ORIGINAL ARTICLE FOOD ALLERGY AND ANAPHYLAXIS

Measurement of Ara h 1-, 2-, and 3-specific IgE antibodies is useful in diagnosis of peanut allergy in Japanese children

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Keywords
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Abstract

Background: Food challenges are time-consuming, expensive, and not always possible to perform. Therefore, new tools to diagnose food allergy are desired. The aim was to evaluate IgE antibodies to peanut allergens in the diagnosis of peanut allergy in Japanese children using ImmunoCAP® and IgE immunoblotting.

Methods: The study included 2–13-yr-old consecutive patients (n = 57) referred to our specialist clinic for investigation of current peanut allergy using food challenge. All children had a previous doctor's diagnosis of peanut allergy and were on elimination diet. Serum samples were analyzed for IgE reactivity to peanut, recombinant (r) Ara h 1, 2, 3, 5, 8, and 9. IgE immunoblotting (n = 23) was performed using extracts from raw and roasted peanut.

Results: Twenty-six of the children failed (allergic group), and 31 passed the peanut challenge (tolerant group). The rAra h 2 ImmunoCAP test was superior in its ability to differentiate between children in the allergic and tolerant groups with a sensitivity and specificity of 88% and 84%, respectively (cutoff, 0.35 kU_A/l). The combination of rAra h 1, 2, and 3 resulted in a higher specificity (94%) when IgE to all of them was the criteria for positivity. ImmunoCAP generally showed a good agreement with immunoblotting using both raw and roasted peanut for IgE reactivity to Ara h 1, 2, and 3.

Conclusions: Measurement of IgE antibodies to rAra h 1, 2, and 3 is useful in the diagnosis of peanut allergy and in the investigation of reactions to raw and roasted peanut.

Peanut allergy is one of the most common allergies in Westernized countries with an estimated prevalence of up to 1.8% (1, 2). Clinical symptoms of peanut allergy mainly involve the oral cavity, skin, and gastrointestinal tract and occasionally include respiratory symptoms (2, 3). In Japan, peanut is ranked among the top five items causing anaphylaxis (4).

Several proteins have been identified as peanut allergens, and the use of recombinant allergens has offered improved possibilities for a more specific and simplified peanut diagnosis (5–7). Ara h 1, Ara h 2, and Ara h 3 belong to the peanut storage proteins and have been designated as major allergens, based on the prevalence of IgE reactivity among allergic patients (6, 8). Ara h 6 is a 2S albumin and shares several IgE epitopes with Ara h 2 (9, 10). The minor peanut allergen Ara h 5 shows homology with pollen profilins and is reported to be recognized by around 10% of peanut-sensitive individuals (8). Ara h 8, a Bet v 1-homologous pathogenesis-related (PR)-10 protein, has been shown as a major allergen for patients with concurrent birch pollen and peanut allergies (11). Lipid transfer protein (LTP), a pan-allergen with a degree of cross-reactivity comparable to profilin, is present in peanut as Ara h 9 (12, 13).

The aim of this study, composed of Japanese children with suspected peanut allergy, was to evaluate ImmunoCAP® tests

with recombinant peanut allergens as useful tools in distinguishing between genuine peanut-allergic and asymptomatic patients. Additionally, the ImmunoCAP results were compared with IgE immunoblotting using extracts from raw and roasted peanuts.

Materials and methods

Study design

The study included 57 consecutive Japanese patients (median age, 6 yrs; range, 2–13 yrs; 16 girls and 41 boys) referred to the Department of Allergy, Clinical Research Center for Allergology and Rheumatology, at the Sagamihara National Hospital in Japan for the investigation of current peanut allergy using an open food challenge as described later. All children had a previous doctor's diagnosis of peanut allergy (at a children's primary care clinic) and were on an elimination diet. Most children (82%) also had other food allergies according to their clinical documentations. All but five peanut allergy diagnoses were based on documented self-reported immediate reactions to peanut in combination with other findings, for example, clinical symptoms and peanut sensitization. Four peanut allergy diagnoses were based on serology testing, and one on doctor's judgment, without a documented history of immediate reaction to peanut. All these five children had objective clinical symptoms and suffered from other food allergies beside peanut allergy. Clinical and laboratory data from the patient records are shown in Table 1. For further serological analysis, sera from all patients were prepared from venous blood samples and frozen at −20°C until use.

Ethical approval was obtained through the Institutional Review Boards at Sagamihara National Hospital. Written, informed consent was given by the child and/or child's parents prior to enrollment.

