

Original article

Serological characteristics of peanut allergy in children

Background: Food challenge is considered an excellent clinical tool for the diagnosis of specific food allergy. However in the case of peanut allergy it may be difficult to perform because of the severity of the reactions. The quantitation of a specific immunoglobulin E (IgE) response to different peanut allergens could also contribute to the improvement of the diagnosis. We characterized the IgE response to a whole peanut protein extract and to Ara h 1 and Ara h 2 in different groups of patients classified according to the severity of their allergic reactions.

Methods: Specific serum IgE were analyzed in 96 children by enzyme-linked immunosorbent assay tests using a whole protein extract or purified peanut proteins and anti-human IgE monoclonal antibodies labeled with acetylcholinesterase.

Results: A parallel was observed between levels of peanut-specific IgE and the classification in five groups and subgroups of patients upon increasing severity of symptoms, especially within the group of highest severity. Moreover, the highest frequency of positive response and the highest levels of specific IgE were observed with whole peanut protein extract.

Conclusion: In a retrospective evaluation of peanut allergy in children, we have shown that quantitation of peanut-specific IgE could be used to avoid a food challenge particularly in the case of severe reactions. When compared to Ara h 1 and Ara h 2, whole peanut protein extract appeared to be the most appropriate allergen to perform the test.

**H. Bernard¹, E. Paty², L. Mondoulet¹,
A. W. Burks³, G. A. Bannon⁴,
J. M. Wal¹, P. Scheinmann²**

¹INRA-Laboratoire d'Immuno-Allergie Alimentaire, CEA-Saclay, Gif sur Yvette, France; ²Service de Pneumologie et d'Allergologie Pédiatriques, Groupe Hospitalier Necker, Paris, France; ³Department of Pediatrics, Arkansas Children's Hospital Research Institute, Little Rock, AR, USA; ⁴Product Characterization Center, Monsanto, St Louis, MO, USA

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Hervé Bernard
INRA-Laboratoire d'Immuno-Allergie Alimentaire
Bâtiment 136
Saclay, 91191 Gif Sur Yvette
France

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Peanut allergy affects ca. 0.6–1% of the US and EU population and it accounts for the majority of fatal and near-fatal food-induced anaphylactic reactions (1–3). Peanut allergy begins early in life, affecting young children. It has been assumed to be rarely outgrown (4, 5) although Skolnick et al. described that it may be outgrown in 21.5% of patients (6).

Considering the severity, prevalence and lifelong persistence of peanut allergy, in addition to the lack of preventive or curative therapy, the identification and characterization of peanut allergens could contribute to the improvement of diagnostic tests and treatment for peanut allergy. Two major allergenic peanut proteins have been identified: Ara h 1 and Ara h 2, which are recognized by 70–90% of patients with peanut allergy (7, 8). Ara h 3, a glycinin protein is recognized by serum immunoglobulin E (IgE) from only about 45% of those patients with a convincing history of peanut sensitivity (9). In addition, there are numerous other peanut allergens that have been

identified. In general, these allergens appear to be isoforms of either Ara h 1, Ara h 2, or Ara h 3. However, Ara h 5 has been identified as having significant homology with profilin and is recognized by serum IgE from about 10% of the peanut sensitive population (10).

Allergic patients generally present specific serum IgE for more than one component of the peanut. The relative contribution of different proteins to the potency of peanut in provoking severe symptoms is not understood. Any correlation of allergen-specific IgE with severity of clinical reactions may help to identify those peanut proteins which are most important for the disease process. With this aim in mind, we have analyzed the peanut-specific IgE binding profiles of 96 peanut allergic children in different groups defined by the severity of the manifestations observed.

