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

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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;117:1423-9.)

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.

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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

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 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× 1:5 [wt/vol] for 1 hour at 4°C) and ethanol: ether (1:3 [vol/vol], 3× 1:5 [wt/vol] for 1 hour at 4°C). After drying, mustard and cabbage were extracted with 0.5 mol/L NaCl (2× 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 H_2O 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 H_2O 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 μm; 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 antibodies[14] and a serum pool from patients allergic to cabbage, and then pooled, freeze-dried, and dissolved in H_2O. The isolated protein was quantified by a commercial bicinchoninic acid test (BCA; Pierce, Cheshire, United Kingdom).

SDS-PAGE was performed by the method of Laemmli[15] 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 Biflex 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 μg/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 μL purified proteins—Bra o 3 or rPru p 3—at 3 μg/mL, or cabbage extract at 15 μg/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.