Oral peanut challenge test

All patients underwent an open peanut challenge which was performed using the identical titration steps as for the double-blinded ones recommended by the American Academy of Allergy and Immunology (14). Briefly, subjects were given increasing amounts of steamed cake that was mixed with powdery roasted peanut, starting at 1/16 of the amount of the highest challenge dose. It was followed by roughly doubling doses every 15 min until the highest ordinary challenge dose was achieved at 3 g of peanut flour or until an objective allergic reaction was observed. In doubtful cases, children were receiving up to 10 g of peanut. Any immediate symptom from skin, gastrointestinal tract, respiratory tract, and oral mucosa was observed and documented. Rescue medication (anti-histamine, nebulized β2-adrenergic

Table 1 Characteristics of patients, all with a previous diagnosis of peanut allergy, who failed (allergic group) or passed (tolerant group) the peanut challenge in the present study including laboratory data

Parameter	Allergic group	Tolerant group	p value
Number of patients	26	31	
Dose at reaction, gram, median (range)	3 (0.19–10)	No reaction*	
Sex, no of boys (%)	22 (85)	19 (61)	NS
Age, years, median (range)	6 (3–13)	6 (2–13)	NS
Total IgE, kU/l, median (range)	1225 (32–13,300)	679 (50–4730)	NS
Eosinophils, count, median (range)	300 (130–1220)†	400 (30–1650)	NS
Allergic heredity, no (%)			
Mother allergic	15 (58)	18 (58)	NS
Father allergic	12 (46)	17 (55)	NS
Both parents allergic	18 (69)	23 (74)	NS
History of immediate reaction to peanut, no (%)	26 (100)	26 (84)‡	NS
Other food allergy diagnoses, no (%)	20 (77)	27 (87)	NS
Allergic complications, no (%)			
Bronchial asthma	14 (54)	14 (45)	NS
Atopic dermatitis	20 (77)	20 (65)	NS
Allergic Rhinitis	13 (50)	13 (42)	NS
Allergic Conjunctivitis	5 (19)	9 (29)	NS
Oral allergy syndrome	1 (3.8)	2 (6.5)	NS
Anaphylaxis to any food	18 (69)	14 (45)	NS
Anaphylaxis to peanut	8 (31)	1 (3.2)	<0.01

NS, not significant.

*Challenged with 3 g (n = 11) or 10 g (n = 20) of peanut flour.

†Data missing from one patient.

‡Of the five patients lacking a documented history of an immediate reaction to peanut, four had a previous peanut allergy diagnosis based on serology testing in combination with clinical symptoms, and one patient had a diagnosis based on clinical symptoms only (doctor's judgment).

agonist, glucocorticoid, and adrenaline) was administered immediately upon detection of an allergic reaction. Subsequently, the children were carefully monitored for 24 h. Anaphylactic reactions were defined in accordance with the recently established guidelines as symptoms affecting at least two major organ systems, starting rapidly within minutes or within hours after food ingestion (15). Diagnosis of peanut tolerance was confirmed by safe routine introduction of peanuts at the outpatient clinic. As all children considered to be peanut tolerant were not receiving 10 g peanut at challenge, sub-analyses were performed to evaluate their impact on the study results.

Allergen-specific IgE and total IgE measurements

The 57 serum samples were analyzed using ImmunoCAP (Phadia, Uppsala, Sweden) for IgE reactivity to peanut extract, recombinant (r) Ara h 1, rAra h 2, rAra h 3, rAra h 8, and rAra h 9, and cross-reactive carbohydrate determinant (CCD), that is, MUXF3 from bromelain. Twenty-four of the patient samples were additionally analyzed for IgE reactivity to rAra h 5 using experimental ImmunoCAP tests developed following the standard method of Phadia. The selection of samples was forced by limited serum volumes and the available amounts of rAra h 5. All but one of these 24 samples were also subjected to IgE immunoblotting as described later. The development and production of rAra h 5 has been described elsewhere (16). All other ImmunoCAP tests are commercially available. Two cutoff levels for positive results were used in the evaluation of the test results: 0.10 and 0.35 kU_A/l. The serum total IgE levels were determined using ImmunoCAP Total IgE assay (cutoff: 2 kU/l).

Peanut extracts

Roasted peanut extract was obtained from roasting whole peanut (Jumbo Runner, Allergon, Ängelholm, Sweden) at 170°C for 20 min prior to grinding in 0.1 M phosphate buffer, pH 7.4, using an Ultra-Turrax type TP 18/10 rotary homogenizer (IKA Werke, Staufen, Germany). The extraction was continued by gently shaking for 2 h at 4°C before centrifugation (5000 ***g***, 30 min) and filtration. Extracts of raw peanut were made as above, except for the grinding that was redundant because it had already been performed by the manufacturer (Allergon). Both extracts were freeze-dried and kept at +4°C until use.

