Evaluation of IgE Antibodies to Recombinant Peanut Allergens in Patients with Reported Reactions to Peanut

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Key Words

Allergy · Ara h 1 · Ara h 2 · Ara h 3 · Ara h 8 · Ara h 9 ·
Peanut · Recombinant allergens · Sensitization · Severe
symptoms

Abstract

Background: Peanut may cause severe reactions in allergic individuals. The objective was to evaluate IgE antibodies to various recombinant (r) peanut and birch pollen allergens in relation to IgE levels to whole peanut extract and severe allergic reactions to peanut. **Methods:** Seventy-four Swedish peanut-allergic patients (age: 14–61 years) reported previous peanut exposure and associated symptoms using a questionnaire. Their IgE reactivity to peanut, birch pollen and individual allergen components was analyzed using ImmunoCAP®. **Results:** Of the 48 subjects sensitized to Ara h 1, 2 or 3, 60% had peanut-specific IgE levels >15 kU_A/l, while 100% of the subjects without detectable IgE to these allergens had low peanut-specific IgE levels (<10 kU_A/l). The levels of IgE to rAra h 8, rBet v 1 and birch pollen were highly

correlated ($r_s = 0.94$, $p < 0.0001$). Fifty-eight patients reported adverse reactions after accidental or deliberate peanut exposure (oral, inhalation or skin) of whom 41 had IgE to rAra h 1, 2 or 3. Symptoms of respiratory distress were associated with sensitization to Ara h 1, 2 or 3 (56 vs. 18%, $p < 0.01$). Two cases of anaphylaxis were reported among the individuals sensitized to Ara h 1–3. IgE to rAra h 8, rAra h 9, profilin or cross-reactive carbohydrate determinants were not associated with severe symptoms. **Conclusions:** The results indicate that IgE reactivity to Ara h 1, 2 and 3 is associated with severe reactions after exposure to peanut in Swedish patients.

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Introduction

Peanut is the most common food involved in fatal allergic reactions in western countries [1–3]. Accidental peanut consumption may cause severe allergic reactions in susceptible individuals. Severe reactions due to food

allergy caused 48 deaths in the UK from 1999 to 2006 [2], for which peanut and tree nuts seemed to be commonly responsible [2, 4]. In contrast to other common food allergies in childhood, peanut allergy is often a lifelong disease [5]. The prevalence of peanut allergy has increased and has been estimated to be between 0.5 and 2% [6, 7]. The symptoms upon peanut ingestion might vary from mild reactions, such as urticaria and oral allergy syndrome (OAS), to respiratory distress and severe systemic reactions needing medical care, such as anaphylactic shock. Peanut sensitization, determined by allergen-specific IgE analysis in blood or skin prick testing to peanut extract, unfortunately has a low positive predictive value since many sensitized individuals are tolerant to peanut [8, 9]. Such imprecision might be due to cross-reactive IgE antibodies displaying low clinical significance like IgE antibodies induced by profilins or PR-10 proteins in pollen that cross-react with their peanut homologues Ara h 5 and Ara h 8, respectively [10–12], or IgE to cross-reactive carbohydrate determinants (CCDs) [13].

The peanut seed storage proteins Ara h 1 (vicilin), Ara h 2 (conglutinin) and Ara h 3 (glycinin) are all major allergens and seem to be responsible for primary sensitization to peanut in susceptible individuals [14, 15]. IgE antibodies to the peanut storage proteins have been suggested as risk markers for severe allergic reactions in peanut-sensitized individuals [16]. Even if double-blind placebo-controlled food challenges are regarded as the gold standard in the diagnosis of food allergy, many individuals with a known peanut sensitization and a history of adverse reactions to peanut are never challenged. These individuals often spend their lives with the fear of severe or even deadly reactions to accidental exposure to peanut. However, not all of them will react with severe symptoms upon peanut exposure and further studies are warranted to find diagnostic tools to identify the patients with the highest risk of fatal reactions. Analysis of the IgE reactivity pattern to various peanut allergens has been suggested as such a tool.

The aim of this study was to analyze IgE antibodies to recombinant allergens from peanut (rAra h 1, 2, 3, 8 and 9) and birch pollen [rBet v 1 and rBet v 2 (profilin)], and CCD in patients with a suspected peanut allergy, mostly young adults living in a birch-abundant region, and to evaluate the association between different IgE reactivity patterns with reported symptoms to peanut and peanut-containing foods.