Material and methods

Study population

A retrospective study was conducted on 96 children who were recruited from the pediatric allergy clinic at the Hôpital Necker-Enfants Malades Paris, France from 1996 to 1999. Eighty-five

Abbreviations: ELISA, enzyme-linked immunosorbent assay; EAST, enzyme allergosorbent test; OFC, oral food challenge; SPT, skin-prick test; PPV, positive predictive value; NPV, negative predictive value.

percent came from atopic families and more than 90% of the children revealed a history of atopic dermatitis while approximately 65% had asthma. The cohort included 64 boys and 32 girls. The patients have experienced an adverse reaction to peanut within the last 12 months. After an extensive history, physical examination and skin-prick testing to suspected foods, two groups were defined based essentially on the objective manifestations that were observed at the last peanut reaction:

Group A: 58 children had a strongly suggestive clinical history of peanut allergy with symptoms occurring in the 30 min after one single ingestion of peanut and positive skin-prick tests (SPTs) to peanut.

Group B: 38 children had an unconvincing clinical history of peanut allergy with symptoms occurring after the consumption of a meal with multiple foods (suggesting possible peanut allergy among polysensitizations to other foods), and with positive SPTs to peanut but also to eggs and/or milk. Symptoms were moderate or mild, no severe reaction was observed.

In addition 50 children formed the control group. Eight were atopic asthmatic children with positive SPT to dust mites and 42 were non-atopic children. All of them had negative SPT to peanut and no history of adverse reactions despite regular ingestion of peanuts.

Children whose parents refused the oral food challenge (OFC) or who presented very severe symptoms (i.e. anaphylactic shock or angioedema with cyanosis) were excluded from the study.

Based on clinical observations, group A was further divided into mild, moderate and severe allergy subgroups using the classifications described by Furlong (11) which was the one currently used at the Hospital at the time of the study.

Children in A1 subgroup ($n = 16$) had mild symptoms: significant skin or gastrointestinal symptoms either alone or in combination with mild symptoms in other systems or any combination of mild symptoms.

Children in A2 subgroup ($n = 16$) had moderate symptoms: wheezing alone or significant involvement of two organs systems.

Children in A3 subgroup ($n = 26$) had severe symptoms: wheezing with evidence of hives or diarrhea/vomiting, or involvement of three organ systems.

Symptoms considered mild included pruritus, conjunctivitis, abdominal pain, nasal congestion, itchy throat and sneezing.

Skin symptoms were the most frequent and included hives, oedema, pruritus and conjunctivitis. Respiratory symptoms were throat tightness, dyspnea, wheezing, chest tightness, coughing, and nasal congestion. Gastrointestinal symptoms were mouth/tongue pruritus, nausea, abdominal pain, vomiting.

Based essentially on the history of patients, group B was further divided into two subgroups, i.e. subgroup B1 ($n = 18$) and B2 ($n = 20$). A cumulative body of evidence permitted to suspect a likely allergy to peanut in children of subgroup B1 while reported symptoms did not bring any strong presumption of such a peanut allergy in children of subgroup B2.

As shown in Table 1, the majority of the studied population was young (mean age: 5.5 years; median: 4.5 years; range: 1.5–16 years). The distribution of ages was not the same in the different groups and subgroups. Medians were 3.6, 5.6, 6.4 years in subgroups A1, A2, A3 respectively; 4.2 and 3.2 years in subgroups B1 and B2 respectively showing that severity of symptoms was generally associated with a greater age of children.

Skin-prick tests

Skin-prick tests were performed on the volar face of the forearm using commercial peanut extracts (Stallergenes, France), a positive

Table 1. Distribution of ages in the different subgroups of patients

Group	Age (years)					
	<2	2 ≤ <4	4 ≤ <6	6 ≤ <8	8 ≤ <10	10 ≤
A1	0	9	3	1	0	3
A2	0	4	7	4	1	0
A3	1	7	2	4	5	7
B1	1	8	4	3	1	1
B2	1	13	3	0	1	2
Total	3	41	19	12	8	13

control (histamine, 10 mg/ml) and a negative control (50% glycerosaline solution). A SPT response was scored as positive if the wheal diameter was ≥ 3 mm and at least 50% greater than that produced by the positive control (12). Dermographic reactions were excluded by testing with a negative control solution.