IgE immunoblotting

Immunoblotting was performed on sera from 14 subjects who failed challenge and from 9 who passed challenge. Freeze-dried extracts of raw and roasted peanuts were dissolved in sample buffer and separated by reducing SDS-PAGE on ExcelGel TM 2-D Homogeneous 12.5% using Multiphor II (GE Healthcare, Uppsala, Sweden) and transferred to nitrocellulose membranes. Blotted nitrocellulose strips were incubated with patient sera diluted to a peanut-specific IgE concentration of 0.5 kU_A/l. The procedure mainly followed a previously described method except that bound IgE antibodies were detected by luminescence using the ECL advanced method (GE Healthcare) (17).

Statistics

Data were analyzed using GRAPHPAD PRISM (version 4.03, GraphPad Software, La Jolla, CA, USA). Mann–Whitney *U* test and Fisher's exact test (both two tailed) were used for pairwise comparisons of continuous parameters (specific IgE concentrations) and categorical data between groups, respectively. Receiver operating characteristic (ROC) analysis was performed to test results in relation to challenge outcome and presented graphically and as area under curve with 95% confidence interval (95% CI). All p values < 0.05 were considered significant.

Results

Oral peanut challenge test

Twenty-six of the children with suspected peanut allergy failed the peanut challenge (allergic group), and 31 passed the challenge (tolerant group). One of the latter had a history of anaphylactic reaction reported to be associated with peanut consumption. However, this was not confirmed in the challenge.

The dose to which the peanut-allergic children reacted varied from 0.19 to 10 g of peanut flour, which was the highest dose in the challenge. The median dose for a positive reaction was 2.75 g, which corresponds to roughly 2–3 peanuts. In the tolerant group, 20 children were challenged with 10 g of peanut and 11 children with 3 g. History of bronchial asthma was more common among the latter than the 10 g challenged children ($p < 0.05$). Tolerance was confirmed in all children who passed the challenge by safe routine introduction of peanuts at the outpatient clinic. Eight of the children who failed the challenge had a history of anaphylactic reactions associated with peanuts, and 6 of them also reacted with anaphylaxis at challenge. In total, eight had anaphylactic reactions during the peanut challenge for which all were successfully treated with using rescue medications. The symptoms documented from 25 (96%) of the children in the allergic group were urticaria (n = 17), erythema (n = 8), abdominal pain (n = 10), vomit (n = 8), diarrhea (n = 1), cough (n = 13), wheeze (n = 5), mucous membrane-related symptoms including throat pruritus and lip swelling (n = 9), and anaphylaxis (n = 8). One patient was classified as peanut allergic because of subjective symptoms only expressed as severe abdominal pain by the challenge test. Within a few months after the challenge, this patient developed convincing objective symptoms (exanthema including hives) by the accidental ingestion of peanut butter and was therefore included in the allergic group.

Serological analysis

When comparing the different ImmunoCAP tests using ROC analysis, the rAra h 2 ImmunoCAP test was superior in its

ability to differentiate between children who failed peanut challenge (allergic group) and children who passed the challenge (tolerant group), with an area under the curve of 0.91 (95% CI, 0.82–1.00; $p < 0.0001$). Neither of the other recombinant allergen ImmunoCAP tests showed an overall performance close to the rAra h 2 test (Fig. 1).

The peanut-allergic children more frequently had IgE antibodies to peanut and rAra h 2 than the tolerant children (Table 2). Likewise, the IgE antibody levels to peanut and rAra h 2 were higher in the children who failed challenge than in the children who passed. A similar difference in the IgE antibody levels was shown for rAra h 1 (Table 2). The IgE reactivity profiles of the five tolerant children with Ara h 2-specific IgE antibodies above the higher study cutoff (0.35 kU_A/l) are shown in Table 3. These children did not differ from the other tolerant children in any parameter studied and had no history of anaphylactic reactions to peanut (data not shown).

Comparable differences between the study groups were obtained when excluding the children in the tolerant group that were challenged with only 3 g of peanut protein. In addition to the differences described for peanut, Ara h 1 and Ara h 2, it was significantly more common with IgE antibodies to rAra h 3 (>0.35 kU_A/l) among the peanut-allergic children (allergic group) than the tolerant children challenged with 10 g ($p < 0.05$, data not shown), and the allergic group had slightly higher specific IgE levels to rAra h 8 (median, 0.22 kU_A/l; range, <0.10–33.9 kU_A/l vs. median, <0.10 kU_A/l; range, <0.10–12.3 kU_A/l; $p < 0.05$).