Material and Methods

Study Design

The study group of 74 patients was composed of mostly young adults (median age: 23 years, range: 14–61 years, 47 females and 27 males) living in the western area of Sweden (Gothenburg). Inclusion criteria were a known peanut sensitization and a history of suspected peanut allergy. Thirty-three of them (A1–A33) originated from a larger previously described cohort of peanut-sensitized children and adolescents followed for more than 15 years [17]. The other 41 individuals (B1–B41) were patients at the Asthma and Allergy Clinic at Sahlgrenska University Hospital. The participants were asked to complete a questionnaire with detailed questions regarding known exposure to peanut and allergic symptoms. The symptom categories were: *Eczema*, *Urticaria*, *Facial Edema* (swelling in neck, lips and face), *OAS* (itching throat and face), *Abdominal Pain* (stomach ache, diarrhea), *Respiratory Distress* (asthma, dyspnea), and *Other Symptoms* (as indicated by the patient). The patients also reported whether they suffered from other allergies (birch pollen, timothy pollen, hazelnut, soy bean, lentil, pea, almond, liquorice, carrot, potato, apple, pear, plum, kiwi and strawberry). The questionnaire was reviewed by an investigator (J.Å.) before database entry. Venous blood samples were collected for serum preparation. The sera were frozen at -20°C until analysis. The two patient groups (A1–A33 and B1–B41) did not significantly differ regarding gender or reported peanut exposure. However, the A1–A33 group (median age: 18 years, range: 14–30 years) was significantly younger than the B1–B41 group (median age: 26 years, range: 15–61 years, $p < 0.0001$). The study was approved by the Research Ethics Committee of the University of Gothenburg.

Allergen-Specific IgE Measurements

The serum samples were analyzed using ImmunoCAP® (Phadia AB, Uppsala, Sweden) for IgE reactivity to recombinant (r) Ara h 1, rAra h 2, rAra h 3, rAra h 8, rAra h 9, rBet v 1, rBet v 2, CCD, peanut extract and birch pollen extract. IgE antibody levels $>0.10 \text{ kU}_A/\text{l}$ were considered positive.

Statistics

The Mann-Whitney U test and Fisher's exact test (both two-tailed) were used for pair-wise comparisons of continuous parameters (specific IgE concentrations) and categorical data between groups, respectively. Spearman's rank correlation test was used to establish the strength of the relationship between IgE antibody levels. All p values <0.05 were considered significant.

Results

IgE Reactivity Patterns

Forty-eight patients (65%) had IgE antibodies to at least one of the recombinant peanut storage proteins rAra h 1, rAra h 2 or rAra h 3 (group positive for Ara h 1–3). Polysensitization to 2 or 3 of these allergens was common, and the IgE levels to these allergens were correlated ($r_s = 0.78\text{--}0.91$, $p < 0.0001$) whereas sensitization to only one

Table 1. Patient data (subjects A1–A33) sorted by peanut exposure (A1–A25), reported respiratory distress (A1–A9), sensitization to Ara h 1, 2 and/or 3 (A1–A18 and A26–A31) and IgE levels to peanut

