

Immunological analysis of allergenic cross-reactivity between peanut and tree nuts

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Summary

Background Peanut and tree nut allergy is characterized by a high frequency of life-threatening anaphylactic reactions and typically lifelong persistence. Peanut allergy is more common than tree nut allergy, but many subjects develop hypersensitivity to both peanuts and tree nuts. Whether this is due to the presence of cross-reactive allergens remains unknown.

Objective The aim of this study was to investigate the presence of allergenic cross-reactivity between peanut and tree nuts.

Methods Western blotting and ELISA were performed using sera from subjects with or without peanut and tree nut allergy to assess immunoglobulin E (IgE) reactivity to peanut and tree nut extracts. Inhibition ELISA studies were conducted to assess the presence of allergenic cross-reactivity between peanut and tree nuts.

Results Western blot and ELISA results showed IgE reactivity to peanut, almond, Brazil nut, hazelnut and cashew nut for peanut- and tree nut-allergic subject sera. Raw and roasted peanut and tree nut extracts showed similar IgE reactivities. Inhibition ELISA showed that pre-incubation of sera with almond, Brazil nut or hazelnut extracts resulted in a decrease in IgE binding to peanut extract, indicating allergenic cross-reactivity. Pre-incubation of sera with cashew nut extract did not cause any inhibition.

Conclusion These results show that multiple peanut and tree nut sensitivities observed in allergic subjects may be due to cross-reactive B cell epitopes present in different peanut and tree nut allergens. The plant taxonomic classification of peanut and tree nuts does not appear to predict allergenic cross-reactivity.

Keywords allergen avoidance, allergy, cross-reactivity, IgE, inhibition ELISA, multiple nut sensitivity, peanut, tree nuts, taxonomic classification

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Introduction

Peanut and tree nut allergy is characterized by a high frequency of life-threatening anaphylactic reactions and typically lifelong persistence [1]. There appears to be a strong clinical association between peanut and tree nut allergy, although reports regarding the prevalence of multiple nut sensitivity have produced widely disparate findings. Two groups analysing subjects from specialist allergy clinics have reported a prevalence of between 35% and 40% [2, 3]. However, in a randomly sampled population of the general community, only 2.4% of peanut- and tree nut-allergic subjects reported symptoms consistent with allergy to more than one variety [4]. A similar lack of certainty relates to the relative prevalence of allergy among the different varieties of tree nuts. In a study by Ewan [2], Brazil nut was the tree nut

to which sensitivity most commonly existed, followed by almond, hazelnut, walnut and cashew, whereas in Sicherer et al.'s [3] group, walnut produced greater than 50% of reactions to tree nuts and Brazil nut was the least commonly sensitizing nut. In both studies, peanut, a groundnut (legume) was the most common sensitizing agent.

Early studies examining the basis of cross-reactivity between foods were able to demonstrate the presence of immunoglobulin E (IgE) cross-reactive proteins in phylogenetically similar plants, but these links were not borne out clinically [5]. More recent studies have concentrated on determining the presence of proteins of homologous structure within peanuts and different varieties of tree nut, although their presence does not necessarily equate with cross-reactivity. The major peanut allergen Ara h 1 and the major walnut allergen Jug r 2 are both members of the vicilin seed storage family, yet they show no significant IgE cross-reactivity on the basis of inhibition immunoblotting experiments [6]. However, in a small study using sera from two walnut-allergic subjects, by pre-incubating with crude peanut extract, IgE binding to a crude walnut extract immunoblot

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was significantly abrogated, suggesting that cross-reactive proteins do occur within these foods [6]. No other studies have examined cross-reactivity between peanut and tree nuts.

In contrast, IgE cross-reactivity between the various tree nuts has been demonstrated by several authors. Inhibition immunoblots using albumin fractions from walnut and hazelnut as inhibitors suggested that significant cross-reactivity exists between this fraction and almond conglutin γ , with lesser cross-reactivity also demonstrated for almond 2S albumin [7]. Partial cross-reactivity between hazelnut and macadamia nut has been demonstrated by Sutherland et al. [8], with sub-total diminution of IgE binding to a 17.4-kDa protein with pre-incubation of sera with hazelnut extract. Hazelnut has also been shown to possess multiple cross-reacting proteins with sesame seed and poppy seed, apart from a unique allergen of approximately 20 kDa, and also with allergens associated with the oral allergy syndrome, tree pollens and stone fruit [9–11]. Pistachio nut appears to cross-react with cashew nut, with RAST inhibition studies suggesting that IgE binding to pistachio was diminished by pre-incubation with cashew extract, although inhibitory concentrations were high [12]. In another study, Brazil nut-allergic individuals were shown to have IgE antibodies to peanut, hazelnut and walnut [13]. Finally, some have suggested that there may be common allergens between pistachio, peanut, walnut, chestnut, almond and cashew nuts as well as pine nut and almond [12, 14, 15].

Although it has been suggested that cross-reactivity may exist between peanut and different tree nut types, whether this is the case for all nuts to which subjects are commonly sensitized remains unknown. Determination of the immunological relationship between peanut and tree nuts is essential for patient management and the development of safe vaccines for nut allergy. This study was undertaken to determine if peanut-specific IgE antibodies from peanut- and tree nut-allergic subjects cross-react with proteins found in the commonly encountered tree nuts.