The extract-based peanut ImmunoCAP test had an excellent clinical sensitivity (100%), but the specificity was very low when using the 0.35 kU_A/l cutoff level (Table 4). The rAra h 2 test, on the other hand, showed a high sensitivity combined with a high specificity (88% and 84%, respectively). When using the criterion that all of the rAra h 1, rAra h 2, and rAra h 3 tests should be positive for the diagnosis of peanut allergy, a slightly higher specificity was obtained (94%) compared to the rAra h 2 ImmunoCAP alone, but the sensitivity of that combination was only 31%. The optimal cutoff levels corresponding to the statistically highest combined sensitivity and specificity of the peanut and rAra h 2 ImmunoCAP tests were obtained from the ROC analysis (Table 4). When using the optimal cutoff for rAra h 2 (0.66 kU_A/L), the high sensitivity was maintained and the specificity was slightly increased compared to the 0.35 kU_A/l cutoff. However, while the specificity increased to similar figures as for rAra h 2 when using the optimal cutoff for the peanut ImmunoCAP, the sensitivity of the test was drastically decreased to 69%. Similar data were obtained when excluding the 11 children in the tolerant group who did not receive all 10 g of peanut at challenge (Table 4).

All eight children that experienced anaphylactic reactions during the peanut challenge had IgE antibodies to rAra h 2. Their Ara h 2-specific IgE levels (median, 11.9 kU_A/l; range, 2.13–46.7 kU_A/l) were significantly higher compared to the children expressing milder symptoms at challenge (median, 1.34 kU_A/l; range, <0.10 – >100 kU_A/l; $p < 0.01$). A similar difference was obtained when also including the two allergic children with a history of anaphylaxis but with milder symptoms at challenge to the former group (data not shown).

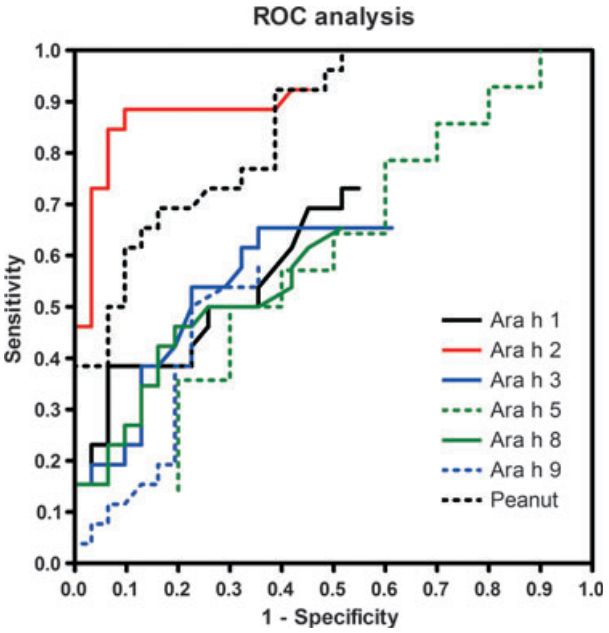


Figure 1 ROC analysis using different ImmunoCAP tests for the diagnosis of peanut allergy.

IgE immunoblotting

IgE immunoblotting with extracts of both raw and roasted peanut was performed with sera from 23 children with the aim of confirming the individual IgE reactivity patterns revealed using ImmunoCAP. IgE reactivity to rAra h 1 was associated with IgE binding to a protein at about 55–70 kDa in immunoblotting with raw and roasted peanut extract (Fig. 2 and 3, e.g. patients #38 and #56) (18, 19). Most children with IgE reactivity to rAra h 2 showed three bands at about 15, 17, and 20 kDa (e.g. patients #23 and #29). The double band at 17–20 kDa corresponds to Ara h 2 (19, 20), and the 15 kDa band is most likely Ara h 6 (9, 10). IgE reactivity to rAra h 3 corresponded to a double band between approximately 37 and 50 kDa (e.g. patients #30 and #38), which is indicative of the acidic subunits of 42 and 45 kDa that have been described as containing the main IgE-binding epitopes of Ara h 3 (19, 21). IgE binding to Ara h 8, with a molecular weight of approximately 17 kDa (11), was difficult to distinguish from Ara h 2 in patients positive for IgE to both rAra h 2 and rAra h 8 in ImmunoCAP. In one of the Ara h 8-sensitized patients lacking IgE reactivity to rAra h 2 (patient #1), a band at 17 kDa in the raw peanut extract most probably represents Ara h 8. This IgE binding was abolished after roasting. IgE binding in the 12–14-kDa region was observed in sera from several patients. A band at approximately 14 kDa revealed for patients #1 and #46 in both raw and roasted peanut probably represents IgE binding

