

Allergy to pistachio: crossreactivity between pistachio nut and other Anacardiaceae

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Summary

Background Anaphylaxis against Anacardiaceae nuts is uncommon and the allergens involved still poorly characterized. For this reason two patients with allergy towards pistachio nut (a member of the Anacardiaceae family) have been studied.

Objective Identification of immunoallergens present in pistachio nut and analysis of crossreactive antigens in other members of the same plant family, specifically cashew and mango.

Methods Presence of specific IgE for pistachio and cashew nut and for mango seed and pulp was determined by skin tests and radioallergosorbent assay (RAST). The allergenic profile of pistachio and cashew was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting. Cross-reactivity between pistachio and the other Anacardiaceae was studied by RAST inhibition.

Results Skin tests were positive for pistachio and cashew in the two children and for mango seed in one. RAST was positive for pistachio and cashew in both patients. On immunoblotting, serum from both patients recognized several pistachio and cashew allergens with a molecular weight ranging from <14·2–70 kDa. RAST inhibition demonstrated common antigenic determinants between pistachio and cashew nut. Crossreactivity was also found between pistachio nut and mango seed but not with mango pulp.

Conclusion Pistachio nut contains several protein allergens able to trigger type I hypersensitivity reactions. These allergens can be found also in cashew nut and mango seed but not in mango pulp.

Keywords: food allergy, nut allergy, pistachio, cashew, mango, pistachio allergens, Anacardiaceae crossreactivity

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Introduction

The incidence of food allergy in children was estimated around 8% in two prospective studies [1,2]. Allergenic foods vary according to the age of patients and eating habits in different areas. For example, while in the United States reactions to milk, soybean, eggs and peanuts predominate [3–5]; in Spain, fish is the third

most important cause of anaphylaxis, after eggs and milk, with nuts ranking fifth in frequency order [6].

Peanut allergy is the most researched [7], and the one that causes more anaphylactic reactions. Nevertheless, allergic reactions to many other nuts including cashew (*Anacardium occidentale*) [8] and pistachio (*Pistacia vera*) [9,10] have been described.

In Spain, ≈100 000 000 kg of nuts are consumed annually. Walnuts, almonds and peanuts account for 60% of the total (20% each). Consumption of pistachios is lower, roughly 10% of the total, while that of cashews

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is minimal [11]. Both pistachio and cashew, together with mango (*Mangifera indica*), belong to the Anacardiaceae family and all of them may cause anaphylaxis [8–10,12]. None of the three is indigenous to Spain [13] and their introduction into the Spanish diet is relatively modern.

We recently had the opportunity to study two children who presented severe anaphylactic reactions after ingestion of pistachio nuts. Given the scarcity of *in vitro* data, we have investigated the allergenic components present in pistachio nut as well as its crossreactivity with other nuts and fruits of the Anacardiaceae family.

Materials and methods

Case reports

Patient 1

At the age of 22 months, a 3-year-old boy had suffered vomiting, facial angioedema, aphonia, generalized urticaria and pruritus, with general malaise immediately after eating pistachio nuts. He was treated at the emergency room with corticosteroids, anti-histamines and adrenaline. His condition improved shortly afterwards and he was asymptomatic within 24 h. He had never eaten cashew nuts or mango before and anamnesis failed to establish whether he had eaten pistachio nuts on other occasions.

He had also suffered skin lesions consistent with atopic dermatitis at the age of 12 months and urticaria with the introduction of eggs in his diet. The rest of his medical history was unremarkable. His mother mentioned that she had pollen allergy.

Physical examination and complementary tests including a haemogram, white-cell count and differential count, blood biochemistry, chest x-ray, hydatid serology, C3 and C4 complement fractions, parasites in faeces and Graham test were normal or negative, except for the presence of 800 eosinophils/mm³ in plasma. Total IgE was 578 kU/L. Skin tests and specific IgE for egg were positive.

Patient 2

A 6-year-old boy who at the age of 3 years had presented lingual pruritus, vomiting, generalized urticaria and dyspnoea on two occasions both immediately post-ingestion of pistachio nuts. He was treated with corticosteroids and anti-histamines and improved within hours. Anamnesis failed to establish whether he had previously tolerated pistachio nuts. He had never eaten cashew nuts or mango.

This patient had had urticaria and angioedema when

eggs and fish were introduced into his diet. From the first year of life, he had suffered episodes of bronchial asthma. There was no family history of atopy and the rest of his personal medical history was unremarkable.

Physical examination and complementary tests including haemogram, white-cell count and differential count, blood biochemistry, chest and paranasal sinuses x-rays, Mantoux test, and parasite study in faeces were normal or negative. Skin tests and specific IgE for fish, egg and *Cladosporium herbarum* were positive. Total IgE was 99 kU/L. The patient is following a diet free of the afore-mentioned foods. He requires inhaled corticosteroids, cromolyn sodium and maintenance bronchodilators to control his asthma.