No.	Questionnaire data				ImmunoCAP analysis, kU _A /l									
	sex/age	peanut exposure	peanut symptoms ¹	other allergies ²	peanut	birch	Ara h 1	Ara h 2	Ara h 3	Ara h 8	Ara h 9	Bet v 1	Bet v 2	CCD
A1	M/17	oral	R, V, A	B	>100	3.69	>100	>100	37.09	1.03	<0.10	3.56	<0.10	<0.10
A2	M/17	oral	R, F, U, O	T, H, A	>100	0.99	44.99	72.23	15.95	0.22	<0.10	<0.10	2.03	0.27
A3	F/25	oral	R, F, O	B, H, S	>100	55.91	99.01	73.50	51.23	12.68	0.23	58.58	0.14	<0.10
A4	F/17	inhalation	R, F	B, T	77.28	<0.10	26.92	40.56	6.49	<0.10	<0.10	<0.10	<0.10	<0.10
A5	M/16	inhalation	R	B, T	57.02	5.75	14.88	14.53	17.86	0.91	<0.10	6.16	<0.10	<0.10
A6	F/19	oral	R, O	T, H	5.25	1.49	0.96	1.90	<0.10	0.31	<0.10	1.29	<0.10	<0.10
A7	F/18	oral	R, F, U, O	S	3.11	11.22	3.58	<0.10	0.18	2.32	<0.10	12.97	<0.10	<0.10
A8	M/18	oral	R, F, U, V, O	B, T	1.05	0.94	<0.10	0.89	<0.10	0.19	<0.10	0.60	<0.10	<0.10
A9	F/19	oral	R, F	B, H, S	0.37	48.62	0.33	<0.10	<0.10	13.04	<0.10	40.89	<0.10	<0.10
A10	M/15	oral ³	unknown	S	>100	5.09	>100	>100	>100	1.28	<0.10	5.75	<0.10	<0.10
A11	M/19	skin	F, O	B, T, S, A	>100	3.18	61.02	33.27	39.61	0.16	<0.10	2.94	<0.10	0.15
A12	M/22	oral	F	B	29.65	46.53	0.58	16.98	<0.10	19.59	<0.10	54.22	<0.10	<0.10
A13	F/15	oral ³	F, O	B, T, H, S, A	27.72	>100	2.57	<0.10	7.92	47.48	<0.10	82.74	<0.10	<0.10
A14	M/25	oral ³	F, U, A	B, T, H, S, A	19.49	<0.10	15.71	5.67	0.57	<0.10	<0.10	<0.10	<0.10	<0.10
A15	M/15	oral	F	B, T	17.51	>100	6.04	<0.10	8.27	20.43	0.64	>100	0.10	0.15
A16	M/15	oral	U, O	–	7.19	0.46	<0.10	1.86	<0.10	<0.10	<0.10	0.30	<0.10	<0.10
A17	F/18	oral	severe OAS	B, T	2.96	11.53	3.00	0.49	<0.10	0.98	0.11	7.40	<0.10	<0.10
A18	F/23	oral	U, A, O	B, T, H, S, A	2.32	28.23	<0.10	1.37	<0.10	7.72	<0.10	26.86	<0.10	<0.10
A19	M/15	oral	O	B, T, H, A	1.75	>100	<0.10	<0.10	<0.10	59.32	<0.10	>100	<0.10	0.24
A20	F/27	oral ³	F, E, O	B, T	1.54	1.97	<0.10	<0.10	<0.10	<0.10	<0.10	0.94	<0.10	0.84
A21	F/26	oral	O	B, T, H	0.63	13.53	<0.10	<0.10	<0.10	2.02	0.94	8.87	<0.10	<0.10
A22	M/15	oral	A, O	B, T, H	0.54	6.29	<0.10	<0.10	<0.10	2.70	<0.10	6.16	<0.10	<0.10
A23	F/14	oral	U	H, A	0.48	28.41	<0.10	<0.10	<0.10	0.17	<0.10	30.75	<0.10	<0.10
A24	M/16	oral	F, O	B, T, H, A	0.17	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
A25	F/22	oral	U, O	B, T, H, A	<0.10	5.49	<0.10	<0.10	<0.10	0.63	<0.10	4.83	<0.10	<0.10
A26	F/18	no exposure	–	B, T	>100	<0.10	59.76	38.87	29.05	<0.10	<0.10	<0.10	<0.10	<0.10
A27	F/18	no exposure	–	T, H	70.94	<0.10	39.50	15.32	13.11	<0.10	<0.10	<0.10	<0.10	<0.10
A28	M/15	no exposure	–	H	9.37	0.16	4.54	4.10	0.31	<0.10	<0.10	0.12	<0.10	<0.10
A29	M/30	no exposure	–	T, A	8.08	<0.10	5.17	4.97	0.21	<0.10	<0.10	<0.10	<0.10	<0.10
A30	M/14	no exposure	–	B, T	4.32	11.54	<0.10	<0.10	1.81	2.09	<0.10	9.20	0.90	<0.10
A31	F/24	no exposure	–	B, T, H	0.97	21.77	<0.10	0.53	<0.10	3.97	<0.10	19.79	<0.10	<0.10
A32	F/23	no exposure	–	–	2.16	1.53	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	1.30
A33	F/30	no exposure	–	T	0.31	25.36	<0.10	<0.10	<0.10	6.61	<0.10	16.47	1.46	<0.10

F = Female; M = male.

¹ Peanut symptoms: A = abdominal pain; E = eczema; F = facial edema; O = oral allergy syndrome; R = respiratory distress; U = urticaria; V = vomiting.

