

Walnut Allergy in Peanut-Allergic Patients: Significance of Sequential Epitopes of Walnut Homologous to Linear Epitopes of Ara h 1, 2 and 3 in Relation to Clinical Reactivity

Leonard Rosenfeld^a Wayne Shreffler^b Ludmilla Bardina^b Bodo Niggemann^a
Ulrich Wahn^a Hugh A. Sampson^b Kirsten Beyer^a

^aDepartment of Pediatric Pneumology and Immunology, University Children's Hospital Charité, Berlin, Germany;

^bDivision of Pediatric Allergy and Immunology and Jaffe Food Allergy Research Institute, Mount Sinai School of Medicine, New York, N.Y., USA

Key Words

Allergen structure • Epitopes • IgE • In vitro tests •
Peanut allergy • Walnut allergy

Abstract

Background: Peanut allergy is a frequent and potentially life-threatening food allergy. Despite the large taxonomic distance between the plants, peanut-allergic patients often react to tree nuts such as walnuts. While the allergens of peanut and walnut have a high degree of homology in their amino-acid sequences, it is unknown whether this similarity is responsible for the observed co-reactivity. Therefore, we analyzed the binding of specific IgE antibodies to sequential epitopes of peanut and walnut in peanut-allergic patients with and without walnut allergy. **Methods:** The IgE binding to previously described sequential epitopes of peanut and the homologous regions of walnut was assessed in 32 peanut-allergic patients using a peptide microarray technology. Twelve patients had a clinically relevant walnut allergy and 20 were tolerant to walnut. Inhibition assays with peanut peptides and corresponding walnut sequences were per-

formed to show specific binding to sequential epitopes. **Results:** No differences in the recognition of sequential epitopes could be found between peanut-allergic patients with or without walnut allergy. Only a few patients showed IgE binding to walnut sequences that corresponded to sequential epitopes of peanut. In the inhibition assays, no relevant cross-reacting IgE antibodies could be detected for the peptides analyzed. **Conclusion:** Our results indicate that although they share a rather high degree of homology with the corresponding regions of walnut allergens, the sequence stretches previously identified as sequential IgE binding epitopes of Ara h 1, Ara h 2 and Ara h 3 have no IgE binding equivalents in walnut allergens.

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Introduction

Peanut and tree nut allergies are common food allergies [1]. In a countrywide computer-based telephone survey in the USA, a prevalence of 1% could be found for peanut and tree nut allergy [2, 3], with a tendency to in-

creasing prevalence [3–5]. Another characteristic of these allergies is that they can elicit especially severe and potentially life-threatening allergic reactions [6]. Among 32 fatal food-allergic reactions observed in the USA between 1994 and 1999, peanuts were the responsible allergen in 62% and tree nuts in 31% of the cases, respectively. The danger of allergic reactions is especially high as doses eliciting a reaction can be very low. It has been shown that 18% of patients react at doses <65 mg of peanut [7, 8]. An aggravating factor is that these allergies are rarely outgrown like other food allergies, but show a persistent course in around 80% of patients [9]. It has been noted that a number of peanut-allergic patients also react against tree nuts [10]. Using the current in vitro diagnostic methods, however, it is not possible to differentiate reliably between tree nut-allergic and tree nut-tolerant peanut-allergic patients [11]. Often peanut-allergic patients must avoid tree nuts in order to prevent possible allergic reactions. Since many processed foods contain nuts or carry a 'may contain' label, dietary measures are often difficult. This leads to significant uncertainty and danger for the patients.

Recently, a great deal of work has been done to develop component-resolved diagnostics as a new paradigm for diagnosing food allergies. The major allergenic proteins of peanut and several tree nuts as well as the linear epitopes of peanut have been identified and characterized [12–17]. Moreover, novel techniques, such as antigen arrays, have facilitated the study of antibody binding to B-cell epitopes.