Table 2 Serological data

Test	Specific IgE data	Allergic group n = 26	Tolerant group n = 31	p value
Peanut	>0.10 kU_A/l, no. (%)	26 (100)	27 (87)	NS
	>0.35 kU_A/l, no. (%)	26 (100)	24 (77)	<0.05
	Level (kU_A/l), median (range)	6.3 (1.4–>100)	1.4 (<0.10–13.4)	<0.0001
rAra h 1	>0.10 kU_A/l, no. (%)	19 (73)	16 (55)	NS
	>0.35 kU_A/l, no. (%)	10 (38)	7 (23)	NS
	Level (kU_A/l), median (range)	0.26 (<0.10–43.4)	0.14 (<0.10–6.0)	<0.05
rAra h 2	>0.10 kU_A/l, no. (%)	24 (92)	14 (45)	<0.001
	>0.35 kU_A/l, no. (%)	23 (88)	5 (16)	<0.0001
	Level (kU_A/l), median (range)	3.2 (<0.10–>100)	<0.10 (<0.10–4.0)	<0.0001
rAra h 3	>0.10 kU_A/l, no. (%)	17 (65)	18 (61)	NS
	>0.35 kU_A/l, no. (%)	11 (42)	6 (19)	NS
	Level (kU_A/l), median (range)	0.34 (<0.10–21.6)	0.14 (<0.10–2.4)	NS
rAra h 5*	>0.10 kU_A/l, no. (%)	12 (86)	8 (80)	NS
	>0.35 kU_A/l, no. (%)	9 (64)	7 (70)	NS
	Level (kU_A/l), median (range)	0.45 (<0.10–23.9)	1.1 (<0.10–26.7)	NS
rAra h 8	>0.10 kU_A/l, no. (%)	17 (65)	14 (52)	NS
	>0.35 kU_A/l, no. (%)	11 (42)	6 (19)	NS
	Level (kU_A/l), median (range)	0.22 (<0.10–33.9)	0.10 (<0.10–12.3)	NS
rAra h 9	>0.10 kU_A/l, no. (%)	15 (58)	6 (35)	NS
	>0.35 kU_A/l, no. (%)	4 (15)	4 (13)	NS
	Level (kU_A/l), median (range)	0.16 (<0.10–4.3)	<0.10 (<0.10–3.0)	NS
CCD†	>0.10 kU_A/l, no. (%)	16 (62)	15 (50)	NS
	>0.35 kU_A/l, no. (%)	7 (27)	6 (20)	NS
	Level (kU_A/l), median (range)	0.17 (<0.10–2.1)	0.10 (<0.10–9.1)	NS

NS, not significant.
*Allergic group, n = 14; tolerant group, n = 10.
†Tolerant group, n = 30.

Table 3 Specific IgE reactivity profiles in children that passed the peanut challenge despite having IgE antibodies to rAra h 2 (>0.35 kU_A/l)

	Specific IgE antibodies (kU_A/l)							
Patient ID	Peanut	rAra h 1	rAra h 2	rAra h 3	rAra h 5	rAra h 8	rAra h 9	CCD
6	0.47	0.10	1.09	0.11	NA	0.12	<0.10	<0.10
25	6.34	2.23	0.68	0.41	NA	<0.10	<0.10	<0.10
29	5.32	0.21	3.98	0.86	0.24	<0.10	2.99	0.37
37	12.10	0.38	0.39	0.36	NA	0.37	1.00	9.13
53	2.83	0.24	0.36	0.14	0.38	<0.10	<0.10	NA

to Ara h 5 (16, 22). In several other cases (e.g. patients #12 and #26), a 12-kDa band was associated with IgE binding to Ara h 2/Ara h 6, suggesting that it could correspond to a breakdown product of a 2S albumin, probably Ara h 6 (23). No band on the immunoblots corresponded to the 9-kDa allergen Ara h 9 (13, 24), although some patients showed IgE reactivity to rAra h 9 in ImmunoCAP (e.g. patients #3 and #29).

Generally, there was a good agreement between IgE reactivity to individual peanut allergens observed using recombinant allergen-based ImmunoCAP tests and IgE immunoblottings with extracts from both raw and roasted peanut. This was valid for both the allergic group (Fig. 2) and the tolerant group (Fig. 3), and especially for IgE reactivity to peanut storage proteins.

Discussion

The present study shows that IgE antibodies to rAra h 2 are superior markers for peanut allergy in Japanese children. The ImmunoCAP results generally showed good agreement with the outcome of the performed IgE immunoblotting study using both raw and roasted peanut.

The allergic and tolerant groups generally had very similar patient characteristics. The children classified as being allergic in the present study (allergic group) all had a history of

Table 4 Clinical efficacy of the peanut and rAra h 2 ImmunoCAP tests and combination of the rAra h 1, rAra h 2, and rAra h 3 tests

