

Measurement of peptide-specific IgE as an additional tool in identifying patients with clinical reactivity to peanuts

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Background: Peanut allergy is one of the most common food allergies, often resulting in severe reactions. Diagnostic decision levels of food-specific IgE antibody concentrations have been described. However, many patients still need to undergo oral peanut challenges because their IgE levels are in the non-diagnostic level.

Objective: The aim of this study was to determine whether differences exist in IgE-binding epitope recognition between sensitized children with and without symptomatic peanut allergy.

Methods: Eight peptides representing the immunodominant sequential epitopes on Ara h 1, 2, and 3 were synthesized on SPOTs membranes. Individual patient labeling was performed with sera from 15 patients with symptomatic peanut allergy and 16 patients who were sensitized but tolerant. Ten of these 16 patients had "outgrown" their allergy.

Results: Regardless of their peanut-specific IgE levels, most patients with symptomatic peanut allergy showed IgE binding to the 3 immunodominant epitopes on Ara h 2. In contrast, each of these epitopes was recognized by < 10% of the tolerant patients. In addition, tolerant patients did not recognize 2 immunodominant epitopes on Ara h 1. At least 93% of symptomatic, but only 12.5% of tolerant patients, recognized 1 of these "predictive" epitopes on Ara h 1 or 2. Moreover, the cumulative IgE binding to the peanut peptides was significantly higher in patients with peanut allergy than in tolerant patients. With up to 50% of patients with peanut-specific IgE levels below diagnostic decision levels still being clinically reactive, oral food challenges could be avoided in ~90% of these patients through determination of peptide-specific IgE.

Conclusions: Determination of epitope recognition provides an additional tool to diagnose symptomatic peanut allergy, especially in children with peanut-specific IgE below diagnostic decision levels. (*J Allergy Clin Immunol* 2003;112:202-7.)

Key words: Peanut, Ara h 1, Ara h 2, Ara h 3, epitope, tolerant, outgrown, IgE

Peanut allergy is one of the most common food allergies in the United States and several European countries, with 1.5 million people being affected in the United States alone.¹ Peanut allergy tends to be more severe in nature than other food allergies and often results in anaphylactic reactions.² Up to 80% of individuals with peanut allergy react to their first known exposure.^{3,4}

The initial diagnosis is frequently based on histories of severe clinical reactions in combination with elevated serum IgE antibody levels against peanut or positive skin prick tests. In less severe cases, double blind, placebo-controlled food challenges are used to confirm the diagnosis.⁵ In addition, many children who are clinically allergic to other foods are placed on a peanut-free diet, because sensitization to peanuts was detected by skin prick test or allergen-specific serum IgE even before peanuts had been introduced into their diet. Although these children require further evaluation of their peanut allergy, food challenges are often put off, because they might lead to severe symptoms.

As opposed to many other childhood food allergies (milk, egg, wheat, and soy) that are outgrown by most patients, peanut allergy tends to persist throughout adulthood. However, it has been reported in recent years with an update in this issue of the *Journal* that up to 20% of the children with peanut allergy lose their allergy.⁶⁻⁹ Therefore, children with peanut allergy need to be re-evaluated on a regular basis.

The double-blind, placebo-controlled food challenge is the "gold standard" for diagnosing food allergy.¹⁰ However, the procedure is time consuming, cost intensive, and stressful to the patient. In addition, it places the patient at risk for a severe anaphylactic reaction. Therefore, in vitro tests predicting the outcome of the food challenges are under investigation. Peanut-specific IgE concentrations in the serum and skin prick test wheal diameters have been correlated with patient histories and the outcome of peanut challenges to generate probability curves that depict the likelihood of patients with a particular peanut-specific IgE levels of reacting to peanut.¹¹⁻¹⁴ In this way, diagnostic decision levels have been identified that help to reduce the requirement for food challenges in a number of patients. However, large numbers of patients still require oral food challenges, because their peanut-specific IgE level or skin prick test wheal diameter are below the established diagnostic decision levels.