² Other allergies: A = almond; B = birch pollen; H = hazelnut; S = soy; T = timothy pollen.

³ Deliberate peanut intake.

of the components was uncommon but existed occasionally (tables 1, 2).

Twenty-six patients (35%) had no detectable IgE to rAra h 1, rAra h 2 or rAra h 3 (group negative for Ara h 1–3). This group of patients did not differ from the group positive for Ara h 1–3 regarding IgE reactivity to rAra h 8.

The patients in the group positive for Ara h 1–3 had significantly higher peanut-specific IgE levels than those in the group negative for Ara h 1–3 (median: 21.0 kU_A/l, range: 0.37–>100 kU_A/l versus median: 0.73 kU_A/l, range: <0.10–9.90 kU_A/l, $p < 0.0001$). Twenty-nine patients (60%)

in the group positive for Ara h 1–3 had peanut-specific IgE levels above the suggested 95% predictive clinical decision point of 15 kU_A/l, i.e. the identified level for a risk of allergic reactions upon oral challenge [18]. None of the patients in the group negative for Ara h 1–3 had peanut-specific IgE levels above the positive decision point ($p < 0.01$).

All subjects with IgE reactivity to rAra h 8 ($n = 52$) also had IgE to birch pollen and all but 1 had IgE to rBet v 1, and the IgE antibody levels highly correlated ($r_s = 0.94–0.98$, $p < 0.0001$). Five peanut-sensitized individuals did not have IgE to any of the peanut components included.

Table 2. Patient data (subjects B1–B41) sorted by peanut exposure (B1–B33), reported respiratory distress (B1–B17), sensitization to Ara h 1, 2 and/or 3 (B1–B14, B18–B26 and B34) and IgE levels to peanut