These antigen arrays can be used to measure clonal diversity [18]. As the antigenic complexity of each arrayed element decreases, fewer epitopes are represented in each array element. When using short peptides as array elements, these theoretically represent single sequential B-cell epitopes that are recognized by defined antibody clones. Several studies have provided evidence for a possible role of these sequential B-cell epitopes in the diagnosis of food allergies. Thus, correlations have been made between antibody diversity and clinically relevant allergy [19, 20]. In other studies, patients recognizing specific sequential epitopes of cow's milk proteins are less likely to outgrow cow's milk allergy in comparison to those who did not show antibody binding towards these IgE binding sites. Furthermore, a correlation between specific IgE diversity and clinical severity as well as in vitro effector cell function could be shown in peanut allergy confirming at the same time again the previously described linear epitopes as relevant IgE binding sites [21]. These observations led to our hypothesis that antibody binding to

shared sequential epitopes could also play a role in the high degree of coincidence of peanut and tree nut allergy.

This hypothesis is based on the fact that despite their taxonomic distance, peanuts and tree nuts have very similar allergenic proteins [14]. These proteins belong to the group of seed storage proteins such as the 2S albumins, and the 7S and 11S globulins. Previous studies have shown that peanut-specific IgE antibodies cross-react with tree nut allergens in inhibition assays [22]. Although the two 7S globulins, Ara h 1 of peanut and Jug r 2 of walnut, were not significantly cross-reactive in inhibition immunoblotting experiments, pre-incubation of walnut-allergic sera with peanut extract did result in a significant decrease in IgE binding to walnut extract [23].

While the sequential epitopes of peanut are known, there is little direct evidence about the role of shared epitopes between peanuts and tree nuts to explain clinical co-reactivity. To investigate whether peanut-allergic patients with walnut allergy recognize linear epitopes in more conserved regions than walnut-tolerant ones, we compared IgE binding to sequential epitopes of peanut and walnut between peanut-allergic patients with and without walnut allergy.

Material and Methods

Peptides, Reagents and Array Production

To study the role of sequence similarities between peanuts and walnuts in clinically relevant co-reactivity, the peanut allergens Ara h 1, Ara h 2 and Ara h 3 and the walnut allergens Jug r 1, Jug r 2 and Jug r 4 were chosen. A set of peptides 15 amino acids in length with an offset of 5 amino acids covering the previously described sequential epitopes of the peanut allergens and the corresponding homologous peptides of the walnut allergens were commercially synthesized (JPT Peptide Technologies, Berlin, Germany) [15, 17, 24, 25] (see 'alignment' in online suppl. fig. 1, www.karger.com/doi/10.1159/000327841). The sequence of Ara h 1 was completely covered. To define the corresponding peptides, the alignment of the primary structure of peanut and walnut allergens was performed using Vector NTI Software (Invitrogen, Carlsbad, Calif., USA). 'Similarity' was defined as similarity in polarity or hydrophily of amino acids. So far, no IgE binding sites have been identified for the basic subunit of Ara h 3. Therefore, the whole basic subunit of Ara h 3 and the corresponding walnut Jug r 4 were synthesized with an offset of 10 amino acids. Following the recommendations of the manufacturer, the peptides were stored at –80°C.

Working dilutions were made in protein printing buffer (TeleChem, Sunnyvale, Calif., USA) to a final concentration of 0.67 µmol/ml and stored in 384-well polypropylene plates (Matrix Technologies, Hudson, N.H., USA). Triplicate spots were printed on epoxy-derivatized slides (SuperEpoxy Substrate,

TeleChem) 350 nm apart using a NanoPrint LM60 Microarrayer (TeleChem). AlexaFluor-labeled protein was used to print positional reference controls. Negative control features (buffer alone and non-binding peptides) were also printed. Anti-IgE monoclonal antibodies (Pharmingen, San Jose, Calif., USA) covalently conjugated to fluorescent markers (AlexaFluor 647) were prepared by reaction with activated N-hydroxysuccinimidyl-ester fluorochromes according to the manufacturer's protocol (Molecular Probes, Eugene, Oreg., USA). The final molar ratio of fluorochrome to immunoglobulin was 5:1.

Patients

Thirty patient sera with documented clinical reactivity to peanut were retrieved from a study on peanuts and tree nuts conducted at the Mount Sinai Pediatric Allergy Clinic [26] and 2 were from the University Hospital Charité (Berlin, Germany). Twenty of these patients were walnut tolerant (age 3–25 years, median 8 years) and 12 were walnut allergic (age 3–23 years, median 7 years). Patients referred for suspected peanut or tree nut allergies answered a questionnaire about their perceived food allergies. Allergen-specific diagnoses were based on the questionnaire, convincing medical history of type 1 reactions, and serologic test evidence of IgE-mediated allergy (ImmunoCAP, Phadia, Uppsala, Sweden; table 1). A patient was considered to have a negative diagnosis if he or she could tolerate ingestion of the food. The patients in the Mount Sinai cohort were highly atopic (57% with atopic dermatitis and 58% with asthma) [26]. Ten non-atopic control sera were taken from healthy volunteers with no history of food allergy. The study protocol was approved by the Mount Sinai Institutional Review Board and the Charité Ethical Commission.