Materials and methods

Subjects

Sera from four subjects recruited from the Alfred Hospital Asthma and Allergy Clinic, Melbourne, Australia were used in this study. These subjects had a clinical history of sensitivity to peanut and tree nuts. All had specific IgE to peanut (RAST score of ≥ 2 , Pharmacia CAP System™, Pharmacia Diagnostics, Uppsala, Sweden). The study was approved by the Alfred Hospital Ethics Committee and informed consent was obtained from all subjects before blood was obtained. The clinical characteristics and nut-specific IgE RAST scores for these subjects are given in Table 1. Sera from two non-peanut- and tree nut-allergic individuals, one atopic (history of grass pollen sensitivity) and one non-atopic, were used as negative controls.

Preparation of crude nut extracts

Peanuts and the tree nuts almond, Brazil nut, cashew and hazelnut were used in this study and the taxonomic

Table 1. Characteristics of peanut and tree nut-sensitive patients

Patient no.	Age (years)	Sex	Atopy history	Known nut sensitivities	Other food sensitivities	Clinical reaction to peanut and tree nuts				RAST scores					
						Clinical features of anaphylaxis	Age at first reaction (years)	Time since last reaction (months)	Total IgE (kU/L)	Peanut	Almond	Brazil nut	Cashew	Hazelnut	Walnut
1	27	F	Eczema	Peanut, almond		Asthma, urticaria, facial oedema	19	11	nd	3	0	0	0	0	nd
2	34	M	Asthma, rhinitis, eczema	Peanut, hazelnut		Asthma, urticaria, facial oedema	8	12	416	2	1	2	2	2	2
3	28	M	Rhinitis, eczema	Peanut, Brazil nut, almond, hazelnut, walnut, cashew, pine nut		GI† upset, laryngeal oedema, facial oedema	1	36	4189	2	0	0	3	2	2
4	54	M	Asthma, eczema	Peanut, walnut	Banana	Asthma, laryngeal oedema, facial oedema	1	120	2492	2	1	0	1	2	1

nd—not done.

Table 2. Taxonomic classification of peanut and tree nut plants (USDA, NRCS, 2001. The PLANTS Database, Version 3.1. National Plant Data Centre)

Sub-class	Order	Family	Genus, species (Common name)
Rosidae	Fabales	Fabaceae (Pea family)	<i>Arachis hypogaea</i> L. (Peanut)
	Proteales	Proteaceae (Protea family)	<i>Macadamia integrifolia</i> (Macadamia nut)
	Rosales	Rosaceae (Rose family)	<i>Ivesia ptyocharis</i> (Pine nut) <i>Prunus dulcis</i> (Almond)
	Sapindales	Anacardiaceae (Sumac family)	<i>Anacardium occidentale</i> L. (Cashew) <i>Pistacia vera</i> L. (Pistachio nut)
Hamamelidae	Fagales	Betulaceae (Birch family)	<i>Corylus avellana</i> L. (Hazelnut)
		Fagaceae (Beech family)	<i>Castanea sativa</i> (European chestnut) <i>Quercus ilex</i> L. (Acorn nut)
	Juglandales	Juglandaceae (Walnut family)	<i>Carya illinoensis</i> (Pecan)
			<i>Juglans regia</i> L. (English walnut)
Dilleniidae	Lecythidales	Lecythidaceae (Brazil nut family)	<i>Bertholletia excelsa</i> (Brazil nut)

classification of these plants is shown in Table 2. The extraction of proteins from peanut and tree nuts was conducted using a method similar to the Pharmacia CAP system. Commercially available almonds, Brazil nuts, cashews, hazelnuts and peanuts (either raw or roasted at 180 °C for 15 min; Naytura, Sydney, Australia) were crushed and defatted by adding acetone. Contents were mixed and centrifuged at 1000g and the pellet was resuspended in diethyl ether. This procedure was repeated five times. After the final extraction, the crushed product was separated by vacuum filtration and dried for 10 min under vacuum. The dried, defatted product was then ground to powder using liquid nitrogen and incubated overnight in phosphate-buffered saline (PBS) alone (for Western blotting and ELISA studies) or PBS with CompleteTM protease inhibitor cocktail (for inhibition ELISA) (Roche Diagnostics, Mannheim, Germany) at 4 °C, with shaking. The extract was further centrifuged at 1000g to pellet debris and at 20 000g to obtain a clear supernatant. The protein concentration of each nut extract was then determined using the Bio-Rad Micro protein assay (Bio-Rad, Hercules, CA, USA) following the manufacturer's instructions. A suitable walnut extract for this study could not be obtained due to unsatisfactory protein yields.

Electrophoresis and Western immunoblotting

Gel electrophoresis and immunoblotting were conducted following established protocols [16]. Briefly, proteins from crude roasted peanut extracts were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) on 16% gels, according to Laemmli [17], under reducing conditions using the Xcell II Mini-Cell apparatus (Invitrogen, Carlsbad, CA, USA) at 125 V for 2 h. Benchmark[®] pre-stained protein ladder (Invitrogen) was also resolved and proteins were stained with Coomassie brilliant blue (CBB; Sigma, St Louis, MO, USA).