	Peanut		rAra h 2		All of 1+2+3*
Assay cutoff	0.35 kU_A/l	4.33 kU_A/l†	0.35 kU_A/l	0.66 kU_A/l†	0.35 kU_A/l
Sensitivity (95% CI)	100% (87–100)	69% (48–86)	88% (70–98)	88% (70–98)	31% (14–52)
Allergic group (n = 26) vs. Tolerant group challenged with 3 g or 10 g of peanut protein (n = 31)					
Specificity (95% CI)	23% (9.6–41)	84% (66–95)	84% (66–95)	90% (74–98)	94% (79–99)
PPV (95% CI)	52% (37–66)	78% (56–93)	82% (63–94)	88% (70–98)	80% (44–97)
NPV (95% CI)	100% (59–100)	76% (59–89)	90% (73–98)	90% (74–98)	62% (46–75)
Allergic group (n = 26) vs. Tolerant group challenged with 10 g of peanut protein (n = 20)					
Specificity (95% CI)	25% (8.7–49)	95% (75–100)	85% (62–97)	90% (68–99)	100% (83–100)
PPV (95% CI)	63% (47–78)	95% (74–100)	88% (70–98)	92% (74–99)	100% (63–100)
NPV (95% CI)	100% (48–100)	70% (50–86)	85% (62–97)	86% (64–97)	53% (36–69)

PPV, positive predictive value; NPV, negative predictive value.

*Positive test results for IgE to all three allergens (rAra h 1, rAra h 2, and rAra h 3).

†Optimal cutoff according to ROC analysis graphically described in Fig. 1.

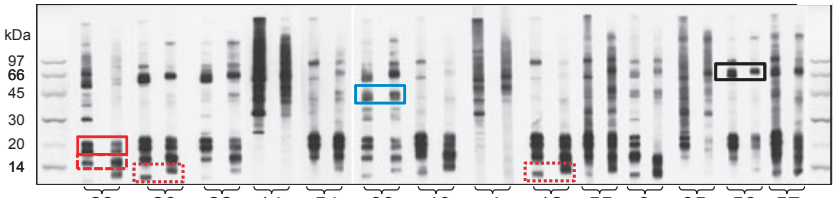
reaction to peanut and failed the peanut challenge performed. The tolerant group consisted of children whose history of peanut reaction could not be confirmed in the challenge. Only objective reactions were regarded as positive reactions, making the diagnosis using results of the challenge persuasive even if the challenge was not conducted as a double-blind placebo-controlled food challenge. The only exception from that rule was a patient, classified as peanut allergic, who expressed severe abdominal pain (regarded as subjective symptom) because of the peanut challenge and who later developed convincing objective symptoms by accidental ingestion of peanuts. A maximum dose of 3–5 g of peanut flour has been regarded as useful at peanut challenges (25, 26). The highest ordinary dose in the present study composed of children aged 2–13 yrs was 3 g of peanut protein, roughly corresponding to three peanuts. However, a majority of the subjects in the tolerant group (i.e. 20 children) received 10 g of peanut, which was given in doubtful cases to rule out false-positive diagnoses.

Two cases of previous anaphylactic reactions to peanut in the allergic group were not confirmed at challenge, and one case in the tolerant group. It is well known that anaphylactic reactions to peanut are often unpredictable (27). Discrepancies between case history and food challenge results might occur and could be due to tolerance development or the need for additional factors for the initiation of severe reactions (28).

Our results from the specific IgE analyses are in line with others showing that Ara h 2 seems to be the most important peanut allergen (5, 6, 19, 20, 29). However, the importance of different peanut allergens for clinical symptoms seems to differ between geographic areas (30). IgE to Ara h 8 is more prominent in peanut-allergic patients exposed to pollen from Fagales trees (e.g. birch), and IgE to Ara h 9 is commonly found in peanut-allergic patients living in the Mediterranean area (13, 30). Ara h 8 belongs to the PR-10 protein family that includes labile food allergens that normally only induce mild allergic reactions such as oral allergy syndrome in sensitized individuals (11, 31). Other explanations why IgE reactivity to Ara h 8 mostly is associated with only mild symptoms or tolerance to peanut could be the relatively low affinity of tree pollen-induced cross-reactive IgE antibodies, and much lower amounts of Ara h 8 in peanut compared to the storage proteins Ara h 1, Ara h 2, and Ara h 3 (11, 18, 23).

