

Predictive value of skin prick tests using recombinant allergens for diagnosis of peanut allergy

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Background: Current diagnosis of peanut allergy relies on natural extracts that lack standardization. Recombinant DNA technology allows production of pure biochemically characterized proteins. Their usefulness for peanut allergy diagnosis is not established.

Objective: This study aimed to evaluate the diagnostic value of the 3 major recombinant peanut allergens.

Methods: Recombinant (r) Ara h 1, rAra h 2, and rAra h 3 were produced according to the recommendations of good manufacturing practice for recombinant allergens. Skin prick tests (SPTs) and IgE ELISA assays were performed in 30 patients with peanut allergy and 30 control subjects without food allergy: 15 nonatopic and 15 sensitized to birch pollen. Disease severity was graded by clinical scoring.

Results: All patients with peanut allergy showed positive SPT results to rAra h 2; 40% reacted with rAra h 1 and 27% with rAra h 3. No control subjects reacted with any of the recombinant allergens. Monosensitization to rAra h 2 was observed in 53% of patients. Neither SPT size nor levels of specific IgE were correlated with the disease severity. However, patients with monosensitization to rAra h 2 had a significantly lower severity score than polysensitized subjects and a lower level of specific IgE against peanut extract and rAra h 2.

Conclusion: Skin prick tests to individual recombinant peanut allergens appear to be a safe and effective diagnostic tool.

Cosensitization to rAra h 2 and rAra h 1 and/or rAra h 3 is predictive of more severe reactions.

Clinical implications: Recombinant peanut allergens can be used by SPTs for diagnosis and evaluation of allergy severity. (J Allergy Clin Immunol 2006;118:250-6.)

Key words: Food allergy, peanut, diagnosis, recombinant allergens

Peanut food allergy (FA) is a major public health problem because of its severity and prevalence, which is estimated to be 0.5% to 1%.^{1,2} Peanut is the most common food to cause fatal and near-fatal food allergy.³⁻⁵ The threshold of clinical reactivity can be very low: 18% of patients react at a dose less than 65 mg.^{6,7} In addition, peanut allergy is characterized by its persistency.⁸

Diagnostic methods (skin prick tests [SPTs], basophil activation tests, and measurement of specific IgE [sIgE]) are based on natural peanut extracts that contain both allergenic and nonallergenic molecules. The extract composition depends on the origin of raw material and extraction, purification, and storage procedures. This leads to a great deal of variability and difficulty of standardization.^{9,10}

Production of recombinant peanut allergens leads to standardized reagents that are biochemically characterized and that can be produced on a large scale. Eight allergens of peanuts have been identified from Ara h 1 to Ara h 8.¹¹ Ara h 1, Ara h 2, and Ara h 3 are the 3 main allergens.¹² Shreffler et al¹² have demonstrated using *in vitro* studies that 97% of subjects with peanut allergy recognized at least 1 of these allergens, whereas 77%, 75%, and 77% recognized rAra h 1, rAra h 2, and rAra h 3, respectively. Before peanut recombinant allergens can be used as a diagnostic tool, their immunologic reactivity and ability to induce skin reactions need to be established and compared with natural allergens. Here, we report the results of this validation step on the basis of *in vitro* and *in vivo* methods.¹³

In vitro methods (CAP-System FEIA, Pharmacia, Sweden; immunoblot methods, or ELISA) are designed to demonstrate the capacity of binding of sIgE of patients with allergy to the allergens. Elevated levels of sIgE characterize patient biological sensitization status. It has been proposed that the risk of FA in patients with atopic dermatitis could be estimated by the level of sIgE: a level higher

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Supported in part by a grant from Allerbio and funding from the Ministry of Higher Education, Lorraine Region, and the Urban Community of Nancy.

Disclosure of potential conflict of interest: A. W. Burks owns stock in Seer Pharmaceuticals, Inc. and has received grant support from the National Institutes of Health, Gerber Foundation, and Food Allergy and Anaphylaxis Network. H. A. Sampson has consultant arrangements with Seer Pharmaceuticals, Inc., owns stock in Seer Pharmaceuticals, Inc., has a patent licensing agreement for Ara h 3, and has received grant support from the National Institutes of Health. The rest of the authors have declared that they have no conflict of interest.

Received for publication February 10, 2006; revised April 11, 2006; accepted for publication April 12, 2006.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2006.04.053

Abbreviations used

AU: Arbitrary unit
CPE: Commercial peanut extract
DBPCFC: Double-blind placebo-controlled food challenge
FA: Food allergy
LAL: Limulus Amoebocyte Lysate
sIgE: Specific IgE
SPT: Skin prick test

than 15 kU/L has a positive predictive value superior to 95%.¹⁴ If sIgE levels are between 0.35 kU/L and 15 kU/L, however, it is not possible to predict accurately the risk of FA. An undetectable peanut-specific IgE level may correspond to a true FA in 27% of the cases, a level of sIgE <2 kU/L to a 33% chance of a true FA, and a level <5 kU/L to a 39% chance of FA.¹⁵ The level of sIgE does not correlate with the severity of allergy and cannot be used as a predictive tool of severity.¹⁶ The difficulty of interpretation of the level of specific IgE may be explained in part by the variability of the natural extract used for the biological tests, the techniques used, and the studied population. These biological tests cannot currently be substituted for the oral challenge test that is the current standard for FA diagnosis. A better standardization of biological tests using recombinant allergens would allow the improvement of their diagnostic value.

