

Anaphylactic reaction to ingestion of *Quercus ilex* acorn nut

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Summary

Background A patient experienced an anaphylactic reaction after eating acorn nuts, fruit of the holm oak (*Quercus ilex*), one of the most abundant trees in Spain. Several urticaria episodes upon ingestion of peanuts were also referred.

Objective To assess the hypersensitivity reaction to acorn and to characterize the allergenic proteins involved.

Methods Cutaneous tests were performed using the skin-prick technique, using a large variety of grass, tree and weed pollens as well as fresh nuts and nut extracts. Specific IgE determination was assessed by RAST. IgE binding bands were determined by SDS-PAGE immunoblotting.

Results Skin-prick tests were strongly positive with acorn and peanut. *Olea europaea*, *Quercus alba*, *Quercus ilex* and grass pollens also elicited a weal higher than negative control. Patient serum had measurable levels of IgE antibodies especially to acorn, peanut and grass pollens. Only one protein band, of 17.9 kDa molecular mass, showed IgE-binding properties in the acorn extract. The possible homology of this strong allergenic protein with the group 1 tree pollen allergens was evidenced by the partial inhibition of the western blot with Bet v 1.

Conclusions We present a case of anaphylaxis to acorn ingestion as demonstrated by *in vivo* and *in vitro* results. A 17.9 kDa IgE-binding band, showing some homology to group 1 pollen tree allergens, was recognized by patient serum.

Keywords: *Quercus ilex* allergy, nut allergy, acorn allergens, Bet v 1

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Introduction

The acorn nut is the fruit of the holm oak (*Quercus ilex*), a tree which belongs to the Fagaceae family with two varieties, *rotundifolia* and *ilex*, indigenous to Spain. In fact, Spain possesses the world's largest areas of holm oak plantations [1], albeit sensitization to the pollen of this tree is rarely found [2].

The acorn is an edible (acorn nuts from other *Quercus* species are not edible), brownish fruit that is more frequently used in animal feeds than for human consumption; this could explain the fact that cases of sensitization to the *Q. ilex* nut have not been previously reported.

We present the case of a man who experienced an

anaphylactic reaction upon ingestion of acorn nuts, and several urticarial episodes after intake of peanuts. We decided to study the case in order to ascertain the acorn sensitization and to characterize in detail the allergens involved, as well as their possible relationship with other known allergens from the Fagaceae family.

Case report

A 45-year-old man with no relevant personal history experienced, on two occasions, a generalized urticaria 60 min after consuming peanuts. Several months later, within 15–20 min after deliberately ingesting three acorn nuts (without no other exacerbating factor involved), the patient presented generalized urticaria and angioedema, vomiting, diarrhoea,

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dizzines and hypotension, requiring assistance in an emergency room. Symptoms subsided after treatment with intravenous methylprednisolone and subcutaneous epinephrine.

Materials and methods

Acorn nut extract

Acorn fruits were collected from holm oak trees (*Quercus ilex*) in the Plasencia area (central western Spain). This plant material was first homogenized to a fine powder, defatted with diethyl ether (soxhlet) and stirred magnetically at 1000 rpm for 12 h at 4°C in 0.1 M phosphate buffer, pH 8.0 at 20% w/v. The extract was clarified by centrifugation at 5600 *g* for 30 min, filtered through a 0.22 µm pore diameter membrane and dialysed by tangential ultrafiltration with a 5 kDa cut-off.

Skin tests

Skin-prick tests were carried out using a battery of usual inhalants (mites, fungi, grass, weed, flower and tree pollens, epithelial derivates and foodstuffs), as well as with specific antigens (*Quercus ilex* pollen, acorn nut, peanut and other dried fruits). Acorn and nut extracts were prepared at 10 mg lyophilized extract/mL. Histamine dihydrochloride (10 mg/mL) and phenolated saline were used as positive and negative controls. The skin tests were read after 15 min and considered positive if a weal of 3 × 3 mm larger than saline control was obtained.

RAST

Solid-phase antigen was obtained by coupling the extract solution at 10 mg/mL to the 6 mm diameter CNBr-activated paper disks. RAST was performed with Phadezym RAST reagents (Pharmacia Diagnostics AB, Uppsala, Sweden) following the manufacturer's instructions.

SDS-PAGE-immunoblotting

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 12.5% polyacrylamide gels according to standard procedures.

