The Relevance of Basophil Allergen Sensitivity Testing to Distinguish between Severe and Mild Peanut-Allergic Children

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Key Words

Basophil sensitivity · Peanut sensitization in children · Recombinant peanut allergens

Abstract

Background: Peanut sensitization is common in children. However, it is difficult to assess which children will react mildly and which severely. This study evaluated the relevance of basophil allergen sensitivity testing to distinguish the severity of peanut allergy in children. **Methods:** Twenty-seven peanut-sensitized children with symptoms varying from mild symptoms to severe anaphylaxis underwent peanut CD63 dose-response curve analysis with the inclusion of basophil allergen sensitivity calculation (CD-sens) and peanut component immunoglobulin E (IgE) testing. **Results:** Eleven children who had experienced anaphylaxis to peanuts showed a markedly higher peanut CD63 response at submaximal allergen concentrations and CD-sens (median 1,667 vs. 0.5; $p < 0.0001$) than 16 children who experienced a milder reaction. Furthermore, a negative or low CD-sens to peanuts unambiguously excluded anaphylactic peanut allergy. Children with anaphylaxis have higher levels of Ara h 1, 2, 3 and 9 IgE, but comparable levels of IgE to Ara h 8 and whole-peanut extract. The diagnostic specificity calculated with a receiver operating characteristic analysis reached

100% for CD-sens and 73% for Ara h 2. **Conclusions:** We demonstrated that severe peanut allergy is significantly associated with higher basophil allergen sensitivity. This cellular test should facilitate a more accurate diagnosis of peanut allergy.

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Introduction

Peanut allergy is one of the most common food allergies. A considerable number of children with positive specific immunoglobulin E (sIgE) and positive skin prick test (SPT) are asymptomatic or have a mild clinical picture [1–4]. This can lead to unnecessary avoidance of peanuts in food [5–7].

IgE analysis of peanut allergen components such as Ara h 1, 2, 3 and 9 can only partially designate patients truly allergic to peanuts [8–10]. Others mainly sensitized to the Bet v 1 analog Ara h 8 express milder or no symptoms and usually have a clinical allergy to pollen allergens [11–14]. A double-blind, placebo-controlled food challenge, which is regarded as the gold standard [14], is still required to confirm peanut allergy. Nevertheless, this challenge is difficult to perform in daily practice because it must be conducted in hospital settings, it is time

consuming and it is associated with the risk of anaphylaxis.

Activation of allergic inflammatory effector cells (mast cells and basophils) is the key event during the development of allergic symptoms. Thus, the basophil activation test (BAT) may offer a clinical answer to an allergen [15] and a complement to the severity of the clinical picture. There are few studies that perform BATs in peanut food allergy [16, 17].

For this reason, we included various peanut-sensitized children, evaluated their basophil allergen sensitivity to peanut allergen and then related this cellular sensitivity to the severity of clinical reaction as well as to the sIgE reactivity to peanut allergen components.

Material and Methods

Study Population

All children were recruited from the Department of Pediatrics of the University Medical Center in Maribor, northeastern Slovenia. The inclusion criteria were children with unequivocal ingestion of peanut and development of clinical symptoms of anaphylaxis defined according to the international criteria reported by Sampson et al. [18], oral allergic syndrome (OAS), eczema worsening, abdominal cramps or other symptoms related to peanut allergy (skin, respiratory, cardiovascular or gastrointestinal symptoms). The symptoms were described by the physician experienced with allergic reactions in children and confirmed in their medical records. A convincing clinical reaction was any of the described symptoms presented within 1 h of peanut ingestion. Additionally, we studied those children with positive sIgE antibodies (sIgE-ab) to peanuts and/or a positive peanut SPT. A total of 27 children were included (median age 9 years, 20 boys), 11 with a history of anaphylaxis [18] and 16 with milder clinical symptoms when they consumed peanuts (e.g., isolated eczema worsening with itching, redness, OAS or abdominal cramps). We also collected data on pollen allergies, early sensitization to foods and atopy in relatives.

The study protocol was approved by the Slovenian National Medical Ethics Committee (KME160/07/10), and all parents gave their informed consent.