The *in vivo* technique of SPT provides information regarding direct activation of skin mast cells by any given allergen mixture, thereby inducing a specific histamine release.

Until now, the diagnostic value of skin tests to recombinant peanut allergens has not been established. The current study defines the recognition profile of peanut recombinant allergens (rAra h 1, rAra h 2, and rAra h 3) *in vivo* (SPT) and *in vitro* (sIgE) and compares native peanuts and commercial extract in 30 patients allergic to peanuts.

METHODS

Patients

Thirty patients (age 3-20 years old) allergic to peanuts, 15 nonatopic subjects, and 15 patients allergic to birch pollen without food allergy were included. The study was reviewed and approved by the local ethical committee, and all patients or their parents provided written informed consent forms.

Food allergy to peanuts was assessed by clinical history, positive SPT, and/or sIgE levels (CAP-System FEIA) greater than 15 kU/L¹⁴ or a positive double-blind placebo-controlled food challenge (DBPCFC).¹⁷

The clinical severity of the allergy reaction was evaluated on a scale between 0 and 5 with slight modifications to that reported by Ewan and Clark.¹⁸ This grading system used in our clinical department is the following: 0, no symptoms; 1, abdominal pain that resolved without requiring medical treatment, rhinoconjunctivitis, urticaria fewer than 10 papulas, rash (eczema onset); 2, one organ involved, abdominal pain requiring treatment, generalized urticaria, nonlaryngeal angioedema, mild asthma (cough or fall of peak

expiratory flow < 20%); 3, two organs involved; 4, three organs involved or asthma requiring treatment or laryngeal edema or hypotension; 5, cardiac and respiratory symptoms requiring hospitalization in intensive care.

Production of recombinant peanut allergens rAra h 1, rAra h 2, and rAra h 3

pET-24 expression vectors containing the sequences of peanut allergens Ara h 1, Ara h 2, and Ara h 3 in fusion with a Histidine (His) tag at the C terminal extremity¹⁹⁻²² were used to transform competent *Escherichia coli* Rosetta (DE3) (Novagen, Madison, Wis). The 3 different *E coli* strains were grown in Lennox Broth medium containing the appropriate antibiotics (kanamycin and chloramphenicol) at 37°C. When OD_{600nm} reached 0.6, the expression of recombinant protein was induced by adding 1 mmol/L isopropylthiogalactoside. After induction, cultures were incubated at 37°C for 4 or 12 hours. The cell pellet was collected by centrifugation and frozen.

Purification of rAra h 1, rAra h 2, and rAra h 3 from *E coli*

His-tagged rAra h 1, 2, 3 fusion proteins overexpressed in *E coli* Rosetta (DE3)/pET-24-Ara h-(His)₆ were purified from inclusion bodies as follows. Cell pellets were resuspended and sonicated in lysis buffer (50 mmol/L sodium phosphate and 50 mmol/L NaCl, pH 7.8) containing protease inhibitor cocktail (Sigma, St Louis, Mo) and lysozyme. Inclusion bodies recovered by centrifugation (12,000g, 20 minutes, 4°C) were incubated in a shaker overnight at room temperature in solubilization buffer (50 mmol/L sodium phosphate containing 300 mmol/L NaCl, 10 mmol/L imidazole, 8 mol/L urea, 10% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride, and 2 mmol/L β-mercaptoethanol, pH 7). The solubilization buffer was exchanged for binding-washing buffer (50 mmol/L sodium phosphate containing 500 mmol/L NaCl, 8 mol/L urea, 20 mmol/L imidazole, 1 mmol/L phenylmethylsulfonyl fluoride, and 2 mmol/L β-mercaptoethanol, pH 7.8) using PD-10 desalting columns (Amersham Biosciences, Buckinghamshire, UK). rAra h 1, 2, and 3 were then purified by liquid chromatography on an Akta Explorer 10 system using His-Trap prepacked columns (Amersham Biosciences). Protein refolding was performed directly on the column by removing urea from the washing buffer. Bound proteins were eluted with an increasing gradient of imidazole. Protein concentrations were determined by using a Bio-Rad Protein Assay commercial kit (Hercules, Calif) on the basis of the method described by Bradford.²³

SDS-PAGE

Recombinant protein purity and stability during storage at -20°C was monitored by SDS-PAGE electrophoresis under reducing conditions using 12% polyacrylamide NuPage Bis-tris gels (Invitrogen, Carlsbad, Calif) followed by Coomassie blue staining.