Skin tests

Skin tests were performed on the volar surface of the forearm. Pistachio, cashew and mango were tested in natural form with the prick by prick technique [14], with a Prick Lancetter (DHS, Bayer, Leverkusen, Germany) device. Briefly, a lancet was pricked into pistachio, cashew and mango in their natural form and immediately thereafter into the skin. The same technique was used in 10 atopic subjects with no history of allergy to Anacardiaceae who served as controls.

Other foods (fish, cod, shellfish, eggs, milk, almond, hazelnut, peanut sunflower seed and walnut) and inhalants (dust, mites, pollen, moulds and animals) were tested in both patients by conventional skin-prick test. Glycerol saline solution and histamine hydrochloride (10 mg/mL) were used as negative and positive control, respectively. All these extracts and controls were purchased from Bencard (Brentford, UK).

Reading was performed after 15 min; the greatest diameter and its perpendicular were measured in the resulting weal. The mean of both diameters was considered as the size of the test. Tests greater than 3 mm were assessed as positive [15].

Extract procedure

Pistachio and cashew nuts were dry-grounded to produce a fine dust, defatted in diethyl ether in a Soxhlet apparatus, and hydrosoluble components extracted by magnetic stirring in modified Coca solution (10 g sodium chloride, 4 g sodium bicarbonate and 0.3 g of human serumalbumin in 1000 mL distilled water) for 2 h at 4°C.

Following centrifugation of the mixture at 5000 rpm for 30 min, a supernatant of the solubilized components was obtained and fractionated by acetone precipitation for subsequent ultrafiltration (Pellicon System, Millipore) with a 5000 Da cut-off. After sterilization by

Table 1. Anacardiaceae skin tests and RAST results

Patient	Test	Pistachio	Cashew	Mango (pulp)	Mango (seed)
1	Prick by prick (mm)	3	4	(-)	(-)
	RAST (PRU)	>17.5	2.32	<0.35	<0.35
2	Prick by prick (mm)	5	3	(-)	4
	RAST (PRU)	1.24	1.22	<0.35	<0.35

filtration through a 0.22 µm pore diameter membrane, the supernatant was frozen and lyophilized.

Two mango extracts from seed and pulp, respectively, were obtained. The process was similar to the above mentioned, but with omission of the defatting and acetone precipitation stages.

Direct RAST

Paper discs (Whatman 42) were activated with cyanogen bromide followed by coupling of the extracts (pistachio and cashew nuts, mango seed and pulp) by the Ceska *et al.* method [16]. Extracts were dissolved in Ceska buffer at 4 mg protein/mL and in a ratio of 0.2 mL/paper disc. Technique development was performed with Phadezym RAST Tracer reagents (Kabi-Pharmacia) following the method of Wide *et al.* [17].

RAST inhibition

Pistachio RAST-inhibition was as in the direct RAST technique, but with the addition of extracts (pistachio, cashew, mango seed and pulp) in liquid phase at concentrations of 100–0.001 mg/mL, according to Yman *et al.* [18]. Fifty microlitres of four dilutions of the extracts were added to solid-phase antigen. Immediately afterwards, 50 µL of sera from the patient were also added. After 12 h incubation, bound IgE was revealed with Phadezym RAST tracer reagents.

SDS-PAGE blotting

Pistachio and cashew SDS-PAGE was performed in 12.5% polyacrylamide gels following the discontinuous buffer technique of Laemmli *et al.* [19]. Protein samples, 20 µg each, were applied to each gel lane and run in duplicate. After running, one set of proteins present in the extracts were stained with Coomassie Brilliant Blue (R250). In the other set of samples, separated proteins were electrically transferred to PVDF membranes (Immobilon P, Millipore) by the technique of Towbin [20], and incubated with sera to be subsequently studied

by immunochemical developing. Sera samples were diluted 1/5 in TTBS (Tween 20 in Tris-buffered saline). Chromogenic enzymatic developing was performed using anti-human IgE peroxidase (rabbit anti-human IgE chain conjugated to horse-radish peroxidase — DAKO) as a conjugate and 4-chloro-1-naphtol as a substrate, according to the method described by Shen *et al.* [21]. Sera from two atopic patients without allergy to the Anacardiaceae family were used as negative control.

A kit of calibrating proteins of known molecular weight was applied in parallel (LMW, Pharmacia Bio-systems) to enable molecular weight estimation of extract proteins. The transferred pattern was stained with Amido Black.

Results

Skin tests and RAST

Skin tests for pistachio and cashew were positive in both patients. Mango seed proved positive only in patient 2, while mango pulp was negative in both (Table 1). Skin tests for other nuts (almond, hazelnut, peanut, walnut and sunflower seed) and pollens were negative. None of the 10 controls had positive skin tests with pistachio, cashew or mango.

