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Gluten-free food as source of hidden allergen (lupine)

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Key words

Food allergen, lupine, gluten-free food

Summary

A woman, 68 yrs, developed an anaphylactic reaction after tasting a few pieces of gluten-free pasta. She was not celiac but was preparing a meal for her celiac nephew. The culprit pasta contained lupine flour and lupine proteins. Prick test with lupine extract was positive. ELISA and immunoblot analysis showed the presence of specific IgE to lupine in patient's serum.

Lupine allergy was first described in 1994 (1). Since then many cases of lupine allergy have been described, probably because the inclusion of lupine flour in food has steadily increased during the last decade. Allergic sensitization to lupine is considered clinically relevant especially in peanut-sensitized individuals, both adults (2) and children (3), although some cases of primary lupine allergy have also been described (4-6). This report describes an adult, without a history of food allergy or of sensitization to peanut, who developed an immediate systemic allergic reaction (anaphylaxis) after eating a few pieces of gluten-free pasta made with lupine flour.

Case report

A 68 year-old woman was referred to our service for allergological evaluation after an episode of generalized urticaria, epigastric pain, ocular itching, periocular oedema and dyspnoea occurring about 30 minutes after tasting 3-4 small pieces of gluten-free pasta ("maccheroncini") while preparing a meal for her celiac nephew.
The allergic reaction was promptly treated at the E.R. of the local hospital and there were no subsequent reactions.
Personal history was unremarkable except for the presence of seasonal rhino-conjunctivitis since the age of 40 yrs.
The label of the culprit pasta (BiAglut PastaMia®) declared the following ingredients: maize starch, potato flour, lupine flour and lupine proteins, fat acids.
SPT with commercial food extracts (cereal mix, legume mix, peanut, soy, peach, tomato, walnut, hazelnut, spices mix, cod, milk, white egg, yolk, almond, potato, shrimp, mussel) and pollens (grass, mugwort, pellitory, birch, hazelnut) (Lofarma S.p.A., Milan, Italy) were all negative except for birch pollen (mean diameter of the wheal 8 mm). SPT with lupine extract was positive (mean diameter of the wheal 10 mm). Histamine 10 mg/ml (positive control) 6 mm.

Patient's serum was positive to lupine extract as assessed by an ELISA IgE assay: 1,7 vs. 0,3 (control serum).
Immunoblot analysis showed that a certain number of components were recognized by patient's serum. More specifically, a wide zone comprised between about 50-100 kDa and a more restricted zone at about 18 kDa, perhaps corresponding to 2S albumins (Figure 1).

Discussion

The patient, according to the results of skin testing, was sensitized to both birch pollen and lupine, but not sensitized to peanuts. ELISA IgE assay and immunoblotting confirmed the presence of specific IgE to lupine in patient's serum. A cross-reactivity between birch pollen and lupine due to a Bet v 1 homolog allergen seems unlikely since Bet v 1 homolog allergens are heat- and pepsin-labile while the patient developed the anaphylactic reaction after the ingestion of pasta boiled at 100 °C for several minutes. Moreover, the patient, although sensitized to birch pollen, has never shown an oral allergic syndrome after the ingestion of fresh fruits (for example apple) which are a well known source of Bet v 1 homolog allergens. A recent study has shown that lupine allergy is more complicated than previously thought because many allergens are involved, both cross-reactive with other legumes and unique for lupine (5). Our immunoblot analysis showed a pattern of multiple recognition by patient's serum. The clinical pattern of the reaction lends support to the hypothesis that a stable allergen, not cross-reacting with peanut or other legumes, was primarily involved.
This case shows that gluten-free foods can be a source of hidden allergens and that their consumption is not exempt from allergological risks. Moreover, since celiac disease and IgE mediated allergy are independent phenomena that can coexist in the same individual, the repeated ingestion of lupine flour in celiac subjects using gluten-free food could be a potential risk for allergic sensitization; further studies are needed to elucidate this point.

Appendix

Preparation of lupine extract

Eight grams of defatted lupine flour was submitted overnight to an aqueous extraction in 100 ml of 0.1M phosphate-buffered saline, pH 7.4 (PBS).

After centrifugation supernatant was harvested and dialyzed against saline by membrane at 3.5 cut off, before to be filtered through a 0.22 μm membrane. Protein content was determined by Bradford's method and resulted 8.2 mg/ml. For SPT preparation, lupine extract was diluted 1:2 with glycerin.
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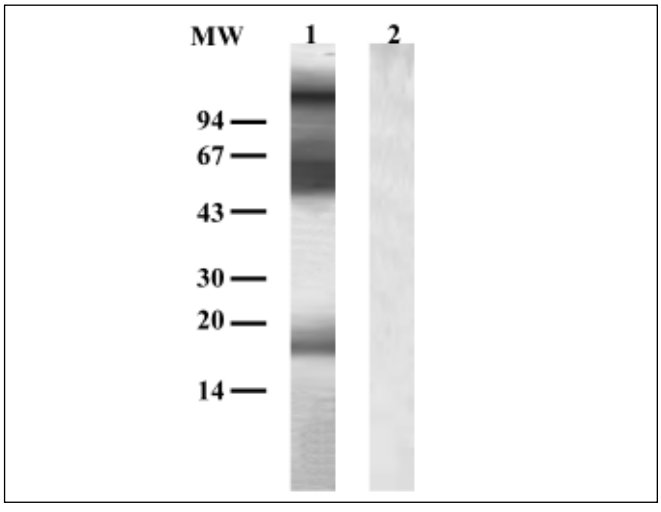
ELISA IgE

Two μg/100 ul (coating buffer: 15 mmol/L Na2CO3 and 35 mmol/L NaHCO3, pH 9.6) of lupine extract, (Maxisorp Nunc, Roskilde, Denmark) were added to 96-microtitre wells for coating phase. After washings, wells were saturated with 2% bovine serum albumin (BSA) in PBS (dilution buffer) for 2 hours at r.t.. Subsequently 100 ul of sera from normal subject and patient were added to wells and incubated for 2 hours at r.t.. Specific IgE was detected by adding a peroxidase-conjugated anti-human IgE goat serum (diluted 1:3500, Biospacific, Emeryville, CA, USA); a colorimetric reaction was induced by using tetramethylbenzidine/H2O2 as substrate. The enzyme reaction was stopped after 20 minutes by the addition of 1 mol/L HCl. Absorbance values (O.D) were read at 450 nm by spectrophotometer. Serum was considered positive when its OD value is at least two times higher than control one.

Figure 1 - IgE reactivity on lupine extract of patient's serum (lane 1) and normal serum (lane 2). M.W.: molecular weight standards



Immunoblotting

Electrophoresis of lupine extract (12 μg per lane) was carried out in a 10% polyacrilamide precast Nupage Bis-Tris gel according to manufacturer instructions (Invitrogen, Milan, Italy) at 180 mA for 1 h. The resolved proteins were transferred onto a nitrocellulose membrane (Protran BA 85, Schleicher and Schuell, Milan, Italy) according to Towbin (7). The membrane was saturated in TBS buffer containing 5% defatted dry milk (saturating buffer) and incubated with patient's serum or control normal serum diluted 1:2 in saturating buffer. Bound specific-IgE were detected by adding of peroxidase-conjugated anti-human IgE goat serum (diluited 1:1500, Biospacific, Emeryville, CA, USA) and ECL western blotting kit (Amersham, Milan, Italy) as substrate.

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