Immunoblotting analysis

After protein transfer and blocking in TRIS-buffered saline with Tween (TRIS 0.1 mol/L, pH 7.5, 0.15 mol/L NaCl and 0.1% Tween) containing 5% defatted dry milk (blocking buffer), membranes were incubated with allergic patient sera diluted in blocking buffer. Bound immunoglobulins were detected by peroxidase-conjugated goat antihuman IgE (Kirkegaard & Perry Laboratories, Gaithersburg, Md) using ECL Plus Western blotting kit (Amersham Biosciences).

Endotoxin determination by Limulus Amoebocyte Lysate

Levels of endotoxin in the purified recombinant proteins solutions were evaluated by the semiquantitative gel-clot method using the

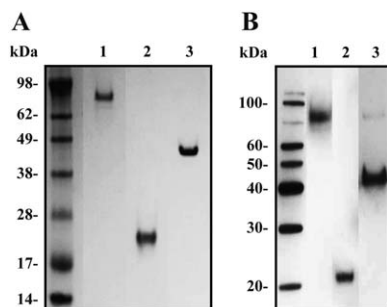


FIG 1. Biochemical characterization of peanut recombinant allergens: control of purity and *in vitro* immunoreactivity. **A**, SDS-PAGE followed by Coomassie staining. Lanes 1 to 3, rAra h 1, rAra h 2, rAra h 3. **B**, IgE immunoblotting with peanut allergic patient serum. Lanes 1 to 3, rAra h 1, rAra h 2, rAra h 3.

End-point gel commercial kit (Endosafe, Charles River Laboratories, Wilmington, Mass). Briefly, samples were incubated 1 hour at 37°C with Limulus Amoebocyte Lysate (LAL). Serial dilutions of sample were mixed in equal volumes with LAL reagent of known sensitivity. After incubation for 1 hour at 37°C, the formation of a firm gel was indicative of an endotoxin amount in the sample at least equal to the LAL reagent sensitivity. The endotoxin concentration was then calculated by multiplying the reciprocal of the greatest dilution of the test solution that gave a positive response by the sensitivity of the LAL reagent. Results were expressed in EU/mL. Endotoxin levels in the purified recombinant proteins solutions were assayed and estimated to be below the authorized limit of 5 EU/kg/h described in European Pharmacopoeia,¹³ thus allowing a clinical study.

SPTs

Skin prick tests to common inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, birch pollen, grass, plantain, artemisia, cat and dog epithelia, and latex) were performed. Main food allergens (milk, egg, fish, soy, wheat), nuts (hazelnuts, macadamia, Brazil nut, walnut, pistachio, almond, pecan, cashew, chestnut), legumes (green peas, beans, lentils, lupine, carob, blond peas, chick peas), and particular foods incriminated by the clinical history of the patient were tested by using the prick-in-prick method.²⁴

Skin prick tests for standardization of peanut recombinant allergens were performed on the back using (1) crushed roasted peanut, (2) commercial protein extract (CPE) of roasted peanut (Allerbio, Varennes en Argonne, France) containing 600 µg/mL of proteins, and (3) rAra h 1, 2, 3 at increasing concentrations: 1, 10, and 100 µg/mL, with the exception of rAra h 1, which could be used only to 60 µg/mL because of limited solubility. The positive control was 9% codeine. The negative control was phenol glycerol diluted in physiological saline solution. A wheal diameter equal to at least 50% of that obtained with the positive control codeine was considered positive.

ELISA

IgE specific binding to CPE (Allerbio, Varennes-en Argonne, France) and recombinant proteins was determined by ELISA. Microtiter plates (Nunc Maxisorp; Nalge Nunc International, Roskilde, Denmark) were coated (100 µL/well) with CPE and recombinant proteins at 0.5 µg/mL in PBS pH 7.4. Plates were blocked with 10% FBS in PBS and incubated with control and patient sera that were diluted in blocking solution. Bound sIgE was detected by peroxidase-conjugated goat antihuman IgE antibody (KPL) and revealed with 3,3',5,5'-tetramethylbenzidine (Pierce, Rockford, Ill). The enzymatic reaction was stopped by 2 mol/L H₂SO₄, and the absorbance was

immediately read at 450 nm with an automated microplate reader. sIgE levels were considered positive when measured OD_{450 nm} was at least equal to 2 times the mean OD obtained with the 30 sera of control subjects. IgE levels were expressed in arbitrary units (AU) and correspond to the OD_{450 nm} corrected by the dilution factor of the serum.

Statistical analysis

Data are expressed as means ± SEMs. Statistical analyses were performed by using the Student *t* test for comparison between 2 groups. A *p* value < .05 was considered statistically significant.

RESULTS

Biochemical characterization of peanut recombinant allergens

rAra h 1, rAra h 2, and rAra h 3 produced in *E coli* appeared as single bands on SDS-PAGE with apparent molecular masses of 72 kD, 20 kD, and 40 kD, respectively (Fig 1, A). This corresponds to their predicted molecular masses. Purity was judged greater than 98% estimated using SDS-PAGE followed by Coomassie staining. IgE-binding capacity to rAra h 1, rAra h 2, and rAra h 3 was investigated by immunoblots. Fig 1, B, shows that sIgE from serum of a patient allergic to peanut recognized all 3 peanut recombinant proteins.