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The major and minor allergens of peanuts have been identified. They consist of the peanut conglutins Ara h 2, 6, and 7^{15,16}; the peanut vicilin Ara h 1¹⁷; and the peanut glycinins Ara h 3 and 4.^{16,18} In addition, the IgE binding sites for Ara h 1, 2, and 3 have been determined.¹⁸⁻²⁰ For Ara h 1, at least 23 B-cell epitopes were identified. Four of these epitopes were found to be immunodominant, defined as those epitopes that are bound by peanut-specific IgE from most patients with peanut allergy and that bind more IgE than any of the other epitopes.¹⁹ Three immunodominant epitopes have been identified for Ara h 2 and one for Ara h 3, respectively.^{18,20}

In the present study, we compared differences in IgE binding to the immunodominant B-cell epitopes on Ara h 1, 2, and 3 between children with symptomatic peanut allergy, children who had outgrown their allergy, and children who are sensitized but clinically tolerant to peanut ingestion with the goal of identifying additional diagnostic markers for peanut allergy.

METHODS

Patient population

Sera from 31 patients were analyzed for IgE-binding patterns to the immunodominant epitopes on Ara h 1, Ara h 2, and Ara h 3 that were identified previously.¹⁸⁻²⁰ Patients were divided into 2 categories: patients with symptomatic peanut allergy ($n = 15$) and patients tolerating peanut although they are sensitized (ie, possess peanut-specific IgE antibodies [$n = 16$]). All patients with symptomatic peanut allergy had either a convincing history of clinical reactivity to peanut with IgE-mediated symptoms and high levels of peanut-specific IgE (group A, $n = 10$), lower peanut-specific IgE levels with a positive physician-supervised challenge to peanut ($n = 4$), or multiple severe reactions ($n = 1$) (group B). The tolerant groups were composed of 10 patients (group C) who were allergic to peanut but had outgrown their allergy and 6 patients (group D) who never had clinical reactions to peanut despite being sensitized. Patients who outgrew peanut allergy were those who had a definitive history of peanut ingestion and clinical reactivity and who subsequently had negative physician-supervised peanut challenges (group C). All patient characteristics are shown in Table I.

Peanut-specific IgE levels were measured using the Pharmacia Cap System FEIA (Pharmacia Diagnostics, Uppsala, Sweden). Sera in the "outgrown" and tolerant group were obtained at the time of clinical nonresponsiveness.

Preparation and labeling of SPOTs membranes for detection of IgE binding to B-cell epitopes

Peptides representing previously identified immunodominant IgE-binding epitopes on Ara h 1 (epitope 1, AA25-34, AKSSPYQKKT; epitope 3, AA65-74, LEYDPLRVYD; epitope 4, AA89-98, GERTRGRQPG; epitope 17, AA498-507, RRYTARLKEG),¹⁹ Ara h 2 (epitope 3, AA27-36, DRRCQSQLEK; epitope 6, AA57-66, YERDPYSPSQ; epitope 7, AA65-74, SQDPYSPSPY),²⁰ and Ara h 3 (epitope 3, AA279-293, VTVRGGLRLSPDRK)¹⁸ were synthesized on SPOTs membranes (Genosys Biotechnologies, Woodlands, Tex), a derivatized cellulose membrane using the 9-fluorenyl-methoxycarbonyl method as described earlier.²¹ The membranes were then blocked and incubated with individual patients' and nonatopic controls' sera diluted 1:5. IgE antibody binding was detected by ¹²⁵I-labeled anti-human IgE (Diamed, Windham, Me).

Membranes were exposed to an X-OMAT film (Kodak, Rochester, NY) with an intensifying screen at -20°C for 48 hours and developed. The optical density of each spot was measured by reflection densitometry. The cutoff level for significant binding was 0.02. This cutoff level was chosen because it provided a clear distinction between specific and nonspecific binding.