Specific IgE Measurements and SPT

SPT is considered valid when the mean wheal diameter is ≥ 3 mm. The levels of sIgE-ab to Ara h 1, 2, 3, 8 and 9 as well as to Bet v 1, Phl p 7 and Phl p 1–5 were quantified using the CAP FEIA system (Phadia, Uppsala, Sweden). A positive test was defined as an IgE-ab level >0.35 kU/l.

Basophil Activation Tests

BATs were performed as described previously [19, 20]. Briefly, whole-blood samples were incubated with various concentrations of an extract PBS of crude peanuts (CP; final concentrations from 3,000 to 0.003 ng/ml) or commercially available peanut allergen (commercial extract, CE; Bühlmann Laboratories, Schönenbuch, Switzerland; final concentrations from 30 to 0.3 ng/ml) and posi-

tive controls, 0.55 μ g/ml of anti-Fc ϵ RI mAb (Bühlmann Laboratories) and 2 μ M fMLP (Sigma-Aldrich, St. Louis, Mo., USA) at 37°C for 15 min.

The basophil allergen-specific sensitivity was evaluated by comparing basophil CD63 responses at submaximal allergen concentrations [21] and by CD-sens analyses [21, 22].

Statistical Analysis

The results are presented as medians and interquartile ranges. Data distribution was calculated using the Shapiro-Wilk test. Depending on the distribution of the data, we used either ANOVA or the Mann-Whitney test. Fisher's exact test was used to compare the categorical data between groups. Correlation coefficients were calculated using Pearson's correlation coefficient. A receiver operating characteristic (ROC) analysis was performed with test results and presented graphically and as area under the curve (AUC) with a 95% confidential interval. Significance was considered as $p < 0.05$. Statistical analysis was carried out using GraphPad Prism 5.

Results

Demographic and Clinical Data

Anaphylaxis and non-anaphylaxis children were comparable according to age, sex, early sensitization to food, pollen allergy, peanut SPT positivity, atopy in relatives, and abdominal cramps, OAS and atopic dermatitis (table 1). Children in the non-anaphylactic group expressed only minor symptoms, they were monosymptomatic or had mostly skin problems (worsening of atopic dermatitis). However, the anaphylaxis group had more oral symptoms after peanut ingestion ($p = 0.033$) and less eczema worsening ($p = 0.008$). The observed children with anaphylaxis had no abdominal cramps, comparable to the non-anaphylactic group (table 2).

CD63 Basophil Response to Peanut Allergens

The CD63 basophil response was performed with CP and CE for the anaphylaxis and non-anaphylaxis groups (table 3; fig. 1, 2). With CP stimulation, the CD63 basophil response was significantly higher for anaphylactic patients (fig. 2a) at concentrations of 0.3 ($p < 0.0001$), 30 ($p < 0.0001$) and 3,000 ng/ml ($p = 0.03$), and the same was true for CE at concentrations of 3 ($p = 0.001$) and 30 ng/ml ($p < 0.0001$) compared to non-anaphylactic patients (fig. 2b). There were no statistical differences in the lowest concentrations of CP and CE ($p = \text{NS}$). The basophil responses to positive control anti-Fc ϵ RI and fMLP were highly comparable between the anaphylaxis [Fc ϵ RI median 94.5, range (Q1–Q3) 88.7–98.6%; fMLP median 34.9, range 28–66.2%] and the non-anaphylaxis group (Fc ϵ RI median 92.1, range 67.5–97.6%; fMLP median 34, range 20.6–49.3%).