No.	Questionnaire data				ImmunoCAP analysis, kU _A /l									
	sex/ age	peanut exposure	peanut symptoms ¹	other allergies ²	peanut	birch	Ara h 1	Ara h 2	Ara h 3	Ara h 8	Ara h 9	Bet v 1	Bet v 2	CCD
B1	M/16	inhalation	R, F	B, T, H, S, A	>100	25.26	50.83	36.65	12.86	5.14	11.29	26.33	1.01	1.03
B2	F/28	oral	R, U, V, D	B	>100	31.26	29.06	>100	17.64	7.30	0.11	24.72	<0.10	<0.10
B3	M/19	oral	R, U, A	A	89.68	<0.10	25.69	19.90	0.74	<0.10	0.24	<0.10	<0.10	<0.10
B4	M/34	oral	AX, R, F, U, A, O	S	72.16	0.94	16.30	46.48	3.24	<0.10	<0.10	0.37	<0.10	<0.10
B5	F/37	oral	R, F, U	B, H, S	64.39	12.10	21.02	47.22	9.81	6.75	<0.10	7.54	<0.10	0.11
B6	F/23	inhalation	R, U	T, H, A	61.84	15.58	11.71	32.96	6.01	2.83	0.22	9.26	0.11	0.11
B7	F/39	inhalation/oral	AX, R, F, U, O	H	41.68	0.47	12.93	23.77	4.56	<0.10	<0.10	0.21	<0.10	<0.10
B8	F/38	oral	R, F, U, A, O	T, H, S, A	35.29	<0.10	18.46	13.58	1.27	<0.10	<0.10	<0.10	<0.10	0.12
B9	F/22	oral	R, F, O	B, T	19.53	59.75	0.14	0.12	0.20	14.17	1.22	50.62	0.22	0.76
B10	F/34	oral	R, F	–	14.48	7.59	4.22	3.61	1.09	1.38	<0.10	6.51	<0.10	<0.10
B11	F/23	oral	R, F, O	–	9.42	<0.10	0.70	3.48	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
B12	F/28	oral	R, F, U, A, E, O	B, T, H, A	0.92	6.66	<0.10	0.61	<0.10	2.80	<0.10	5.90	<0.10	<0.10
B13	M/22	oral ³	R, F, O	H, A	0.91	1.54	<0.10	0.16	<0.10	0.17	<0.10	1.21	<0.10	<0.10
B14	M/38	skin	R, F	B, T, H, A	0.79	0.85	<0.10	0.81	<0.10	<0.10	<0.10	0.49	<0.10	<0.10
B15	M/58	oral	R, A	B, T, S	0.75	0.54	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.65
B16	F/35	oral	R, F, A, O	B, H, A	0.63	13.27	<0.10	<0.10	<0.10	2.41	<0.10	14.77	<0.10	<0.10
B17	F/25	oral	R, F, U, A, O	B, T, H, A	0.27	12.84	<0.10	<0.10	<0.10	0.17	<0.10	7.62	<0.10	<0.10
B18	F/15	oral	F, O	B, T, S	>100	35.14	66.26	>100	20.80	5.83	0.16	26.30	0.11	0.17
B19	F/24	inhalation	F, U	–	>100	7.45	63.09	37.66	13.93	1.42	<0.10	3.69	<0.10	<0.10
B20	M/17	oral	F, U	S	>100	1.26	>100	>100	54.08	0.13	<0.10	0.94	<0.10	<0.10
B21	F/22	skin	U, O	B, T, S, A	45.04	11.08	4.95	25.83	2.52	1.91	<0.10	7.70	<0.10	<0.10
B22	F/25	oral	O	–	22.46	2.49	5.07	8.36	2.60	<0.10	<0.10	2.01	<0.10	<0.10
B23	F/28	inhalation	O	B, T	16.37	51.98	4.48	6.82	<0.10	12.84	<0.10	42.36	<0.10	<0.10
B24	F/37	oral	F, U, A, O	–	13.07	<0.10	1.11	8.42	0.51	<0.10	<0.10	<0.10	<0.10	<0.10
B25	F/33	inhalation/oral	F, U, O	B, T, H, A	0.55	17.64	0.19	<0.10	<0.10	5.95	<0.10	12.17	<0.10	<0.10
B26	F/28	oral	F, U, A, O, V	A	0.45	0.10	<0.10	0.32	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
B27	F/34	oral ³	F, O	B, H, A	4.80	1.90	<0.10	<0.10	<0.10	0.12	0.11	1.56	<0.10	<0.10
B28	F/26	oral	F, O	B, T, H, A	2.92	>100	<0.10	<0.10	<0.10	31.09	1.29	47.47	<0.10	<0.10
B29	F/26	oral ³	F, E, O	B, T, H, A	1.87	>100	<0.10	<0.10	<0.10	42.59	0.12	>100	<0.10	<0.10
B30	M/27	oral ³	F, U, E, O	B, H, A	0.79	13.94	<0.10	<0.10	<0.10	2.99	<0.10	9.60	0.24	<0.10
B31	F/26	oral	F, O	B, T, H, S, A	0.77	3.28	<0.10	<0.10	<0.10	<0.10	2.54	2.20	0.85	<0.10
B32	F/23	oral	O	B, T, H, S, A	0.72	40.30	<0.10	<0.10	<0.10	5.42	<0.10	32.84	<0.10	0.14
B33	F/24	oral ³	U, A	B, T, H, S	0.22	18.71	<0.10	<0.10	<0.10	3.44	<0.10	10.45	<0.10	<0.10
B34	F/19	no exposure	–	B, T	16.76	44.84	3.37	7.74	0.14	2.90	<0.10	16.34	0.84	<0.10
B35	F/17	no exposure	–	B, T, H, A	9.90	>100	<0.10	<0.10	<0.10	64.65	0.36	>100	<0.10	0.30
B36	M/18	no exposure	–	B, T, A	4.69	97.30	<0.10	<0.10	<0.10	35.66	<0.10	>100	<0.10	<0.10
B37	M/25	no exposure	–	S	1.60	49.88	<0.10	<0.10	<0.10	11.35	<0.10	45.10	<0.10	<0.10
B38	F/61	no exposure	–	B, S	1.32	29.82	<0.10	<0.10	<0.10	10.99	<0.10	15.52	<0.10	<0.10
B39	F/29	no exposure	–	B, T, H, A	0.55	3.78	<0.10	<0.10	<0.10	1.59	1.24	1.84	<0.10	0.13
B40	M/20	no exposure	–	T	0.26	0.18	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.14	<0.10
B41	F/29	no exposure	–	B, T	0.21	65.50	<0.10	<0.10	<0.10	4.42	<0.10	48.56	<0.10	<0.10

F = Female; M = male.

¹ Peanut symptoms: AX = anaphylaxis; A = abdominal pain; D = dizziness; E = eczema; F = facial edema; O = oral allergy syndrome; R = respiratory distress; U = urticaria; V = vomiting.