RAST was positive for pistachio and cashew in the two patients, whereas specific serum IgE was not detected for mango fractions (Table 1) or other nuts or pollens (not shown).

SDS-PAGE

At least 18 protein bands with a molecular weight ranging from <14.4 kDa to 71 kDa were detected in pistachio extract. Those of greater intensity corresponded to molecular weight of 13.7, 23.6, 28.0, 35.5 and 42.8 kDa. The cashew extract presented nine bands in the same molecular weight range as those of pistachio; those of 19.4, 21.2 and 38.5 kDa showed greater intensity (Fig. 1).

SDS-PAGE immunoblotting

Sera of the two patients recognized proteins in pistachio and cashew. When proteins of the pistachio extract, separated in SDS-PAGE, were incubated with serum from patient 1, six IgE binding bands with molecular weights between <14.4 and 70.9 kDa were observed (Fig. 2). After incubation with serum from patient 2, most of the binding bands had the same molecular weight as in patient 1. In both patients binding was stronger between 30 and 35 kDa.

In cashew, four IgE binding bands with a molecular weight between 20.1 and 67 were revealed in patient 1 (Fig. 2). Binding in patient 2 was very similar. The strongest binding took place at 32 kDa in both patients. No binding was detected from control sera.

RAST inhibition

Pistachio extract coupled to BrCN activated cellulose discs was used as solid phase. Both mango seed and cashew were seen to produce significant RAST inhibition to pistachio (Fig. 3). In patient 2, inhibitory concentrations of cashew and mango seed extracts were very high. However, the regression lines obtained with them were parallel to the one obtained with pistachio. This finding argues in favour

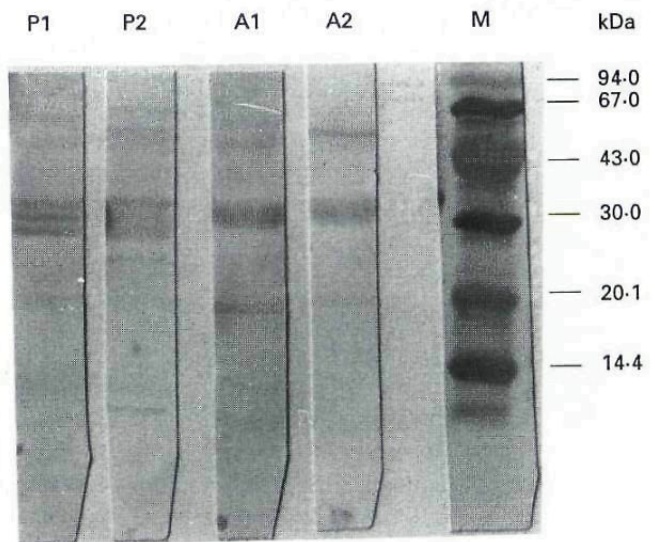


Fig. 2.

of crossreactivity. No significant inhibition was obtained with mango pulp.

Discussion

Poison ivy is the member of the Anacardiaceae family most frequently involved in hypersensitivity reactions, often causing contact dermatitis [22]. Although anaphylactic sensitization to members of this family is less common, Sampson *et al.* described two cases of death after eating cashew, a nut of the Anacardiaceae family, in a series of 13 patients with fatal or near-fatal food-related anaphylactic reactions [23].

Pistachio is the Anacardiaceae most commonly consumed in Spain, and here we describe two children who suffered serious anaphylactic reactions after eating this nut. In both cases, pistachio-specific IgE was detected by skin tests and RAST confirming an IgE-mediated mechanism of their symptoms. Immunoblotting experiments showed that several of the proteins present in this nut behaved as allergens, binding IgE present in patients' serum. The IgE binding pattern differed in the two patients, but in both of them binding was strongest in three bands comprised between 30 and 41 kDa. Parra *et al.* [10] recently described three adult cases of anaphylaxis to this nut. Interestingly, in two of these patients IgE binding occurred mainly in proteins with an apparent molecular weight of 34 kDa. It is therefore possible that the main allergenic constituents in extracts of pistachio nut are located in the 30–40 kDa molecular weight range.

It was not possible to establish a positive history of prior pistachio nut ingestion in either of the two cases.