Statistical analysis

All analyses of data were performed with nonparametric tests (Mann-Whitney U test for comparison between groups) using GraphPad Prism 3.03 software (GraphPad Software, San Diego, Calif). Differences associated with P values $< .05$ were considered significant.

RESULTS

This study was performed to test for differences in IgE-binding epitope recognition between children with symptomatic peanut allergy and those who were sensitized but clinically tolerant. Therefore, IgE binding to immunodominant B-cell epitopes on the peanut allergens Ara h 1 to 3 were compared between the 2 patient groups. The first group was composed of 15 patients with symptomatic peanut allergy subdivided into patients with high and low peanut-specific IgE levels, and the second group was composed of 16 children who tolerated peanut despite being sensitized. Ten of the 16 tolerant children were previously symptomatic to peanut but outgrew their allergy, whereas the remaining 6 never had clinical reactions after peanut consumption (Table I).

Fig 1 shows the IgE binding to the 8 immunodominant epitopes on Ara h 1, Ara h 2, and Ara h 3 synthesized on SPOTs membrane for 3 representative patients from each group. The overall percentage of patients from each group binding to these 8 immunodominant epitopes is shown in Fig 2. All 3 immunodominant epitopes on Ara h 2 were recognized by most patients with symptomatic peanut allergy but only by 10% of patients who outgrew their allergy and by no individuals who never experienced clinical reactions. The immunodominant epitope on Ara h 3 was recognized by 87% of patients with symptomatic allergy but also by 31% of patients who could eat peanut (Fig 2). Surprisingly, the 4 immunodominant epitopes on Ara h 1 were recognized only by 20% to 40% of patients with symptomatic peanut allergy. However, IgE antibodies from clinically tolerant patients did not bind 2 of these 4 epitopes (epitope 3 and 4) (Fig 2).

One could argue that the differences in epitope binding between the patients with symptomatic peanut allergy and those who were tolerant is due to differences in their peanut-specific IgE levels. Therefore, the patients with symptomatic peanut allergy were further subdivided into patients with high and low levels of peanut-specific IgE. As shown in Fig 2, B, the epitopes were recognized well even by symptomatic patients with low peanut-specific IgE levels. Moreover, the overall intensity of binding to all immunodominant epitopes was significantly lower in the clinically tolerant patients who had outgrown their allergy or who had never shown clinical reactions compared with the allergic group, regardless of their peanut-specific IgE levels (Fig 3).

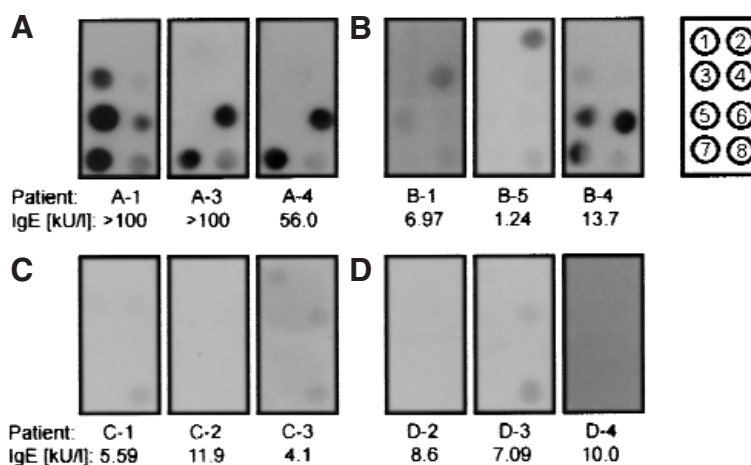


FIG 1. Eight immunodominant epitopes of Ara h 1 (Spots 1-4 representing epitope 1, AA25-34; epitope 3, AA65-74; epitope 4, AA89-98; and epitope 17, AA498-507), Ara h 2 (Spots 5-7 representing epitope 3, AA27-36; epitope 6, AA57-66; and epitope 7, AA65-74), and Ara h 3 (Spot 8 representing epitope 3, AA279-293) were synthesized on SPOTs membranes. Shown is the IgE labeling with 3 representative patients from each group of (A) patients with peanut allergy with high levels of peanut-specific IgE, (B) patients with peanut allergy with low levels of peanut-specific IgE, (C) patients who had outgrown their peanut allergy, and (D) patients who were sensitized but never clinically reactive to peanut.

