

ORIGINAL PAPER

Children with peanut allergy recognize predominantly Ara h2 and Ara h6, which remains stable over time

A. E. Flinterman*, E. van Hoffen*, C. F. den Hartog Jager*, S. Koppelman^{*†1}, S. G. Pasmans*, M. O. Hoekstra[‡], C. A. Bruijnzeel-Koomen*, A. C. Knulst* and E. F. Knol*

*Department of Dermatology/Allergology, University Medical Centre Utrecht, The Netherlands, [†]TNO Quality of life, Zeist, The Netherlands and [‡]Department of Paediatrics, University Medical Centre Utrecht, The Netherlands

Clinical and Experimental Allergy

Summary

Background In peanut-allergic adults, IgE is mainly directed to Ara h1 and Ara h2. More recently, a role for Ara h6 has been suggested. In contrast to adults, IgE in children can fluctuate over time. Therefore, children may have a more dynamic reactivity to peanut.

Objective To examine the IgE reactivity to major peanut allergens in peanut-allergic children at two subsequent time-points.

Methods Twenty children (3–15 years old) with peanut allergy, confirmed by a double-blind placebo-controlled food challenge (DBPCFC), were included. Just before and 20 months after DBPCFC, IgE reactivity to purified Ara h1, Ara h2, Ara h3 and Ara h6 was studied by immunoblots and skin prick tests (SPTs).

Results Before DBPCFC, all peanut-allergic children showed IgE reactivity to Ara h2; Ara h6 was recognized by 16 children, and Ara h1 and Ara h3 by 10 children. After 20 months, peanut-specific IgE levels (median 23 kU/L) and the individual recognition of major allergens were comparable with the levels and recognition before challenge (median 28.2 kU/L). SPT with Ara h2 and Ara h6 was positive in most children, whereas SPT with Ara h1 and Ara h3 was positive in approximately half of the children. Ara h6 induced the largest weals. None of the parameters were related to the severity of peanut allergy.

Conclusion Ara h2 and Ara h6 are the most frequently recognized major peanut allergens in children. The individual reactivity to the major peanut allergens remained stable over time, despite DBPCFC.

Keywords Ara h2 and Ara h6, children, IgE reactivity, peanut allergy, stable individual recognition

Submitted 28 December 2006; revised 30 March 2007; accepted 11 May 2007

Correspondence:

Dr E. F. Knol, Department of Paediatrics, University Medical Centre, G02.124, PO Box 85500, 3508 GA Utrecht, The Netherlands.
E-mail: e.f.knol@umcutrecht.nl

Introduction

Peanut allergy is among the most important food allergies in westernized countries [1–4].

Several proteins in peanut have been implicated as allergens. Ara h1, belonging to the vicilin family, and Ara h2, a member of the conglutin family, were thought to be the most important, because these allergens are recognized by IgE in 70–90% of patients with peanut allergy [5–9]. Ara h3, a glycinin protein, is recognized by only 45% of patients with a convincing history of peanut sensitivity [10]. Ara h6 shows homology with Ara h2. In

recent studies, we showed that adult peanut-allergic patients recognized Ara h6 to a similar extent as Ara h2, indicating that Ara h6 should be considered a major peanut allergen as well [11, 12]. Inhibition experiments demonstrated that IgE-binding to Ara h6 was cross-reactive with Ara h2. The diversity of IgE allergen binding rather than binding to one specific allergen has been related to the severity of the reaction to peanut [13].

Most studies investigating the reactivity to major peanut allergens were conducted in adults. It is assumed that IgE of children binds to similar proteins as IgE of allergic adults. In children with presumed peanut allergy, IgE specific for Ara h1, Ara h2 and Ara h3 has been described [5, 6, 8, 9, 14]. These studies were conducted with whole

¹Present address: HAL Allergy, Haarlem, The Netherlands.

peanut extract or with only purified Ara h1 and Ara h2. Moreover, the finding that Ara h6 is an important allergen has not yet been confirmed in children. We think it is important to investigate IgE reactivity in children to all these purified major allergens simultaneously, and to illustrate this with *in vivo* reactivity to purified allergens by skin prick tests (SPTs) in children.

Sensitization to peanut in children may be more dynamic than in adults. In children, the level of peanut-specific IgE can fluctuate over time, independent of exposure to peanut [15]. This is in line with the occurrence of fluctuating allergic sensitization described for several other food allergens in young children [16]. No study has recorded the IgE reactivity to the major peanut allergens over time so far.

The aim of the present study was to investigate the reactivity to peanut extract as well as to purified major peanut allergens (Ara h1, Ara h2, Ara h3 and Ara h6) by immunoblots and SPT in children with peanut allergy, confirmed by a double-blind placebo-controlled food challenge (DBPCFC). To analyse the dynamics of IgE reactivity over time, we reinvestigated the reactivity to the major peanut allergens 20 months after DBPCFC.