For Western immunoblotting, peanut proteins separated by 16% SDS-PAGE were transferred onto nitrocellulose membranes (0.45 µm; Schleicher and Schuell Inc., Dassel, Germany) at 25 V for 2 h using Xcell II blotting apparatus (Invitrogen) according to Towbin and Gordon [18]. Membranes were blocked in 1% bovine serum albumin (BSA) in PBS for 1 h and subsequently washed once with PBS containing 0.05% Tween-20 (PBS-T) and twice in PBS alone. IgE reactivity of peanut proteins was determined by incubat-

ing the membranes in subject and control sera diluted 1:5 with 0.5% BSA in PBS. Membranes were then incubated in rabbit polyclonal anti-human IgE antibody (1:200; DAKO, Carpinteria, CA, USA) and horseradish peroxidase (HRP)-labelled goat anti-rabbit IgG antibody (1:2000; Promega, Madison, WI, USA) each for 1 h, with washes conducted between incubations as described above. IgE binding was detected using the substrate 4-chloro-1-naphthol (Sigma) in the presence of hydrogen peroxide [16].

Serum IgE ELISA and inhibition ELISA

ELISA studies were performed according to established methods [19]. Briefly, raw and roasted nut extracts were adjusted to 1 µg/mL using 50 mM bicarbonate buffer, pH 9.6, dispensed into 96-well polystyrene plates (50 µL/well; Costar, Acton, MA, USA), and incubated overnight at 4 °C. Plates were washed with PBS-T and blocked with 5% skim milk powder (SMP) in PBS-T (200 µL/well) for 1 h at 37 °C. After washing with PBS-T, 50 µL of subject and control sera, diluted with PBS-T containing 1% SMP, were added to the wells and incubated at 37 °C for 2 h. Plates were washed with PBS-T and incubated with rabbit polyclonal anti-human IgE antibody (1:1000; 50 µL/well; DAKO) for 1 h at 37 °C, followed by HRP-labelled goat anti-rabbit IgG antibody (1:1000; 50 µL/well; Promega), incubated similarly for 1 h at 37 °C, with PBS-T washes in between incubations. IgE binding was detected using *O*-phenylenediamine tablets (Sigma) dissolved in 0.05 M phosphate-citrate buffer (50 µL/well; Sigma). The reaction was stopped after 10 min with the addition of 4 M hydrochloric acid (50 µL/well), and the absorbance (OD) in each well was measured at 490 nm. The absorbance in control wells containing no antigen was subtracted from the absorbance in antigen-coated wells to account for non-specific binding. Assays were performed in triplicate and mean values with standard deviation are shown.

Inhibition experiments were conducted by coating 96-well polystyrene plates with roasted peanut extract (1 µg/mL), and blocking as described above. Subject and control sera (diluted with 1% SMP in PBS-T for an OD 490 nm reading of ~1.0 for peanut extract) were pre-incubated with nut extracts or, as a control, keyhole limpet haemocyanin (KLH; Sigma) in the presence of protease inhibitors (as described before) at a final concentration of 0.2, 1, 5, 25 and 125 µg/mL, at room temperature for 2 h. The inhibition mixtures (including sera

with no inhibitor as positive controls) were then dispensed into wells (50 µL/well) and incubated at 37 °C for a further 2 h. The assay was then continued as described above. Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = 100 - \left(\frac{\text{OD}_{490} \text{ of serum with inhibitor}}{\text{OD}_{490} \text{ of serum without inhibitor}} \times 100 \right)$$

Lower inhibitor concentrations (0.04, 0.008, 0.0016 and 0.00032 µg/mL) were also used for roasted peanut extract to enable the calculation of the concentration required for 50% inhibition of IgE binding (IC₅₀).

Results

Western blot analysis of serum IgE reactivity to roasted peanut extract

Roasted peanut extract was separated by SDS-PAGE and transferred onto nitrocellulose membranes. Membrane strips were incubated with sera from four peanut- and tree nut-

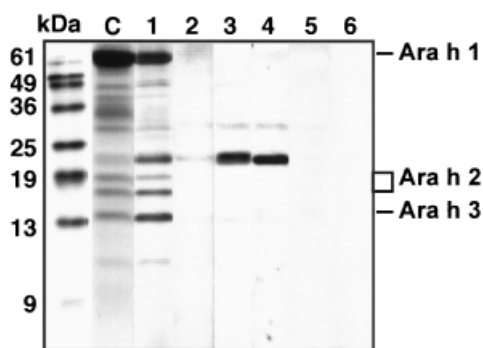


Fig. 1. Immunoblotting for IgE binding to crude roasted peanut extract. Crude roasted peanut extract was resolved by 16% SDS-PAGE as shown by Coomassie staining (C). Proteins were transferred onto nitrocellulose membranes and probed for IgE binding using sera from peanut-allergic (1–4), atopic, non-nut-allergic (5) and non-atopic (6) patients.

allergic subjects to examine IgE reactivity to peanut allergens (Fig. 1). Subject 1 showed IgE reactivity to bands corresponding to the molecular mass of the known peanut allergens Ara h 1, Ara h 2 and Ara h 3. All four subjects reacted to a 23-kDa protein that has not been described before. Serum from two control individuals (one atopic and one non-atopic) did not show IgE binding to roasted peanut proteins. Western immunoblotting using almond, Brazil nut, cashew and hazelnut extracts was also performed with these patient sera. Several allergenic proteins were detected for each tree nut; however, the pattern of IgE binding differed for each patient (data not shown).