Patients

Thirty patients with peanut allergy (12 female and 18 male subjects) were enrolled (Table I). The mean age was 9.2 ± 0.8 years (range, 3-20 years). The clinical features occurring after accidental ingestion were scored for 3 patients at grade 1, six others at grade 2, three at grade 3, and seven at grade 4. The DBPCFC induced symptoms of grade 1 in six patients, grade 2 in three, grade 3 in five, and grade 4 in three.

Twenty patients presented cross-sensitization to other nuts, 10 patients to others legumes. Seven patients were monosensitized to peanuts.

SPT

No subject from the control group reacted with any recombinant allergen. One control subject with sensitization to birch pollen reacted by SPT to native peanut, with negative SPT result to CPE and recombinant allergens. On the other hand, rAra h 1, 2, and 3 were all able to induce cutaneous reactions characteristic of mast cell degranulation in patients allergic to peanut (Fig 2, A). Intensity of this reaction as determined by the wheal diameter increased concomitantly with the concentration of the preparation (Fig 2, B). At the highest concentration tested, all patients with peanut allergy reacted by SPT to rAra h 2, 40% to rAra h 1, and 27% to rAra h 3 (Fig 2, C).

Native peanut gave a positive SPT result with papules significantly larger than those observed with CPE (15.4 ± 1.2 vs 7.6 ± 0.7 mm; *P* < .001). The size of the wheal resulting from the SPT with rAra h 2 was comparable to that of the size of the wheal resulting from the SPT with CPE (Fig 3, A).

TABLE I. Clinical data for patients with peanut allergy

Patient no.	Age (y)	Sex	Atopy clinical manifestations	Foods sensitization*	Clinical history of peanut allergy		CD (mg)	DBPCFC to peanut		PslgE (kU/L)
					Symptoms	Clinical score		Symptoms	Clinical score	
1	20	F	AD, RC, A	n, l	U, LAO	4	965	U, OAS, G, A	—	67.8
2	3	M	AD	n	U	1	500		—	5.87
3	10	F	AD, RC	n		—	965	P, G, AST	1	1.83
4	15	F	AD	l	U	2	7000	U, G	3	91.9
5	7	M	AD		U	2	44.4		—	1.82
6	4	M	AD	n		—	44.4	A	2	15.3
7	15	F	AD	n	U, RC, G, A	4	3500	U, G, RC	4	45.6
8	7	M	AD	n		—	7000	G	2	2.18
9	5	M	AD	n	U	1	44.4		—	2.69
10	7	M	AD, RC, A	n		—	500	G, RC	—	14.3
11	14	F	AD, RC, G	n, l	U, G, LAO	3	500	G, OAS	1	100
12	4	M	AD			2	500	P, G, RC	4	3.87
13	9	F	AD, G, A	n	U, A	2	965	U, OAS	1	9
14	6	M	AD	n	U, RC	3	44.4		—	2
15	19	F	AD, RC, A			—	500	G, A	3	42.3
16	6	M	AD, RC	n, e	U	2	215	A	1	39.90
17	5	F		e, m, mu		—			—	100
18	6	M	AD	n, e, l		—	500	PL, G, RC, AST	4	100
19	6	M	AD, RC	n, l	A	3	1500	G, AST	3	100
20	11	M	AD, RC	e, f	G, AS	4	265	PL, OAS	2	42.3
21	3	F	AD, A	e, m		—	500	U, G, AST	1	95
22	13	F	AD, A	n, l	U	2	44.4		—	10.4
23	8	M	AD, RC, A	n, e, m, w		—	15	RC	—	28
24	10	M	A	l	A	4	44.4		—	66.3
25	8	F	AD, RC, A	n, l	A	4	215	G	—	13
26	10	M	AD, A	n		—	500	U, PL	3	4.80
27	10	M	AD, RC	n, f, l	U	4	265	U	—	7.62
28	9	F	AD	n, l	U	1	500		—	1.31
29	11	M	AD, RC		U, RC, A	4	215	U, RC	3	14.5
30	14	M	AD	n, ef, f, s		—	65	G	1	25.5

M, Male; F, female; Symptoms: AD, atopic dermatitis; RC, rhinoconjunctivitis; A, asthma; G, gastrointestinal symptoms; U, urticaria; LAO, laryngeal angioedema; AS, anaphylactic shock; OAS, oral allergy syndrome; AST, asthenia; P, pruritus; PL, laryngeal prurit. Food allergy: e, egg; ef, exotic fruits; f, fish; l, legumes; m, milk; mu, mustard; n, nuts; s, sesame; w, wheat; CD, Cumulative dose; PslgE, peanut-specific IgE, determined using the CAP-RAST system (Pharmacia).

