

Epitope specificity and immunoaffinity purification of the major peanut allergen, *Ara h* I

A. Wesley Burks, MD [\[MEDLINE LOOKUP\]](#)

Gael Cockrell, BS [\[MEDLINE LOOKUP\]](#)

Cathie Connaughton, BS [\[MEDLINE LOOKUP\]](#)

Ricki M. Helm, PhD [\[MEDLINE LOOKUP\]](#)

- Little Rock, Ark.

Sections

- [Abstract](#)
- [Methods](#)
- [Results](#)
- [Discussion](#)
- [References](#)
- [Publishing and Reprint Information](#)
- [Articles with References to this Article](#)

Abstract

The antigenic and allergenic structure of *Ara h* I, a major allergen of peanuts, was investigated with the use of seven monoclonal antibodies obtained from BALB/c mice immunized with purified *Ara h* I. Previous work with monoclonal antibodies produced to allergens has primarily been done with inhalant allergens. Only recently have the major allergens of various foods been determined so that investigations with monoclonal antibodies into the allergenic epitopes could begin. When used as a solid phase in an ELISA, these monoclonal antibodies captured peanut antigen, which bound human IgE from patients with positive results to challenges to peanuts. The *Ara h* I monoclonal antibodies were found to be specific for peanut antigens when binding for other legumes was examined. In ELISA inhibition studies with the monoclonal antibodies, we identified four different antigenic sites on *Ara h* I. In related studies with pooled human IgE serum from patients with positive results to challenges to peanuts, we identified three similar IgE-binding epitopes. As a means of purifying the *Ara h* I allergen, we prepared an immunoaffinity column with monoclonal antibody 8D9. We eluted from this column the allergen *Ara h* I, which had a mean molecular weight of 63.5 kd and which bound human IgE from individual and pooled serum of patients with peanut sensitivity. (J ALLERGY CLIN IMMUNOL 1994;93:743-50.)

DBPCFC	Double-blind, placebo-controlled, food challenge
PBS	Phosphate-buffered saline
SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

In the past several years multiple allergens, which stimulate IgE production and cause IgE-mediated disease in human beings, have been identified.¹ The identification and purification of these allergens is essential for the structural and immunologic studies necessary to understand how these molecules stimulate IgE antibody formation.¹ Significant information on identification and purification of inhaled allergens from a wide variety of sources including pollens, dust mites, animal danders, insects, and fungi has been accumulated.¹ In comparison, little scientific work has been completed on the food allergens that are known to cause IgE-mediated reactions.²⁻⁴

Peanuts are considered one of the most allergenic foods.⁵ Sensitive individuals may experience symptoms ranging from urticaria to anaphylaxis.⁵ Multiple cases of fatal and near-fatal anaphylaxis have been reported.^{6,7} The actual prevalence of peanut hypersensitivity is unknown, although one study suggests that up to 10% of allergic patients may be sensitive.⁸ This sensitivity appears early in life and in many patients, persists indefinitely.

Monoclonal antibodies have been used increasingly to define the allergenic portion of many allergens. Several allergens, including the dust mite allergen, *Der f I*⁹ and the grass allergen, *Lol p I*,¹⁰ have been studied with the use of monoclonal antibodies.

In this report we have investigated the epitope specificity of *Ara h I*¹¹ by using monoclonal antibodies as probes for mapping the antigenic determinants. We have produced and characterized a panel of monoclonal antibodies against purified *Ara h I*. The epitope specificities of these monoclonal antibodies allowed us to define at least four antigenic sites of *Ara h I*. Inhibition assays were used to determine the IgE-binding sites on *Ara h I*. Immunoaffinity chromatography with one of the monoclonal antibodies to *Ara h I* was then used to purify *Ara h I*.