Immunolabeling

An area around the printed grids was encircled with a hydrophobic PAP Pen (DAKO, Glostrup, Denmark) and blocked for 1 h with 400 µl PBS with 0.5% Tween 20 (PBS-T) with 1% human serum albumin (HSA) at room temperature. Diluted patient serum (1:5 in PBS-T/HSA) was applied to the array and was incubated overnight on a rotator at 4°C and protected from light. Titration and kinetic experiments (data not shown) were previously performed to determine optimum conditions. Slides were then washed with PBS-T and incubated for 1 h with 250 µl anti-IgE (1:10,000 PBS-T/HSA) at room temperature in the dark. All incubations were performed in a humidity chamber (Binding Site, San Diego, Calif., USA). Subsequently, the slides were washed 3 times for 3 min in PBS-T in a washing station (TeleChem), rinsed with distilled water and spun dry. The slides were scanned on a ScanArray Gx Scanner (Perkin Elmer Life and Analytical Sciences, Waltham, Mass., USA).

Inhibition Experiments

Immunolabeling was done according to the above-mentioned protocol. In the inhibition experiments, we used pooled sera from 5 peanut- and walnut-allergic patients. Before applying the pooled serum to the array area, it was pre-incubated with increasing amounts of peptide. The molar ratio of peptide:peanut-specific IgE antibody was calculated in the different dilutions from 1:1 up to 10,000:1. To test for potential cross-reactivity, the sera were pre-incubated concomitant with different highly homologous peanut and walnut peptides, with highly conserved regions with strong binding to the peanut sequences being selected.

Table 1. Patient sera with clinical manifestations

Patient	Peanut symptoms	Walnut symptoms	Peanut IgE, kU/l	Walnut IgE, kU/l
1A	U/AE/F	AE	7.4	6.79
2A	U/F	U/F/OA	38.20	11.80
3A	U/F/OA/E/C	AE/F/OA/E/C	>100	6.93
4A	U/OA/G/D/Bs/L	E/Co/D/Bs	24.20	15.80
5A	U/AE/OA/E/D/Bs	E/D/Bs	>100	7.5
6A	U/G/E/BP/S	U/AE/F/R	100	32.1
7A	U/F	U/OA	0.74	1.13
8A	U/F/AE/AD/Co/D/Bs/R/C/L	D/Bs	53.40	5.83
9A	U	OA	>100	10.4
10A	AD/Di	U/E/Bs	6.29	42.7
11A	AE/F	E	5.28	12
12A	AE	U/BP	4.92	6.51
1B	U/AE	0	2.34	<0.35
2B	U/C	0	19.9	<0.35
3B	U/F	0	34.3	<0.35
4B	U/F	0	2.56	0.55
5B	U/AE	0	>100	1.31
6B	U/AE	0	>100	1.13
7B	Co/D/Bs	0	58.9	<0.35
8B	F/E	0	2.62	0.56
9B	U/AE	0	97.7	<0.35
10B	U/AE	0	18.7	<0.35
11B	U/Co/Bs	0	27.7	<0.35
12B	AE/F/G/E/Di/Co	0	6.69	<0.35
13B	U/AE/F/E/Co	0	35.3	<0.35
14B	AE/D/R	0	>100	
15B	E	0	100	0.35
16B	U	0	17.2	<0.35
17B	U/AE/F/AD/OA/E/D/Bs/L	0	100	NA
18B	U/G/E/D/R/S	0	25.9	NA
19B	U/AE/OA/G/E/Co/D/Bs/R/C/L/BP/S	0	100	NA
20B	U/AE/F/C	0	>100	<0.35

U = Urticaria; AE = angioedema; F = flush; AD = worsening of eczema; OA = itching and tingling sensation on lips, oral cavity and throat; G = gastric pain and/or burning abdominal pain; E = emesis; Di = diarrhea; Co = cough; D = dyspnea; Bs = bronchospasm/wheezing; R = rhinitis; C = conjunctivitis; L = laryngoedema/throat tightness; BP = drop in blood pressure; S = shock, passing out; NA = not assessed. All clinical reactions were type 1 reactions and occurred within 2 h after ingestion of the food.

