

IgE cross-reactivity between the major peanut allergen Ara h 2 and tree nut allergens

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Received 21 December 2005; accepted 21 February 2006

Available online 31 March 2006

Abstract

Allergy to peanut and tree nuts is characterised by a high frequency of life-threatening anaphylactic reactions and typically lifelong persistence. Although peanut is the most common cause of nut allergy, peanut allergic patients are frequently also sensitive to tree nuts. It is not known if this is due to cross-reactivity between peanut and tree nut allergens. In this study, the major peanut allergen Ara h 2 was cloned from peanut cDNA, expressed in *E. coli* cells as a His-tag fusion protein and purified using a Ni-NTA column. Immunoblotting, ELISA and basophil activation indicated by CD63 expression all confirmed the IgE reactivity and biological activity of rAra h 2. To determine whether or not this allergen plays a role in IgE cross-reactivity between peanut and tree nuts, inhibition ELISA was performed. Pre-incubation of serum from peanut allergic patients with increasing concentrations of almond or Brazil nut extract inhibited IgE binding to rAra h 2. Purified rAra h 2-specific serum IgE antibodies also bound to proteins present in almond and Brazil nut extracts by immunoblotting. This indicates that the major peanut allergen, Ara h 2, shares common IgE-binding epitopes with almond and Brazil nut allergens, which may contribute to the high incidence of tree nut sensitisation in peanut allergic individuals.

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Keywords: Allergy; Peanut; Tree nuts; IgE cross-reactivity; Ara h 2

1. Introduction

Food allergies are a common cause of allergen-induced anaphylaxis but peanuts and tree nuts account for the majority of food-related anaphylaxis in children and adolescents (Sampson et al., 1992). Peanut and tree nut allergy differ from other types of food allergies in that they typically persist beyond childhood (Bock and Atkins, 1989). At present, there is no available specific form of treatment for peanut and tree nut allergy with a requirement for ongoing avoidance of the offending food. However, difficulties arise with the increasing use of peanuts and tree nuts as food additives, resulting in accidental exposures which occur in high frequency among peanut allergic individuals (Bock and Atkins, 1989; Sicherer et al., 1998; Vander Leek

et al., 2000). Consequently, there is a growing need to further improve the management and treatment for this type of food allergy.

Peanut allergy is more prevalent than tree nut allergy although co-sensitisation is common (Ewan, 1996). Allergenic tree nuts include almond (Poltronieri et al., 2002), Brazil nut (Borja et al., 1999), cashew (Quercia et al., 1999), hazelnut (Pastorello et al., 2002), macadamia (Sutherland et al., 1999), walnut (Asero et al., 2004) and pine nut (Ano et al., 2002). We have previously demonstrated the presence of cross-reactive allergens in peanut and the tree nuts almond, Brazil nut and hazelnut (de Leon et al., 2003) although the identity of the offending allergens is not known. A number of peanut allergens have been identified and characterised, and two of these, namely Ara h 1 and Ara h 2, have been classified as major allergens with >90% reactivity in peanut allergic subjects (Burks et al., 1991, 1992, 1995). However, of these two major peanut allergens, Ara h 2 has been described as functionally more potent and

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more relevant than Ara h 1 in the peanut allergic population on the basis of specific IgE and ability to induce histamine release in basophils from allergic subjects (Koppelman et al., 2004; Palmer et al., 2005). Ara h 2, a glycoprotein allergen, was previously cloned and sequenced (Stanley et al., 1997), with amino acid sequence comparisons showing approximately 40% sequence identity with conglutin- δ , a sulphur-rich protein from the lupine seed (Gayler et al., 1990). Consequently, Ara h 2 was classified as a member of the conglutin family of seed storage proteins (Stanley et al., 1997). Currently, two isoforms of Ara h 2 have been identified (Chatel et al., 2003) which confirmed the presence of Ara h 2 as a protein doublet in peanut extract, at 17–19 kDa by SDS-PAGE analysis (Burks et al., 1992).

Conglutin-type proteins in almond have also been identified as being IgE reactive (Poltronieri et al., 2002). Given the allergenic importance of Ara h 2 in the peanut allergic population and the allergenicity of conglutin proteins in some tree nuts, this study sought to determine whether Ara h 2 is involved in allergenic cross-reactivity with tree nut proteins, thus explaining the observed common co-sensitisation to peanut and tree nuts.

2. Materials and methods

2.1. Subjects

Seventeen peanut allergic subjects and eight atopic, non-peanut allergic subjects were recruited from The Alfred Hospital, Allergy and Asthma Clinic. The peanut allergic subjects in this study had clinical symptoms of IgE-mediated peanut hypersensitivity and a peanut-specific IgE CAP score of ≥ 2 (≥ 0.7 kU_A/l; Pharmacia CAP SystemTM, Pharmacia Diagnostics, Uppsala, Sweden). The three peanut allergic subjects studied in detail had known clinical sensitivity and/or specific IgE (CAP score ≥ 2) to at least two of the tree nuts examined in this study, namely almond, Brazil nut, cashew and hazelnut (Table 1). The atopic, non-peanut allergic subjects in this study had no clinical history of IgE-mediated hypersensitivity to peanut and had negligible levels of peanut-specific IgE as measured by ELISA (data not shown). This study was approved by the Alfred Ethics Committee and informed written consent was obtained from each subject.