Methods

Study population

Twenty children with peanut allergy (six female and 14 male; age 3–15 years) were recruited from the Department of Paediatric Dermatology/Allergology at the University Medical Centre Utrecht. These children participated in an earlier study to determine eliciting doses (ED) for peanut and were a representative sample from the population of peanut-sensitized children in our outpatient clinic with regard to specific IgE levels and severity of previous allergic reactions to peanut [17]. Inclusion criteria consisted of a positive DBPCFC with peanut, elevated peanut-specific IgE and positive SPT. Besides peanut allergy, 19 children had other food allergies, mainly nut allergy. All had a history of atopic dermatitis: 15 children of allergic asthma and eight children of seasonal rhinoconjunctivitis. Parents of these children were asked to document the (inadvertent) ingestion of food products labelled 'may contain peanut' prospectively, and describe any allergic reaction in a diary. All parents gave written informed consent before enrolment in the study. The study was reviewed and approved by the Central Committee of Human based Research in the Netherlands (CCMO, The Hague, the Netherlands).

Clinical evaluation

DBPCFC was performed as described previously [17]. In short, increasing amounts of defatted peanut flour were

given at time intervals of 15–30 min: 0.01, 0.1, 0.3, 0.5, 1, 10, 100, 1000 and 3000 mg. Four placebos were interspersed randomly. An open challenge of 10 peanuts was the last step in the procedure. The challenge was discontinued after the occurrence of objective symptoms. After the occurrence of subjective symptoms (oral allergy, abdominal pain and nausea), the DBPCFC was continued after the disappearance of these symptoms. For determination of the ED, the first dose to provoke symptoms, either subjective or objective, was used.

The severity of allergic reactions was divided according to Muller into mild (grade 0 and 1), moderate (grade 2) and severe (grade 3 and 4) [18]. Mild reactions were classified as symptoms of the oral cavity, the skin and/or mucous membranes (urticaria, angio-oedema, rhinitis, conjunctivitis), moderate reactions included gastrointestinal symptoms (diarrhoea, vomiting, nausea, abdominal pain) and severe reactions consisted of respiratory symptoms (bronchoconstriction, stridor, hoarseness) and/or cardiovascular symptoms.

Peanut-specific immunoglobulin E levels

Serum samples were collected for each patient at the day of challenge ($t=0$) and 20 months after ($t=20$). Peanut-specific IgE was determined in all samples by CAP-system FEIA according to the manufacturer's instructions (Phadia, Uppsala, Sweden). IgE > 0.35 kU/L was considered to be positive.

Purified major allergens

Previously developed purification protocols were used for the preparation of Ara h1 [19], Ara h2 [11], Ara h3 [20] and Ara h6 [11], with purity of >95% as judged by SDS-PAGE electrophoresis and Coomassie brilliant blue staining. Individual peanut allergens were sterilized and stored as described before [7].

Sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoblot

SDS-PAGE was performed using a BioRad Mini Protean II system (BioRad, Hercules, CA, USA) with 15% acrylamide gels (15 × 10 cm) according to the manufacturer's instructions. Crude peanut extract (CPE, 2 µg) and all four purified major peanut allergens (0.4 µg per allergen) were loaded in separate lanes. After protein separation, the proteins were transferred to polyvinylidene difluoride sheets (Immobilon-P, Millipore Corp. Bedford, MA, USA). Membranes were blocked in 4% dried milk (Protifar Plus, Nutricia, Cuijk, the Netherlands) in PBS o/n at 4 °C. Patient serum of both time-points ($t=0$ and $t=20$ months) was diluted 1:50 in dilution buffer (PBS, containing 0.5% dried milk and 0.02% Tween-20) and applied on the

membranes for 1 h at room temperature. Bound IgE was detected using peroxidase-conjugated goat-anti-human IgE (Kirkegaard Perry Limited, Guildford, UK) diluted 1:30 000 in dilution buffer and a subsequent staining reaction for peroxidase activity using the ECL technique (Amersham Life Science, Amersham, UK) according to the manufacturer's instructions. We scored the IgE binding from – (no visible bands) to ++++ (very broad and thick bands) by visually analysing all blots, with three investigators reaching consensus.

