

Carob is not allergenic in peanut-allergic subjects

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Summary

Background The antigenic potential of proteins from the carob bean, a member of the legume family used as a food additive, have not so far been investigated and legumes share antigenic proteins with peanut, a potent trigger of anaphylaxis.

Objective To assess the carob protein determinants of sensitization in peanut-allergic children.

Methods In a prospective, double-blind, placebo-controlled study 12 patients (median age 9.5 years) with a history of hyperreactivity to peanut (anaphylaxis) were assessed. Skin prick tests with a commercial peanut allergen, raw carob pulp, raw and cooked carob cotyledon formula were used to confirm the history. RAST for peanuts and cooked carob were used to evaluate sensitization to these proteins. Carob-specific IgE were identified by immunoblotting analyses. Allergic reactivity was evaluated during double-blind placebo-controlled food challenges (DBPCFC; 5.5 g carob extract and cooked carob cotyledon formula).

Results Peanut allergen-induced skin prick test positivity in all children (confirmed during double-blinded challenge in 6/12 patients), carob pulp in 3/12 patients, raw carob bean in 6/12, and cooked carob cotyledon formula in none. RAST were positive for peanut in all cases but negative for carob beans in 9/12 cases. Immunoblot analyses found peanut-specific IgE in all cases and raw carob bean-specific IgE in eight cases. Carob allergens were identified in the 17.5, 48, and 66 kDa MW bands. The least allergenic density was found for cooked carob proteins. There was no clinical reactivity with either raw or cooked carob during DBPCFC.

Conclusions These data suggest that carob-specific sensitization, apparent both *in vitro* and in SPTs, can be concordant with peanut allergy and that cooked carob can be ingested by children who are allergic to peanuts. That heat-processing deactivates carob protein allergenicity has dietary implications for polyallergic children.

Keywords: carob allergy, cross-reactivity, double-blind placebo-controlled food challenge, immunological tolerance, peanut allergy

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Introduction

Peanut can trigger fatal or near-fatal food anaphylaxis [1–3]. Although it shares antigens with the major genera of Leguminosae, clinically relevant cross-reactivity among legumes is considered uncommon [4]. Although the locust

tree belongs to this botanical family, hypersensitivity to its bean proteins among peanut-allergic children is not documented, however, respiratory symptoms have been attributed to carob exposure [5–7]. Both carob flour (E410) and carob galactomannan are common additives in processed foods and the former has recently been introduced as a thickening agent in antiregurgitation formulas [8]. The main goal of this study was to investigate whether children who are allergic to peanuts can cope with carob proteins in

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their diet. Immunological sensitization to carob and clinical tolerance to carob-specific allergens were thus prospectively assessed in a consecutive series of 12 children with a history of anaphylactic reaction to peanuts.

Patients and methods

Patients admitted for immediate onset of allergic symptoms after peanut exposure between 1 July 1994 and 30 June 1995 were considered when presenting for follow-up visits. Informed parental consent was obtained for participation and blood tests after institutional review of the ethics of the study aim and protocol. Two girls and 10 boys (median age 9.5 years; range 4.12–14 years) were recruited. Presenting allergic symptoms were asthma ($n = 1$), lip oedema and asthma ($n = 1$), urticaria ($n = 2$), urticaria and laryngeal stridor ($n = 4$), allergic rhinitis and urticaria ($n = 2$), lip oedema and laryngeal stridor ($n = 2$). A peanut extract (Bayer, Baranzate, Milan, Italy) solubilized in glycerol 50% at 1 mg/mL concentration and toasted native peanuts were used in the skin tests [9]. double-blind, placebo-controlled food challenges (DBPCFC) were used to confirm the diagnoses of peanut allergy. Peanut capsules (Lofarma, Milan, Italy) were administered in doubling doses of 0.2, 0.4, 0.8, 1.6 g at 30 min intervals for 4 h. Children with swallowing difficulties were given capsule contents in apple juice (additive-free except for ascorbic acid) after a negative skin test for apple juice. DBPCFC with carob was performed on a second visit. The raw challenge food was fresh, raw, peeled carob cotyledons ground after removing the embryo. Cooked carob cotyledon formula (CCC; Nestargel, Nestlé, Vevey, Switzerland), a powdered carob bean weaning food industrially processed by heating at 60°C for 20 min, was the cooked challenge food. Both challenge meals were masked in the same apple juice which was used as placebo. Challenge was discontinued at the first onset of symptoms or with a negative response after the eighth dose.