Table 1
Specific IgE CAP scores to tree nuts for three peanut allergic subjects

Subject	Known peanut/tree nut allergies	IgE CAP score (kU _A /l)			
		Almond	Brazil nut	Cashew	Hazelnut
A7	Peanut, cashew, hazelnut	ND	2 (1.20)	2 (0.88)	1 (0.39)
A8	Peanut	2 (1.79)	2 (1.44)	1 (0.39)	3 (8.45)
A11	Peanut, almond, walnut, cashew	1 (0.39)	1 (0.49)	3 (6.17)	2 (2.31)

ND, not done.

2.2. Antigens

Recombinant Ara h 2 was cloned based on the published sequence [Stanley et al., 1997; GenBank accession no. L77197] and expressed as a His₆-tagged protein as we have described previously (Glaspole et al., 2005). The expressed protein was affinity purified using Ni-NTA agarose (Qiagen, Doncaster, Australia) as described by the manufacturer but using a modified denaturing wash buffer (50 mM NaH₂PO₄, 300 mM NaCl, 50 mM imidazole and 8 M urea, pH 8.0) and elution buffer (50 mM NaH₂PO₄, 300 mM NaCl, 500 mM imidazole and 8 M urea, pH 8.0).

Peanut (de Leon et al., 2003), tree nut (de Leon et al., 2003), house dust mite (Gardner et al., 2004) and rye grass pollen (Burton et al., 2002) proteins were extracted in phosphate buffered saline (PBS). The protein concentration of these extracts was determined using the BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA).

2.3. Electrophoresis and Western immunoblotting

Recombinant Ara h 2 was resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 14% gels under reducing conditions as described previously (de Leon et al., 2003). Proteins were stained with Coomassie brilliant blue (Sigma, St. Louis, MO, USA).

For Western immunoblotting, rAra h 2 was transferred onto nitrocellulose membranes following 14% SDS-PAGE as described by de Leon et al. (2003). Membranes were incubated in subject sera, diluted 1/5 in 1% skim milk powder (SMP) in PBS, overnight at room temperature. IgE binding was detected using rabbit anti-human IgE (DAKO, Carpinteria, CA, USA) and HRP-labelled goat anti-rabbit IgG (Promega, Madison, WI, USA) antibodies.

2.4. Affinity purification of rAra h 2-specific antibodies

The 96-well polystyrene plates (Costar, Acton, MA, USA) were coated with rAra h 2, diluted to 1 μ g/ml in 50 mM bicarbonate buffer (50 μ l/well), and incubated overnight at 4 °C. Plates were washed with PBS containing 0.05% Tween (PBS-T) and blocked with 5% SMP in PBS-T for 1 h at 37 °C. After washing with PBS-T, sera, diluted 1/10 with 1% SMP in PBS-T were added to the wells and incubated at 37 °C for 2 h. Plates were washed with PBS-T and antibodies eluted by incubation with 0.2 M glycine buffer (pH 2.6; 50 μ l/well) to the wells followed by incubation for 10 min at room temperature. The antibody solution from each well was collected and neutralised to pH $\sim$ 7.4 using 2 M NaOH.

2.5. Direct IgE and inhibition ELISA

Direct IgE ELISA was performed as described by de Leon et al. (2003). The 96-well polystyrene plates (Costar) were coated with rAra h 2 (1 μ g/ml in 50 mM bicarbonate buffer, pH 9.6, 50 μ l/well) and incubated overnight at 4 °C. Plates were washed with PBS-T and blocked with 5% SMP in PBS-

T. For the inhibition ELISA, subject sera diluted with 1% SMP in PBS-T for an $OD_{490\text{nm}}$ reading of ~ 1.0 for rAra h 2, were pre-incubated with peanut or tree nut extracts or, as positive and negative controls respectively, rAra h 2 and keyhole limpet hemocyanin (KLH, Sigma) at room temperature for 2 h. The inhibition mixtures or whole sera (diluted 1/10 in 1% SMP in PBS-T) were added to the rAra h 2-coated plate and incubated at 37°C for 2 h. Plates were washed with PBS-T and incubated with rabbit anti-human IgE antibody (1:1000; DAKO) followed by HRP-labelled goat anti-rabbit IgG antibody (1:1000; Promega), both for 1 h at 37°C , with PBS-T washes after each incubation. IgE binding was detected using *o*-phenylenediamine tablets (Sigma) dissolved in 0.05 M phosphate-citrate buffer (Sigma) and the reaction was stopped with the addition of 4 M HCl. The absorbance in each well was measured at 490 nm and non-specific binding of antibodies was eliminated by subtracting the absorbance for control wells containing no antigen from the absorbance for antigen-coated wells. Percentage inhibition of IgE binding was calculated using the following formula: % inhibition = $100 - (\text{OD}_{490\text{nm}}$ of serum with inhibitor / $\text{OD}_{490\text{nm}}$ of serum without inhibitor $\times 100$).