Table 2. Inhibition of IgE binding to peanut peptides

Sequence	Similarity	Identical amino acids	Inhibition	
			peanut peptide	walnut peptide
Ara h 1.024 DLSNNFGKLFVKPD Jug r 2.024 SYSNQFGQFFEACPE	60%	40%	+	–
Ara h 1.032 KQAKDLAFPGSGEQV Jug r 2.032 REAKELSFNMPREEI	60%	33%	+	–
Ara h 2.021 QQFKRELPNLPQQCG Jug r 1.021 EEMVQSARDLPNECQ	40%	33%	+	–
Ara h 3.004 IFSGFTPEFLEQAFQ Jug r 4.004 VFSGFDADFLADAFN	73%	53%	+	(+)
Ara h 3.008 EEYDEDEYDEEDR Jug r 4.008 RKERERERESEERR	40%	33%	+	–
Ara h 3.024 VIDNLPEEVVANSYG Jug r 4.024 AIRALPEEVLATAFQ	67%	47%	+	–

Analysis

The fluorescence signal was digitalized with ScanArray Express 4.0 software (Perkin Elmer) using the 'adaptive threshold' algorithm. Artifacts were manually eliminated. The subsequent calculations were done by Excel (Microsoft, Renton, Wash., USA) and a custom-made program (Contec Vision, Waltham, Mass., USA). This was used to relate the grid data to the corresponding peptide identity and to calculate the means and SDs of the triplicates. Array elements with coefficients of variation >30% were excluded from the analysis. The mean plus 2 SD log-transformed of the negative controls (buffer alone) was used as an intraslide cutoff to establish background. An interslide cutoff was calculated as the mean plus 2 SD intensity of the corresponding signal from the control sera. For calculation of the percentage of patients with positive IgE binding, both intra- and interslide cutoffs had to be exceeded.

The following analysis was done with SPSS 11 for Mac OS X. Additional analysis and graphical display were performed using Prism (GraphPad Software, San Diego, Calif., USA).

To compare the frequency of binding to peanut and walnut peptides between walnut-allergic and walnut-tolerant patients, χ^2 tests were calculated for each peptide. We then performed a Bonferroni correction for multiple testing. The number of peptides recognized by the patients in the different groups was compared and significance was tested by the Mann-Whitney U test.

Results

IgE antibodies from patients allergic to peanuts and walnuts were compared to those allergic to peanut but not walnut with respect to binding of certain peanut or corresponding walnut peptides (fig. 1). The two groups did

not show significant differences with respect to peanut-specific IgE (in walnut-allergic patients: from 0.74 to >100 kU/l, median: 31.2 kU/l, and in walnut-tolerant ones: 2.34 to >100 kU/l, median: 34.8 kU/l). The walnut-specific IgE was higher in the walnut-allergic group (in walnut-allergic patients: 1.13–42.7 kU/l, median 8.95 kU/l, and in walnut-tolerant ones: <0.35–1.31 kU/l, median: <0.35 kU/l, $p < 0.05$).

Comparing the frequency of IgE binding to either peptides of peanut allergens or walnut allergens, no peptide could be identified that discriminated between the two groups. Moreover, there was no difference in the number of either peanut or walnut epitopes recognized by either patient population (fig. 2). On the contrary, most of the IgE binding to peanut peptides appeared in regions with lower homology.

Walnut peptides had been chosen as they corresponded to known peanut peptides. There was very little recognition of these walnut peptides by either walnut-allergic or walnut-tolerant patients. Only 1 walnut peptide (Jug r 1.013) was recognized by >20% of patients in either group (fig. 1).