Blood samples

Venous blood (5 ml) was collected from each patient and allowed to clot at room temperature for 1 h, then was centrifuged and the serum was aliquoted and stored at -20°C .

Food challenge

Except five highly allergic children who had experienced most severe reaction to peanut and who were put in subgroup A3, all the children underwent the open OFC. All parents signed a consent form indicating their agreement that their child should undergo the test. Children were hospitalized and staid under intensive medical observation during the challenge and during 4 h following the challenge. Each child had an intravenous catheter inserted just before the test. The same doctor performed all the challenges and the reactions were scored as to type time of onset and duration. The initial dose of peanut hidden in apple compote was 5 mg. Thereafter, increasing doses of peanut were given every 30 min (10 mg, 50 mg, 100 mg, 200 mg, 400 mg, 800 mg, 1600 mg, 3 g, 6 g) until a dose of 12 g was reached. Children then staid under observation during 4 h. The food challenge was considered positive as soon as one of the following symptoms was noted in the different organs: rhinitis, sneezing, cough, breathing difficulty, wheezing, erythema, pruritus, labial, facial or generalized urticaria, diarrhea, vomiting or abdominal pain.

In vitro studies

Specific IgE were determined by an original enzyme allergosorbent test (EAST) using immobilized whole peanut protein extract or purified Ara h 1 or Ara h 2 for capture and anti human IgE monoclonal antibodies (clone BS17) labelled with Acetylcholinesterase (AChE) as a tracer.

Reagents and materials

Unless otherwise stated, all reagents were of analytical grade and from Sigma (St Louis, MO). Buffers and reagents as well as anti-human IgE monoclonal antibodies (mAb BS17) Acetyl cholinesterase conjugate were prepared as previously described for milk specific IgE determination (13, 14).

Solid-phase enzyme immunoassays were performed in 96-well microtiter plates from Nunc (Roskilde, Denmark) using automatic Titertek microtitration equipment (washer, dispenser, and reader) from Labsystem (Helsinki, Finland).

Allergen preparation

Whole peanut extract. Proteins from commercial Virginian roasted peanuts were extracted as described by Moutete et al. (15).

Purified Ara h 1 and Ara h 2. Ara h 1 protein was purified from crude peanut extracts as previously described (16). Ara h 2 was prepared by precipitation using ammonium sulphate and then purified using anion exchange chromatography and hydrophobic interaction liquid chromatography and characterized by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

Enzyme immunoassays

Microtiter plates were coated by passive adsorption with whole peanut protein extract or with purified peanut proteins at a concentration of 5 µg/ml in 25 mM ethylenediaminetetraacetic acid pH 9.3. After 24-h incubation, the plates were washed and human serum albumin was used as saturating agent to avoid nonspecific binding. Plates were washed just before use.

Fifty micro liters of individual serum at serial dilutions were dispensed per well. After 24-h incubation at room temperature and extensive washing, 50 µl of a solution of the BS17-AChE conjugate (3 Ellman units/ml) were added per well. Following an overnight incubation, 200 µl of Ellman's reagent used as enzyme substrate were dispensed into each well and absorbance was measured at 414 nm.

Sera from nonallergic patients or allergic patients to dust mites were used as negative controls and allowed to determine the non-specific binding. Moreover sera from peanut allergic patients were also tested on micro plates coated with bovine serum albumin as complementary negative controls.

Quantification of specific IgE was made by comparison with dose-response curves obtained with a total IgE assay performed with known standards of human IgE. The corresponding immunometric assays of total and specific IgE were performed under identical conditions, an anti-human IgE mAb (clone LE27), complementary to the BS17-AChE tracer, being used as capture antibody for the total IgE assay, instead of the immobilized allergen used for specific IgE determination (13, 14).