2.6. Basophil activation

This assay was conducted as described by Drew et al. (2004). Whole blood was incubated for 20 min with rAra h 2 or, as positive controls, roasted peanut extract, rabbit anti-human IgE antibody and *N*-formyl-met-leu peptides (Sigma), or stimulation buffer alone as a negative control. Cells were incubated with goat anti-human IgE-FITC (Caltag Laboratories, Burlingame, CA, USA) and mouse anti-human CD63-PE (Caltag Laboratories). Red blood cells were lysed by adding lysis buffer (39 mM NH_4Cl , 2.5 mM KHCO_3 , 0.2 mM EDTA). Cells were subsequently washed and analysed using a FACScalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and Cell Quest software (Becton Dickinson). 7-Aminoactinomycin D (Sigma) was added to the samples to exclude non-viable cells from analysis.

3. Results

3.1. Western immunoblotting and ELISA for serum IgE reactivity to rAra h 2

rAra h 2 which had been purified using nickel resin under denaturing conditions, showed a major protein band with a molecular mass of $\sim 20\text{ kDa}$ by SDS-PAGE (Fig. 1). Western immunoblotting using peanut allergic serum indicated that the purified rAra h 2 was IgE reactive (Fig. 1, lane 2). Negligible IgE binding was obtained using serum from an atopic, non-peanut allergic subject, demonstrating the specificity of IgE reactivity. Minor low molecular weight protein bands were also detected by SDS-PAGE which bound IgE antibodies from peanut allergic serum. These may represent breakdown products of rAra h 2 obtained during protein expression. High molecular weight proteins also bound IgE antibodies. Some of these proteins may

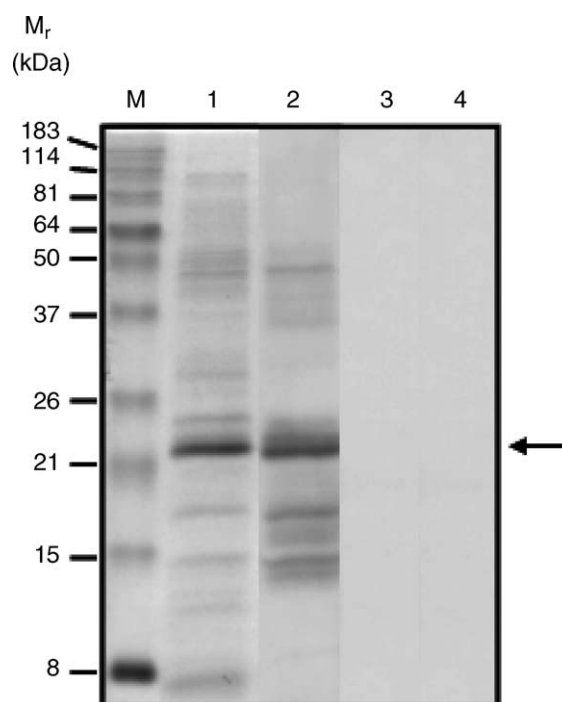


Fig. 1. IgE reactivity of purified rAra h 2 by Western immunoblotting. rAra h 2 (2 μg) was resolved by 14% SDS-PAGE under reducing conditions and proteins were stained with Coomassie brilliant blue (lane 1). IgE reactivity was assessed by Western immunoblotting using sera from peanut-allergic subject A8 (lane 2) and non-peanut allergic subject NA4 (lane 3). Membranes were also incubated with the secondary and tertiary antibodies only as a control (lane 4). Arrow indicates the position of the rAra h 2 monomer. M, molecular mass markers.

be aggregates of rAra h 2 as they bound the anti-His₆ antibody (data not shown).

The IgE reactivity to rAra h 2 was further investigated by ELISA using sera from 17 peanut allergic subjects and 7 atopic, non-peanut allergic control subjects. The cut-off for positive IgE binding was taken as 2 S.D. above the mean $OD_{490\text{nm}}$ scores of the atopic, non-peanut allergic subjects. As shown in Fig. 2, 13/17 (76%) peanut allergic subjects were positive for IgE binding.

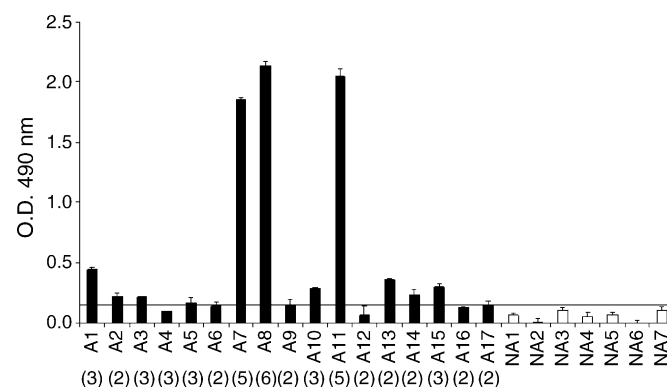


Fig. 2. IgE reactivity to rAra h 2 by ELISA. Sera from 17 peanut allergic subjects and 7 non-peanut allergic subjects were assayed for IgE binding to rAra h 2. Error bars indicate standard deviation. The horizontal line indicates the cut-off for positive IgE binding as indicated by the mean $OD_{490\text{nm}}$ readings for the non-peanut allergic subjects plus 2 S.D. Peanut-specific IgE CAP scores are shown in parenthesis.

ing to rAra h 2. This confirms the observation that Ara h 2 is a major peanut allergen (Burks et al., 1991, 1992, 1995; Kleber-Janke et al., 1999).