Methods

Patients with positive results to peanut challenge

Approval for this study was obtained from the Human Use Committee at the University of Arkansas for Medical Sciences. Nine patients (mean age, 4.2 years) with atopic dermatitis and a positive immediate prick skin test result to peanut had either a positive double-blind, placebo-controlled food challenge (DBPCFC) to peanut or a convincing history of peanut anaphylaxis (the allergic reaction was potentially life-threatening, i.e., laryngeal edema, severe wheezing, and/or hypotension). Details of the challenge procedure and interpretation have been previously discussed.¹¹ Five milliliters of venous blood was drawn from each patient and allowed to clot, and the serum was collected. An equal volume of serum from each donor was mixed to prepare a nine-person peanut-specific IgE antibody pool.

Monoclonal antibodies

Mouse hybridoma cell lines were prepared by standard hypoxanthine, aminopterin, and thymidine selection after polyethylene glycol-mediated cell fusion as described by de St. Groth and Scheidegger.¹² P3×63-Ag8.653 mouse/myeloma cells were fused with immune splenocytes from female BALB/c mice hyperimmunized with *Ara h I*.¹¹ Hybridoma cell supernatants were screened by ELISA, and cell lines were cloned by limiting dilution.¹³ The antibodies secreted by the monoclonal hybridoma cell lines were isotyped according to the directions provided (ScreenType, Boehringer Mannheim, Indianapolis, Ind.). Ascites fluid produced in pristane-primed BALB/c mice was purified with Protein C Superose, as outlined by the manufacturer (Pharmacia, Uppsala, Sweden). Purified monoclonal antibodies from selected cell lines were used in an ELISA, an ELISA inhibition, and an immunoblot analysis for affinity purification of *Ara h I*.

ELISA

A biotin-avidin ELISA was developed to quantify anti-peanut (IgE) protein antibodies with modifications from an assay described previously.¹⁴ The upper two rows of a 96-well microtiter plate (Gibco, Santa Clara, Calif.) were coated with 100 µl each of equal amounts (1 µg/ml) of anti-human IgE monoclonal antibodies 7.12 and 4.15 (kindly provided by Dr. A. Saxon) in coating buffer (0.1 mol/L sodium carbonate–bicarbonate buffer, pH 9.5). The remainder of the plate was coated with 100 µl of *Ara h I* at a concentration of 1 µg/ml in coating

buffer. The plate was incubated at 37° C for 1 hour and was washed five times with rinse buffer (phosphate-buffered saline [PBS], pH 7.4, containing 0.05% Tween 20 [Sigma Chemical Co., St. Louis, Mo.]) immediately and between subsequent incubations. In the upper two rows, a secondary IgE reference standard, ranging from 0.05 to 25 ng/ml, was used to generate a curve for IgE.

The peanut challenge-positive serum pool and patients' serum samples were diluted (1:20 vol/vol bovine serum albumin) and dispensed in duplicate in the lower portion of the plate. After incubation for 1 hour at 37° C and washing, biotinylated, affinity-purified goat anti-human IgE (KPL, Gaithersburg, Md.) (1:2500 vol/vol bovine serum albumin) was added to all wells. Plates were incubated for 1 hour at 37° C and washed; 100 µl of horseradish peroxidase-avidin conjugate (1:2500 vol/vol PBS) (Vector Laboratories, Burlingame, Calif.) was added for 5 minutes. After washing, the plates were developed by the addition of a buffer containing o-phenylenediamine (Sigma). The reaction was stopped by the addition of 100 µl 2N-hydrochloric acid to each well, and absorbance was read at 492 nm with a Microplate Reader (model 450; Bio-Rad Laboratories, Chemical Div., Richmond, Calif.). The standard curve was plotted on logit graph by means of simple linear regression analysis, and the antigen-specific values for the pool and for individual patients were read from the curve as a percent of the peanut-positive antibody pool.

An indirect ELISA was used to determine the ability of the various monoclonal antibody preparations to capture peanut antigen that could bind human IgE directed toward *Ara h* I. In a 96-well microtiter plate (Gibco) 100 µl of the monoclonal antibody (at varying concentrations) was incubated in coating buffer (carbonate buffer, pH 9.6) for 1 hour at 37° C. After washing, crude peanut extract was added in diluent buffer (2% bovine serum albumin and 0.05% Tween 20) for 1 hour at 37° C. Next, human serum containing IgE antibodies to *Ara h* I was added (either the pooled peanut-positive serum or serum from individual patients) for 1 hour at 37° C. After washing, biotinylated, affinity-purified goat anti-human IgE was added for 1 hour at 37° C. The plate was then developed as in the ELISA described previously.