Skin prick test with whole peanut extract and purified major allergens

SPT were performed on the patient's back or on the flexor side of the forearm with a prick needle and commercial peanut extract (ALK-Abelló, Nieuwegein, the Netherlands). Purified Ara h1, Ara h2, Ara h3 and Ara h6 were used in serial 10-fold dilutions, ranging from 100 to 1 µg/mL. The dilutions were prepared using a diluent containing 50% glycerol (v/v), 0.9% NaCl (w/v), 0.4% phenol (w/v) and 0.3% human serum albumin (w/v) in PBS. Histamine dihydrochloride (10 mg/mL) and the glycerol diluent of the SPT extracts served as positive and negative controls, respectively. Because peanut induces larger weals in children than in adults (data not shown), we started with 1 µg/mL in the first group of children out of safety precautions. The next groups were tested with higher concentrations (10 and 100 µg/mL) as no serious reactions occurred. The order in which the children were tested was random. SPT reactivity was measured after 15 min and transferred with a transparent adhesive tape onto a record sheet. The area of the SPT weal was determined by computer scanning [21]. SPT responses were standardized by dividing the weal area of the peanut prick by that obtained from the histamine control. SPT ratios ≥ 0.25 were considered to be positive [12].

Statistics

All analyses were performed with non-parametric tests (Mann-Whitney *U* test and Kruskal-Wallis test for comparison between groups and correlations with Spearman's rank coefficient). For quantitative analysis of the immunoblots, we labelled the values from 0 (corresponding to –) to 4 (corresponding to ++++). Calculations were performed using SPSS (version 12, SPSS Inc., 2001, Chicago, IL, USA). *P*-values < 0.05 were considered to be significant.

Results

Peanut allergy and dietary management during 20 months

The severity of the reaction and the ED determined by DBPCFC are displayed in Table 1. All 20 children devel-

oped an objective reaction, ranging from mild to severe. Mild reactions were oral symptoms with abdominal pain and vomiting, whereas the most severe reaction consisted of generalized urticaria, rhinoconjunctivitis, severe bronchospasm and tachycardia. The ED per individual ranged from 10 mg to more than 3 g.

During the 20 months after DBPCFC, all children continued to eliminate peanut from their diet. Parents of 13 children indicated to be less strict with the elimination of peanut than advised. Four children reported OAS after the ingestion of products that might contain peanut. Two children with a less strict diet experienced an objective allergic reaction to peanut within these 20 months.

Peanut-specific immunoglobulin E recognizes predominantly Ara h2 and Ara h6

Sera were collected before DBPCFC and analysed by IgE-immunoblotting. Figure 1 shows representative examples of immunoblots. Ara h2 and Ara h6 were recognized by the majority of the children, whereas Ara h1 and Ara h3 were recognized by IgE from the serum of approximately half of the children, as shown in Fig. 2. Eight children recognized all four major peanut allergens, three children recognized three major allergens (Ara h2 and Ara h6, together with either Ara h1 or Ara h3), six children recognized two major allergens (in five cases Ara h2 and Ara h6 and in one case Ara h1 and Ara h2) and three children recognized only Ara h2. The group with strict elimination showed similar recognition of major allergens as the group who followed a less strict dietary management. The number of recognized allergens correlated with the level of peanut-specific IgE ($r = 0.68$; $P = 0.001$). No correlation between the number of recognized allergens and the age of the children was found ($r = -0.37$; $P = 0.104$).

The extent of IgE binding, determined by visually analysing the bands, varied widely between the different individuals and the different allergens (Table 1). The IgE binding to Ara h2 and Ara h6 was significantly more intense than the IgE binding to both Ara h1 and Ara h3 (all $P < 0.05$). No difference in IgE binding was shown between Ara h1 and Ara h3 or between Ara h2 and Ara h6.

Recognition of major peanut allergens by immunoglobulin E remains stable 20 months after challenge

Sera were also obtained 20 months after the challenge (Table 1). Peanut-specific IgE levels at that time-point (range 1.0–616 kU/L; median 23.0 kU/L) were comparable with the levels before challenge (range 1.0–815 kU/L; median 28.2 kU/L; $P = 0.218$).

At $t = 20$ months Ara h2 was still recognized by all children (100%), Ara h6 by 17 (85%), Ara h3 by 11 (55%) and Ara h1 by nine (45%).

Table 1. Age and results of DBPCFC per subject, followed by the peanut-specific IgE and the IgE reactivity to major peanut allergens on blot at $t=0$ and $t=20$ months