3.2. Biological activity of rAra h 2

The biological activity of rAra h 2 was measured using the basophil activation test. Whole blood from a peanut allergic subject (A7) with high serum IgE reactivity to rAra h 2 was incubated with rAra h 2 and the percentage of activated basophils, as indicated by CD63 expression, was determined by flow cytometry (Fig. 3). Viable basophils were gated based on forward scatter and side scatter, 7AAD exclusion and IgE^{hi} staining. The expression of CD63 on IgE^{hi} cells was then determined as an indicator of basophil activation. A high percentage of activated basophils (82%) was obtained following incubation of peanut allergic whole blood with roasted peanut extract positive control. Incubation of whole blood with rAra h 2 also resulted in a high percentage of activated basophils (87%), indicating that the rAra h 2 preparation in this study was biologically active. In contrast, there was a much lower percentage of activated basophils in the no antigen negative control (34%)

As a control for the specificity of basophil activation, rAra h 2 was also used to stimulate basophils from a HDM allergic, non-peanut allergic subject (Fig. 3(b)). Incubation of whole blood

from this subject with rAra h 2 resulted in minimal activation of basophils in comparison to the peanut allergic subject (0.3%). In contrast, stimulation with HDM resulted in a high percentage of activated basophils (83%). Minimal basophil activation (4%) was obtained in the absence of antigen stimulation.

3.3. Cross-reactivity of rAra h 2 with tree nut allergens

The ability of rAra h 2 and peanut and tree nut extracts to non-specifically inhibit IgE reactivity was first assessed by testing inhibition of latex-specific IgE binding to the latex allergen, rHev b 6.01 (de Silva et al., 2004). Serum from a latex, non-peanut/tree nut allergic subject was pre-incubated with increasing concentrations of rAra h 2. There was minimal inhibition of IgE binding by rAra h 2 to the similarly expressed rHev b 6.01 in comparison to the rHev b 6.01 positive control (data not shown). In the same way, negligible non-specific inhibition was also demonstrated for the roasted peanut, roasted almond, raw Brazil nut, roasted cashew and roasted hazelnut extracts (data not shown).

The ability of tree nut extracts to inhibit IgE binding to rAra h 2 was assessed using sera from three peanut allergic subjects (A7, A8 and A11) previously demonstrated to have high levels of specific IgE to rAra h 2 ($OD_{490\text{ nm}} \geq 1$) as well as to some tree nuts (Table 1). High levels of inhibition were obtained with the rAra h 2 positive control in all subjects (Fig. 4), demon-

