16 of 17 (94%) individual sera from patients allergic to cabbage, as determined by ELISA (Fig 2). All positive sera showed also specific IgE to Pru p 3, and 15 of them to the crude cabbage extract in the same ELISA tests; meanwhile, specific IgE neither to cabbage nor to these purified allergens were detected in the pool of sera from control subjects allergic to dust mite.

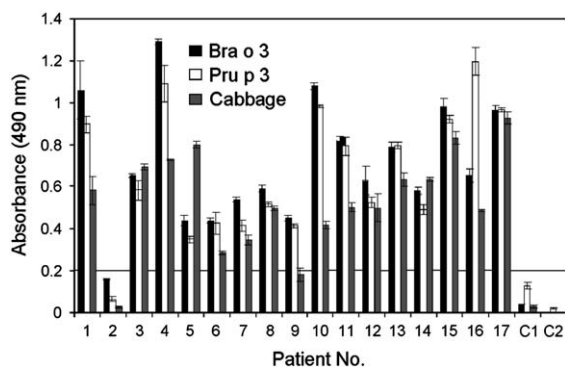


FIG 2. Specific IgE to nBra o 3, rPru p 3, and crude cabbage extract of 17 individual sera from patients with cabbage allergy. A serum pool of subjects with allergy to mites (C1) and BSA in solid phase (C2) were tested as negative controls. Means ($n = 3$) and SDs (bars) are represented. Specific IgE levels were considered positive when greater than 0.2 optical density units.

CAP-inhibition assays with the pool of sera from patients allergic to cabbage and cabbage as solid phase (Fig 3) indicated that a high proportion of the IgE was directed toward LTP allergens. Thus, when used as inhibitor, Bra o 3 manages to inhibit as much as 70% of the total IgE binding, Pru p 3 maximum inhibition was in the same range (73%), Art v 3 reached 48%, and mustard and peach commercial extracts reached 82% and 84%, respectively. Estimated concentrations of the purified allergens necessary to inhibit 50% of the total IgE binding to cabbage were 1.8 $\mu\text{g/mL}$, 0.8 $\mu\text{g/mL}$, and 36 $\mu\text{g/mL}$ for Bra o 3, Pru p 3, and Art v 3, respectively. A milk extract was used as negative control for this assay, showing inhibitions lower than 15% at the maximum concentration. In addition, preincubation of the sera with Bra o 3 also significantly inhibits the IgE binding to other extracts used in solid phase, such as broccoli (73%), cauliflower (57%), mugwort pollen (61%), and peach (32%; not shown).

DISCUSSION

Regarding food IgE-mediated allergy, it is now well known that cross-reactions among allergens from different sources, as a result of similarities among their IgE epitopes, are responsible for very significant clinical disorders. A clear example is the pollen-food allergy syndrome, which is the most common food hypersensitivity in the adult population, and includes several distinct clinical associations, such as birch pollen with Rosaceae fruit, ragweed pollen with melon, and mugwort pollen with celery allergies.²²

These pollen-food cross-reactions seem to be the consequence of the structural homology among allergens belonging to a very limited number of protein families. Among them, both Bet v 1 homologous, a group of plant-defence or pathogenesis related proteins, and profilins, which are cytoskeletal proteins in the eukaryotes, seem to play a critical role.²³ More recently, another group of pathogenesis related proteins known as LTP has been

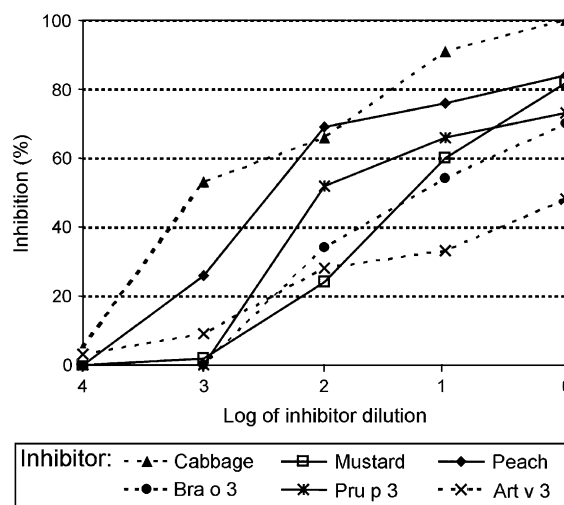


FIG 3. CAP-inhibition assay using cabbage in solid phase. Inhibitors included the crude cabbage extract, commercial mustard and peach extracts, and purified LTPs Bra o 3, Art v 3, and Pru p 3. Maximum inhibitor concentrations used were 2 mg/mL for the crude extract and 20 $\mu\text{g/mL}$ for the purified proteins.

identified as food-pollen cross-reacting allergens, especially in the Mediterranean area.²¹