ELISA for serum IgE response to crude peanut and tree nut extracts

Direct IgE ELISA was performed to confirm the IgE reactivity of crude peanut, almond, Brazil nut, cashew and hazelnut extracts for use in inhibition assays. The difference in IgE reactivity between raw and roasted peanut and tree nut extracts was also assessed to investigate the effects of roasting on the allergenicity of these extracts. Fig. 2 shows the IgE reactivity of raw and roasted peanut and tree nut extracts, with different levels of IgE binding observed in all four peanut- and tree nut-allergic subjects. Subjects 2 and 3 (Figs 2b,c) showed IgE binding to all raw and roasted peanut and tree nut extracts, although the latter showed minimal IgE reactivity to raw and roasted Brazil nut extracts. Sera from subjects 1 and 4 (Figs 2a,d) had IgE antibodies to raw and roasted almond, hazelnut and peanut extracts, with minimal or no IgE binding to Brazil and cashew nut extracts. Negligible IgE binding was obtained with non-nut allergic and non-atopic control sera (Figs 2e,f). Titration of serum IgE was also conducted for subjects 2 and 3 to determine if there were any changes in IgE binding after roasting of the peanut and tree nut extracts (Fig. 3). In both subjects, the level of IgE binding to the raw and roasted extracts did not differ between serum dilutions. This was further confirmed by Western immunoblotting studies where minimal differences in IgE reactivity were observed for raw and roasted peanut and

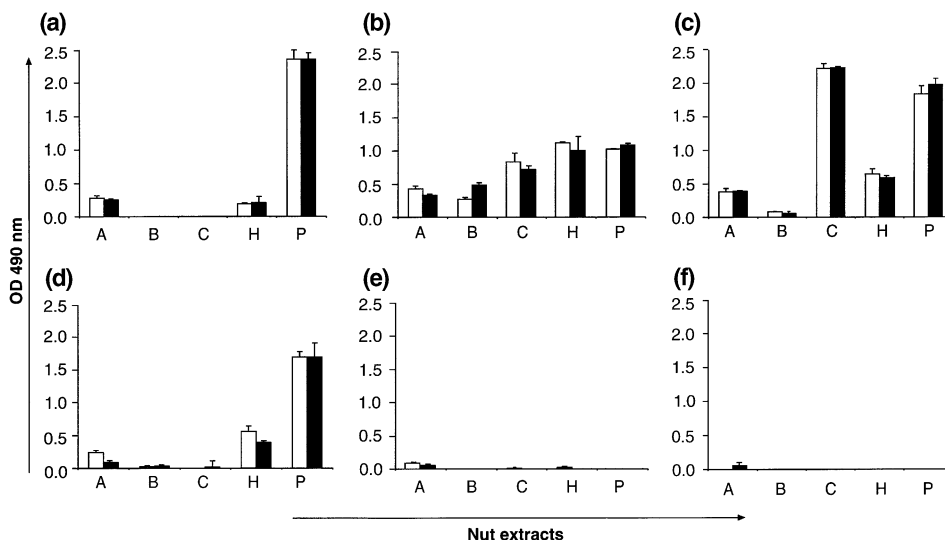


Fig. 2. ELISA for serum IgE binding to different nut extracts. ELISA plate was immobilized with raw (empty bars) and roasted (solid bars) nut extracts (1 µg/mL) including almond (A), Brazil nut (B), cashew (C), hazelnut (H) and peanut (P). IgE binding was assessed among four multiple nut-allergic patients (a–d), one atopic, non-nut-allergic patient (e) and one non-atopic patient (f), at a serum dilution of 1 : 10. The absorbance in control wells containing no antigen was subtracted from antigen-coated wells. The mean values for triplicates are shown and the standard deviation is indicated by error bars.