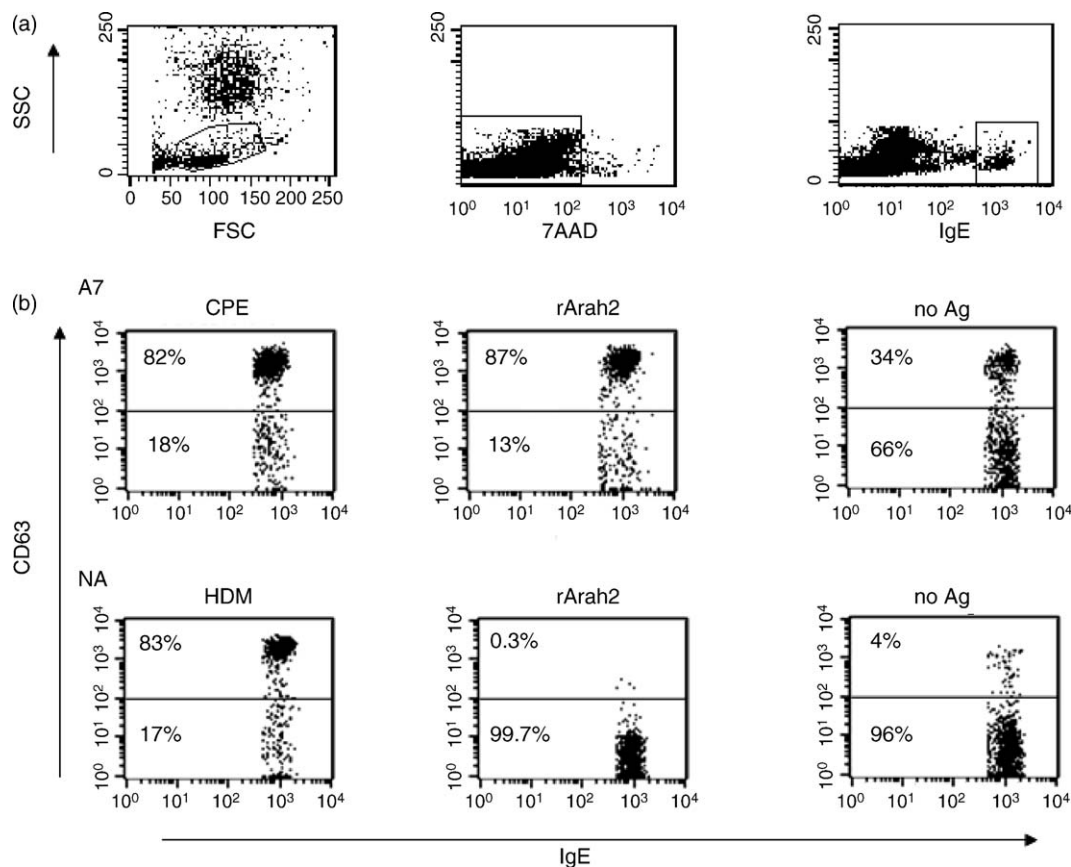


Fig. 3. Biological activity of rAra h 2. Whole blood from one peanut allergic subject (A7) and one HDM allergic, non-peanut allergic subject (NA) was incubated with 1 $\mu\text{g/ml}$ of roasted peanut extract (CPE), house dust mite extract (HDM) or rAra h 2 and basophil activation was analysed. (a) Live basophils were gated based on the scatter profile, 7AAD exclusion and high IgE staining. (b) The percentage of activated basophils was calculated as the proportion of IgE^{hi} cells expressing CD63. The cut-off for CD63 positive staining was determined from control samples.

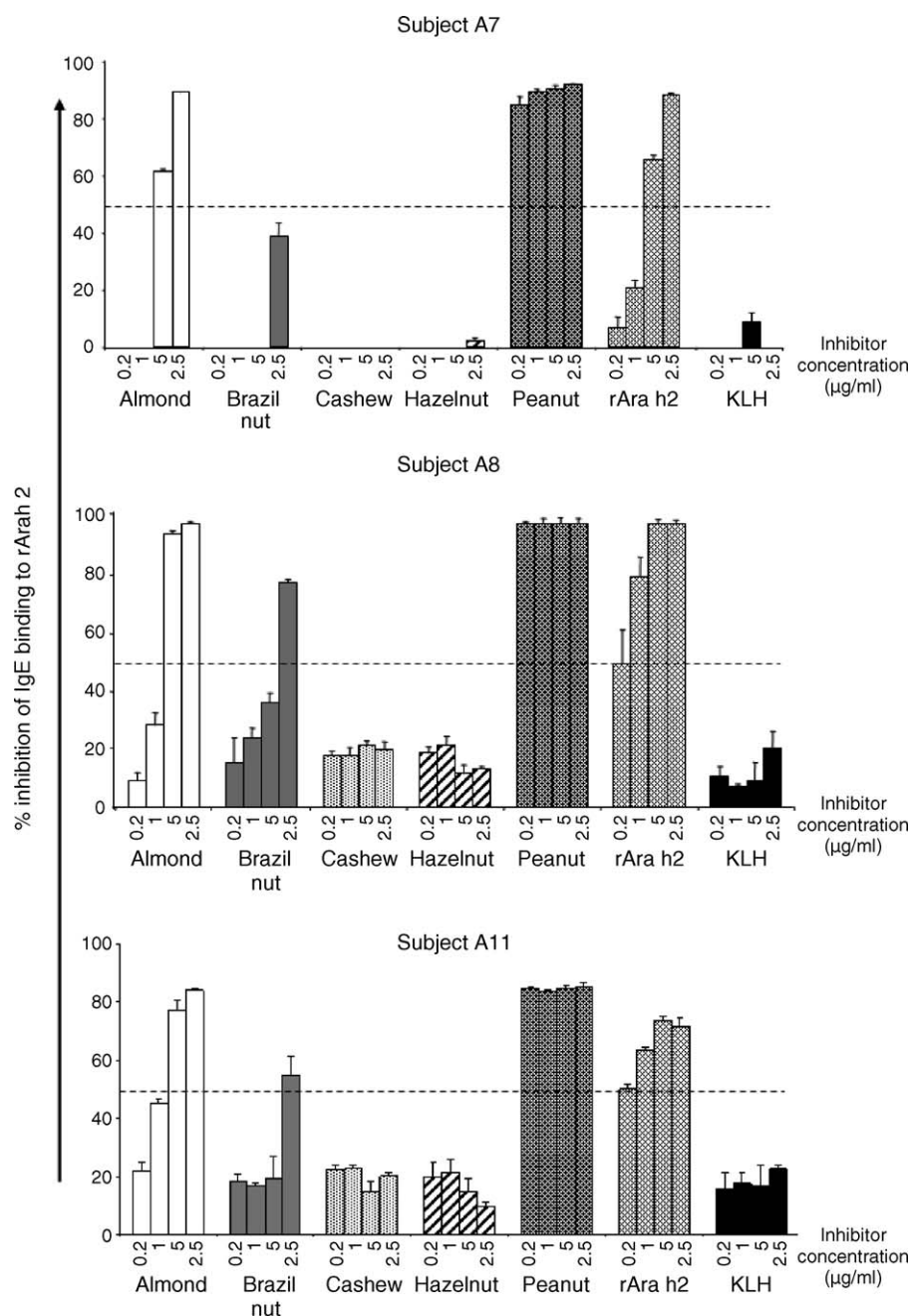


Fig. 4. Inhibition of IgE binding to rAra h 2 by peanut and tree nut extracts. Sera from three peanut and tree nut allergic subjects (A7, A8 and A11) were pre-incubated with different concentrations of roasted almond, raw Brazil nut, roasted cashew, roasted hazelnut and IgE binding to rAra h 2 immobilised on ELISA plates was measured. rAra h 2 and roasted peanut extract were used as positive controls and KLH was included as a negative control extract. Mean values for triplicates are shown and the standard deviation is indicated by error bars. Dotted line shows 50% inhibition of IgE binding to rAra h 2.