SPT and RAST

Raw carob pulp and the raw ground cotyledons (re-suspended in glycerol 50% at 1 : 10 wt:vol), and reconstituted CCC were used for skin testing. Weal diameters wider than negative controls by at least 3 mm on a millimetre calibrated calliper disk up to 30 min after pricking were considered positive [10]. Ten mg/mL histamine phosphate in 50% glycosaline and glycosaline alone were used as positive and negative controls, respectively. A RAST (Pharmacia kit, Uppsala, Sweden) for peanut allergen, raw and cooked carob bean was carried out using a whole blood sample. RAST with carob were prepared as follows: 0.5–2 mg carob extract were suspended in 1 mL phosphate buffer solution,

shaken for 4 h, centrifuged and filtered on Millex 0.8 and 0.22 mm (Millipore, Bedford, MA, USA). Samples were then loaded on to paper disks activated by cyanogen bromide for binding. Allergen-specific IgE titres above 5 UI/mL defined RAST positivity [11].

DBPCFC

Challenge meal volume was based on the highest protein amount set for office procedure [12,13]. CCC 50 g was used in the cooked carob challenge to make up 250 mL of reconstituted formula at the recommended concentration of 20 g/100 mL. As CCC contains 11.2% proteins, the cooked carob challenge meal was composed of 5.6 g cotyledon proteins in apple juice administered in doubling doses of 0.33, 0.67, 1.33 and 2.67 g. The raw carob bean cotyledon is 15.5% proteins [14] and 36 g of dry carob cotyledons pounded in a mortar were used to administer the raw carob challenge in doses refracted in 2.4, 4.8, 9.6 and 19.2 g increments. The same apple juice was added as a mask and used as a placebo. Placebo and allergen challenges were administered on separate days over 4 h at 30-minute intervals.

Immunoblotting

The spectrum of IgE binding to peanuts and carob bean-specific component proteins was determined by immunoblot analyses of patients' sera. Proteins were transferred from an SDS-PAGE gel onto polyvinylidene difluoride (PVDF, Immobilon P, Millipore, Bedford, MA, USA) by electrophoretic elution. The transfer buffer was TRIS 25 mmol/L, glycine 193 mmol/L and methanol 20%. To verify the protein transfer, a solution of 0.2% ponceau S (in 3% TCA and 3% sulphosalicylic acid) was used; protein absence in blotted gel was verified using staining procedure [15]. After de-staining, PVDF membranes were blocked with 1% gelatin and washed three times with 0.25% gelatine solution (Bio Rad, Milan, Italy) in 150 mmol/L NaCl, 5 mmol/L EDTA, 50 mmol/L TRIS, and 0.05% Triton-X. The membranes were immersed in 0.25% gelatine solution 10 mL with 0.3 mL of allergic patients' serum. Antigen-IgE complex was detected using goat antihuman IgE antibodies labelled with alkaline phosphatase. After incubation in bromochloroindolylphosphate/nitroblue tetrazolium (BCIP/NBT) solution, an intense precipitate developed at the site of the enzyme binding.

Results

The history of peanut allergy was confirmed during challenge among six children only, as patients with laryngospasm were not challenged for fear of inducing anaphylactic shock. Skin tests to raw bean proteins were positive in half

Table 1. Positive results (*n*) at SPT, RAST, immunoblotting and DBPCFC outcome.

	SPT commercial extract	SPT fresh sample	RAST	Immunoblot	DBPCFC
Peanuts	12	12	12	12	6*
Raw carob pulp		3	2	†	†
Raw carob bean		6	3	8	0
Cooked carob bean‡		0	0	2	0

*Not performed because six patients reported previous laryngospasm. †not performed. ‡CCC (Nestargel™).

the cases and RAST in three, while no reactions were induced during DBPCFC. There was no evidence of sensitization (SPT, RAST, DBPCFC) to cooked carob. A single patient showed carob-specific IgE with cooked carob at immunoblotting (Table 1). Peanut-specific IgEs were identified by serum immunoblot analyses and carob-specific IgE (from raw carob) were also found in eight patients' sera. Peanut-specific IgE were foremost in the 63.5 and 17.5 bands in a position consistent with the reported molecular weights of the Ara h 1 and the Ara h 2 peanut allergens (Fig. 1). The 46 kDa band shows clearly and intermediate lanes also appear. Raw carob immunoblots display a minor allergenic density, with the same band visible at 17.5 kDa. Lanes appear at 48 and 66 kDa in the carob samples. In two cases CCC exhibits the identical 17.5 kDa band, although density is extremely reduced.