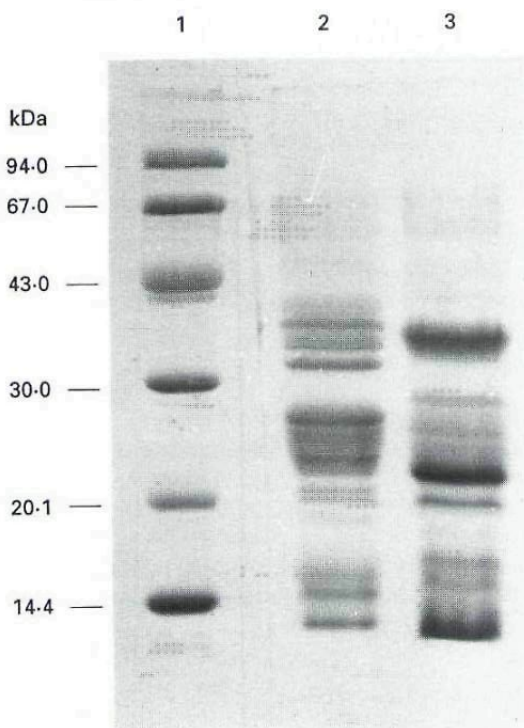


Fig. 1.

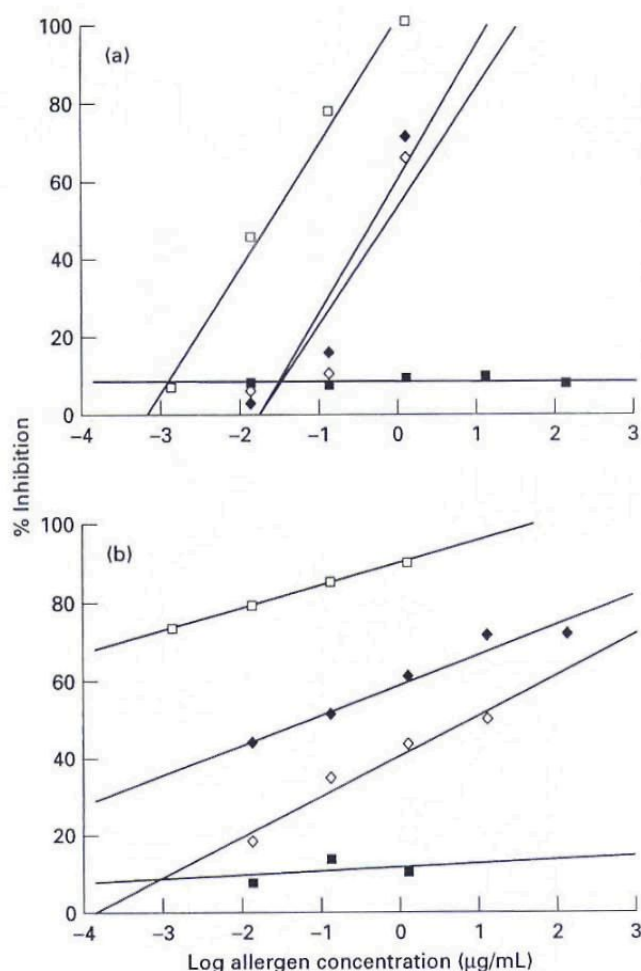


Fig. 3.

There was no record of exposure to other Anacardiaceae and both patients tolerated other nuts, fruits, legumes and vegetables and RAST assays were negative for non-Anacardiaceae nuts. Therefore, we think sensitization was likely produced by a prior exposure to pistachio which took place unknowingly (pistachio may be an ingredient in other foods such as ice-cream, oriental dishes, confectionery, etc.). Presentation of symptoms at 22 months of age, as in patient 1, is uncommon in most cases of nut allergy and sensitization may have occurred after very few exposures to pistachio. Therefore, the sensitizing potential of pistachio nut may be very high in atopic children. This early onset resembles to some extent the case of peanut allergy, in which 50% of peanut allergic children are sensitized before the age of 2 [24].

In view of the above and the severity of symptoms, we believe that pistachio should be included in the list of foods to be tested in the study of anaphylactic reactions of unknown origin in children.

Crossreactivity among different members of the same family is an important aspect to be assessed in food allergies. Pistachio, cashew and mango all belong to the Anacardiaceae family. The two patients described here possessed specific IgE for cashew, which was demonstrated *in vivo* and *in vitro*, and neither had had previous contact with it. Allergenic homology between pistachio and cashew was confirmed by experiments of RAST inhibition. Similar results have been found by others [10]. Thus, faced with a history of anaphylaxis to one of these nuts, both should be avoided.

Jansen *et al.* [25] reported three patients that had developed Anacardiaceae anaphylaxis (one to pistachio and two to mango). One of the two mango allergic patients and the one allergic to pistachio had specific IgE for both mango and pistachio, which pointed towards the existence of common allergens between them. However, confirming crossreactivity studies were not performed.

Two parts of Mango were also studied: pulp and seed, which is the one botanically closer to pistachio nut. RAST inhibition experiments revealed crossreactivity between pistachio and mango seed (but not pulp) in both patients. It is possible that patients may tolerate mango since only the pulp is usually consumed. However, more studies are necessary to unravel cross-reactivity between mango fruit and nuts of the Anacardiaceae family.

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