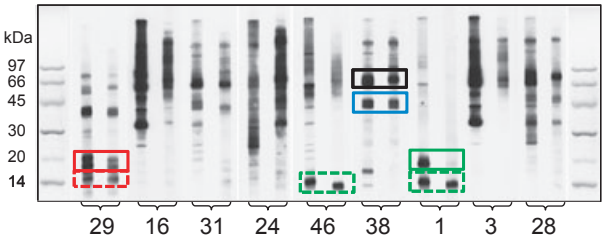
In a Swedish study by Asarnoj et al. (32), IgE to Ara h 8 was associated with asymptomatic sensitization to peanut while IgE to Ara h 2 was associated with allergic reactions to peanut. The Japanese peanut-allergic children in our study had IgE reactivity profiles similar to Swedish allergic children, with a high prevalence of IgE to Ara h 2 (88%) and a bit lower prevalence of IgE to Ara h 1, Ara h 3, and Ara h 8 (38–42% in the present study when using the 0.35 kU_A/l cutoff). In a recent British study by Nicolaou et al. (7), an even better performance of the rAra h 2 ImmunoCAP test was found. As much as 97.5% of the 80 included peanut-sensitized children were correctly classified as peanut allergic or tolerant when using the 0.35 kU_A/l assay cutoff. Using higher cutoff levels than the traditionally used 0.35 kU_A/l increases the specificity of the test. With the statistically optimal cutoff for the peanut ImmunoCAP in the present study (4.33 kU_A/l), the specificity increased from 23 to 84%. However, the sensitivity decreased from 100 to 69%. For rAra h 2 ImmunoCAP, the specificity slightly increased to 90% when using the optimal cutoff (0.66 kU_A/l) without affecting the high sensitivity of the test (88%). In our study population, a positive predictive value of 82% was obtained for the rAra h 2 ImmunoCAP test (>0.35 kU_A/l), indicating that only the presence of IgE antibodies to Ara h 2 is in most cases not enough for a clear peanut diagnosis. Thus, the diagnosis should always be based on the combination of serology testing and evaluation of case history and, if applicable, food challenges.

The observed Ara h 8 sensitization is probably due to cross-reactivity of IgE antibodies induced to Aln g 1, the PR-10 allergen in alder pollen that is common in the study area (33). The high prevalence of IgE to Ara h 5 (profilin) also indicates an impact of cross-reactive IgE antibodies. The prevalence of IgE to Ara h 9 of 14% is very similar as shown



CAP (sIgE)	23	26	22	14	54	30	40	4	12	55	9	35	56	57
Peanut	*2.29*	*84.3*	*25.6*	*5.09*	*1.98*	*19.9*	*13.5*	*1.39*	*23.6*	*9.65*	*8.52*	*2.53*	*30.7*	*71.9*
rAra h 1	**<0.10**	**12.8**	**3.21**	**0.18**	**<0.10**	**5.87**	**0.22**	**<0.10**	**0.13**	**0.31**	**0.30**	**0.19**	**8.59**	**7.73**
rAra h 2	**0.83**	**42.2**	**12.0**	0.13	**2.13**	**6.27**	**11.78**	<0.10	**15.7**	**7.38**	0.66	**1.73**	**28.8**	**46.7**
rAra h 3	<0.10	0.35	0.43	0.49	<0.10	**5.38**	0.34	<0.10	0.25	0.27	0.57	0.20	0.36	2.29
rAra h 5	<0.10	**23.9**	2.06	0.18	<0.10	**9.83**	1.72	0.16	0.36	0.82	**5.19**	0.53	0.36	0.13
rAra h 8	<0.10	0.48	**5.81**	0.12	<0.10	0.18	**4.44**	<0.10	0.26	**9.51**	**24.5**	**15.8**	1.02	<0.10
rAra h 9	**<0.10**	**0.99**	**<0.10**	**0.15**	**<0.10**	**0.22**	**0.17**	**<0.10**	**0.22**	**0.59**	**0.20**	**0.24**	**0.22**	**<0.10**
CCD	<0.10	0.36	<0.10	0.15	<0.10	0.29	0.55	0.26	0.15	0.21	0.40	0.21	0.32	<0.10

Figure 2 IgE immunoblotting with sera from children who failed peanut challenge (allergic group) using extract from both raw (left lane) and roasted peanut (right lane). Patient IDs are indicated together with individual ImmunoCAP results. Dominating IgE reactivities defined as an IgE ratio (recombinant allergen test/peanut extract test) of >0.25 and an IgE concentration of >0.35 kU_A/l are indicated (bold). Examples of estimated positions of Ara h 1 (black solid square), Ara h 2 (red solid square), Ara h 3 (blue solid square), Ara h 6 (red dashed square), and a presumed breakdown product of 2S albumin (red dotted square) are indicated in the immunoblot as mentioned in the Results section.



CAP (sIgE)	29	16	31	24	46	38	1	3	28
Peanut	*5.32*	*3.81*	*2.49*	*2.50*	*4.07*	*13.4*	*3.64*	*0.63*	*<0.10*
rAra h 1	0.21	0.19	0.57	0.26	<0.10	**6.02**	<0.10	0.14	<0.10
rAra h 2	**3.98**	<0.10	<0.10	0.13	<0.10	<0.10	<0.10	0.18	<0.10
rAra h 3	0.86	<0.10	0.17	0.29	0.35	2.39	<0.10	0.14	<0.10
rAra h 5	0.24	**7.04**	<0.10	**3.14**	**26.69**	1.48	**12.83**	**0.74**	<0.10
rAra h 8	<0.10	<0.10	<0.10	**4.04**	**11.33**	0.27	**12.32**	0.25	<0.10
rAra h 9	**2.99**	**0.11**	**<0.10**	**0.31**	**0.12**	**0.68**	**<0.10**	**0.59**	**<0.10**
CCD	0.37	<0.10	<0.10	0.15	0.26	0.11	<0.10	0.31	<0.10