Table 1. Clinical characteristics of the patients

Patient No.	Age, years	Time from the last symptom onset, months	Anaphylaxis	Adrenalin treatment, n	Atopic dermatitis worsening	Abdominal cramps	OAS	Admitted to hospital, n	Peanut diet
1	11	8	yes	3	no	no	no	3	yes
2	16	6	yes	2	no	no	yes	2	yes
3	11	34	yes	6	no	no	yes	3	yes
4	3	2	yes	4	no	no	yes	4	yes
5	5	37	yes	2	no	no	no	2	yes
6	1	2	yes	1	no	no	yes	1	yes
7	6	4	yes	1	no	no	yes	1	yes
8	7	56	yes	1	no	no	no	1	yes
9	14	120	yes	1	no	no	yes	1	yes
10	1	2	yes	1	yes	no	no	1	yes
11	1	2	yes	1	no	no	no	1	yes
12	2	13	no	no	yes	no	no	1	yes
13	2	3	no	no	yes	no	no	1	yes
14	1	4	no	no	yes	no	no	1	no
15	1	4	no	no	yes	no	no	1	yes
16	3	6	no	no	yes	no	yes	2	yes
17	10	3	no	no	yes	no	yes	1	no
18	4	8	no	no	no	yes	no	2	yes
19	12	5	no	no	no	yes	no	3	yes
20	14	9	no	no	no	yes	no	1	no
21	15	14	no	no	no	yes	no	3	yes
22	11	6	no	no	yes	no	no	1	no
23	18	7	no	no	no	yes	no	0	yes
24	13	5	no	no	yes	no	no	0	yes
25	13	2	no	no	no	yes	no	2	yes
26	12	3	no	no	yes	no	no	0	no
27	10	7	no	no	yes	no	no	2	no

CD-Sens Results

In the anaphylaxis group, CD-sens was markedly higher, with a median of 1,667 [range (Q1–Q3) 1,000–333,333] with CP stimulation, and with CE stimulation, there was a median of 10 (range 6.2–21.7). With CP stimulation, the non-anaphylaxis group showed a median of 0.5 (range 0.2–0.88), and with CE stimulation, the median was 0 (range 0–2.4; $p < 0.0001$ for CP and CE). CD-sens in non-anaphylactic children was 3,000 times (for CP) and 10 times (for CE) lower than in anaphylactic children. Interestingly, there were no Ara h 2 negative children in the anaphylaxis group; all belonged to the non-anaphylaxis group and had a lower CD-sens than Ara h 2 positive children (CD-sens for CP: Ara h 2 positive, median 1,333, range 52.8–25,625, vs. Ara h 2 negative, median 0.25, range 0.25–0.5; $p < 0.0001$) (fig. 3).

We also analyzed the correlation between CD-sens results and Ara h 2 levels. With CP stimulation, there was

no correlation between Ara h 2 and CD-sens ($r = 0.3$, $p = 0.2$), but we confirmed a positive correlation for stimulation with CE ($r = 0.7$, $p = 0.0026$). There was also a significant correlation between CD-sens for stimulation with CP and CE ($r = 0.54$, $p = 0.02$).

Finally, we performed ROC analysis for CD-sens (fig. 4). For CP stimulation, the AUC was 0.96 and the p value < 0.0001 . The optimal recalculated threshold was > 27.8 , with a sensitivity of 88% and a specificity of 100%. For CE stimulation, the AUC curve was 0.98 and the p value < 0.0001 . The optimal recalculated threshold was > 5.3 , with a sensitivity of 94% and a specificity of 91%.

Peanut Allergen Components

In component analysis, all children with anaphylaxis showed positive sIgE reactivity to Ara h 2 and were more often positive to Ara h 1 and 3. They also had significantly higher sIgE-ab levels to those components ($p < 0.05$).

Table 2. Characteristics of the patients studied

	Anaphylaxis		P
	positive (n = 11)	negative (n = 16)	
Age, years	6.2 [5.1–11.8]	10.7 [2.95–13.75]	NS
Males	7 (64)	13 (81)	NS
Patients with pollen allergy	5 (45)	11 (68.5)	NS
Early sensitization to food	6 (55)	9 (56)	NS
Eczema	1 (9)	10 (62.5)	0.008
Abdominal cramps	0 (0)	6 (37.5)	NS
Oral symptoms after peanut	6 (50)	2 (12.5)	0.033
sIgE to peanut, kU/l	67 [1.8–100]	6.9 [4.2–8.8]	NS
Peanut prick positive	8 (89)	5 (62.5)	NS
Atopy in relatives	10 (83)	9 (56)	NS
rAra h 1, kU/l			
Median	5.82 [0.0–37.3]	0.0 [0.0–0.0]	0.004
Positive results	6 (55)	2 (12.5)	0.011
rAra h 2, kU/l			
Median	57.9 [2.1–100]	0.0 [0.0–2.9]	0.0003
Positive results	11 (100)	5 (31.3)	0.0015
rAra h 3, kU/l			
Median	1.2 [0.0–24.1]	0.0 [0.0–0.0]	0.0013
Positive results	7 (58)	1 (6.25)	0.0025
rAra h 8, kU/l			
Median	0.0 [0–4]	0.0 [0.0–4.6]	NS
Positive results	4 (33.3)	7 (43.75)	NS
rAra h 9, kU/l			
Median	0.0 [0.0–0.4]	0.0 [0.0–0.0]	NS
Positive results	3 (25)	0 (0)	0.0267
rPhl p 7, kU/l			
Median	0	0	NS
Positive results	0 (0)	0 (0)	NS
rPhl p 1–5, kU/l			
Median	67.2 [22.2–95.7]	100 [62.5–100]	NS
Positive results	4 (33.3)	11 (68.75)	NS
rBet v 1, kU/l			
Median	0.6 [0.0–18.3]	2.0 [0.0–32.3]	NS
Positive results	5 (41.7)	8 (50)	NS
Values are given as n (%) or medians [Q1–Q3].			