Inhibition experiments were also carried out to test for potential cross-reactivity. While specific inhibition of IgE binding to the peanut peptides could be obtained by pre-incubating with peanut peptides, this was not possible with the corresponding walnut sequences for most of the tested peptides (table 2). Only the walnut sequence VFSGFDADFLADAFN led to a dose-dependent inhibi-

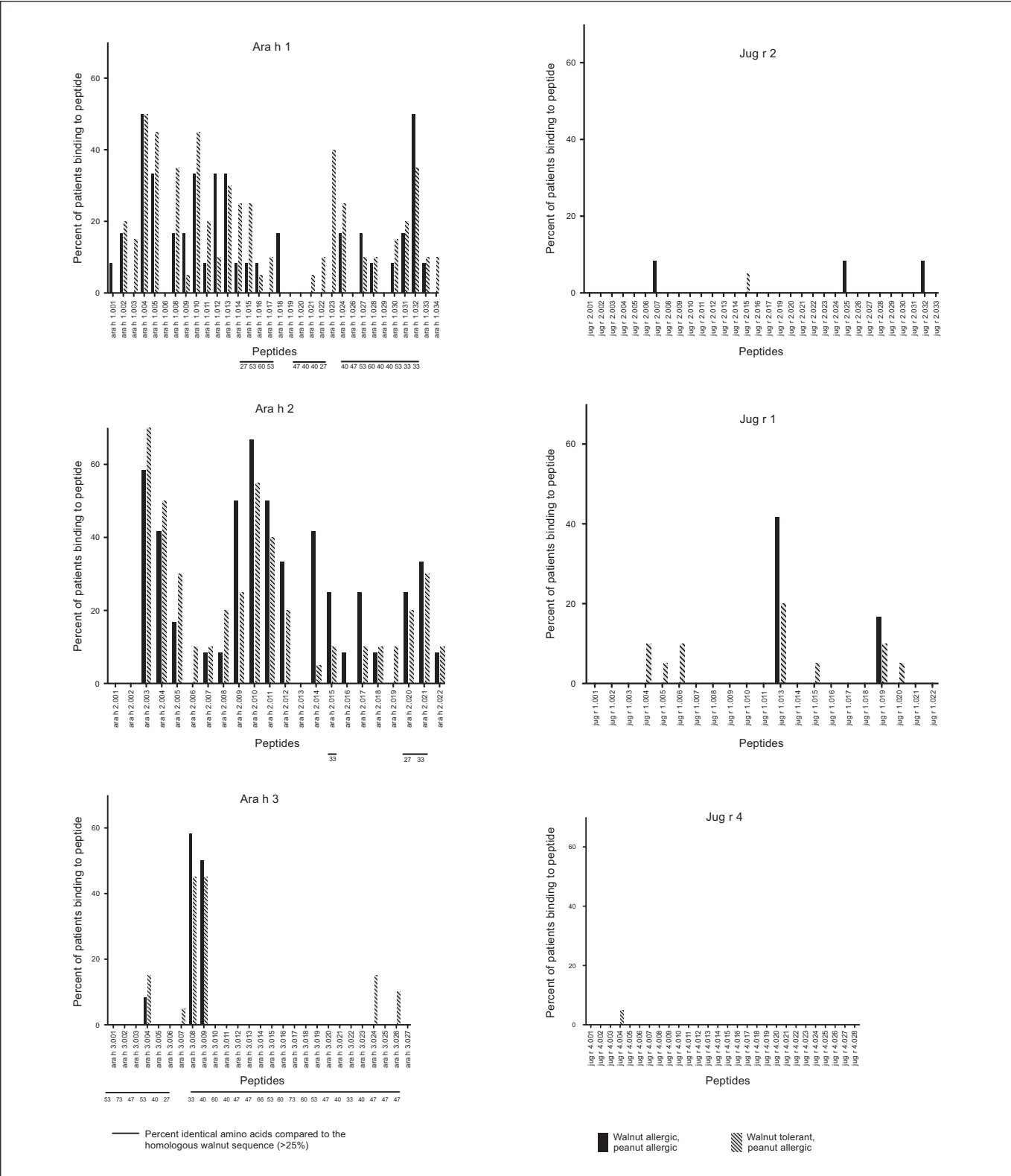


Fig. 1. IgE binding pattern of peanut-allergic patients with and without walnut allergy to chosen peanut and walnut epitopes. Bars represent percentage of IgE binding to the specific sequences in the different groups. Numbers of walnut sequences of Jug r 2, 1 and 4 correspond to homologous sequences of peanut allergens Ara h 1, 2 and 3, respectively.