No.	Age	DBPCFC		Immunoblot $t=0$ months						Immunoblot $t=20$ months					
		Severity*	ED	IgE [†]	Ara h1	Ara h2	Ara h3	Ara h6	Tot	IgE [†]	Ara h1	Ara h2	Ara h3	Ara h6	Tot
1	12	1	1000	3.3	—	+	—	—	1	2.1	—	+	—	+	2
2	4	1	1000	15	—	++	++	+	3	24	—	+++	++	+	3
3	10	1	300	61	+	+++	+++	++	4	46	—	+++	+++	++	3
4	6	1	10	527	+++	++++	+++	++++	4	616	+++	++++	++++	++++	4
5	7	1	10	163	++	++++	—	+++	3	309	+++	++++	++	++++	4
6	14	2	>3000	3.0	+	+	—	—	2	7.8	+	+	—	—	2
7	7	2	>3000	21	++	++	+	+++	4	44	+	++	+	+++	4
8	6	2	3000	6.9	—	+	—	+	2	5.0	—	+	—	+	2
9	15	2	3000	36	—	+	—	+	2	23	—	++	—	+	2
10	5	2	3000	4	—	+	—	+	2	3.8	—	+	—	+	2
11	7	2	3000	35	—	++	—	—	1	46	+	+	—	—	2
12	4	2	1000	1.0	—	+	—	+	2	1.0	—	+	—	+	2
13	3	2	1000	7.5	+	+	+	+	4	9.5	—	++	+	++	3
14	12	2	300	21	—	+++	+	+++	3	18	—	+++	+	+++	3
15	4	2	300	2.5	—	+	—	+	2	1.9	—	+	—	—	1
16	5	2	100	250	+	++++	+	+++	4	67	+	++++	+	+++	4
17	12	2	100	3.3	—	+	—	—	1	1.9	—	+	—	+	2
18	4	3	1000	815	+++	++++	++++	++++	4	606	+++	++++	++++	++++	4
19	5	3	1000	99	+	+++	+	+++	4	54	+	+++	+	+++	4
20	6	3	1000	183	+	++++	++	+++	4	163	+	++++	++	+++	4

*Specific IgE in kU/L.

[†]Severity: 1 = mild; 2 = moderate; 3 = severe.

ED, eliciting dose (mg peanut flour); Tot, total number of allergens recognized by each individual; DBPCFC, double-blind placebo-controlled food challenge.

The recognized number and the extent of IgE binding to Ara h1, Ara h2, Ara h3 and Ara h6 on the blots at $t=20$ months did not differ between both time-points (Fig. 3). Four major allergens were investigated in each child ($n=20$); hence, 80 tests were performed at both time-points. The recognition at $t=0$ was comparable with $t=20$ months in 73 of the 80 cases (91%). Six children showed minor changes between $t=0$ and $t=20$ months. An example of such a change is shown in Fig. 1c. These changes were not related to age, level of specific IgE or dietary management during the 20 months (strict, less strict). One child (No. 15) clearly recognized Ara h3 after 20 months (++) in contrast to $t=0$ (—), as is shown on the immunoblot in Fig. 1d. This child had a strict elimination diet with no apparent ingestion of peanut, and reported no reactions to peanut during the observation period of 20 months.

Skin prick test is mainly positive for Ara h2 and Ara h6

The SPT ratios at $t=20$ months (median 6.9; range 0.8–25) were not significantly different from SPT ratios at $t=0$ months (median 4.3; range 2.3–23; $P=0.25$).

SPT for the major peanut allergens were performed at $t=20$ months at concentrations of 1, 10 and 100 µg/mL. There was a clear dose-response relationship for all

purified allergens tested, with larger weals at higher concentrations (Fig. 4). The largest SPT reactivity was induced by Ara h6, followed by Ara h2.

With the lowest concentration (1 µg/mL), SPT was mainly positive for Ara h6 (12/20) and in a minority positive for Ara h1 (3/20) and for Ara h2 (4/20). At a concentration of 10 µg/mL, SPT was again mainly positive for Ara h6 (13/15), but also for Ara h2 (9/15) and in a minority for Ara h1 (3/15) and for Ara h3 (1/12). At the highest concentration (100 µg/mL), SPT was positive for Ara h2 (9/9) and for Ara h6 (7/8), and in half of the subjects for Ara h1 (4/9) and for Ara h3 (5/9).

SPT were less often positive than the immunoblots, especially at the lower concentrations (1 and 10 µg/mL). At the highest concentration (100 µg/mL), the *in vivo* reactivity to major allergens detected by SPT was similar to the recognition of these allergens on immunoblot in 26 of the 35 performed SPT (74%). At this concentration, three children had a positive SPT for Ara h1, Ara h3 or Ara h6, whereas no recognition was observed on the immunoblot for these allergens.

No clinical implications of major allergen recognition

It has been suggested that the severity of peanut allergy is related to the number of allergens recognized [12, 13].