ELISA inhibition

An ELISA inhibition assay was developed to examine the site specificity of the monoclonal antibodies generated to *Ara h* I. One hundred microliters of *Ara h* I protein (1 mg/ml) was added to each well of a 96-well microtiter plate (Gibco) in coating buffer (carbonate buffer, pH 9.6) for 1 hour at 37° C. Next 100 µl of differing concentrations (up to 1000 times excess) of the seven monoclonal antibodies was added to each well for 1 hour at 37° C. After washing, a standard concentration of the biotinylated monoclonal antibody preparation was added for 1 hour at 37° C. The assay was developed by the addition of the avidin substrate as in the ELISA described previously.

A similar ELISA inhibition was performed with the peanut-positive serum IgE pool instead of the biotinylated monoclonal antibody to determine the ability of each monoclonal antibody to block specific IgE binding.

Preparation of anti-peanut-specific IgE₁ immunoaffinity columns

Purified monoclonal antibody preparations from four cell lines were used to prepare immunoaffinity columns. Ten grams of freeze-dried cyanogen bromide-activated Sepharose (Sigma) was swollen and washed in 2 L of 1 mmol/L HCl for 2 hours at room temperature. Swollen beads were collected in a scintered glass funnel and washed two times with an additional 2 L of 1 mol/L HCl to form a moist cake. The activated beads were then added to a monoclonal antibody solution (5 to 10 mg protein per milliliter of gel) dissolved in coupling buffer (0.1 mol/L NaHCO₃, 0.5 mol/L NaCl, pH 8.3) at room temperature for 2 hours. The unbound supernatant fraction was collected by centrifugation (1500 rpm for 10 minutes) and saved for residual antibody concentration analysis. The antibody-coupled gel was mixed with 100 ml 0.2 mol/L glycine at room temperature for 2 hours. The unbound supernatant fraction was again separated from the gel by centrifugation and saved for analysis. The immunoaffinity gel was then equilibrated with digestion buffer (1 mol/L NaCl, 20 mmol/L NaH₂PO₄). All supernatant fractions used to prepare the immunoaffinity column were analyzed for antibody (280 nm absorption), and the binding efficiency was determined by subtracting the unbound concentration from the total applied antibody concentration divided by total antibody times 100%.

Affinity purification of *Ara h* I

One hundred milliliters of crude peanut extract (10 to 20 mg/ml) in digestion buffer was added (2 ml/min) to a peanut-specific immunoaffinity gel column (160 × 30 mm) incorporated into the fast protein liquid

chromatography system (Pharmacia). Digestion buffer was passed through the column until the protein absorption (280 nm) reached baseline. *Ara h* I was eluted with 100 mmol/L triethylamine (pH 11.5) at a flow rate of 1 ml/min into test tubes containing 100 μ l of 1 mol/L NaH₂PO₄ buffer to neutralize the eluate. *Ara h* I eluted in this manner from multiple runs was pooled, dialyzed against 100 mmol/L ammonium bicarbonate buffer, and lyophilized for storage before analysis.

Electrophoresis and immunoblotting

The electrophoresis procedure¹⁵ was a modification of that of Sutton et al.¹⁶ Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with a 12.5% polyacrylamide separating gel and a stacking gel of 3%. Twenty microliters of a 1 mg/ml solution of each protein was applied to each well. Electrophoresis was performed for 4 hours at 0.030 A per gel (E-C Apparatus Corp., St. Petersburg, Fla.) for the 14 × 12 cm gels. To assure proper protein separation and visualization, Coomassie Brilliant Blue (Imperial Chemical Industries, Ltd., Macclesfield, Cheshire, England) stains were done on gels.

Proteins were transferred¹⁷ from the separating gel to a nitrocellulose membrane in a transfer buffer (tris-glycine) with 10% sodium dodecylsulfate and 40% methanol. The procedure was done in a transblot apparatus (Hoefer Scientific Instruments, San Francisco, Calif.) for 30 to 60 minutes (0.15 A). An amido black stain (Sigma) was done to assure transfer of the protein.