Figure 3 IgE immunoblotting with sera from children who passed peanut challenge (tolerant group) using extract from both raw (left lane) and roasted peanut (right lane). Patient IDs are indicated together with individual ImmunoCAP results. Dominating IgE reactivities defined as an IgE ratio (recombinant allergen test/peanut extract test) of >0.25 and an IgE concentration of >0.35 kU_A/l are indicated (bold). Examples of estimated positions of Ara h 1 (black solid square), Ara h 2 (red solid square), Ara h 3 (blue solid square), Ara h 5 (green dashed square), Ara h 6 (red dashed square), and Ara h 8 (green solid square) are indicated in the immunoblot as mentioned in the Results section.

for Swedish patients (30) and is clearly lower than observed in populations living in the Mediterranean area (13, 24).

The present study is the first on IgE reactivity patterns to peanut allergens in Japanese children. In another study comprising 31 Chinese children by Chiang et al. (34), a prevalence of 87% for IgE antibodies to Ara h 1 and Ara h 2 was reported, indicating that the importance of Ara h 2 observed in our study might be similar in several Asian countries. An added value of our Japanese study is that the allergy diagnosis was performed after challenge with peanut, while the Chinese children were regarded as peanut allergic based on questionnaire data only.

The combination of test results from the rAra h 1, rAra h 2, and rAra h 3 ImmunoCAP tests resulted in a slightly higher specificity when concomitant IgE positivity to all three allergens was the criterion. Previous studies have shown that poly-sensitization to Ara h 1, Ara h 2, and Ara h 3 is associated with severe reactions to peanut (5, 32). The present study included too few peanut-allergic patients to draw any similar conclusions. Sensitization to Ara h 2 may also be a marker for severe allergic reactions to peanut as recently shown in a questionnaire-based study (35). In line with that observation, all eight patients with anaphylaxis at peanut challenge in the present study had IgE reactivity to rAra h 2 and at higher serum levels than the allergic children with milder symptoms.

No major differences were observed between raw and roasted peanut regarding IgE binding to the identified allergens in the immunoblots. However, IgE binding to Ara h 2 slightly decreased, while the IgE binding to the presumed Ara h 6 at 12 kDa slightly increased after roasting. Moreover, in one patient, the binding to a 17-kDa band, most probably the heat-labile Ara h 8 (11), was abolished after roasting the peanuts. In accordance with these results, other studies have shown that the antibody-binding patterns to Ara h 1, Ara h 2, Ara h 3, Ara h 5, and Ara h 6, as studied by immunoblotting, are only slightly affected by roasting (10, 22, 36). Interestingly in a recent study by Vissers et al. (37), it was shown that most IgE-binding capacity of soluble 2S albumins (Ara h 2/6) isolated from roasted peanut was preserved compared with Ara h 2/6 from raw peanut, while heating up Ara h 2/6 in solution to 110°C caused denaturation of the allergens and a greatly reduced capacity of IgE binding and basophil degranulation. IgE reactivity to Ara h 5 (profilin), Ara h 8 (PR-10 protein), and Ara h 9 (LTP), all having molecular weights between 9 and 17 kDa (11, 13, 16,

22, 24), was more difficult to elucidate by immunoblotting partly because of overlapping binding patterns for the low molecular weight allergens. Another explanation for observing less IgE binding to Ara h 5, Ara h, 8 and Ara h 9 could be their suboptimal concentration in conventionally prepared peanut extracts (11, 13, 22). Furthermore, small proteins <10 kDa such as Ara h 9 might migrate out from conventional SDS-PAGE gels that are optimized for proteins with higher molecular weights. Generally, the agreement between the allergen-specific IgE reactivity observed using ImmunoCAP and immunoblotting was good, especially for Ara h 1, 2, and 3.

In conclusion, similar to several other populations, peanut-allergic Japanese children are frequently sensitized to the peanut storage proteins, especially Ara h 2. It was found that the rAra h 2 ImmunoCAP with a sensitivity of 88% and specificity of 84% was superior in the diagnosis of peanut allergy in comparison with the conventional peanut extract-based test that had a very low specificity. By combining the rAra h 2 and the rAra h 1 and rAra h 3 ImmunoCAP tests, it was possible to obtain an even higher specificity (94%). A good agreement between ImmunoCAP and IgE immunoblotting with both raw and roasted peanut indicates that recombinant peanut allergen tests can be used in the diagnosis of peanut allergy to both highly processed (roasted peanut) and more mildly processed forms of peanut.

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