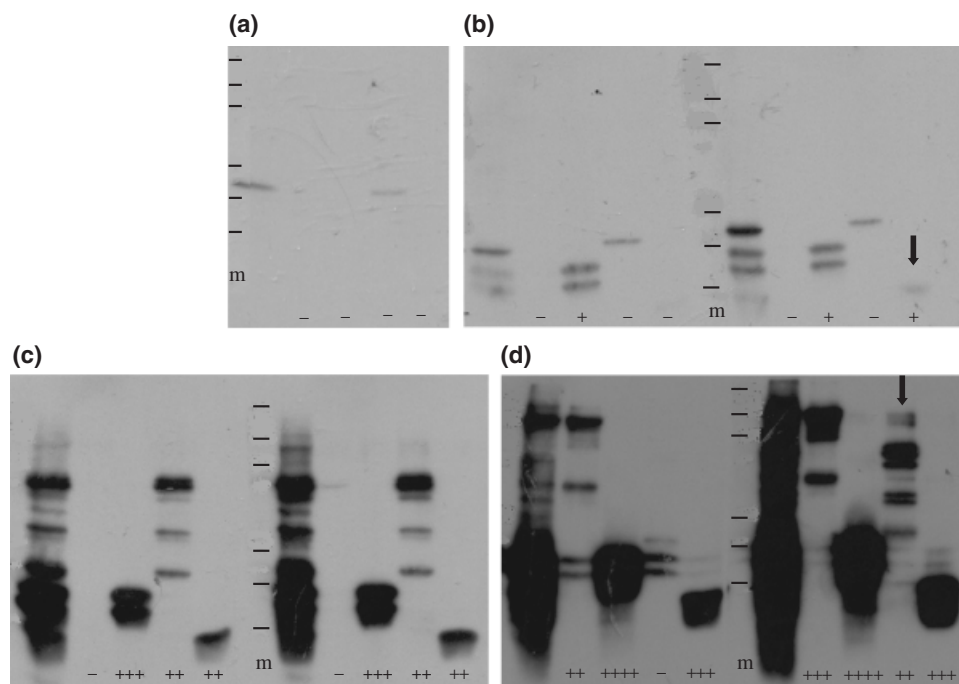


Fig. 1. Examples of immunoblots: a control subject without peanut sensitization (a), peanut-allergic subjects with low peanut-specific IgE (No. 17; 3.3 and 1.9 IU/mL), with a moderate level of specific IgE (No. 2; 15 and 23.5 IU/mL), or with high levels of specific IgE (No. 5; 163 and 309 IU/mL) at $t=0$ and $t=20$ months (b–d, respectively). The extent of the IgE reactivity is described below each lane on the immunoblot. The lanes represent crude peanut extract, Ara h1, Ara h2, Ara h3 and Ara h6 from left to right. Marker lanes are indicated with 'm', representing proteins at 250, 100, 50, 25, 20 and 15 kDa. Differences between $t=0$ and $t=20$ months are indicated with an arrow. Lanes of Ara h1 and Ara h3 in panel D, $t=0$, show a minor reactivity at the height of Ara h2. This is most likely due to a minute overflow of the Ara h2 lane occurred during loading of the gel. The impurity of the Ara h1 and Ara h2 is not likely based on the lack of reactivity at the height of Ara h2 in the Ara h1 and Ara h3 lanes in panel D, $t=20$.

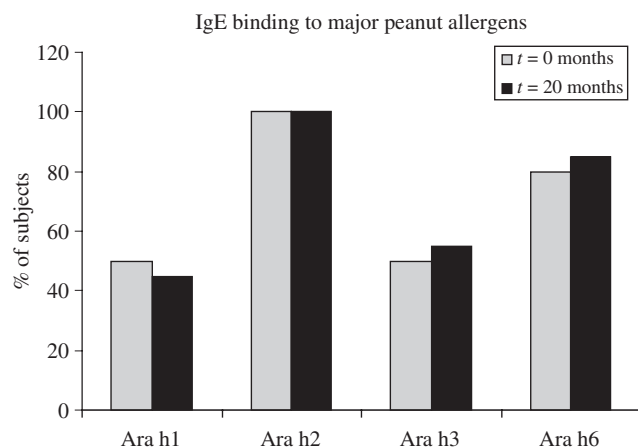


Fig. 2. IgE reactivity to major peanut allergens on immunoblot. The percentage of individuals ($n=20$) recognizing the specific major allergens (Ara h1, Ara h2, Ara h3 and/or Ara h6) are displayed for $t=0$ and $t=20$ months.

It appeared that the severity of the allergic reaction to peanut determined by DBPCFC was not correlated with the extent of allergen recognition, nor with the number of allergens recognized, either by immunoblot or by SPT. The ED was not correlated with these parameters either.

Discussion

Ara h2 and Ara h6 appear to be the most frequently and most strongly recognized major peanut allergens in peanut-allergic children, as determined both by immunoblot and SPT. The recognition of all major allergens remained stable 20 months after DBPCFC with peanut.

In our opinion, this is the first study to investigate the IgE reactivity to four major peanut allergens simultaneously in children with confirmed peanut allergy. Ara h1 and Ara h2 have long been regarded as the most potent peanut allergens, as they are recognized by the majority of peanut-allergic subjects [5–9]. Comparative studies between both allergens have acknowledged the importance of Ara h2 over Ara h1 [5, 7, 8]. In the current study, Ara h6 seems to have a similar allergenic IgE-binding potential as Ara h2. This is in line with recent studies in adults from our group [11, 12]. In contrast to other studies, we used purified allergens in addition to CPE [6, 9]. In this way, we were able to differentiate the IgE reactivity to these major allergens on the immunoblots, especially in the region of 14–22 kDa where several peanut allergens are located. IgE reactivity to Ara h2 (17 and 20 kDa) and Ara h6 (15 kDa) was present in almost all children with peanut allergy.