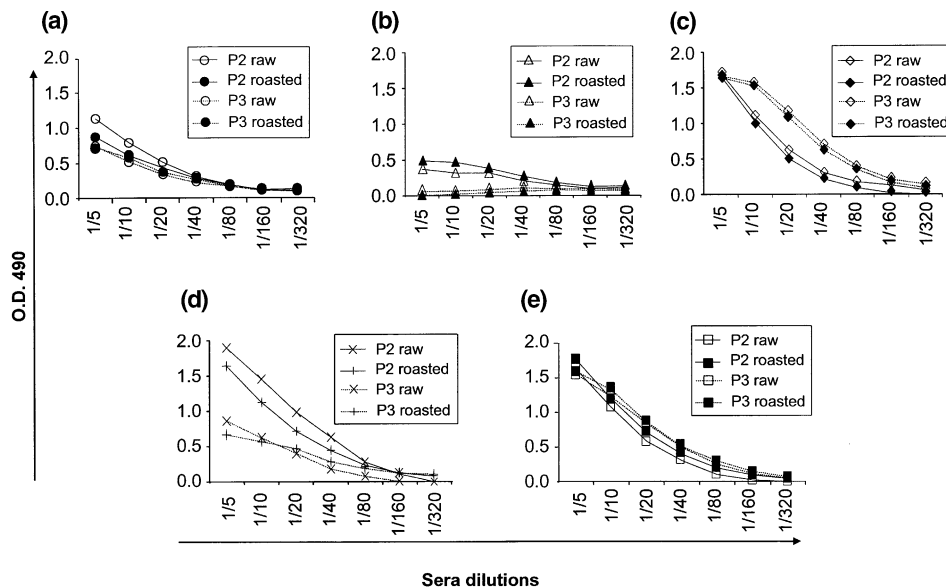


Fig. 3. ELISA for titration of serum IgE against raw and roasted nut extracts. ELISA plate was immobilized with raw and roasted almond (a), Brazil nut (b), cashew (c), hazelnut (d) and peanut (e) extracts. Sera from patient nos. 2 (P2) and 3 (P3) were serially diluted and IgE binding was assessed. The mean values for triplicates are shown. The absorbance in control wells containing no antigen was subtracted from antigen-coated wells.

tree nut proteins (data not shown). Therefore, roasting appears to have little or no effect on the allergenicity of peanut and tree nuts.

Inhibition ELISA

Inhibition ELISA was performed to determine whether cross-reactive allergens were present in the peanut and tree nut extracts. In this assay, the capacity of roasted almond, raw Brazil nut, roasted cashew and roasted hazelnut extracts to inhibit IgE binding to roasted peanut extract immobilized on an ELISA plate was assessed. As only minimal differences in allergenicity were observed between raw and roasted extracts (Figs 2 and 3), the form (raw or roasted) in which the extracts were used as inhibitors was determined according to that most commonly consumed.

The results of the dose-dependent ELISA inhibition assays for all four peanut- and tree nut-allergic subjects are shown in Fig. 4. The specificity of this assay was demonstrated by the strong inhibition obtained with roasted peanut extract (positive control) in all the subject sera, while the negative control extract (KLH) induced minimal or no inhibition of IgE binding to roasted peanut extract. To assess the specificity of the inhibitions observed, a non-specific inhibition ELISA was performed. In this assay, the non-specific inhibitory effect of the peanut and tree nut extracts was evaluated in a latex-glove-extract-specific IgE ELISA. Negligible inhibition of IgE binding to the glove extract was observed when serum from a latex, non-nut allergic patient was pre-incubated with 0.2–125 µg/mL of peanut and tree nut extracts (data not shown), further validating the specificity of this assay. Of the tree nut extracts used in this study, roasted almond showed the highest level of inhibition followed by raw Brazil nut and roasted hazelnut extract. These data are consistent with the presence of cross-reactive allergens in almond, Brazil nut, hazelnut and peanut. In contrast, cashew extract did not demonstrate a dose-dependent inhibition of IgE binding to roasted peanut extract, with minimal inhibition at the maximum inhibitor concentration of

125 µg/mL, indicating the lack of cross-reactive allergens in this extract. The differences in the degree of inhibition observed with the peanut and tree nut extracts were further investigated by determining the IC_{50} to roasted peanut extract, as shown in Table 3. As expected, the roasted peanut extract (positive control) gave the lowest IC_{50} in all subjects, with concentrations ranging from 0.006 to 0.058 µg/mL. All four subjects also achieved 50% inhibition of IgE binding to roasted peanut with roasted almond extract as the inhibitor, although IC_{50} values were much higher than the positive control, ranging from 0.6 to 45 µg/mL. Raw Brazil nut extract showed 50% inhibition of IgE binding to roasted peanut extract in three out of four subjects, while two out of four subjects showed 50% inhibition with roasted hazelnut extract within the range of inhibitor concentrations used in this study. In contrast, both roasted cashew extract and KLH demonstrated negligible inhibition levels similar to that obtained in the non-specific inhibition ELISA (data not shown).

Discussion

Allergy to at least one tree nut is a common clinical observation in the peanut-allergic population, and limited immunological studies suggest the presence of cross-reactive allergens [9, 12, 14, 15, 20]. Here we demonstrate serum IgE cross-reactivity between allergens present in peanut, almond, Brazil nut and hazelnut using inhibition ELISA. No IgE cross-reactivity was detected between cashew and peanut.

In this study, four subjects who were allergic to peanut as well as one or more tree nuts were studied in detail. The RAST scores for almond, Brazil nut, cashew, hazelnut and peanut in all four subjects correlated well with the IgE levels obtained in the serum IgE ELISA. However, in some cases, subject clinical history did not correlate with RAST scores. Subjects 1 and 3, both with a history of sensitivity to almonds, were positive for IgE to almond by ELISA, but negative by RAST. There are no published reports of