strating the specificity of this assay, with minimal inhibition observed with the negative control extract, KLH. Interestingly, higher levels of inhibition were obtained with roasted peanut extract compared to rAra h 2, indicating that serum IgE antibodies from all three subjects may have a higher affinity for the natural form of Ara h 2 present in crude peanut extract. Of the tree nut extracts tested for IgE cross-reactivity, roasted almond showed strong inhibition of IgE binding to rAra h 2 (84–98%) and raw Brazil nut extract showed weaker inhibition (39–79%). Negligible inhibition, similar to the KLH negative control, was obtained for roasted cashew and roasted hazelnut extracts.

3.4. Identification of cross-reactive tree nut allergens by immunoblotting

Affinity-purified antibodies specific for rAra h 2 were used to identify the proteins responsible for the observed cross-reactivity between Ara h 2 and the tree nuts, almond and Brazil nut. These antibodies were affinity purified from peanut allergic subject A8 serum by incubation with rAra h 2 immobilised on plates. Ara h 2 specificity of the purified antibodies was confirmed by immunoblotting on roasted peanut extract (Fig. 5(a), lane 3); IgE antibodies bound to a protein doublet with a molec-

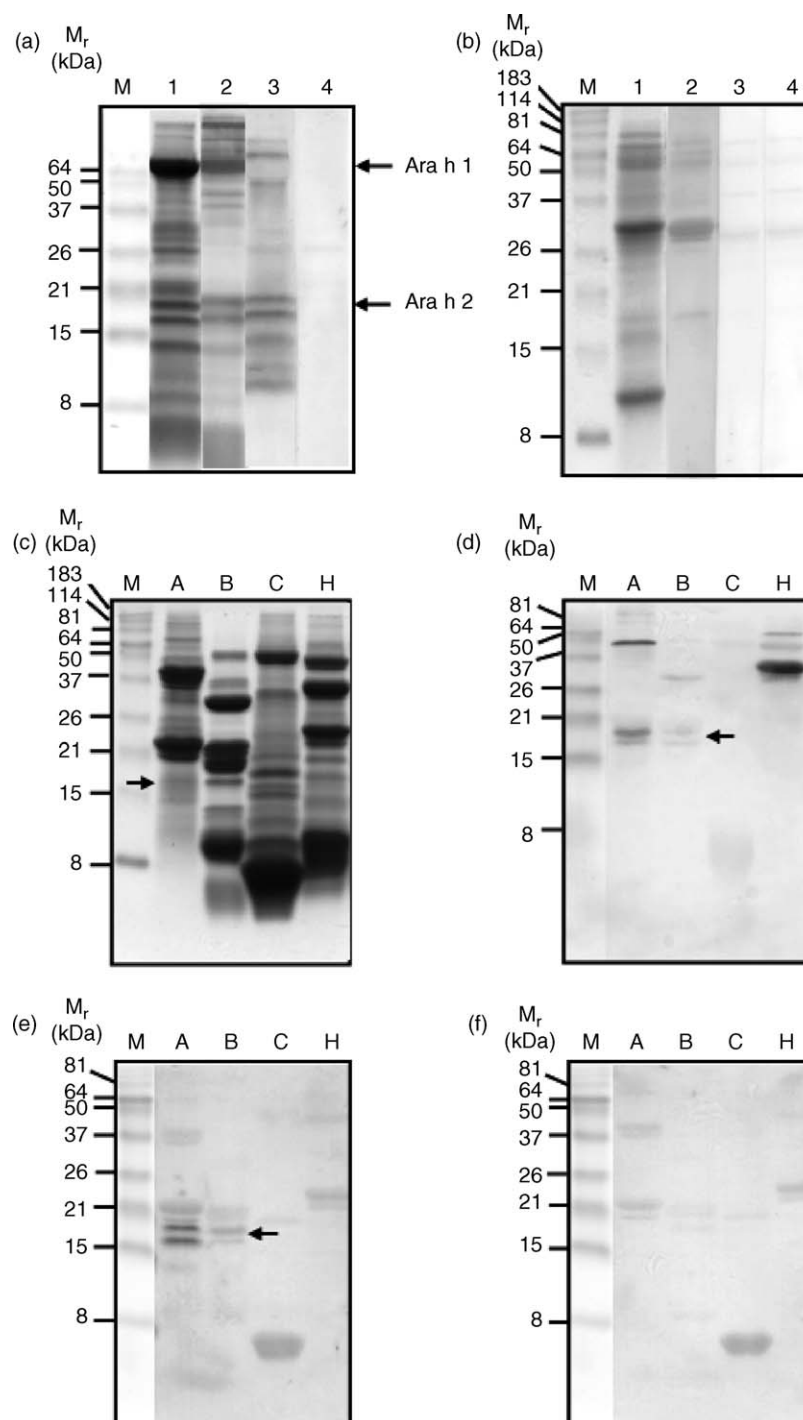


Fig. 5. Detection of cross-reactive allergens in tree nuts using affinity-purified rAra h 2 antibodies. Anti-rAra h 2 antibodies were purified from subject A8 serum and the specificity of the eluted IgE antibodies was tested by incubation with (a) roasted peanut extract and (b) RGP nitrocellulose strips. Arrows indicate position of Ara h 1 and the Ara h 2 doublet. Lanes: M, molecular mass markers (M_r); 1, Coomassie-stained gel; 2, whole serum (diluted 1/10); 3, anti-rAra h 2 antibodies (neat; equivalent to 1/10 dilution of whole serum); 4, no serum control blot. Cross-reactive tree nut allergens were identified by incubation of anti-rAra h 2 antibodies with roasted almond (A), raw Brazil nut (B), roasted cashew (C) and roasted hazelnut (H) extracts immobilised onto nitrocellulose membranes followed by detection of IgE binding. (c) Coomassie-stained gel of tree nut extracts. (d) Incubation of tree nut extracts with subject A8 whole serum (diluted 1/10). (e) Incubation of tree nut extracts with anti-rAra h 2 antibodies (neat; equivalent to 1/10 dilution of whole serum). (f) No serum negative control blot. Arrows indicate position of cross-reactive allergens. M, molecular mass markers (M_r).

ular mass of 17–19 kDa which corresponds to the molecular mass of Ara h 2 (Burks et al., 1992). IgE binding was also detected to other peanut proteins of differing molecular masses but not to Ara h 1. As for rAra h 2 in Fig. 1, these proteins

may represent aggregates or breakdown products of Ara h 2 or Ara h 2-homologous proteins. Unlike whole serum from subject A8, incubation of the purified antibodies with RGP nitrocellulose strips (as a negative control) did not exhibit any IgE

binding (Fig. 5(b)), confirming the specificity of the purified antibodies.