The outcomes of DBPCFC with both raw and cooked carob beans were negative.

Discussion

There are adult reports of rhinoconjunctivitis, asthma and urticaria induced by exposure or contact with carob-specific allergens [5–7] but a Medline literature search failed to identify reports of allergy to ingested carob. This is the first investigation of carob bean allergenicity through a description of its antigens. In this limited population, allergy symptoms were not induced on formal oral challenge. Tolerance to both heated bean proteins was seen on challenge despite evidence of hypersensitivity to the raw endosperm proteins. Carob-specific IgE were found in peanut-sensitive children, but carob antibodies did not

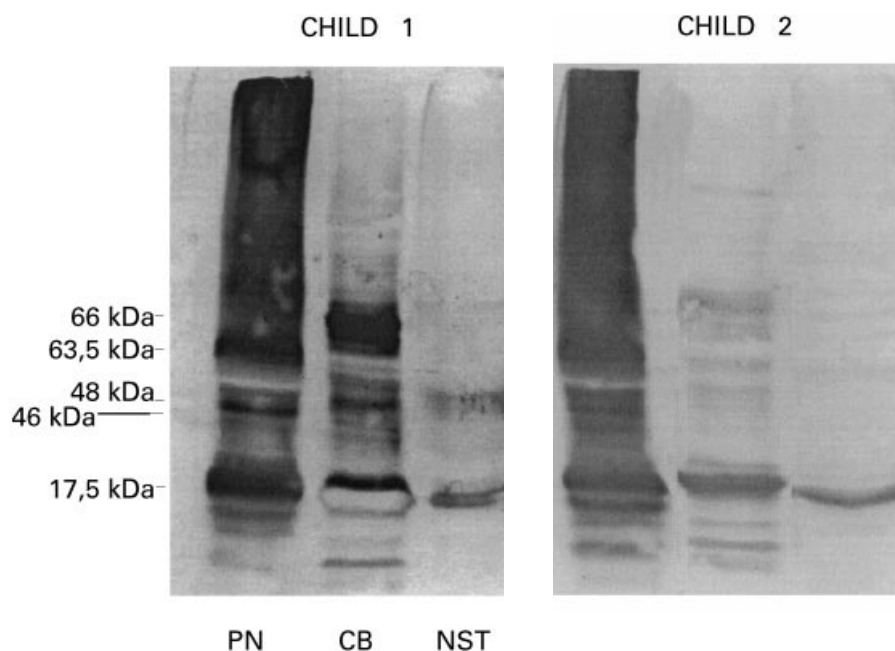


Fig. 1. Immunoblotting at autoradiography for peanut (PN) and carob (CB) allergens and cooked carob cotyledon formula (CCC) in two sera.

prime a skin response to cooked bean extract. Thus, raw and cooked carob panels were probed with the sera of peanut-sensitive children to detect specificity of binding. Carob IgEs were found in positions close to Ara h 2 antigens (Fig. 1). As Ara h 1 and Ara h 2 are found bonded to peanut acid glycoproteins [16,17], we infer that the carob bean contains glycoproteins with a similar allergenic potential. There is evidence that sensitization to legumes does not always translate into clinical reactivity, however, up to 94% of SPTs have yielded false positives for soybean [4,18,19]. The present data support the idea of a similar phenomenon occurring with CCC but, owing to sample size, no generalization should be inferred. Heat processing inhibited both hypersensitivity and the expression of serum carob-specific IgE on immunoblot panels when a cooked carob preparation was used in the challenge. In this context, the allergenic behaviour of carob closely resembles that of the soybean. Separated peanut proteins retain their allergenicity even when partially denaturated by freezing to -18°C and heating to 177°C [20]. We infer from this that heat processing reduces the allergenicity of carob proteins, and also does so more effectively than is the case when peanut is heated. If heat processing allows carob to be used by children with other allergies (and especially with peanut allergy) this may ease dietary restrictions [21]. Results from this limited series suggest that, when concomitant allergy to legume protein subunits is suspected, cooked carob beans may become a valuable protein source. Carob may thus also be acceptable as an additive in processed foods, antiregurgitation and weaning formulas as avoidance of its proteins cannot be recommended on the basis of crossreactivity, which is considered low between Leguminosae. Furthermore, as sensitization to legumes such as pea and lentil has been described in absence of sensitization to peanut [4,22], we cannot exclude that allergic reactions can follow ingestion of carob by children.

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