² Other allergies: A = almond; B = birch pollen; H = hazelnut; S = soy; T = timothy pollen.

³ Deliberate peanut intake.

Fig. 1. Reported other allergies (a) and reported symptoms to peanut in exposed patients (b) in relation to sensitization to Ara h 1, 2 and 3. ■ = Positive for Ara h 1–3, sIgE levels to peanut >15 kU_A/l; ▨ = positive for Ara h 1–3, sIgE levels to peanut <15 kU_A/l; □ = negative for Ara h 1–3, all patients had sIgE levels to peanut <15 kU_A/l. * $p < 0.05$, ** $p < 0.01$.

Table 3. Prevalence of IgE reactivity to peanut, birch pollen and individual allergenic components in the two groups of patients included in the present study

Group	Peanut	Ara h 1	Ara h 2	Ara h 3	Ara h 8	Ara h 9	Birch	Bet v 1	Bet v 2	CCD
A1–A33, %	97.0	57.6	57.6	48.5	69.7	12.1	84.8	75.8	15.2	18.2
B1–B41, %	100.0	48.8	56.1	41.5	70.7	29.3	87.8	82.9	19.5	24.4
Total, %	98.6	52.7	56.8	44.6	70.3	21.6	86.5	79.7	18.9	21.6

No significant differences were observed using Fisher's exact test.

All of them had low levels of IgE to whole peanut extract (range: 0.17–2.16 kU_A/l). Three of them had IgE to CCD and one had IgE to profilin (rBet v 2). One subject was peanut negative (<0.10 kU_A/l) but had low IgE levels to rAra h 8 (0.63 kU_A/l). Concordantly, several subjects in the group negative for Ara h 1–3 had low IgE levels to peanut while they had high IgE levels to rAra h 8 (tables 1, 2).

There was no significant difference between the A1–A33 and B1–B41 patients included in the present study regarding their IgE levels to peanut (tables 1, 2) or the prevalence of IgE reactivity to individual allergenic components (table 3).

Reported Symptoms

Besides their peanut allergy, most patients also reported birch and timothy pollen allergy and other food allergies (tables 1, 2). It was more common with self-reported birch pollen allergy and apple allergy in the group negative for Ara h 1–3 than in the group positive for Ara h 1–3 (fig. 1a).

According to the questionnaires, 58 patients had had an adverse reaction after they had eaten peanut or peanut-containing foods accidentally or deliberately (13 cases), or had been exposed to peanut via skin contact or inhalation. Forty-one (71%) of them belonged to the group positive for Ara h 1–3. The time since the last

Fig. 2. Flow-chart of sensitization pattern and reported severe or mild symptoms.

known reaction to peanut varied from <1 to 24 years prior to the blood sampling (median: 5 years, 25% percentile: <1 year, 75% percentile: 12.5 years) and did not significantly differ between the group positive for Ara h 1–3 and the negative group (data not shown). The reported exposure dose varied from just a taste of peanut-containing food (spitting it out), inhalation of peanut dust or skin contact with peanut, to ingestion of larger amounts of peanut-containing food. However, it was impossible to recall the actual amount of peanut protein in each exposure.

Twenty-six cases (45%) of respiratory distress were reported (considered as severe symptom). Most of them belonged to the group positive for Ara h 1–3 (i.e. 56 vs. 18% of the patients in the group positive for Ara h 1–3 and the negative group, respectively, $p < 0.01$, fig. 1b). No association was observed between respiratory distress caused by peanut exposure and pollen-induced asthma (data not shown). Other common symptoms reported were facial edema (64%), OAS (62%) and urticaria (43%). No association was observed between sensitization to Ara h 1, Ara h 2 or Ara h 3 and these symptoms (considered as mild symptoms) following peanut exposure (fig. 1b). Twelve patients in the group positive for Ara h 1–3 but none in the negative group ($p < 0.05$) reported other symptoms which included 2 cases of anaphylaxis (fig. 1b). Also when studying the subgroup of patients with IgE levels to peanut below the 95% predictive clinical decision point of 15

kU_A/l, IgE to rAra h 1, 2 and/or 3 was statistically associated with severe symptoms (60 vs. 18% of the patients in the group positive for Ara h 1–3 and the negative group, respectively, $p < 0.05$, fig 1, 2).