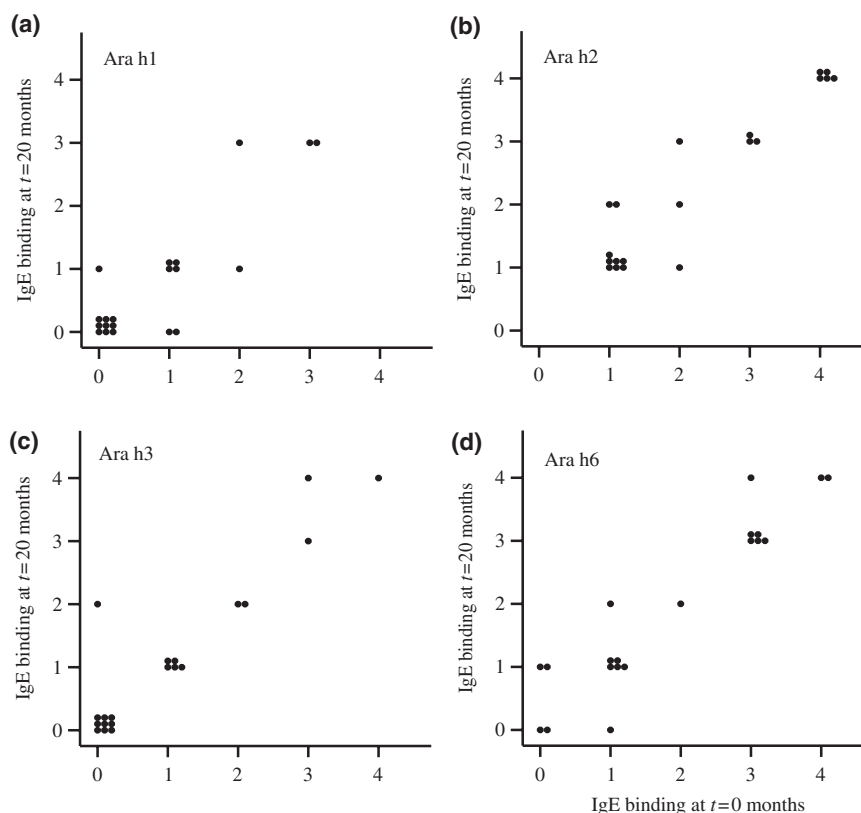


Fig. 3. IgE reactivity at $t=0$ months compared with $t=20$ months for Ara h1 (a), Ara h2 (b), Ara h3 (c) and Ara h6 (d). Each dot represents one individual.

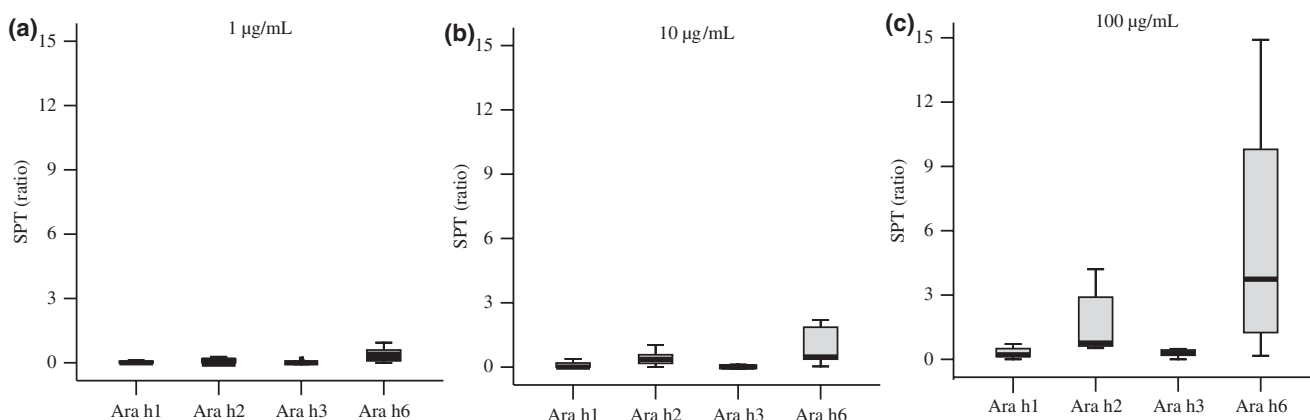


Fig. 4. Skin prick test (SPT) with major peanut allergens. The interquartile ranges of SPT ratios (area peanut allergen/area histamine) at $t=20$ months (Ara h1, Ara h2, Ara h3 and Ara h6) are displayed for (a) 1 $\mu\text{g/mL}$ ($n=20$); (b) 10 $\mu\text{g/mL}$ ($n=16$); and (c) 100 $\mu\text{g/mL}$ ($n=10$).