TABLE I. Patient characteristics

Patient group	No.	Age (y): median [range]	Peanut-specific IgE: median [range]	Past clinical reactions	Oral food challenge
A	10	8.5 [5-37]	100 kU/L [30-100 kU/L]	Respiratory and gastrointestinal symptoms, hives, angioedema, anaphylaxis	Not done
B	5	10 [8-45]	4.08 kU/L [1.24-13.7 kU/L]	Respiratory and gastrointestinal symptoms, hives, angioedema, anaphylaxis	Positive*
C	10	6.5 [4-11]	3.93 kU/L [0.84-11.9 kU/L]	Respiratory and gastrointestinal symptoms, hives, angioedema	Negative
D	6	5.5 [4-11]	4.1 kU/L [1.24-8.6 kU/L]	None	Not done

*One patient did not undergo an oral challenge test because of multiple severe reactions.

Our results indicate that determination of IgE binding to the immunodominant peanut epitopes, especially epitopes 3, 6, and 7 on Ara h 2 and epitopes 3 and 4 on Ara h 1, could be useful in distinguishing patients with symptomatic peanut allergy from those who are clinically tolerant to peanut.

DISCUSSION

In this study, we were able to show differences in IgE epitope recognition between patients with symptomatic peanut allergy and those who were sensitized but had either outgrown their allergy or were never clinically reactive. The most striking difference was seen for epitopes on Ara h 2. In previous studies by Burks et al¹⁵ and Stanley et al,²⁰ Ara h 2 was shown to be recognized by serum IgE from > 90% of patients with peanut allergy and to contain 10 sequential epitopes, 3 of which were immunodominant and recognized by all of the patients tested. In this study, these 3 immunodominant epitopes were recognized by 60% to 73% of the patients

with peanut allergy but only by 6% of the individuals tolerant to peanut. The epitope recognition in the allergic patients is lower than in the study by Stanley et al but is in good agreement with other reports evaluating IgE binding to Ara h 2 in larger patient populations, which showed only an overall recognition rate of 71% to 85%.^{16,22,23}

In addition to the Ara h 2 allergenic epitopes, 2 epitopes on Ara h 1 were not recognized by any of the tolerant patients in this study. However, these epitopes were recognized by only 20% to 40% of the patients with peanut allergy. In previous studies defining the epitopes on Ara h 1, the 4 immunodominant epitopes were bound by IgE from at least 80% of the 10 patients with peanut allergy tested¹⁹ using the same method. However, the frequency of IgE binding to the whole protein varies widely throughout the literature, reflecting differences in patient populations and methods used. Using immunoblots, Burks et al²⁴ found IgE binding to both native and recombinant Ara h 1 in > 90% of the 18 patients tested. In contrast, studying IgE binding to crude peanut extract on SDS-PAGE in 89