Food allergy to members of the Brassicaceae family, especially to mustard, has been recently identified as a relatively common—at least in certain areas—and potentially severe condition. As a consequence, mustard has been included in the list of 12 potential allergenic ingredients to be labeled in the new European Union directive on food labeling.²⁴ Curiously, allergy to other members of this family, such as cabbage, had been rarely reported.^{4,5} However, we have recently studied a group of patients with mustard allergy, all of them showing sensitization to other members of the same family, and most of them with clinical allergy to cabbage leaves, peach fruit, and mugwort pollen.⁹ This cross-reactivity profile cannot be explained adequately by the previously identified 2S and 11S mustard allergens,¹⁰⁻¹² which accumulate specifically in seeds. Therefore, we decided to search for potential cross-reacting allergens widely distributed throughout the plant, and to select cabbage leaves as the target food. This approach has allowed us to detect a protein of around 9 kD as a putative candidate by preliminary IgE immunodetection assays, and to identify it as a LTP, named Bra o 3, the cabbage homologous with the major allergens from peach fruit (Pru p 3) and *Artemisia* pollen (Art v 3).

Furthermore, in the current study, we have managed to demonstrate fully the relevance of Bra o 3 in cabbage allergy, both *in vivo* and *in vitro*. More specifically, 94% of our patients showed specific IgE to Bra o 3, as detected by ELISA, and in 86% of them, a positive SPT to Bra o 3 was found. In CAP-inhibition assays, although performed with pooled serum, which could lead to overestimation of the immunologic properties of those sera with higher specific IgE, as much as a 70% inhibition of the total IgE binding to cabbage, used in solid phase, was reached when the

pool of our patients' sera was preincubated with Bra o 3. Therefore, Bra o 3 is clearly a major cabbage allergen, and this food is to be added to the growing list of plant foods and pollens in which LTPs have been identified as relevant allergens.²¹

Moreover, the very close botanical relationship among cabbage, cauliflower, broccoli, and Brussels sprouts (all of them classified as varieties of *Brassica oleracea*), together with the significant inhibition of the IgE-binding to broccoli and cauliflower extract (>50%) by Bra o 3, suggests that all of these plant foods could share similar allergens. The same seems to be true for mustard, which showed a reactive band of around 9 kd. Thus, our data point to LTPs—and not to storage proteins—as the major allergens responsible for cross-reactions among Brassicaceae foods, at least in our population.

It is noteworthy that in our series of patients with mustard allergy previously described, we had found very significant associations with mugwort pollen allergy, as well as with several plant food allergies, such as other Brassicaceae, Rosaceae fruit, nut, legumes, and corn.⁹ Consequently, we have proposed a new mugwort pollen–mustard allergy syndrome, completely different from the mugwort-celery-spice allergy syndrome formerly reported in Central Europe.²⁵ Interestingly, the pattern of plant food sensitizations that we have observed resembles those described for LTP-hypersensitive patients,^{26,27} thus suggesting that LTPs may be responsible for this new clinical syndrome.

In this line, LTPs have been reported as relevant allergens not only in mugwort pollen but also in most plant foods implicated in the syndrome, namely Rosaceae fruit, nuts, and corn,²¹ and now also in Brassicaceae foods. Moreover, the majority of our patients with cabbage allergy showed not only associated allergies to mugwort pollen and peach but also positive SPT to Pru p 3 in 100% and to Art v 3 in 86% of patients tested. Finally, Pru p 3 was able to inhibit more than 70% of the total IgE binding to cabbage in CAP-inhibition assays, demonstrating cross-reactivity among LTPs from Brassicaceae foods and Rosaceae fruits. In the same way, Bra o 3 managed to inhibit significantly the IgE binding to mugwort pollen and several plant foods used in solid phase.

Another finding also deserves consideration. It is known that Art v 3 is a major mugwort allergen, and in some patients with IgE to both Art v 3 and Pru p 3, the former behaves as the primary sensitizing agent.²⁸ The fact is that, although most of our patients with mugwort pollen–food allergy showed positive SPT to Art v 3, all 8 mugwort pollen–sensitized but not food-sensitized controls showed negative SPT to this purified protein. On the basis of these preliminary data, we can suggest that a simple SPT with the mugwort pollen LTP, Art v 3, could be enough to distinguish between mugwort pollen–sensitized patients who are not at risk of having food allergy from patients who can develop severe food IgE-mediated allergic reactions to certain plant foods. This is a clear example of a possible application of component-resolved diagnosis to food allergy. However, more experimental data on the possible

role of pollen LTP in pollen–food cross-reactivity would be expected.

In conclusion, we describe a group of patients with cabbage allergy and characterize Bra o 3, a LTP, as a major cabbage allergen that cross-reacts with mugwort pollen and some plant foods.

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