Statistical analysis

As a result of the non-Gaussian distribution of eliciting threshold doses in the open food challenge and of specific IgE levels, medians and ranges were calculated and analysis were performed using nonparametric statistical methods (Wilcoxon signed rank test, Mann-Whitney rank sum test and Spearman rank correlation test).

Results

Open OFC

During the food challenge, cutaneous, respiratory and/or gastrointestinal symptoms were noted. The first objective reactions were often abdominal pain associated with conjunctivitis or sneezing or urticaria. The challenge was

then stopped and corticosteroids and anti-histamine were given when necessary.

In Group A, all the tested children had a positive open OFC. A SPT to peanut was performed and blood was collected just before the food challenge. The size of the wheals were ≥ 6 mm. Specific peanut IgE determinations in the collected serum samples were performed later on and results are presented below.

In subgroup A1: most children presented cutaneous manifestations, then abdominal pain or respiratory symptoms. One child showed anaphylactic reaction with abdominal pain, vomiting, wheezing, generalized urticaria after eating 800 mg peanut which required the administration of epinephrine in two separate injections. The mean threshold that elicited clinical symptoms for all the patients tested was 2590 mg (min: 25 mg; max: 12 000 mg). Three children had undetectable anti peanut IgE. However their clinical manifestations and SPT results were not different of those of the other children of the group.

In subgroup A2: wheezing and dyspnea were observed and administration of epinephrine was required in two cases. Clinical reactions were observed at a lower threshold of peanut ingestion than in group A1 (mean: 1120 mg; min: 100; max: 8000 mg).

In subgroup A3: clinical reactions during the open OFC were observed at low doses of peanut (mean threshold: 380 mg, min: 75; max: 1600 mg). Children presented gastro-intestinal reactions, cutaneous reactions or wheezing and dyspnea. Administration of epinephrine was required in two cases.

In group B the open OFC was positive in 18 of 38 (47%) patients which confirmed the diagnosis of peanut allergy. In subgroup B1 ($n = 18$), the reactions were mild or moderate (mean threshold: 2600 mg with a minimum at 5 mg and a maximum at 12 000 mg). In subgroup B2 ($n = 20$), no reaction was observed during the challenge and the patients could eat peanuts without any reaction which ruled out the presumption of possible peanut allergy.

Statistical analysis showed significant differences (at least $P < 5\%$) in open OFC threshold doses between group A3 (Median = 400 mg) and the other groups with positive open OFC. In spite of difference in threshold doses distribution (Table 2), no significant statistical difference between groups A1, A2 and B1 was observed (Median = 1600, 800 and 1300 mg respectively).

IgE ELISA validation

No significant signal was observed with any of the negative control groups. The detection limit of the specific IgE, calculated as the dose corresponding to the average nonspecific binding (e.g. background) plus $3 \times$ standard deviations, was 0.1 kU/l. The IgE were determined in the range of 0.1–10 kU/l with a good linearity of the standard curve as shown in Fig. 1. Inter- and intra-assay coefficients of variation were always $< 5\%$.

Table 2. Distribution of the threshold doses eliciting reactions in the different subgroups of patients during open oral food challenge (OFC)

Group	Threshold (mg)				
	<200	200 ≤ <800	800 ≤ <3000	3000 ≤ <12 000	12 000 ≤
A1	1	4	4	6	1
A2	1	7	7	1	0
A3*	6	12	3	0	0
B1	3	2	7	4	2
Total	11	25	21	11	3

* Five highly allergic children didn't undergo open OFC.