The purified rAra h 2-specific antibodies were incubated with nitrocellulose strips of roasted almond, raw Brazil nut, roasted cashew and roasted hazelnut to identify cross-reactive allergens. The secondary and tertiary antibodies showed some weak reactivity to tree nut proteins as demonstrated by the no serum control blot (Fig. 5(f)) and consequently this was used as the cut-off to determine positive serum and purified antibody reactivity to tree nut proteins. As shown in Fig. 5(e), rAra h 2-specific IgE antibodies bound to protein doublets present in roasted almond and raw Brazil nut extract with molecular masses of approximately 16–18 kDa. This is similar to the molecular mass of the Ara h 2 doublet in the peanut extract although these almond and Brazil nut proteins are of low abundance in the extracts upon examination of the Coomassie stained gel (Fig. 5(c)). These proteins also bound IgE antibodies from subject A8 whole serum with similar intensity to the purified antibodies (Fig. 5(d)), indicating that the majority of serum IgE binding to these almond and Brazil nut allergens may be attributed to cross-reactive Ara h 2-specific IgE antibodies. The similarity in IgE-binding intensity of the purified rAra h 2-specific antibodies to Ara h 2 in roasted peanut extract (Fig. 5(a), lane 3) and the protein doublet in roasted almond extract (Fig. 5(e)) confirms the high level of IgE cross-reactivity detected in the inhibition ELISA. The weaker binding of the purified rAra h 2-specific antibodies to raw Brazil nut extract is consistent with the weaker inhibition of rAra h 2-specific IgE by this extract in the inhibition ELISA. Minimal IgE binding was observed with roasted cashew and roasted hazelnut proteins, confirming the observed absence of cross-reactivity in inhibition ELISA. These data demonstrate that IgE cross-reactivity between peanut and the tree nuts almond and Brazil nut is due to cross-reactive allergens that may be homologues of the major peanut allergen, Ara h 2.

4. Discussion

The major peanut allergen, Ara h 2, was first described by Burks et al. (1992) and the high-level expression and purification of the recombinant form of this peanut allergen has been described by Lehmann et al. (2003). In this study, Ara h 2 was successfully expressed in *E. coli* cells and the expressed protein shown to be biologically active and reactive with serum IgE in subjects with confirmed peanut allergy. Additionally, IgE antibodies specific for Ara h 2 were found to cross-react with proteins present in almond and Brazil nut which may contribute to the prevalence of co-sensitisation to peanut and tree nuts in peanut allergic individuals.

The frequency of IgE reactivity to Ara h 2 in the peanut allergic population has consistently been reported to be >50%, both for the natural and recombinant forms (Burks et al., 1992; Kleber-Janke et al., 1999; Koppelman et al., 2004), thus classifying Ara h 2 as a major allergen. In the current study, the recombinant form of Ara h 2 bound serum IgE antibodies from 76% of peanut allergic subjects, confirming the importance of this allergen. As an in vitro correlate of clinical reactivity, the basophil activation assay was used to demonstrate biological

activity of the rAra h 2 preparation. In vitro assays such as histamine release or basophil activation have been widely used to ascertain the biological relevance of recombinant allergen preparations (Boutin et al., 1997; Iacovacci et al., 2002; Diaz-Perales et al., 2003; Westphal et al., 2003). In this study, rAra h 2 was able to activate basophils in whole blood from a peanut allergic subject but not a HDM allergic, non-peanut allergic subject. Although it could be argued that rAra h 2 expressed in a prokaryotic system is not an accurate reflection of the natural counterpart in peanut, the recognition of the recombinant form by serum IgE from peanut allergic individuals and its activation of basophils from allergic subjects confirm the presence of clinically relevant IgE epitopes.