To illustrate IgE binding with *in vivo* reactivity to these peanut allergens, we also performed SPT with these purified allergens. This procedure turned out to be a safe and relatively easy technique in children. SPT was mainly positive for Ara h2 and Ara h6, of which Ara h6 induced the strongest reactivity. Ara h6 can be seen as a truncated isoform of Ara h2, missing one IgE-binding epitope, and is therefore believed to be less potent. However, it has been observed before that Ara h6 shows a unique capacity of inducing SPT reactivity, which may be explained

by a better exposure of IgE-binding epitopes in the 3D structure of our purified Ara h6 than predicted by amino-acid sequence [12]. Discrepancies between immunoblot and SPT were noted in a minority of the tests (26%). A possible explanation is the difference in the number of epitope binding between mast cells and immunoblot. Mast cells need at least two epitopes on the same allergen for cross-linking of IgE, whereas one epitope for IgE binding on the blot is sufficient [22]. On the other hand (conformational), epitopes may be lost

during the electrophoresis used for immunoblotting, rendering only the SPT positive. Overall, SPT confirmed the importance of Ara h2 and Ara h6 as was found by immunoblotting.

Both Ara h2 and Ara h6 are 2S albumins and show great homology; therefore, IgE binding epitopes may be similar [23]. Cross-reactivity between Ara h2 and Ara h6 has been suggested before [11]. The immunodominant epitopes in Ara h2 and Ara h6 are very resistant to enzymatic digestion, which may enhance their allergenicity [24, 25]. In general, IgE reactivity to 2S albumins is a risk factor for more serious reactions [26]. As has been shown in this study, these allergens in peanut show profound IgE binding and SPT reactivity and therefore the development of future therapeutic intervention strategies may be focused predominantly on Ara h2 and Ara h6.

Previous studies have suggested that the number of allergens recognized, so-called 'promiscuity of IgE', was a risk factor for more serious reactions [12, 13]. No correlation between the severity of the reaction during DBPCFC and the number of purified allergens recognized was found in this study, either by immunoblot, or by SPT. The total performed SPT at 100 µg/mL in our study may be a limiting factor. We did observe a correlation between the number of major allergens recognized and the level of specific IgE, which is in accordance with the study of Lewis et al. [13].

Despite the single exposure to peanut during DBPCFC, specific IgE levels remained stable. Moreover, the individual IgE binding pattern to Ara h1, Ara h2, Ara h3 and Ara h6 turned out to be highly comparable between before and 20 months after DBPCFC. During the observation period, 13 children had a less strict dietary management, making exposure to peanut rather likely. This is in accordance with the study of van Odijk et al. [15], who reported stable peanut-specific IgE levels irrespective of exposure to peanut. At least six children reported a reaction after ingestion of peanut-containing products. Neither the dietary management nor the accidental reactions to peanut were related to any changes in IgE reactivity. In six children, we noticed minimal differences in IgE reactivity between the two time-points. Because analyses of both time-points were performed simultaneously on a similar blot, differences were attributed to individual changes rather than changes in the procedure. An explanation could be that the recognition of major allergens on the blot was around the detection limit, resulting in no (–) or very low (+) IgE reactivity. Only one child developed marked IgE binding to Ara h3 after 20 months. As far as the history allowed, no different dietary management or accidental ingestions of peanut were reported in this child. Therefore, we conclude that IgE reactivity remained stable in all children after 20 months, despite DBPCFC.

This suggests that after an initial increase in specific IgE levels early in life, the levels and also the recognition of

the specific major peanut allergens remain highly stable. For future research on the development of initial sensitization to peanut and possible interference by therapy, it may be interesting to investigate the sensitization to Ara h2 and Ara h6 in younger children.

Conclusion

Ara h2 and Ara h6 are the most frequently recognized major peanut allergens in children with peanut allergy, which is comparable with adults. The individual recognition pattern remained stable after challenge and subsequent elimination during 20 months.

Acknowledgements

The authors thank Dr Jonas Lidholm (Phadia, Uppsala, Sweden) for the kind gift of anti-IgE used to set up the immunoblotting experiments.

Funding: Wilhelmina Research Foundation, Utrecht, the Netherlands.