Only children with anaphylaxis also had positive responses to Ara h 9. There were no differences in Ara h 8, Phl p 1–5, Phl p 7 (all negative) and Bet v 1 levels ($p = \text{NS}$; table 2).

In the Ara h 2 ROC analysis, the AUC was 0.9 and the p value 0.0004. An optimal recalculated threshold was

>5.3 kU/l, with a sensitivity of 94% and a specificity of only 73%. For Ara h 1 and 3 testing, the AUC was 0.74 and 0.8 ($p \leq 0.3$), and the recalculated threshold was >3.6 and >1 kU/l, with a sensitivity of 100% but a specificity of only 55%, respectively (fig. 4). ROC analysis for peanut sIgE showed an AUC of 0.68 ($p = \text{NS}$), and the recalculated threshold was >15 kU/l, with a sensitivity of 81% and a specificity of only 64%.

Discussion

This study investigated the relation between clinical allergy and basophil sensitivity to peanuts. We showed that increased basophil allergen sensitivity significantly correlates with the severity of the clinical reaction and that very low or negative basophil sensitivity completely excludes clinically relevant peanut allergy. Furthermore, we also demonstrated that BAT has higher diagnostic specificity with comparable sensitivity than IgE testing for major peanut allergen components.

In daily clinical practice, the diagnosis of peanut allergy is generally based on the case history and the presence of peanut-specific IgE or a positive SPT. Peanut sIgE or the SPT is a proof of sensitization to peanuts but does not equate with clinical allergy. Previous studies related clinical symptoms and the higher level of sIgE/SPT to peanuts with peanut allergy, but this prediction performs suboptimally [23, 24]. Therefore, sIgE to peanut components Ara h 1, 2, 3, 8 and 9 were recently introduced in clinical practice, with Ara h 2 as the clinically most important predictor [4, 8–11, 25]. Nevertheless, a recent update of a diagnostic prediction model for peanut allergy showed that even after the addition of peanut components, up to 50% of patients still require a food challenge [26].

Studies that evaluate the clinical relevance of BAT in food allergies have been described in recent years [15, 16, 27]. Therefore, we investigated whether BAT could increase our diagnostic accuracy by improving the discrimination between mild or irrelevant and severe peanut allergy. In our study, BAT was used to analyze the cellular sensitivity to peanuts by comparing the responses between different submaximal allergen concentrations [17, 19] and by calculating CD-sens [15, 28]. In this study, we used two allergen preparations: CP (raw peanuts bought at a store) and CE. Overall, these two allergens gave us comparable clinical and laboratory results. However, there was a high variation in CD-sens for the anaphylaxis group between both extracts used

Fig. 1. Flow cytometry dot plot analysis of basophil activation (CD123+ and HLA-DR-) according to the surface expression of CD63 in selected patient No. 11 after stimulation with CP (concentration 0.3 ng/ml) and CE (concentration 3 ng/ml).