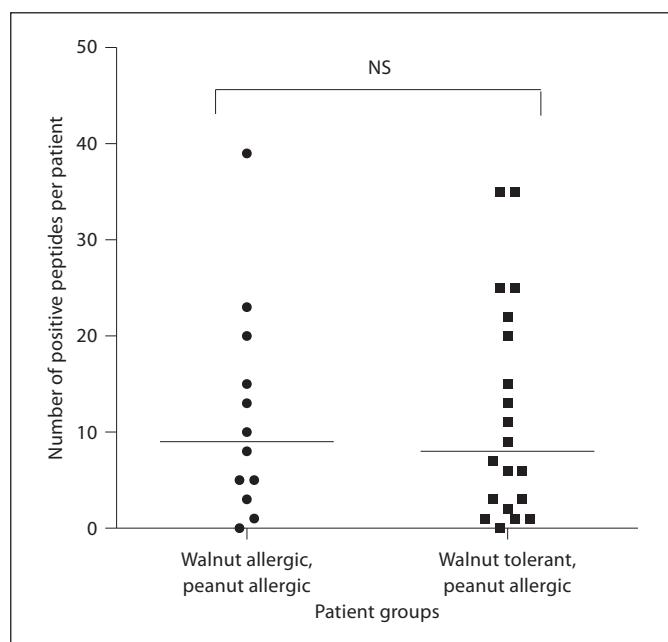


Fig. 2. Number of peptides (peanut + walnut) recognized by each patient comparing walnut-allergic versus walnut-tolerant peanut-allergic patients. NS = Nonsignificant.

tion of IgE binding towards the corresponding peanut sequence IFSGFTPEFLEQAFQ; 1,000-fold higher concentrations of walnut peptide were needed to obtain similar inhibition compared to peanut peptide.

Discussion

We hypothesized that peanut-allergic patients with or without walnut allergy could be distinguished by their peptide binding patterns in the regions of the previously described peanut epitopes. Peanut-allergic patients with coincident walnut allergy were expected to bind to a greater number of walnut sequential epitopes than those without walnut reactivity. We expected these patients to bind to epitopes with high sequence similarity in the primary structure. By identifying discriminating peptides, we sought to provide a basis for a diagnostic in vitro test that could differentiate between peanut-allergic patients and in vitro evidence of walnut sensitivity with or without accompanying clinical walnut allergy. However, in our experiments, we were unable to show any significant differences between the two groups.

Our results indicate that despite the rather high degree of homology shared with the corresponding regions of

walnut allergens, the sequence stretches previously identified as sequential IgE binding epitopes of Ara h 1, Ara h 2 and Ara h 3 have no IgE binding equivalents in walnut allergens.

However, linear epitopes located in other regions of the protein may well be shared. As only previously described sequential epitopes of peanut allergens and the corresponding homologous peptides of the walnut allergens were analyzed, there are some areas of Ara h 2 and 3 and the corresponding walnut peptides which are not covered and which may contain cross-reactive IgE binding sites. Furthermore, other peanut or walnut allergens beside the major allergens or seed storage proteins could be responsible for the coincidence of walnut and peanut allergy.

Cross-reaction between different nut extracts has been shown, whereas the results for cross-reactivity between peanuts and tree nuts are inhomogeneous [22, 27]. The 7S globulins Ara h 1 and Jug r 2 showed no cross-reactivity in inhibition experiments [23]. However, in a three-dimensional computer model, sequential epitopes on 7S globulins of peanut and homologous regions of different tree nuts show a similar three-dimensional conformation which might enable clinical cross-reactivity [28]. We examined the whole sequences of Ara h 1 and Jug r 2 and found no evidence for shared sequential epitopes.

Similar computer models of the three-dimensional structure of the 2S albumins support the lack of cross-reactivity found in our study [29]. Immunoblot experiments with whole 2S peanut and walnut proteins are needed to investigate the cross-reactivity of whole proteins.

IgE binding epitopes of Ara h 3 and Jug r 4 have also been studied in a computer model [30]. The structural homology found in this study might be responsible for clinical cross-reactivity, but we could not find a correlate to this in our experiments. The three-dimensional modeling of the allergens supports the hypothesis that conformational epitopes on the surface of the allergens built by several linear epitopes might be responsible for the observed common IgE binding activity instead of single linear IgE binding epitopes as exclusive basis for IgE cross-reactivity.