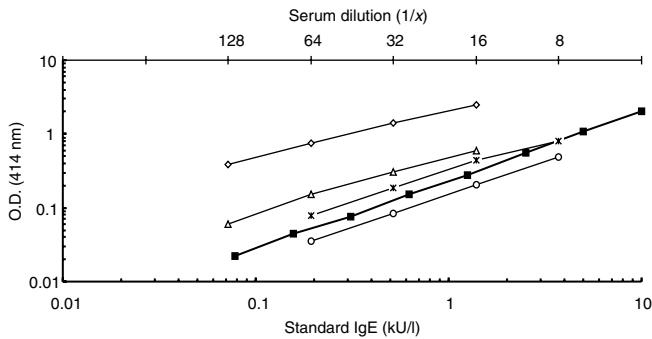


Figure 1. Standard curves obtained with total immunoglobulin E (IgE) and with dilutions of allergic sera to peanut (e.g. specific peanut IgE). The parallelism between the curves allow the quantification of specific anti whole peanut protein IgE. ■, Standard human IgE; △, patient no. 110; ○, patient no. 62; ✕, patient no. 65; ◇, patient no. 101.

Standard curves plotted in EAST of both total and specific IgE, appeared to be parallel (Fig. 1) which allowed a reliable quantitative determination of anti-peanut protein specific IgE. No change in the determinations of peanut-specific IgE was observed whether the determination was directly performed on sera of allergic patients as described or on the sera after they had been IgG-depleted by immunopurification, indicating that specific anti peanut IgG did not interfere with IgE determination (data not shown).

Moreover the peanut-specific IgE binding could be inhibited by addition of increasing amounts of the corresponding allergens in solution (data not shown), further demonstrating the specificity of the assay and the absence of major conformational changes induced by the coating of the allergen.

Specific IgE determination

In the total population of 96 allergic children 85 sera demonstrated a positive IgE response to whole peanut protein extract. Among the 11 children who had undetectable peanut specific IgE, three were in subgroup A1, one in subgroup A2, one in subgroup B1 and six in

subgroup B2. No significant IgE response to Ara h 1 or Ara h 2 was detected in the 11 sera negative to whole peanut protein extract. Among the 85 sera with specific IgE to the whole peanut protein extract, 14 recognized only the whole extract but not the purified Ara h 1 and Ara h 2 while 71 sera had IgE to one or to the two purified allergens. Among these 71 sera, five had IgE to Ara h 1 but not to Ara h 2. Inversely, 23 patients had IgE against Ara h 2 but not against Ara h 1. Significant IgE response to both Ara h 1 and Ara h 2 was observed in 43 cases. Within the whole population of peanut allergic patients, 66 and 48 patient sera showed an IgE response to Ara h 2 and Ara h 1, respectively (i.e. 77 and 56%).

Predictive values were estimated using criteria described by Sampson (17). The IgE responses measured on the whole population of 96 patients were taken into account and calculations were made for different concentrations of specific IgE, i.e. 0.1, 0.35, 1, 5 and 15 kU/l. For each allergen, each individual serum was classified either as a true positive, a false positive, a true negative or a false negative depending on the measured concentration of IgE and on the results of the open OFC. Clinical sensitivity, clinical specificity, positive and negative predictive values (PPV and NPV) appear in Table 3. The PPV were roughly similar for the three allergens whereas clinical sensitivity and clinical specificity were more divergent. The main difference was observed for clinical specificity, which was twofold lower for whole peanut protein extract than for the two purified peanut allergens. It must be noticed that those characteristics, including clinical sensitivity and NPV, have been estimated from the data obtained on the population of 96 allergic children, without including any value from children of the control group. Taking into account true negative results obtained with control individuals would have increased the value of clinical specificity and NPV and thus improved the performances of the test. The highest value of clinical sensitivity was obtained with whole peanut protein extract which is in line with the high number of patient sera recognizing this whole extract in the study population. With regards to the different groups and subgroups of the study population, no false positive results were observed in group A and subgroup B1 with positive OFC. Consequently PPV values are 100% in each of these group/subgroup. As far as whole group B is concerned (i.e. including children of subgroup B2 with negative OFC), PPV value is 55%. More interesting are the values of clinical sensitivity that represent the ratio of allergic subjects tested positive (i.e. with positive OFC and positive specific IgE) on all allergic subjects (i.e. with positive OFC and either positive or negative specific IgE). Data calculated with a cut off of 0.1 kU/l specific IgE, for whole peanut protein extract, Ara h 1 and Ara h 2, in the different groups and subgroups are presented in Table 4.