Thirteen subjects (76%) of those 17 who reported symptoms after peanut exposure in the group negative for Ara h 1–3 were sensitized to Ara h 8. Eleven (85%) of them reported OAS after peanut exposure, 5 (38%) urticaria, 4 (31%) abdominal pain and 2 (15%) respiratory distress. OAS was the only symptom that tended to be found more frequently among the Ara h 8-sensitized subjects in the group negative for Ara h 1–3 compared with the subjects in the group positive for Ara h 1–3 although the difference did not reach the level of significance ($p = 0.057$). All these 13 Ara h 8-sensitized subjects also stated that they suffered from hazelnut allergy, an allergy that was associated with Ara h 8 (without Ara h 1–3) sensitization (75%, $n = 20$ versus 39%, $n = 54$, $p < 0.01$).

Of the 16 subjects sensitized to Ara h 9, 6 reported severe symptoms after peanut exposure. All of them belonged to the group positive for Ara h 1–3. Details about sensitizations and reported symptoms for individual patients are given in tables 1 and 2, and a summary is presented in figure 2.

There was no significant difference between the A1–A33 and B1–B41 patients included in the present study regarding any symptom parameter studied (data not shown).

Discussion

Allergen components have made it possible to study patients' IgE reactivity profiles on a molecular level. The major peanut allergens Ara h 1, Ara h 2 and Ara h 3 are potentially dangerous proteins as they are highly resistant to heating (roasting) and gastrointestinal digestion [19–23]. Similarly, nonspecific lipid transfer proteins (e.g. Ara h 9 in peanut) are known to be very stable proteins [24]. That is quite contrary to PR-10 allergens, such as Ara h 8 in peanut, which are sensitive to heat and proteolytic enzymes [12, 24]. Profilins (e.g. Ara h 5 in peanut) are relatively labile allergens as well [24].

In the present study, we show that 45–57% of mainly young adults with suspected peanut allergy had IgE antibodies to rAra h 1, rAra h 2 and rAra h 3. Most common was IgE to Ara h 2, to which 73–100% of allergic subjects usually are sensitized (depending on the study population and assay technique) [25–28]. The high prevalence of birch pollen sensitization in the patients living in western Sweden is consistent with the finding of a high prevalence of IgE reactivity to the birch pollen-related allergen Ara h 8 (70%). A minority was sensitized to Ara h 9, profilin and CCD. The relatively low prevalence of 22% for IgE reactivity to rAra h 9 is in line with a recently published study exhibiting a prevalence of 15% for Ara h 9 sensitization in peanut-allergic patients living outside the Mediterranean area [29]. Another peanut allergen not included in the present study is Ara h 6, a storage protein that shows substantial cross-reactivity with regard to IgE-binding capacity with Ara h 2 [23, 30]. Studies have demonstrated that Ara h 6 is a major peanut allergen, indicating that it might be an added value to also include Ara h 6 in the diagnosis of peanut allergy [28, 30, 31].

The patients with IgE to any of rAra h 1, 2 or 3 (Ara h 1–3-positive group) showed significantly higher IgE levels to peanut extract (i.e. peanut ImmunoCAP) than the patients in the group negative for Ara h 1–3, and the majority of them had peanut-specific IgE levels above the suggested 95% predictive clinical decision point of 15 kU_A/l for peanut allergy [18]. However, clinical decision points for specific IgE could be dependent on the geographical region and hence the sensitization pattern of the patient, and should not be used in a dichotomous way [32]. Furthermore, other clinical decision points for peanut allergy have been proposed [33, 34].

High IgE levels to rAra h 8 were not associated with high peanut test results. This discrepancy has been explained by the finding that Ara h 8 is present in only low concentrations in peanut extract [12]. As a consequence

this birch pollen-related allergen is present in suboptimal amounts in the peanut ImmunoCAP test and is not able to bind all Ara h 8-specific IgE antibodies.

Severe food reactions are difficult to predict. Interestingly, 23 (56%) of the 41 subjects in the present study belonging to the group positive for Ara h 1–3 reported severe reactions (i.e. respiratory distress) after ingestion of peanut or peanut-containing food, or after inhalation or skin exposure to peanut. Recent studies have shown that asthma-like respiratory distress is often involved in mortality due to food allergy [35]. Two cases of anaphylaxis with unconsciousness were also reported in the group positive for Ara h 1–3. In contrast, just 3 (18%) of the 17 subjects lacking detectable IgE to these allergens (group negative for Ara h 1–3) suffered from severe reactions when exposed to peanut. These results, together with newly published data, indicate that IgE antibodies to the major peanut allergens Ara h 1, Ara h 2 or Ara h 3 are associated with severe reactions after exposure to peanut in children and young adults [26]. Our study suggests that such association is valid regardless of high or low peanut extract-specific IgE levels (i.e. higher or lower than the clinical decision point of 15 kU_A/l).