Strong support for the hypothesis that linear epitopes have little impact on the IgE binding capacity to the whole allergen is given by Albrecht et al. [31]. Linear epitopes of Ara h 2 showed little contribution to the IgE binding to the whole allergen in inhibition assays.

The IgE binding to sequential epitopes in our study as well as the observed heterogeneity of binding are consistent with other microarray studies [21, 24]. In our study,

strongly binding epitopes have previously been described as immunodominant epitopes in immunoblot experiments [17]. The heterogeneity of binding observed in our as well as other microarray studies might explain why we do not find a single discriminating peptide that distinguishes between different patient groups.

The previously identified major linear epitope of Jug r 1 is represented by Jug r 1.019 in our experiments [32]. Even more patients bound to Jug r 1.013 (GYDEDN-QRQHFRQCC). The pathogenetic role of this sequence is however unclear as binding to this sequence was also observed in walnut-tolerant patients.

A limitation of our study is that our patients have been recruited based on convincing medical history of type 1

reactions and serologic testing and not based on double-blind placebo-controlled food challenges.

In summary, our data cannot show that the sequence homologies in the regions of the known sequential epitopes of peanut and the basic subunit of Ara h 3 are responsible for a coincidence of clinical peanut and walnut allergy.

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7S Globulins

		1				70
Ara h 1	(1)	MRGRVSPLMLLLGILVLSVSA	HAKSSP	--YQKKT	ENPCAQRCLQSCQ QEPDDLKQKA	CE\$RCK LEYD
Jug r 2	(1)	-----RGRDDDD	DEENPRD	PREQYRQCQ	EYCR	RQGQGORQQQ--Q-CQIRCEERLEEDQRS
		71				140
Ara h 1	(69)	PRCVYD	PRGHTGTTNQR	SPP GERTRGRQPG	-----	DYDDRRQPRREEGGRWG PAGP---
Jug r 2	(53)	QEERERRRGRD	VDDQNPRD	PEQRYEQCQQQC	ERQRRGQEQ	TLCRRRCEQRRQQEERERQGRDRQDPQQQ
		141				210
Ara h 1	(121)	---	R---	EREREEDWRQPR	EDWRRP	SHQQPRKIRPEGRE GEQEWGTPGSHVREETS
Jug r 2	(123)	YHRCQRR	CQIQEQSPERQ	RQ-CQQR	CERQYKEQQGR	EGP-EASPRRESRGREEEQQR-HNPYYFHSQSI
		211				280
Ara h 1	(183)	STRYGNQNGRIRV	LQRFQ	RSRQFQNLQ	NHRIVQIEAKP	NTLVLPKHADADN
Jug r 2	(190)	RSRHESEE	GEVKYLERF	TERTELLRGI	ENYRVVILDAN	PNTSMLPHHKDAESVAVVTRGRATLTLVSQET
		281				350
Ara h 1	(253)	RKSFNLDEGH	ALRIPSGFIS	YILNRHDNQN	LRVAKISMPVNT	TPGQFEDFFP ASSRDQ-SS SYLQGF SRNTL
Jug r 2	(260)	RESFNLECGD	VIRVPAGAT	VYVINQDSNER	LEMVKLLQPV	NNPGQFREYYAAGAKSPDQSYLRVFSNDIL
		351				420
Ara h 1	(322)	EAA FNAEFNEIRR	VLLLENAGG	EQEERGQRRW	STRSEN	NEGVIVKVSKEHVEELTKHAKSVSK KGSEEE
Jug r 2	(330)	VAALNTPRDR	LERFFDQEQ	-----	REGV	IRASQEKLRALSQHAMSAG-QRPWGR
		421				490
Ara h 1	(392)	GDI	TNPINLRE	GE	PDL SNNFGKLF	EVK PDKKNPQLQDLDMMLTCVEIKEGALMLPHFNSKAMVIVVWNG
Jug r 2	(380)	RSSGGPISL	KSESPSY	SNQFGQ	FFEACPEE	HR-QLQEMDVLVNYAEIKRGAMMVPHYNSKATVVVYVEG

		491		560
Ara h 1	(462)	TGNLELVAV RKEQQQGRGRRREEEDEDDEEEEGSNREV RRYTARLKEG DVFI MPAAHPVAINASS-- ELHLL		
Jug r 2	(449)	TGRYEMACPHVSSQSYEGQGRREQEEEEESTGR---FQKVTARLARGDIFVIPAGHPIAITASQENENLRLL		