Separated protein bands were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore, Milford, MA, USA), and immunostained with 2.5 mL of undiluted human serum (16 h at 4°C). The blots were then incubated with 1:25 diluted rabbit immunoglobulins to human ε-chains (DAKO, Glostrup, Denmark) conjugated to peroxidase. Bound peroxidase was detected by the addition of freshly prepared 0.06%

w/v of 4-chloro-1-naphtol solution (Bio-Rad Lab, Richmond, CA, USA) and 0.01% (v/v) H₂O₂ in TBS. Analysis of electrophoretic components was performed by image analysis in the BIO-IMAGE System (Millipore).

Specific detection of group 1 allergen was carried out by using a monoclonal antibody against Bet v 1 termed Bip 1, which was a kind gift from Drs Ruth Fritsch and Christof Ebner (Institute für Allgemeine & Experimentelle Pathologie, Universität Wien, Austria). Blot developing in this case was carried out by using a 1:1000 dilution of rabbit immunoglobulins to mouse IgG (DAKO, Glostrup, Denmark) conjugated to peroxidase.

SDS-PAGE immunoblotting inhibition was carried out by preadsorption of patient serum for 10 min with an equal volume of Bet v 1 (0.2 mg/mL) purified by electroelution from a *Betula verrucosa* pollen extract.

Results

Skin tests

Skin-prick tests with acorn and peanut were strongly positive (10 × 5 mm and 6 × 6 mm, respectively). Weals slightly smaller, but also positive were obtained with olive (*Olea europaea*, 3 × 3 mm), holm oak (*Quercus robur*, 4 × 3 mm), grasses and compositae pollen extracts. Prick-prick test using fresh acorn and peanut samples were also positive to a similar extent.

Immunoblottings

The presence of positive specific IgE levels to acorn, peanut, grass pollen and *Quercus ilex* pollen extracts was revealed by RAST (class 2 score).

SDS-PAGE immunoblotting of acorn nut and tested extracts, revealed with patient serum, is shown in Fig. 1. Only an IgE binding component was detected in acorn nut extract, at 17.9 kDa. On the contrary, the *Q. ilex* pollen extract showed seven faint IgE binding bands at 108.2 (extrapolated), 83.7, 44.4, 17.7, 16.9, 16.1 and 15.0 kDa. A strong IgE binding band at 37.3 kDa could be observed in the *L. perenne* blot, together with some minor bands at 98.1 (ext.), 88.0, 69.8 and 34.6 kDa. Two intense and close IgE binding proteins were resolved in the peanut extract at 20.3 and 18.6 kDa. No bands were observed in the rest of dried fruits tested (chestnut, hazelnut, almond and pistachio). Bands were proved to be specific as control membranes incubated with negative sera did not presented detectable bands in any case.

Preadsorption of human serum with isolated Bet v 1 produced a 62% inhibition of the IgE binding reaction (Fig. 2), as quantified in terms of optical density in Bio-image analyser. Bip 1 antibody recognized several bands in

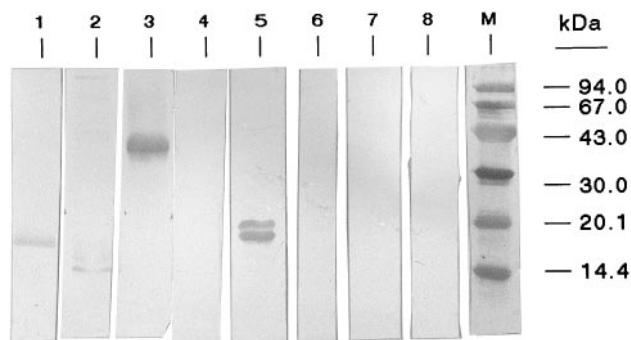


Fig. 1. SDS-PAGE immunoblotting of the following allergenic extracts: (1) Acorn nut, (2) *Quercus ilex* pollen, (3) *Lolium perenne* pollen, (4) chestnut, (5) Peanut, (6) Hazelnut, (7) Almond and (8) Pistachio. All of them immunorevealed with the patient serum. (M) mol. wt. markers stained with Amido Black B.

the *B. verrucosa* pollen extract with the main reactivity at 17 kDa; however, no bands could be detected in the acorn extract incubated with the same monoclonal antibody.

Discussion

This clinical case report describes a patient who experienced an anaphylactic reaction after acorn ingestion, fruit from the holm oak tree (*Quercus ilex*). The results demonstrated the presence of significant levels of specific IgE to acorn nut and also to peanut, the nut most frequently reported as inductor of allergic reactions [3].