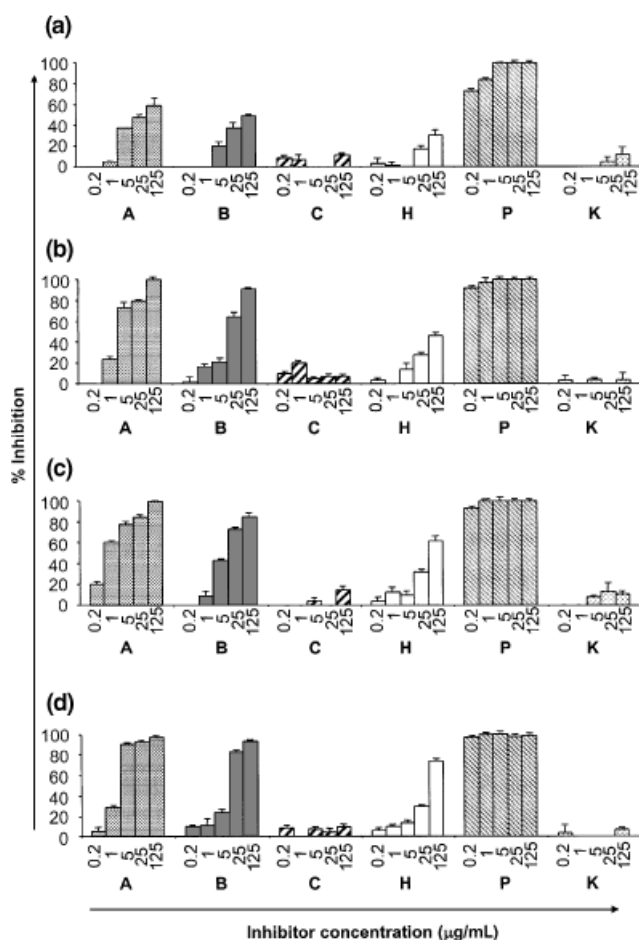


Fig. 4. ELISA for inhibition of serum IgE binding to peanut by other nut extracts. Potential inhibitors of serum IgE binding to plate-immobilized roasted peanut extract include crude roasted almond (A), raw Brazil nut (B), roasted cashew (C), roasted hazelnut (H), roasted peanut positive control (P) and KLH negative control (K) extracts. Inhibition of IgE binding was assessed using four multiple nut-allergic patients (a–d). The mean values for triplicates are shown and the standard deviation is indicated by error bars.

Table 3. Inhibitor concentration required for 50% inhibition of IgE binding to roasted peanut extract

Patient no.	Inhibitor concentration (µg/mL)					KLH†
	Roasted almond	Raw Brazil	Roasted cashew	Roasted hazelnut	Roasted peanut*	
1	45.0	‡	§	‡	0.058	§
2	3.1	18.6	§	‡	0.006	§
3	0.6	8.2	§	82.0	0.015	§
4	2.4	14.0	§	71.0	0.015	§

*Positive control inhibitor.

†Negative control inhibitor.

‡Did not reach 50% inhibition at the maximum inhibitor concentration of 125 µg/mL.

§Did not show inhibition above the levels obtained in non-specific inhibition assay.

the correlation between RAST and ELISA for detection of nut-specific IgE, but our results suggest that ELISA testing is more sensitive than RAST. RAST testing for peanut-specific IgE has previously demonstrated a false-negative rate of

approximately 15% [21]. Within our study, several subjects demonstrated a positive RAST score and ELISA in the absence of a clinical history. While this finding may represent false-positive results, the peanut and tree nut sensitivity data are derived from clinical history rather than oral food challenge, and therefore our specific IgE data may reflect clinically unrecognized nut sensitivities.

The effect of roasting on allergenicity of peanut and tree nut proteins was also investigated in this study. At high temperatures, reducing sugars present in peanuts may react with proteins by the Maillard reaction producing protein-sugar end-products with enhanced allergenicity [22–24], and another study showed loss of IgE binding to a low molecular weight protein of almond following roasting [25]. In our study, IgE binding to almond, Brazil nut, cashew, hazelnut and peanut proteins did not differ between the raw and roasted forms, indicating that the IgE-binding epitopes are heat-stable, possibly contributing to their allergenicity. Whether this is due to heat-stable tertiary structures or the presence of linear epitopes in heat-labile allergens is not known and requires further investigation.

Given that tree nut allergy is a common occurrence among peanut-allergic individuals, information regarding the level of allergenic cross-reactivity between peanut and other tree nuts is critical but currently lacking. In this study, inhibition assays demonstrated that IgE cross-reactivity occurs between peanut, almond, Brazil nut and hazelnut, which are the most common causes of peanut and tree nut allergy [2]. This suggests that there may be similarities in the IgE-binding epitopes of proteins present in these foods. There are, however, differences in the degree of inhibition observed between peanut and the different tree nuts as shown by the IC_{50} for inhibition of peanut reactivity. Almond inhibits IgE binding to peanut at lower concentrations than Brazil nut and in turn hazelnut, although these values were considerably higher when compared to the IC_{50} for peanut control extract.