References

- Emmett SE, Angus FJ, Fry JS, Lee PN. Perceived prevalence of peanut allergy in Great Britain and its association with other atopic conditions and with peanut allergy in other household members. *Allergy* 1999; 54:380–5.
- Sicherer SH, Furlong TJ, Munoz-Furlong A, Burks AW, Sampson HA. A voluntary registry for peanut and tree nut allergy: characteristics of the first 5149 registrants. *J Allergy Clin Immunol* 2001; 108:128–32.
- Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001; 107:191–3.
- 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.
- Bernard H, Paty E, Mondoulet L *et al.* Serological characteristics of peanut allergy in children. *Allergy* 2003; 58:1285–92.
- Clarke MC, Kilburn SA, Hourihane JO, Dean KR, Warner JO, Dean TP. Serological characteristics of peanut allergy. *Clin Exp Allergy* 1998; 28:1251–7.
- Koppelman SJ, Wensing M, Ertmann M, Knulst AC, Knol EF. Relevance of Ara h1, Ara h2 and Ara h3 in peanut-allergic patients, as determined by immunoglobulin E Western blotting, basophil-histamine release and intracutaneous testing: Ara h2 is the most important peanut allergen. *Clin Exp Allergy* 2004; 34:583–90.
- Palmer GW, Dibbern DA Jr, Burks AW *et al.* Comparative potency of Ara h1 and Ara h2 in immunochemical and functional assays of allergenicity. *Clin Immunol* 2005; 115:302–12.
- Burks W, Sampson HA, Bannon GA. Peanut allergens. *Allergy* 1998; 53:725–30.
- Rabjohn P, Helm EM, Stanley JS *et al.* Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J Clin Invest* 1999; 103:535–42.

- 11 Koppelman SJ, de Jong GA, Laaper-Ertmann M *et al*. Purification and immunoglobulin E-binding properties of peanut allergen Ara h 6: evidence for cross-reactivity with Ara h 2. *Clin Exp Allergy* 2005; 35:490–7.
- 12 Peeters KA, Koppelman SJ, van Hoffen E *et al*. Does skin prick test reactivity to purified allergens correlate with clinical severity of peanut allergy? *Clin Exp Allergy* 2007; 37:108–15.
- 13 Lewis SA, Grimshaw KE, Warner JO, Hourihane JO. The promiscuity of immunoglobulin E binding to peanut allergens, as determined by Western blotting, correlates with the severity of clinical symptoms. *Clin Exp Allergy* 2005; 35:767–73.
- 14 Restani P, Ballabio C, Corsini E *et al*. Identification of the basic subunit of Ara h 3 as the major allergen in a group of children allergic to peanuts. *Ann Allergy Asthma Immunol* 2005; 94:262–6.
- 15 van Odijk J, Bengtsson U, Borres MP, Hulthen L, Ahlstedt S. Specific immunoglobulin E antibodies to peanut over time in relation to peanut intake, symptoms and age. *Pediatr Allergy Immunol* 2004; 15:442–8.
- 16 Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J Allergy Clin Immunol* 1999; 103:1173–9.
- 17 Flinterman AE, Pasmans SG, Hoekstra MO *et al*. Determination of no-observed-adverse-effect levels and eliciting doses in a representative group of peanut-sensitized children. *J Allergy Clin Immunol* 2006; 117:448–54.
- 18 Mueller HL. Diagnosis and treatment of insect sensitivity. *J Asthma Res* 1966; 3:331–3.
- 19 de Jong EC, Van ZM, Spanhaak S, Koppelman SJ, Pellegroni H, Penninks AH. Identification and partial characterization of multiple major allergens in peanut proteins. *Clin Exp Allergy* 1998; 28:743–51.
- 20 Koppelman SJ, Knol EF, Vlooswijk RA *et al*. Peanut allergen Ara h 3: isolation from peanuts and biochemical characterization. *Allergy* 2003; 58:1144–51.
- 21 Poulsen LK, Liisberg C, Bindslev-Jensen C, Malling HJ. Precise area determination of skin-prick tests: validation of a scanning device and software for a personal computer. *Clin Exp Allergy* 1993; 23:61–8.
- 22 Knol EF. Requirements for effective IgE cross-linking on mast cells and basophils. *Mol Nutr Food Res* 2006; 50:620–4.
- 23 Breiteneder H, Radauer C. A classification of plant food allergens. *J Allergy Clin Immunol* 2004; 113:821–30.
- 24 Sen M, Kopper R, Pons L, Abraham EC, Burks AW, Bannon GA. Protein structure plays a critical role in peanut allergen stability and may determine immunodominant IgE-binding epitopes. *J Immunol* 2002; 169:882–7.
- 25 Lehmann K, Schweimer K, Reese G *et al*. Structure and stability of 2S albumin-type peanut allergens: implications for the severity of peanut allergic reactions. *Biochem J* 2006; 395:463–72.
- 26 Pastorello EA, Pompei C, Pravettoni V *et al*. Lipid transfer proteins and 2S albumins as allergens. *Allergy* 2001; 56 (Suppl. 67):45–7.