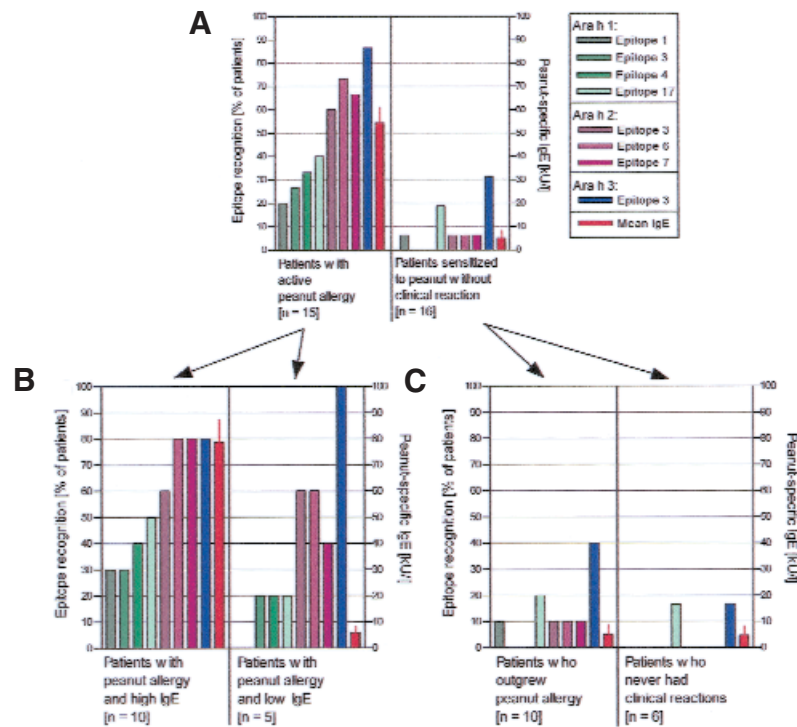


FIG 2. Shown are the percentages of patients recognizing the 8 immunodominant epitopes on Ara h 1, Ara h 2, and Ara h 3 in (A) patients with symptomatic peanut allergy and patients sensitized to peanut without clinical reactions. Patients with peanut allergy were further subdivided into (B) symptomatic patients with high and low peanut-specific IgE and sensitized patients who were further subdivided into (C) patients who had outgrown their symptomatic peanut allergy and those who never showed clinical reactions.

individuals, Clarke et al²² reported that Ara h 1 was recognized by 73% of the patients. Even lower frequencies were found by Kleber-Janke et al,¹⁶ who showed that rAra h 1 on immunoblot was bound by serum IgE from 65% of 40 patients. Using protein microarray technology and sera from 90 patients with peanut allergy, we recently found a frequency of Ara h 1-specific IgE of 75%.²³

In this study, 87% of patients with peanut allergy recognized an immunodominant epitope on Ara h 3 (AA279-293), which is a much higher frequency of recognition than previously reported. In the study by Rabjohn et al,¹⁸ only 8 of 18 (44%) individuals with peanut allergy had IgE binding to rAra h 3, with 8 of 8 of these Ara h 3-allergic patients recognizing the epitope AA279-293. However, in our previous study, rAra h 3 was recognized by 74% of 90 patients with peanut allergy.²³

At present, treatment of peanut allergy consists of total avoidance of peanuts, recognition of early symptoms of an allergic reaction, and management of anaphylaxis.⁵ The disruption of daily life and the permanent risk of a life-threatening event are hard on patients and their families. Therefore, the accurate diagnosis of peanut allergy is essential for the patients with allergy and their families. Although only approximately 20% of patients with peanut allergy become tolerant,⁶⁻⁹ it is necessary to re-evaluate patients with peanut allergy on a regular basis to determine the actual state of their clinical reactivity. It has been shown that measurement of peanut-specific IgE concentrations in the serum or skin prick wheal diameter is help-