Using whole peanut protein extract, the EAST determination of specific IgE has a very high clinical sensitivity and PPV, especially for the severe subgroup of the study

Table 3. Performance characteristics of the enzyme allergosorbent test for determination of specific immunoglobulin E (IgE) using different peanut allergens

IgE level (kU/l)	Whole Ara				Ara h 1				Ara h 2			
	C Sens	C Spec	PPV	NPV	C Sens	C Spec	PPV	NPV	C Sens	C Spec	PPV	NPV
0.1	93	30	83	54	58	85	93	36	80	75	90	50
0.35	93	40	85	60	54	95	97	35	71	85	94	44
1	87	45	88	45	47	95	97	32	64	95	98	41
5	63	90	96	39	35	100	100	28	50	100	100	34
15	52	100	100	36	24	100	100	25	41	100	100	31

C Sens = clinical sensitivity = % of allergic subjects with a positive test = allergic subjects tested positive/all allergic subjects.

C Spec = clinical specificity = % of nonallergic subjects with a negative test = nonallergic subjects tested negative/all nonallergic subjects.

PPV = positive predictive value = probability for patients tested positive of being really allergic = allergic subjects tested positive/all subjects tested positive.

NPV = negative predictive value = probability for patients tested negative of being really nonallergic = nonallergic subjects tested negative/all subjects tested negative. According to Sampson (17).

Table 4. Clinical sensitivity (%) of specific immunoglobulin E determination (cut off = 0.1 kU/l) calculated in each group of patients for different allergens

Allergen group	Whole peanut proteins	Ara h1	Ara h 2
A1	81	50	75
A2	94	44	81
A3	100	85	100
A	93	64	88
B1	94	39	55.5

population. Taking the lowest detectable concentration of specific IgE would lead to a clinical sensitivity of 100% in the most severe subgroup (i.e. A3) and 81% in the subgroup A1. The sensitivity is much decreased when purified allergens are used, i.e. Ara h 2 and especially Ara h 1 for which the percentage of sensitized children is lower than for whole peanut proteins while, on the opposite, the clinical specificity is then much better. It appears that taking into account a concentration of 5, or even 15 kU/l anti whole peanut protein IgE as a decision point could be a valuable compromise which would lead to a still acceptable sensitivity with high specificity and PPV. Using these threshold values would avoid the need of a food challenge in 24 patients (92%) or 21 patients (81%) respectively in the severe group of the study population (A3).

As seen in Table 5, the highest specific IgE response was observed for the whole peanut protein extract then for Ara h 2 while Ara h 1 provided the lowest IgE response. The same differences in specific IgE levels were also observed within each group and subgroup. The highest mean and median values of specific IgE levels were observed in each (sub)group with the whole peanut protein extract. The lowest specific IgE levels were those against Ara h 1.

Statistical analysis using intra-group comparison of specific IgE levels confirmed these differences, particularly in the subgroup A3. In all groups IgE response to whole peanut protein extract was significantly higher ($P < 0.05$) than that to purified Ara h 1 and Ara h 2.