The patient was skin-prick-test positive to acorn and also to some other pollen and nut allergens, presenting significant IgE levels to *Q. ilex* pollen and *L. perenne* pollen (Fig. 1). Although *Quercus ilex* is widely distributed in the world, and especially in Spain, cases of hay fever caused by its pollen have rarely been reported [2]. The study of the IgE binding pattern produced by the patient serum in different pollen and food extracts to which positive cutaneous and/or RAST response had been obtained could yield some valuable information. As can be seen in Fig. 1, only one allergen could be detected in the acorn nut extract (which does not exclude the existence of some other allergens labile to electrophoretic conditions). A coincident band at 17.7 kDa could be also observed in the *Q. ilex* pollen blot, albeit less intense. This could concur with the already known crossreactivity between components from pollens and seeds/fruit of the same plant [4]. It has been also substantiated that homologous protein from pollen and fruit of different plants are responsible for crossreactivity reactions [5–7].

The patient studied was also sensitized to peanut, although not experiencing such a severe response as that to acorn nut. However, the two IgE binding bands detected

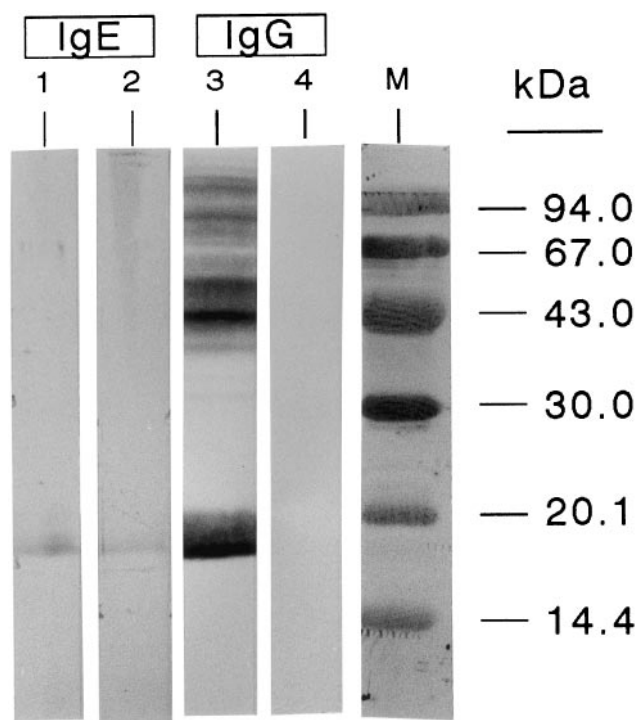


Fig. 2. SDS-PAGE immunoblotting of acorn nut (membranes 1–3) and *Betula verrucosa* pollen extract (4) incubated with the patient serum (IgE blot) and the monoclonal antibody Bip 1 (IgG blot). (1) non-inhibited blot. (2) blot partially inhibited with electroeluted Bet v 1 allergen. (M): mol. wt. markers stained with Amido Black B.

in the peanut blot were not coincident with the one of the acorn nut extracts, suggesting the presence of different allergens. Furthermore, preadsorption of patient serum with peanut extract did not inhibit the acorn blot (figure not shown).

The molecular weight of the only acorn allergen (17.9 kDa), as well as the presence of a similar IgE binding band in the holm oak pollen extract, led us to investigate whether this protein could be homologous to the group 1 pollen allergens described in other trees from the Fagaceae family, i. e. Bet v 1, Aln g 1, Car b 1 and Que a 1, present in allergenic extracts of birch (*Betula verrucosa*), alder (*Alnus glutinosa*), hornbeam (*Carpinus betulus*) and white oak (*Quercus alba*), first reported by Ipsen and Hansen [8], and later identified as a pathogenesis related protein with RNase activity by Bufe *et al.* [9].

In order to demonstrate the possible homology between the acorn nut allergen and the group 1 representative allergen, Bet v 1, an immunoblotting experiment was carried out using a specific monoclonal antibody against Bet v 1, termed Bip 1 [10] and a purified preparation of Bet v 1 to inhibit the IgE binding (Fig. 2). Apparently, controversial results were obtained, since the monoclonal

antibody Bip 1 could not recognize any protein band from the acorn nut extract, as did from the *B. verrucosa* extract, whilst the purified Bet v 1 inhibited 62% of the IgE binding reactivity of the 17.9 kDa acorn band. These results evidence the existence of a significant degree of homology between the acorn and birch allergens, best ascertained by the polyclonal IgE response of the patient serum than through the use of a monoclonal antibody, probably specific towards a Bet v 1 epitope located at a somewhat more differentiated region of their structures. A similar finding was reported by Hirschwehr *et al.* [4] with the 18 kDa protein from hazelnut extract.

This case therefore constitutes a first description of sensitization to acorn nut, the edible fruit of the holm oak (*Quercus ilex*), with detection of a 17.9 kDa allergen which presents a significant degree of homology with the pathogenesis related proteins detected as allergens in pollen tree sources.

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