Interestingly, we found no association between severe symptoms to peanut and IgE reactivity to rAra h 9 in this Swedish study population. This is similar to a recently published study by Krause et al. [36] in which Ara h 9 was reported to be an important allergen in Italian peanut-allergic patients with a prevalence of IgE reactivity of 45%. In that study, most Ara h 9-sensitized patients had a history of mild or only moderate to severe symptoms. An even higher prevalence of 90% of Ara h 9 sensitization was recently reported by Lauer et al. [29] in Spanish patients. However, in that study neither the patients' symptom history nor their IgE reactivity pattern to individual peanut allergens was reported. Nevertheless, particularly for patients living in Mediterranean countries, Ara h 9 should be included in the test profile [29, 36, 37].

A trend towards more reported symptoms of OAS to peanut was found in the small group of 13 patients with IgE reactivity to rAra h 8 and without concomitant Ara h 1, 2 or 3 sensitization. However, some of these patients also reported systemic symptoms such as urticaria and gastrointestinal symptoms, and 2 of them even reported severe symptoms. It should be noted that all 13 patients in this subgroup also reported allergy to hazelnut.

Half of the studied subjects reported reactions to peanut that had occurred within 5 years prior to blood sampling, but few patients had avoided all contact with peanut for 1 or 2 decades due to an allergic reaction in early

childhood. Naturally, this leads to some caution when interpreting the data. One weakness of the present study, based on questionnaires only for the documentation of clinical reactivity, is the causal role of peanut as the offending food. To minimize this dilemma, the questionnaires were reviewed by an investigator before database entry. However, this still does not eliminate the possibility that the offending food in some cases could in fact have been something else than peanut, for example traces of hazelnut added to some food. Furthermore, the association between the severity of reported symptoms and the actual exposure dose cannot be investigated in the present study even if the exposure route was documented. The issue of accuracy of the severity assessment is also a major limitation of the study. It could be questioned whether the patients' symptom descriptions are uniform and in agreement with a doctor's diagnosis. Some subjects could have exaggerated or understated their symptom descriptions when answering the questionnaire, especially when a long time has passed since the occasion. However, it is unlikely that the accuracy in the symptom description would differ between the group positive for Ara h 1–3 and the negative group since the time elapsed between the reaction to peanut and blood sampling did not significantly differ between the two groups. Furthermore, some of the patients might have developed tolerance to peanut and their IgE reactivity profile might have changed since the last reaction. However, data from Flinterman et al. [28] indicate that the IgE reactivity profile to peanut components might be rather stable over time.

Several studies have pointed to the usefulness of allergen components in the diagnosis of peanut allergy [26, 27,

31, 38]. Furthermore, short peanut peptides covering the most immunodominant linear IgE epitopes of Ara h 1, Ara h 2 and Ara h 3 might be an alternative to allergen components in in vitro testing [39, 40]. Interestingly, patients with more severe peanut allergy display a higher diversity in IgE reactivity as reflected by the number of epitopes recognized [39, 41]. Similarly, it has been shown that the risk of severe reactions to peanut increases with the number of peanut storage proteins recognized by the patient's IgE while in most cases IgE only to Ara h 8 is associated with mild symptoms [26, 27, 31]. Our study is in line with these findings. However, double-blind placebo-controlled food challenge remains the gold standard in the diagnosis of peanut allergy, and further work using physician-supervised oral food challenges is warranted to confirm the link between IgE to Ara h 1, 2, or 3 and the severity of reactions.

We conclude that IgE antibodies to rAra h 8 are connected with birch pollen sensitization and seem to be associated with a low risk of severe reactions to peanut in Swedish peanut-allergic patients (represented by mostly young adults with a suspected peanut allergy in our study). On the other hand, IgE to rAra h 1, rAra h 2 and rAra h 3 is associated with high IgE levels to peanut, and in the present study IgE reactivity to any of them was associated with a history of severe reactions to peanut.

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