Table 5. Specific immunoglobulin E levels in the different groups and subgroups

Group	Allergen		
	Whole peanut proteins (kU/l)	Ara h 1 (kU/l)	Ara h 2 (kU/l)
A1 ($n = 16$)			
Mean	25.0	2.1	12
Max	158	25	100
Min	0	0	0
Med	7.5	0.1	1.35
A2 ($n = 16$)			
Mean	84	12.1	43
Max	800	120	370
Min	0	0	0
Med	7.5	0	4.6
A3 ($n = 26$)			
Mean	179	29	122
Max	710	140	375
Min	1.8	0	0.3
Med	126	12.5	72
B1 ($n = 18$)			
Mean	55	9	47.8
Max	515	51	520
Min	0	0	0
Med	1.9	0	0.45
B2 ($n = 20$)			
Mean	1.85	0.06	0.14
Max	6.4	1	1.5
Min	0	0	0
Med	1.4	0	0

In subgroup A3, this difference is even highly significant ($P < 0.001$). Similarly IgE response to Ara h 2 was significantly higher than that to Ara h 1 ($P < 0.001$ and $P < 0.05$ in subgroup A3 and in all other groups respectively).

Correlation between specific IgE responses to two different peanut allergens were calculated by the Spearman rank correlation. This analysis revealed high correlation between the IgE responses against the three different allergens ($r > 0.75$). The highest correlation

Table 6. Inter-group comparisons of specific immunoglobulin E levels (Mann–Whitney rank sum)

Group/group	Allergen		
	Whole peanut proteins	Ara h 1	Ara h 2
A1/A2	NS	NS	NS
A1/A3	***	***	***
A2/A3	**	**	**
B1/A1	NS	NS	NS
B1/A2	NS	NS	NS
B1/A3	***	**	***
B2/A1	**	**	**
B2/A2	***	*	***
B2/A3	***	***	***
B2/B1	*	NS	*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

NS, not significant.

was found between anti-whole peanut protein extract and anti-Ara h 2 IgE levels ($r = 0.9$).

For each allergen, specific IgE levels were statistically compared between the five different groups and subgroups (Table 6). Independently of the allergen, differences between the group with negative open OFC (B2) and those with positive open OFC and convincing allergic reactions (A1, A2, and A3 subgroups) were always significant ($P < 0.05$) or generally highly significant ($P < 0.001$). Differences between B1 and B2 subgroups were also observed excepted in the case of anti-Ara h 1 IgE levels. Furthermore, specific IgE levels in the subgroup A3 were significantly higher than those observed in the other groups or subgroups (generally $P < 0.001$ or $P < 0.01$).

Discussion

We have developed an original quantitative enzyme-linked immunosorbent assay (ELISA) for determination of specific IgE to whole peanut protein extract and to Ara h 1 and Ara h 2 in a large population of allergic children to peanut, classified in five groups and subgroups depending on the severity of their clinical symptoms. Specific IgE response may allow a first discrimination between patients according to the severity of symptoms and between allergens according to their prevalence and potency.

Skin-prick tests are recommended by the AAAAI (18) because of their safety and ease of use. They are sensitive but of low specificity and PPV. Only 59% of the children with positive SPTs to peanuts have been described to react significantly to the food challenge (19). Moreover intensity of SPTs does not well correlate with symptoms experienced during a food challenge (20). Consequently positive SPTs must be taken into account together with other considerations such as specific IgE determinations and food challenge (21).

The double-blind, placebo-controlled food challenge (DBPCFC) is considered the gold standard for the definite diagnosis of peanut allergy (22). As already noticed by Warner some clinically relevant sensitivities might be missed under controlled-challenge conditions (23) and the symptoms observed during the open OFC were not always the same as those originally described as to occur in every day life conditions. Interestingly one patient classified in group A has developed severe symptoms during the open OFC which has then been stopped. It also appeared difficult to get a strict correlation between the threshold of reactivity and the originally described symptoms or the clinical manifestations occurring during the food challenge in all the different groups.