		561		630
Ara h 1	(530)	GFGIN AENN HRIFLAGDKD NV IDQIEKQAKDLAFPGSGE QVEKLIK QKESHFVSARP QSQSQSPSS PEK		
Jug r 2	(516)	GFDINGENNQRDFLAG-QNNIINQLEREAKELSFNMPREEIEEIFESOMESYFVPTERQSRRGQ-----		

		631		658
Ara h 1	(600)	ESPEKED QEEENQGGKGPLLSILKAFN-		
Jug r 2	(579)	-----GRDHPLASILDFAFF		

2S Albumins

		1		70
Ara h 2	(1)	---MAKLTI LVALALFLAA HASARQQWELQGD RRCQSQ LERAN LRPCEQHLMQKIQR DEDSYER DPYSP		
Jug r 1	(1)	-----AALLVALLFVANAAAFRTTITITMEIDEDIDNPRRRGEGCREQIQRQQNLNHCQYYLRQ-QSR		

		71		140
Ara h 2	(68)	SQDPYS-PS PYDRRGAGSSQHQERCC NE LNEFENN QRC M CEALQ QIMENQSDRL QGRQ-QE QQFKREL RN		
Jug r 1	(62)	SGG-----YDEDNQR--QHFRQCCQQLSQMD--EQCQCEGLRQVVRQQQQ QGLRGEEMEEMV QSARD		

		141		161
Ara h 2	(136)	LPQCCGLRAP QRCDLDVE SGG		
Jug r 1	(121)	LPNECGIS-SQRCEIRRSWF-		

11S Globulins

		1		70
Ara h 3	(1)	-----RQQPE---ENACQFQRLNAQRPDNRIESEGGY		
Jug r 4	(1)	MAKPILLSIYLFLIVALFNGCLAQSG-----GRQQQQ---FGQCQLNRLDALEPTNRIEAEAGV		
		71		140
Ara h 3	(30)	IETWNPNNQEFECAG VALSRLVLRNALRRPFYSNAPQEIFIQQGRGYFGLIFPGCPRHYEEPHTQGRRS		
Jug r 4	(57)	IESWDPNNQQFQCAGVAVRRRTIEPNGLLLPOYSNAPQLVYIARGRGITGVLFPGCPETFEEES-QRQSQQ		
		141		210
Ara h 3	(100)	QSQRPPRRLQGEDQSQQQRDSHQKVHRFDEGDLIAVPTGVAFWLYNDHDTDVVAVSLTDTNNNDNQLDQF		
Jug r 4	(126)	GQ-----SREFQQDRHQKIRHFRREGDIIAFPAQVAHWSYNDGSNPVVAISLLDTNNNANQLDQN		
		211		280
Ara h 3	(170)	PRRFNLAGNTEQEFLRYQQQSRQSRRRSLPYSPYSPQSQPRQEEREFSPRGQHSRRERAGQEEENEG GNI		
Jug r 4	(185)	PRNFYLAGNPDDFRPQGQQE-----YEQHRRQQQ-----RQQRPGEHGQQ-QRGLGNV		
		281		350
Ara h 3	(240)	FSGFTPEFLEQA FQVDDRQIVQNLRGETESEEEGAI VTVRGG-LRILSPDRK RRRADEEEE--Y DEDEYEF		
Jug r 4	(234)	FSGFDADFLADAFNVDTETARRLQS---ENDHRRSIVRVEGRQLQVIRPRWSREEQEREERKERERERES		
start of basic subunit ↓				
		351		420
Ara h 3	(307)	DEEDRR RGRGSRGRGNGIEETICTASAKKNIGNRSPDIYNPQAGSLKTANDLNLLILRWLGPSAEYGNL		
Jug r 4	(301)	ESERRQSRRGGR-DDNGLEETICTLRLRENIGDPSRADIYTEEAGRISTVNSHTLPVLRWLQLSAERGAL		
		421		490
Ara h 3	(377)	YRNALFVAHYNTNAHSIIYRLRGRAHVQVVDNSNGNRVYDEELQEGHVLVVPQNFVAVAGKSQS-ENFEYVA		
Jug r 4	(370)	YSDALYVPHWNLNAHSVVYALRGRAEVQVVDNFGQTVFDELREGQLLTIPQNFVAVVKRARN-EGFEWVS		