*Determined using SPT. Clinical scores were defined as described in Methods.

Sixteen patients reacted only to rAra h 2 and 14 to more than 1 recombinant allergen. Out of 8 theoretical possible combinations, 4 were observed (Fig 4, A). The profile of sensitization did not differ between patients monosensitized to peanut and those polysensitized to legumes or other nuts.

There was no correlation between the size of the SPT of rAra h 2 and the clinical score of severity (data not shown). However, patients who were monosensitized to rAra h 2 had a significantly lower severity score than polysensitized patients ($P < .02$; Fig 4, C).

Specific IgE to peanuts

The profile of recognition of recombinant allergens by sIgE exhibited a similar pattern to that of the SPT. All patients had sIgE against rAra h 2, 50% against rAra h 1, 20% against rAra h 3 (Fig 2, C). The mean levels of sIgE recognizing CPE, rAra h 2, and rAra h 1 were comparable,

whereas they were lower for rAra h 3 (Fig 3, B). sIgE binding to recombinant proteins followed a distribution pattern similar to that resulting from SPT (Fig 4, B). There was no correlation between the level of sIgE and severity of allergy (data not shown). However, patients monosensitized to rAra h 2 had a less severe disease than patients who were polysensitized (Fig 4, D). The levels of sIgE were significantly higher in polysensitized than in monosensitized patients for both CPE (29.2 ± 8.3 vs 5.2 ± 3.9 AU; $P < .02$, respectively) and rAra h 2 (17.3 ± 5.1 vs 4.4 ± 3.4 AU; $P < .04$, respectively).

DISCUSSION

Currently, the only way to assess a peanut sensitization is the use of native peanut or commercial extracts derived from native peanut. Because of variability of the raw

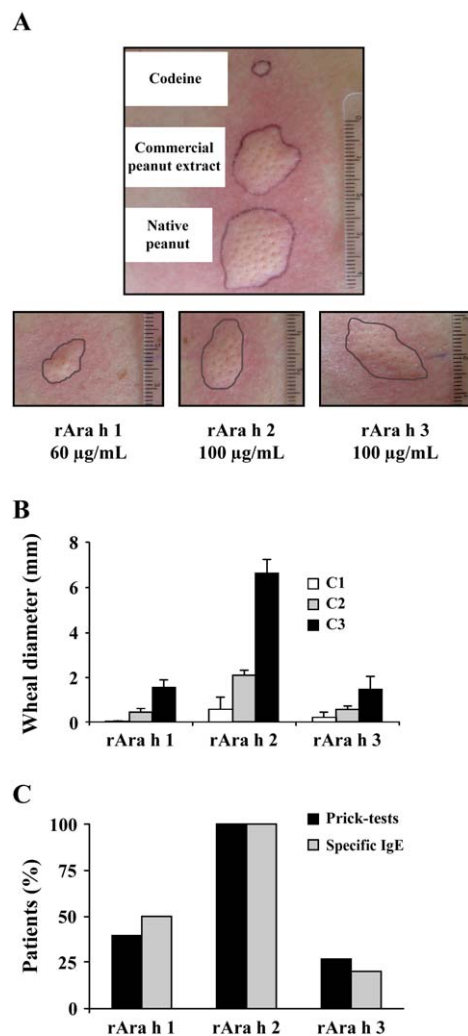


FIG 2. Peanut recombinant allergen reactivity. **A**, Representative SPT reactions of 1 patient allergic to peanut. **B**, Wheal diameters (mm). C1, 1 µg/mL; C2, 10 µg/mL; C3, 60 µg/mL for rAra h 1 and 100 µg/mL for rAra h 2-3. n = 30. **C**, Patients (%) with positive SPT and sIgE to recombinant allergens. n = 30.

material linked to its origin and conditions of production and storage, investigators are confronted with a lack of standardization of the material used both in skin and biological testing.^{9,10} The technology of recombinant allergens appears a promising way to produce biological material with consistent and standardized properties. All patients with peanut allergy included in this study reacted to rAra h 2, and no false positives were observed. Thus, this recombinant protein appears to be the most interesting candidate for use in the diagnosis of peanut allergy. The high sensitivity and specificity of SPT to rAra h 2 needs to be confirmed in a larger population. Skin reactivity to rAra h 2 at the concentration of 100 µg/mL is comparable to that to CPE. The higher responsiveness of SPT to native peanut compared with commercial extracts has been previously observed,²⁵ which could be explained either by the cross-reactivity with carbohydrate determinants and some pollen or vegetable allergens.