Moreover bias occur in food challenge because of ethical reasons that preclude to perform the test on children presumed to be highly reactive. They also may occur because of the part of subjectivity from the patient or his parents and from the physician, in the feeling and the assessment of the severity of the symptoms and in the decision to stop the challenge. This could make the results of food challenge difficult to interpret. However, it must be noticed that most of the the open OFC of this study have been performed on young children (see Table 1) for whom DBPCFC may not be required as the reaction to placebo has been described to be low, i.e. only 0.2% (24). The possible bias was also minimize because the decision to stop the challenge was based on the observation of objective clinical signs and not on subjective complaints. Such subjective complaints are frequently reported in the placebo group besides few mild manifestations (25). In addition DBPCFC is much time consuming and requires several health professionals to take care of the different steps of the test, which is not always compatible with the number of patients waiting to undergo a food challenge in the Hospital. Open OFC may thus appear to be a good alternative for DBPCFC when an OFC is required. In spite of the possible bias discussed above, the statistically lowest threshold which elicited reactions was observed with the patients classified in the severe group, confirming that oral challenges may be optional or contraindicated as also suggested by Sicherer *et al.* (26) In this case, *in vitro* tests and especially ELISA detection of specific IgE, appear to be useful.

Specificity, sensitivity and detection limit of the original ELISA we have developed were shown to be appropriate for detection and quantification of IgE against whole peanut protein extract and against purified Ara h 1 and Ara h 2.

As the isolation and the characterization of a major allergenic fraction by Sachs *et al.* (27), a large number of peanut allergens have now been described (8, 15, 28, 29). Ara h 1 and Ara h 2 are often considered major allergens with frequencies of specific IgE over 65% (7, 8). In the present study, some sera with a specific IgE response to whole peanut protein extract failed to present anti-Ara h 1 or anti-Ara h 2 specific IgE. These results suggest that

other allergens play a role in allergy to peanut as observed by Kleber-Janke *et al.* (10).

A high correlation between the different anti-allergen IgE was observed in all the groups but IgE responses to whole peanut protein extract were generally higher than those to Ara h 2 that were themselves higher than those to Ara h 1 in terms of both frequency and intensity. Whole peanut protein extract thus appears the most efficient allergen for characterizing patients allergic to peanut. However, standardization of whole peanut protein extract seems difficult because the quantity and quality of the proteins vary depending on the varieties and on the conditions of extraction and storage (30, 31).

The PPV and clinical specificity we have estimated using a cut off value of 0.1 kU/l are close to those obtained by Sampson using the CAP system technique with a cut off value of 0.35 kU/l (17). The differences in NPV of the two studies can be explained by the composition of the study population, i.e. the number of control non allergic subjects included in the study and in the calculation of parameters. However using a threshold value of 5 or 15 kU/l anti peanut IgE can be a valuable decision point which could allow to avoid the need of a food challenge in an important part of the children with severe symptoms (i.e. 92 or 81%).

Hourihane *et al.* when studying both young and older children, failed to observe a correlation between radio-allergosorbent test positivity and the severity of the clinical manifestation (20). In the present study on a population of younger patients than in Hourihane's

study, no statistical difference in specific IgE levels between the mild and moderate groups was observed but patients with severe reactions presented the highest anti-whole peanut proteins IgE response. The median value of serum specific IgE concentration in this group was 15-fold higher than those observed in the moderate and mild groups. Quantification of specific IgE thus appears to have a predictive value at least for patients highly reactive, with severe symptoms.

As a consequence of these observations and particularly of the high PPV and sensitivity values of the ELISA test of specific IgE in the severe group, we have now decided not to perform any more open OFC in children who recently had a reaction to peanut with severe symptoms when SPTs are positive and peanut specific IgE are high. If the peanut reaction has been observed several years ago, it is necessary to perform new SPTs and peanut IgE determination before any OFC. The *in vitro* ELISA test for sensitive determination of specific IgE developed in this study appears complementary to food challenge and could serve as a substitute when confirming allergy in patients with moderate or severe suspected reactions. The simplicity of this test allows to avoid patients hospitalization and also appears to be less expensive. The use of whole peanut protein extract appears to be the most appropriate for the sensitivity of the test but it has to be applied to other allergens which are present in peanut to follow up the evolution of the immune response in terms of intensity and specificity during the life of patients.

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