The incidence of tree nut allergy in the peanut allergic population prompted the investigation of allergenic cross-reactivity between Ara h 2 and tree nut allergens in this study. Cross-reactivity studies using inhibition ELISA demonstrated reduced IgE binding to rAra h 2 upon pre-incubation of rAra h 2-reactive subject sera with almond and Brazil nut extracts. Immunoblotting studies using rAra h 2-specific antibodies revealed the presence of potentially homologous Ara h 2 proteins in almond and Brazil nut extract with similar molecular mass to the Ara h 2 doublet present in peanut extract. Ara h 2 is a member of the conglutin family of seed storage proteins which have also been reported to contribute to the allergenicity of almonds (Poltronieri et al., 2002). Poltronieri et al. identified an IgE reactive 45 kDa almond protein and N-terminal sequencing showed 40% identity with conglutin from white and narrow-leaved blue lupine. Typically, seed conglutins are processed into two subunits consisting of a 28–30 kDa N-terminal subunit and a 17 kDa C-terminal subunit (Kolivas and Gayler, 1993). The potential Ara h 2 homologues identified in almond and Brazil nut extract have molecular masses ranging from 17 to 19 kDa and thus may correspond to the C-terminal subunit. Further studies involving molecular cloning and sequence analysis of these potential Ara h 2 homologues are required to confirm this. To date, no Brazil nut allergens have been identified as belonging to the conglutin protein family, however SDS-PAGE clearly reveals allergenic proteins with similar molecular mass to Ara h 2.

A recent study by Barre et al. mapped linear IgE-binding epitopes of Ara h 2 (Stanley et al., 1997) using a homology-based molecular model. Their findings revealed that the linear epitopes mapped on the surface of Ara h 2 were not structurally homologous to the corresponding regions in allergens from walnut (Jug r 1), pecan (Car i 1) and Brazil nut (Ber e 1) (Barre et al., 2005). It is, however, not known if these linear epitopes mapped on the surface of Ara h 2 represent conformational epitopes which are likely to be far more relevant for antibody binding in vivo. Our study clearly demonstrates the existence of allergenic cross-reactivity between Ara h 2 and Brazil nut proteins and therefore homology between linear epitopes may not necessarily be useful in predicting cross-reactivity.

Although the cross-reactive tree nut proteins were of low abundance in the extracts as revealed by Coomassie staining, there was still unexpectedly a high degree of cross-reactivity between these proteins and Ara h 2, which may be attributed to epitope similarity and high antibody affinity. The extent to

which these two factors contribute to allergenic cross-reactivity is largely determined by the degree of sequence homology. A high level of overall sequence homology is likely to result in IgE-binding epitopes that are of high sequence similarity, leading to high affinity, cross-reactive IgE antibody interactions. In contrast, low sequence similarity may yield fewer, low affinity cross-reactive allergen-IgE antibody interactions. Further studies involving molecular cloning, sequence analysis and epitope mapping of the potential Ara h 2 homologues in almond and Brazil nut are necessary to determine the degree of sequence similarity with Ara h 2 and subsequently, the basis for this allergenic cross-reactivity.

Carbohydrate moieties have previously been shown to contribute to IgE cross-reactivity between different allergen sources (Aalberse et al., 1981; van Ree et al., 2000; Hemmer et al., 2004) and may be responsible for allergenic cross-reactivity between peanut and tree nuts. However, the results from our basophil activation and cross-reactivity studies using a prokaryotic recombinant peanut allergen indicate that carbohydrate groups are not essential for biological activity and/or cross-reaction. rAra h 2 was shown to share similar IgE binding epitopes with proteins present in almond and Brazil nut, demonstrating that there are some cross-reactive IgE-binding epitopes that are not carbohydrate in nature. This study, however, was limited since a comparison of cross-reactivity using purified natural and recombinant allergens was not conducted. This would be necessary given that Ara h 2 has been previously classified as a glycoprotein (Stanley et al., 1997). Therefore, the role of carbohydrate groups in peanut and tree nut cross-reactivity cannot be completely excluded without further testing but the results of our study show that there are cross-reactive epitopes in Ara h 2 and tree nut allergens that are not carbohydrate in nature.

5. Conclusions

Our study has demonstrated that the major peanut allergen, Ara h 2, shares similar IgE-binding epitopes with allergens from almond and Brazil nut which may contribute to the high frequency of co-sensitisation to peanut and tree nuts in the peanut allergic population. This information will lead to improved patient management and diagnosis and may provide avenues for the development of specific therapy for this type of food allergy which is currently lacking.

Acknowledgements

Financial support from the National Health and Medical Research Council of Australia and The Alfred Research Trusts is gratefully acknowledged.

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