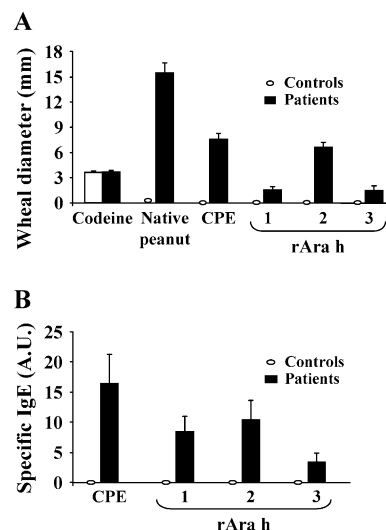


FIG 3. Reactivity to peanut extracts and peanut recombinant allergens. **A**, Wheal diameters (mm) obtained by SPT. rAra h 1, 60 µg/mL; rAra h 2 and 3, 100 µg/mL. **B**, Levels of sIgE determined by ELISA (n = 30 subjects/group).

Another main problem encountered with SPT to peanut is that positive SPT results to peanut are observed in pollen-sensitized patients.²⁶ In a recent study, Mortz et al²⁷ underlined the high rate of peanut sensitization associated with pollen sensitization. The rate of sensitization reaches a level of 5.8% for sIgE (CAP-System FEIA) and 3.4% for SPTs, whereas the diagnosis of true allergy is estimated at 0.5%. The authors show that 96% of these adolescents are sensitized to grass pollen and attribute the high rate of sensitization to peanut detected by sIgE and SPT to the cross-reactivity between peanut and pollen allergens.

Cross-reactivity between peanut and pollen can be a result of carbohydrate determinants or homology of allergenic proteins. This could explain the positive skin test to native peanut in 1 control subject sensitized to birch pollen. Indeed, cross-reactivity with birch pollen allergen (Bet v 1, Bet v 2) has been demonstrated with Ara h 5 and Ara h 8.²⁸ Recombinant allergens produced in *E coli* lack carbohydrate determinants, thereby eliminating the risk of false-positive results because of these determinants.²⁹ Moreover, the absence of carbohydrate determinants could affect refolding and thus the tertiary structure of recombinant proteins. This would modify the recognition of recombinant allergens by sIgE compared with the wild-type allergen. This could explain why there is no cross-sensitization with birch pollen allergen. The skin reactivity and recognition of recombinant allergens by sIgE is more reflective of epitope peptides, and it is not surprising that our findings agree with those of Beyer et al³⁰ and Shreffler et al¹² concerning clinical reactivity and severity.

One of the main problems for physicians is having diagnostic tools sufficiently effective to evaluate the severity of the disease without the use of oral food challenge tests that require a specialized clinical platform and that are time-consuming. Sampson¹⁴ has defined

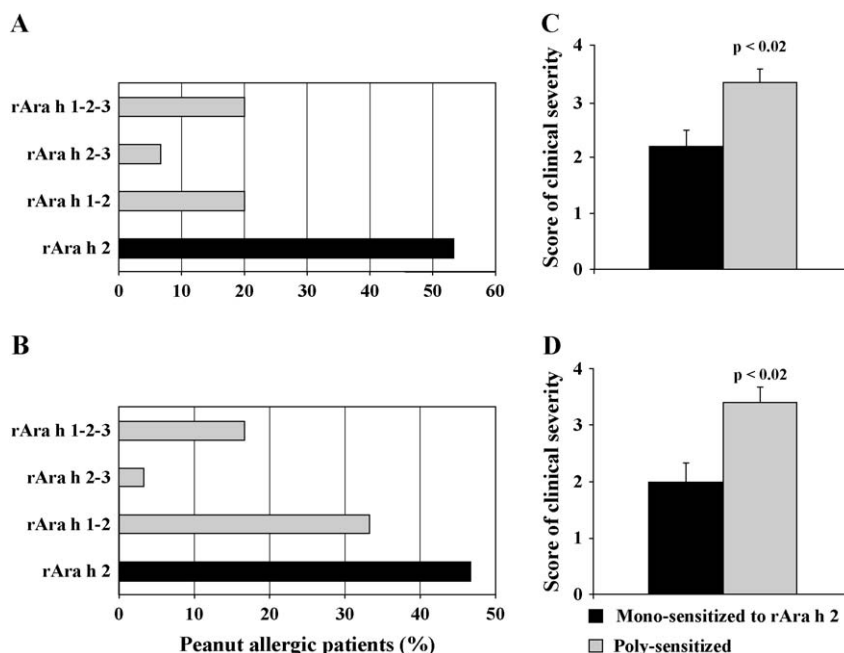


FIG 4. Spectrotypes of recombinant allergen reactivity and clinical score of patients monosensitized to rAra h 2 and polysensitized. Spectrotypes as defined by SPT (**A**) and by sIgE (**B**). Peanut allergy severity in rAra h 2 monosensitized and polysensitized patients as defined by SPT ($n = 16$ rAra h 2 monosensitized and $n = 14$ polysensitized patients; **C**) and sIgE ($n = 14$ rAra h 2 monosensitized and $n = 16$ polysensitized patients; **D**).