The high inhibition of IgE binding to peanut by the peanut extract positive control compared with the tree nut extracts suggests that the level of cross-reactivity between peanut and tree nuts is low. This may reflect differences in the abundance of cross-reactive allergens or epitopes in peanut and tree nut extracts and/or differences in the affinity of peanut-specific IgE antibodies for proteins in almond, Brazil nut and hazelnut extracts [26]. Although the observed cross-reactivity between peanut and tree nuts is low, it is of high clinical relevance. This study does not address the identity of the cross-reacting proteins between peanut and tree nuts, but it seems likely that sensitivity to Ara h 1 is not the basis for this phenomenon. Teuber et al. [6] demonstrated that IgE cross-reactivity between walnut and peanut is not likely to occur between their respective major allergens Jug r 2 and Ara h 1, despite their 36.1% amino acid sequence identity and belonging to the vicilin seed storage family. Other authors have suggested that 70% amino acid sequence homology is required for immunological cross-reactivity to occur between proteins; structures likely to be shared by peanut and the tree nuts that may show this degree of similarity include the panallergens lipid transfer protein and profilin, along with the other seed storage proteins [27]. Despite being the most probable sources of cross-reactivity between peanut and tree

nuts, the relative concentrations of these proteins, their degree of sequence similarity, their extent of IgE cross-reactivity and the functional significance of these features remain undetermined.

Carbohydrate moieties of allergens may also contribute to IgE cross-reactivity; however, the clinical significance of these antibodies is doubtful and they were not examined in this study. In a report by van der Veen et al. [28], 29 of 32 grass pollen-sensitized individuals had IgE antibodies directed at N-linked carbohydrate groups of peanut proteins, but only one of four patients with a positive history and diagnosis of peanut allergy showed such reactivity. Importantly, the grass pollen-allergic individuals with cross-reactive IgE antibodies to carbohydrate determinants did not exhibit clinical symptoms of peanut allergy, and concentrations of peanut allergens that induced basophil histamine release for these subjects were 1000-fold higher than control pollen allergens.

This study demonstrated a lack of cross-reactivity between peanut and cashew, in contrast to past studies demonstrating IgE cross-reactivity between cashew, and other tree nuts [12, 15]. Cashew allergy is rare among peanut- and tree nut-allergic individuals. The major cashew allergen Ana o 1 is a member of the poorly cross-reactive vicilin seed storage family [6, 29]. Although Ana o 1 shows 45% amino acid sequence similarity with Ara h 1, no common IgE-binding epitopes are identified. The absence of other panallergens may also contribute to the observed lack of IgE cross-reactivity between peanuts and cashew nuts.

Peanut-allergic subjects often have serum IgE antibodies that cross-react with other legumes such as soybean. However, ingestion of such legumes does not necessarily induce a clinical allergic reaction in these subjects, suggesting that this cross-reactivity is clinically irrelevant [5, 30]. In our experience, the overwhelming majority of peanut-allergic subjects also have tree nut clinical allergy, suggesting that the cross-reactivity we have observed between peanut and tree nuts is clinically relevant. Nevertheless, the ability of cross-reactive peanut-specific IgE antibodies to induce a type I hypersensitivity response to tree nut allergens should be tested.

From a taxonomic perspective, peanuts and tree nuts are distantly related. In this study, the observed level of cross-reactivity between peanut, almond, Brazil nut, cashew and hazelnut did not correlate with the plant taxonomic relationship. Peanut and cashew, both belonging to the Rosidae subclass, did not show IgE cross-reactivity. Similarly, a previous study found no evidence of cross-reactivity between peanut and macadamia [8], which also belongs to the same subclass. Unlike grass pollen allergy, where cross-reactivity between different grasses correlates highly with taxonomic classification [31], peanut and tree nut cross-reactivity cannot be predicted safely by a taxonomic relationship.

In summary, this study has provided evidence that cross-reactive allergens are present in peanuts and tree nuts including almond, Brazil nut and hazelnut. This may explain the high frequency of tree nut sensitivity among peanut-allergic individuals. However, unanswered questions remain, including the sites at which cross-reactivity occur, the reasons for differing levels of cross-reactivity and the biological relevance of the cross-reactive IgE antibodies. Inhibition

studies and sequence comparisons of different allergens from peanut, walnut and cashew nuts suggest that vicilin seed storage proteins do not play a role in the cross-reactivity of peanut and tree nuts; differences in the relative concentrations of other possible panallergens may be the basis for the different degrees of cross-reactivity observed in this study [27]. Although the findings of the current study contribute to better patient management and diagnosis, further studies allowing the molecular identification and characterization of other potential cross-reactive nut allergens as well as the corresponding IgE-binding epitopes will be a major contribution to improved diagnosis and treatment of peanut and tree nut allergy.