Table 3. Response in BAT on stimulation with CP and CE

	CP concentration, ng/ml				CE concentration, ng/ml		
	0.003	0.3	30	3,000	0.3	3	30
Anaphylaxis group	8.87 (5.6–3.3) ¹	68.9 (61.1–86.7)***	85.6 (78.7–92.5)***	91.1 (87–96)*	1.4 (0.7–5.6) ¹	22.3 (3.9–38.8)**	82.4 (65.1–91)***
Non-anaphylaxis group	6.025 (2.1–8.2)	6.89 (3.5–13.3)	17.76 (8–24.8)	83.56 (53.7–96.4)	2.7 (2.1–3.3)	2.95 (1.8–4.2)	4.7 (2.5–13.8)

Data are median CD63 responses given as percentages, with ranges (Q1–Q3) in parentheses. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$.

¹ Not significant.

(median up to 160 fold). This variation could be explained by the differences in allergenic components in each extract. Namely, the correlation studies between CD-sens and the level of Ara h 2 IgE reactivity suggest that the commercial extract is probably based on a higher abundance of the Ara h 2 component than the CP extract. In the future, it will be important to better standardize the peanut extract and also to evaluate the

threshold level for clinically relevant positivity of peanut BAT.

The major finding of this study was that the basophil sensitivity (i.e., CD-sens) to peanuts was markedly higher in anaphylactic than in non-anaphylactic children and that a negative or very low CD-sens unambiguously excludes severe peanut allergy. This observation is in some accordance with the study by Thyagarajan et al. [27]

Fig. 2. CD63 basophil response curves after stimulation with CP (concentration 0.003–3,000 ng/ml) and CE (concentration 0.3–30 ng/ml) in the anaphylaxis (a) and non-anaphylaxis groups (b).

Fig. 3. CD-sens after stimulation with CP (a) and CE (b). Open squares represent patients who were negative for Ara h 2. Horizontal lines represent the median with interquartile range.

where suppression of basophil CD63 response after peanut oral immunotherapy was demonstrated. In the non-anaphylactic group, there are patients who do not adhere to a strict peanut-free diet which could lead to possible desensitization and significantly lower CD63 response. When we separately analyzed patients maintaining a peanut-free diet in the non-anaphylactic group, no change in this observation was found.

Furthermore, when we investigated the diagnostic value of CD-sens, we showed a larger AUC for CD-sens than for Ara h 2 IgE testing. Consequently, the diagnostic specificity of CD-sens was 18 (for CE) to 27% (for CP) higher and it reached 100%, comparable to the specificity of Ara h 2 testing (73%). Glaumann et al. [17] also demonstrated that a negative CD-sens to peanuts excluded peanut allergy and further suggested that the evaluation of basophil sensitivity could be a safe and time-saving complement to provocation testing. It seems that

Fig. 4. ROC curve analysis of the sensitivity and specificity of CD-sens after stimulation with CP and CE and Ara h 2 and sIgE to peanut testing.

CD-sens demonstrates better testing discrimination ability than peanut component testing, and thus, the inclusion of this test in diagnostic prediction may further reduce the need for peanut challenges. In addition, recalculated threshold levels for Ara h 2 were higher than in studies to date because they were calculated only in patients with severe symptoms (such as anaphylaxis) [29]. Thresholds for Ara h 1 and 3 have the same value and could help in deciding whether to perform or abandon provocation tests.

The clinical and immunological pattern of IgE sensitization to peanut components was typical for central European countries [11]. Thus, IgE antibodies to Ara h 2, and to a lesser extent to Ara h 1 and 3, were detected in 59% of children. About one third of Ara h 1–3 positive children did not show systemic reactions. The sensitization rate to the Bet v 1 homolog was evident in 41% of

children and also in one third of anaphylactic children. Isolated Ara h 8 sensitization was only associated with mild symptoms. Interestingly, 3 anaphylactic children were also sensitized to the lipid transfer protein Ara h 9, which is typical for the Mediterranean allergic population [12]. Overall, these data confirmed the allergenicity and importance of Ara h 2 sensitization in the region around Slovenia. In addition, we excluded the importance of sIgE peanut testing.

In conclusion, we have shown that the evaluation of peanut basophil sensitivity might become an important diagnostic tool for discriminating patients with positive sIgE to peanuts, SPT or even sIgE to Ara h 1–3. However, they may have only mild or irrelevant clinical reactions. Future validation studies are needed to reproduce the importance of BAT and CD-sens in peanut allergy.

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