levels of sIgE with a positive predictive value of 95%. This threshold of sIgE is useful to avoid food challenge tests in patients with higher levels. The remaining problem is to determine whether a patient with a sIgE level less than 15 kU/L has or does not have a true allergy to peanut. In the same way, the threshold of diameter of SPTs with a predictive positive value of 95% has been defined as ≥ 8 mm for commercial extract.³¹ However, no relationship has been demonstrated between the size of SPT or sIgE and the severity of the disease. The fact that polysensitization could be associated with the severity of symptoms has previously been proposed by Beyer et al.³⁰ Using synthetic peptides, the authors demonstrated that the cumulative IgE binding to the peanut peptides was significantly higher in patients with peanut allergy than in tolerant patients. The recent study published by Lewis et al.³² showed that the total number of bands recognized by sIgE per patient is correlated with challenge score. Our current data fully support this conclusion: sensitization established either by SPT or sIgE to rAra h 1 and/or rAra h 3 in addition to rAra h 2 is associated with more severe symptoms. This observation offers the possibility of developing reagents predictive of disease severity that can be used by the practitioner. By bioinformatic annotation of sequences closely related to currently identified immunodominant allergenic epitopes,^{20,22,33} we located epitopes predominantly on Ara h 2, whereas Ara h 1 and Ara h 3 contained fewer of these. This could explain why all subjects with clinically proven peanut allergy indeed reacted to rAra h 2. Additional reactivity to others,

rAra h 1 and rAra h 3, is associated with high clinical scores, suggesting that disease severity is related to epitope spreading. These data are consistent with the higher epitope diversity found by Shreffler et al.¹² in patients with a history of more severe allergic reactions.

One of the main public health problems of FA to peanuts is its persistency: the spontaneous recovery rate is estimated between 18% and 21.5%,^{8,34} and the recurrence rate after recovery at 7.9%.³⁵ The severity of symptoms, the deterioration of the quality of life, the risk of recurrence, and the weak percentage of spontaneous recovery have prompted different research teams to develop therapeutic strategies other than avoidance diet and emergency kits containing self-injectable epinephrine.³⁶ The previous trials of immunotherapy using peanut extract increase the tolerance of patients with peanut allergy to oral ingestion of peanuts, but the high rate of repeated severe systemic reactions in most patients, even during maintenance injections, led to the cessation of this kind of treatment.³⁷ For allergy immunotherapy, a modified peanut product with reduced allergenic activity is needed.^{22,38} The use of recombinant allergens allows the definition of the spectrotype of sensitization of each patient and the design of individual immunotherapy adapted to their sIgE recognition profile without the injection of antigenic components not implicated in the allergic reaction. Immunotherapy trials using modified recombinant allergens selected for inducing a tolerance without adverse reaction can be envisioned. The knowledge of the profile of skin and biological recognition of recombinant allergens is the preamble to

the development of vaccines based on modified recombinant allergens and targeted on the basis of the spectrophotype of the patient.³⁹

We thank Christelle Belcourt and Ludivine Parisot for their excellent technical assistance. We thank Allerbio and Virginie Leduc for providing us with peanut protein extracts. We are most grateful to Dr Frances Yen Potin for very useful scientific discussions and her assistance in revision of the manuscript.