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References

- 1 Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992; 327:380–4.
- 2 Ewan PW. Clinical study of peanut and nut allergy in 62 consecutive patients: new features and associations. *BMJ* 1996; 312:1074–8.
- 3 Sicherer SH, Burks AW, Sampson HA. Clinical features of acute allergic reactions to peanut and tree nuts in children. *Pediatrics* 1998; 102:e6.
- 4 Sicherer SH, Munoz-Furlong A, Burks AW, Sampson HA. Prevalence of peanut and tree nut allergy in the US determined by a random digit dial telephone survey. *J Allergy Clin Immunol* 1999; 103:559–62.
- 5 Bernhisel-Broadbent J, Taylor S, Sampson HA. Cross-allergenicity in the legume botanical family in children with food hypersensitivity. II. Laboratory correlates. *J Allergy Clin Immunol* 1989; 84 (5 Part 1):701–9.
- 6 Teuber SS, Jarvis KC, Dandekar AM, Peterson WR, Ansari AA. Identification and cloning of a complementary DNA encoding a vicilin-like proprotein, Jug r 2, from English walnut kernel (*Juglans regia*), a major food allergen. *J Allergy Clin Immunol* 1999; 104:1311–20.
- 7 Poltronieri P, Cappello MS, Dohmae N. Identification and characterisation of the IgE-binding proteins 2S albumin and conglutin gamma in almond (*Prunus dulcis*) seeds. *Int Arch Allergy Immunol* 2002; 128:97–104.
- 8 Sutherland MF, O'Hehir RE, Czarny D, Suphioglu C. Macadamia nut anaphylaxis: demonstration of specific IgE reactivity and partial cross-reactivity with hazelnut. *J Allergy Clin Immunol* 1999; 104 (1 Part 4):889–90.
- 9 Vocks E, Borge A, Szliska C. Common allergenic structures in hazelnut, rye grain, sesame seeds, kiwi, and poppy seeds. *Allergy* 1993; 48:168–72.
- 10 Luttkopf D, Muller U, Skov PS. Comparison of four variants of a major allergen in hazelnut (*Corylus avellana*) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. *Mol Immunol* 2002; 38: 515–25.
- 11 Hirschwehr R, Valenta R, Ebner C et al. Identification of common allergenic structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J Allergy Clin Immunol* 1992; 90 (6 Part 1):927–36.

- 12 Fernandez C, Fiandor A, Martinez-Garate A, Martinez Quesada J. Allergy to pistachio: crossreactivity between pistachio nut and other Anacardiaceae. *Clin Exp Allergy* 1995; 25:1254–9.
- 13 Arshad SH, Malmberg E, Krapf K, Hide DW. Clinical and immunological characteristics of Brazil nut allergy. *Clin Exp Allergy* 1991; 21:373–6.
- 14 de las Marinas D, Vila L, Sanz ML. Allergy to pine nuts. *Allergy* 1998; 53:220–2.
- 15 Parra FM, Cuevas M, Lezaun A, Alonso MD, Beristain AM, Losada E. Pistachio nut hypersensitivity: identification of pistachio nut allergens. *Clin Exp Allergy* 1993; 23:996–1001.
- 16 Levy D, Davies J, O'Hehir R, Suphioglu C. A rapid and efficient two-step gel electrophoresis method for the purification of major rye grass pollen allergens. *Electrophoresis* 2001; 22:1900–5.
- 17 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680–5.
- 18 Towbin H, Gordon J. Immunoblotting and dot immunobinding – current status and outlook. *J Immunol Methods* 1984; 72: 313–40.
- 19 Suphioglu C, Blaher B, Rolland JM et al. Molecular basis of IgE-recognition of Lol p 5, a major allergen of rye-grass pollen. *Mol Immunol* 1998; 35:293–305.
- 20 Teuber SS, Peterson WR. Systemic allergic reaction to coconut (*Cocos nucifera*) in 2 subjects with hypersensitivity to tree nut and demonstration of cross-reactivity to legumin-like seed storage proteins: new coconut and walnut food allergens. *J Allergy Clin Immunol* 1999; 103:1180–5.
- 21 Sampson HA, Rosen JP, Selcow JE et al. Intradermal skin tests in the diagnostic evaluation of food allergy. *J Allergy Clin Immunol* 1996; 98:714–5.
- 22 Chung SY, Champagne ET. Allergenicity of Maillard reaction products from peanut proteins. *J Agric Food Chem* 1999; 47:5227–31.
- 23 Maleki SJ, Chung SY, Champagne ET, Raufman JP. The effects of roasting on the allergenic properties of peanut proteins. *J Allergy Clin Immunol* 2000; 106:763–8.
- 24 Chung SY, Maleki S, Champagne ET, Buhr KL, Gorbet DW. High-oleic peanuts are not different from normal peanuts in allergenic properties. *J Agric Food Chem* 2002; 50:878–82.
- 25 Venkatachalam M, Teuber SS, Roux KH, Sathe SK. Effects of roasting, blanching, autoclaving, and microwave heating on antigenicity of almond (*Prunus dulcis* L.) proteins. *J Agric Food Chem* 2002; 50:3544–8.
- 26 Aalberse RC, Kleine Budde I, Stapel SO, van Ree R. Structural aspects of cross-reactivity and its relation to antibody affinity. *Allergy* 2001; 56 (Suppl 67):27–9.
- 27 Aalberse RC. Structural biology of allergens. *J Allergy Clin Immunol* 2000; 106:228–38.
- 28 van der Veen MJ, van Ree R, Aalberse RC et al. Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J Allergy Clin Immunol* 1997; 100:327–34.
- 29 Wang F, Robotham JM, Teuber SS, Tawde P, Sathe SK, Roux KH. Ana o 1, a cashew (*Anacardium occidentale*) allergen of the vicilin seed storage protein family. *J Allergy Clin Immunol* 2002; 110:160–6.
- 30 Eigenmann PA, Burks AW, Bannon GA, Sampson HA. Identification of unique peanut and soy allergens in sera adsorbed with cross-reacting antibodies. *J Allergy Clin Immunol* 1996; 98:969–78.
- 31 Suphioglu C, Singh MB, Knox RB. Peptide mapping analysis of group I allergens of grass pollens. *Int Arch Allergy Immunol* 1993; 102:144–51.