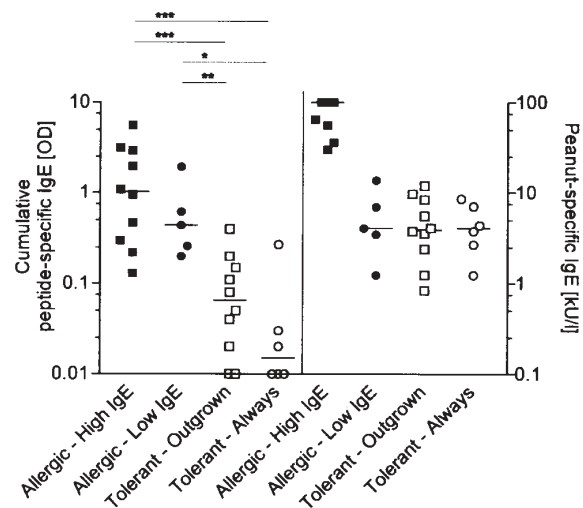


FIG 3. Shown are peanut-specific IgE levels (kU/L) measured by the Pharmacia CAP-System FEIA and the cumulative peptide-specific IgE (OD) measured on the SPOTs membrane. **P* < .05, ***P* < .01, and ****P* < .001.

ful in determining the clinical reactivity of the patients.¹²⁻¹⁴ However, many patients still require an oral food challenge, because their IgE levels or their skin prick test wheal diameters are below the diagnostic decision levels. With decreasing IgE levels, patients need to undergo oral food challenge to determine whether they developed toler-

ance. Interestingly, differences in IgE-binding epitope recognition observed in this study between patients with clinically active peanut allergy and those who were tolerant was evident despite the presence of high or low peanut-specific IgE levels. Therefore, evaluating peptide recognition patterns in addition to peanut-specific IgE levels seems to be useful, especially in patients with peanut-specific IgE levels below the diagnostic decision levels. This might obviate the need to perform oral challenges to confirm their status of clinical reactivity. Up to 50% of patients with peanut-specific IgE levels below the diagnostic decision levels might be clinically symptomatic after peanut ingestion, with approximately 90% of these children recognizing the "predictive" peanut epitopes, as suggested in this study. Studies are now underway linking these "predictive" peanut peptides to a commercial matrix to conduct large-scale studies to confirm or refute the current findings. In addition, these studies will attempt to compare epitope-specific IgE binding in patients before and after the development of tolerance.

Previously, we demonstrated that the recognition of certain linear IgE-binding epitopes correlates with the persistence of milk allergy.^{25,26} In contrast to aeroallergens, in which IgE binding occurs primarily to conformational epitopes located on the surface of the protein, it seems that sequential epitopes also play an important role in food allergens. This might be explained by the fact that these epitopes are more likely to survive digestion intact. In fact, it had been shown for Ara h 1 that its structure protects the IgE-binding epitopes from degradation.²⁷ Similarly, all 3 immunodominant epitopes of Ara h 2 are located on a 10-kd protease-resistant fragment of Ara h 2.²⁸ Moreover, Maleki et al,²⁹ in the current issue of the *Journal*, show that Ara h 2 functions as a trypsin inhibitor protecting itself further from digestion and that roasting enhances this function. In younger children with immature guts that allow increased absorption of larger macromolecules, conformational epitopes might also play an important role. Striking differences in recognition of conformational epitopes have been seen for ovomucoid between younger and older patients.³⁰ It seems possible that children with food allergy initially recognize predominantly conformational epitopes. After maturation of the gut, including its enzymatic activity, more sequential epitopes become recognized if epitope spreading occurs. Epitope spreading involves the acquired recognition of new epitopes within the same molecule and epitopes residing in proteins that are associated in the same macromolecular complex.³¹ Epitope spreading is well described in several autoimmune diseases but is also known as a common feature of the natural immune responses mounted against some pathogens.³¹ Although we have increasing evidence that disease persistence and clinical activity of food allergy are associated with epitope recognition, the basis for this mechanism remains poorly understood. Prospective studies in humans, as well as epitope studies in animal models, will be necessary to investigate the time course and role of epitope spreading in food allergic diseases.

In conclusion, we have demonstrated differences in peanut allergenic epitope recognition between patients with symptomatic peanut allergy and those who are sensitized but clinically tolerant. Consequently, the addition of epitope-specific IgE determinations might prove to be an especially valuable tool for diagnosing symptomatic peanut allergy in patients with low levels of peanut-specific IgE.

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