REFERENCES

- Kanny G, Moneret-Vautrin DA, Flabbee J, Beaudouin E, Morisset M, Thevenin F. Population study of food allergy in France. *J Allergy Clin Immunol* 2001;108:133-40.
- Sicherer SH, Munoz-Furlong A, Burks AW, Sampson HA. Prevalence of peanut and tree nut allergy in the US determined by a random digit dial telephone survey. *J Allergy Clin Immunol* 1999;103:559-62.
- Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001;107:191-3.
- Moneret-Vautrin DA, Morisset M, Flabbee J, Beaudouin E, Kanny G. Epidemiology of life-threatening and lethal anaphylaxis: a review. *Allergy* 2005;60:443-51.
- Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992;327:380-4.
- Moneret-Vautrin DA, Kanny G. Update on threshold doses of food allergens: implications for patients and the food industry. *Curr Opin Allergy Clin Immunol* 2004;4:215-9.
- Morisset M, Moneret-Vautrin DA, Kanny G, Guenard L, Beaudouin E, Flabbee J, et al. Thresholds of clinical reactivity to milk, egg, peanut and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. *Clin Exp Allergy* 2003;33:1046-51.
- Skolnick HS, Conover-Walker MK, Koerner CB, Sampson HA, Burks W, Wood RA. The natural history of peanut allergy. *J Allergy Clin Immunol* 2001;107:367-74.
- Akkerdaas JH, Wensing M, Knulst AC, Krebitz M, Breiteneder H, de Vries S, et al. How accurate and safe is the diagnosis of hazelnut allergy by means of commercial skin prick test reagents? *Int Arch Allergy Immunol* 2003;132:132-40.
- Hefle SL, Helm RM, Burks AW, Bush RK. Comparison of commercial peanut skin test extracts. *J Allergy Clin Immunol* 1995;95:837-42.
- Allergen Nomenclature—The Official "List of Allergens." W.H.O./I.U.I.S. Allergen Nomenclature Sub-Committee. 2006. Available at: <http://www.allergen.org>. Accessed March 15, 2006.
- Shreffler WG, Beyer K, Chu TH, Burks AW, Sampson HA. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *J Allergy Clin Immunol* 2004;113:776-82.
- Cromwell O, Suck R, Kahlert H, Nandy A, Weber B, Fiebig H. Transition of recombinant allergens from bench to clinical application. *Methods* 2004;32:300-12.
- Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001;107:891-6.
- Fleischer DM, Conover-Walker MK, Christie L, Burks AW, Wood RA. The natural progression of peanut allergy: resolution and the possibility of recurrence. *J Allergy Clin Immunol* 2003;112:183-9.
- Hourihane JO, Kilburn SA, Dean P, Warner JO. Clinical characteristics of peanut allergy. *Clin Exp Allergy* 1997;27:634-9.
- Moneret-Vautrin DA, Rance F, Kanny G, Olsewski A, Gueant JL, Dutau G, et al. Food allergy to peanuts in France: evaluation of 142 observations. *Clin Exp Allergy* 1998;28:1113-9.
- Ewan PW, Clark AT. Long-term prospective observational study of patients with peanut and nut allergy after participation in a management plan. *Lancet* 2001;357:111-5.
- Burks AW, Cockrell G, Stanley JS, Helm RM, Bannon GA. Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity. *J Clin Invest* 1995;96:1715-21.
- Rabjohn P, Helm EM, Stanley JS, West CM, Sampson HA, Burks AW, et al. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J Clin Invest* 1999;103:535-42.
- Rabjohn P, West CM, Connaughton C, Sampson HA, Helm RM, Burks AW, et al. Modification of peanut allergen Ara h 3: effects on IgE binding and T cell stimulation. *Int Arch Allergy Immunol* 2002;128:15-23.
- Stanley JS, King N, Burks AW, Huang SK, Sampson H, Cockrell G, et al. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch Biochem Biophys* 1997;342:244-53.
- Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- Dreborg S. Skin test in diagnosis of food allergy. *Allergy Proc* 1991;12:251-4.
- Rance F, Juchet A, Bremont F, Dutau G. Correlations between skin prick tests using commercial extracts and fresh foods, specific IgE, and food challenges. *Allergy* 1997;52:1031-5.
- van der Veen MJ, van Ree R, Aalberse RC, Akkerdaas J, Koppelman SJ, Jansen HM, et al. Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J Allergy Clin Immunol* 1997;100:327-34.
- Mortz CG, Andersen KE, Bindslev-Jensen C. The prevalence of peanut sensitization and the association to pollen sensitization in a cohort of unselected adolescents. The Odense Adolescence Cohort Study on Atopic Diseases and Dermatitis (TOACS). *Pediatr Allergy Immunol* 2005;16:501-6.
- Mittag D, Akkerdaas J, Ballmer-Weber BK, Vogel L, Wensing M, Becker WM, et al. Ara h 8, a Bet v 1-homologous allergen from peanut, is a major allergen in patients with combined birch pollen and peanut allergy. *J Allergy Clin Immunol* 2004;114:1410-7.
- van Ree R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. *Int Arch Allergy Immunol* 2002;129:189-97.
- Beyer K, Ellman-Grunther L, Jarvinen KM, Wood RA, Hourihane J, Sampson HA. Measurement of peptide-specific IgE as an additional tool in identifying patients with clinical reactivity to peanuts. *J Allergy Clin Immunol* 2003;112:202-7.
- Roberts G, Lack G. Diagnosing peanut allergy with skin prick and specific IgE testing. *J Allergy Clin Immunol* 2005;115:1291-6.
- Lewis SA, Grimshaw KE, Warner JO, Hourihane JO. The promiscuity of immunoglobulin E binding to peanut allergens, as determined by Western blotting, correlates with the severity of clinical symptoms. *Clin Exp Allergy* 2005;35:767-73.
- Burks AW, Shin D, Cockrell G, Stanley JS, Helm RM, Bannon GA. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. *Eur J Biochem* 1997;245:334-9.
- Hourihane JO, Roberts SA, Warner JO. Resolution of peanut allergy: case-control study. *BMJ* 1998;316:1271-5.
- Fleischer DM, Conover-Walker MK, Christie L, Burks AW, Wood RA. Peanut allergy: recurrence and its management. *J Allergy Clin Immunol* 2004;114:1195-201.
- Pons L, Palmer K, Burks W. Towards immunotherapy for peanut allergy. *Curr Opin Allergy Clin Immunol* 2005;5:558-62.
- Nelson HS, Lahr J, Rule R, Bock A, Leung D. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J Allergy Clin Immunol* 1997;99:744-51.
- King N, Helm R, Stanley JS, Vieths S, Luttkopf D, Hatahet L, et al. Allergenic characteristics of a modified peanut allergen. *Mol Nutr Food Res* 2005;49:963-71.
- Bannon GA, Cockrell G, Connaughton C, West CM, Helm R, Stanley JS, et al. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy. *Int Arch Allergy Immunol* 2001;124:70-2.