After removal from the transblot apparatus, the nitrocellulose was placed in blocking solution overnight (0.5% gelatin, 0.05% Tween 20, thimerosal). The nitrocellulose blot was then washed three times with rinse buffer (PBS with 0.05% Tween 20) and incubated with the pooled serum (1:20 vol/vol) overnight at 4° C with shaking. After washing again with PBS three times, alkaline phosphatase–conjugated goat anti-human IgE (1:1000 vol/vol PBS, 0.5% gelatin, thimerosal; KPL) was added and incubated at room temperature with shaking for 2 hours. After washing with PBS three times, the blot was developed by the addition of 250 μ l nitroblue tetrazolium (Bio-Rad) and 250 μ l 5-bromo-4-chloro-3-indolyl phosphate (Bio-Rad) solutions in 25 ml carbonate buffer (BioRad) at room temperature for 15 minutes. The reaction was then stopped by decanting the nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate solution and incubating the nitrocellulose for 10 minutes with distilled water. The blot was then air dried.

Similar immunoblots were used for the screening of the monoclonal antibodies for *Ara h* I. After the nitrocellulose was removed from the transblot apparatus and placed in blocking solution overnight, the supernatant from the hybridoma-secreting cell line was incubated overnight at 4° C with shaking. The rest of the development procedure was identical to that for the IgE immunoblot.

Results

Hybridomas specific for *Ara h* I

Cell fusions between spleen cells obtained from female BALB/c mice immunized with *Ara h* I and the mouse myeloma cells resulted in a series of hybridomas specific for *Ara h* I. Thirteen monoclonal antibody–producing lines, which originated in five separate microtiter wells during the initial plating, were chosen for further study. In preliminary studies all 13 hybridoma-secreting cell lines had antibodies that bound *Ara h* I, as determined by ELISA and immunoblot analysis. On the basis of different binding studies, seven of these hybridoma cell lines were chosen for further studies. As determined by isotype immunoglobulin-specific ELISA, all seven hybridoma-secreting cell lines typed as IgG₁.

ELISA with monoclonal antibody as solid phase

Seven monoclonal antibody preparations, 8D9, 8F10, 2E9, 7B3, 1B6, 6B5, and 6F9, were used as capture antibodies in an ELISA with *Ara h* I as the antigen. Serum from individual patients, who had positive challenge results to peanut, was used to determine the amount of IgE binding to each peanut fraction captured by each specific monoclonal antibody (Table I). A reference peanut-positive pool was used as the control serum for 100% binding. Six patients were chosen who had positive DBPCFCs to peanut. All six patients had elevated levels of anti-peanut–specific IgE to the peanut antigen presented by each of the seven monoclonal antibodies compared with the control sera (no. 7, patient without peanut sensitivity who

had elevated serum IgE values; no. 8, patient without peanut sensitivity who had normal serum IgE levels). Titration curves were performed to show that limited amounts of antigen binding were not responsible for similar antibody binding. The amount of anti-peanut-specific IgE antibody to each peanut antigen presented individually by each monoclonal antibody did not differ significantly. However, there were individual patient differences in response to each set of peanut antigens presented by the monoclonal antibodies.

TABLE I. Peanut-specific IgE to antigen presented by seven monoclonal antibodies

Patient No.	<u>Capture antibody (%)</u>						
	8D9	8F10	2E9	7B3	1B6	6B5	6F9
1	38	28	35	32	188	214	121
2	228	156	282	164	75	148	240
3	61	82	38	27	36	68	84
4	18	14	13	13	7	6	17
5	21	24	38	23	86	155	62
6	57	71	56	64	45	87	124
7	7	7	0	0	0	0	10
8	7	1	4	3	0	1	9

Ara h I monoclonal antibodies used as capture antibodies in ELISA with *Ara h I* as the antigen. Values are expressed as a percent of binding compared with challenge-positive peanut pool. Patients 1 to 6 had positive DBPCFC results to peanut; patient 7 is the patient without peanut sensitivity with elevated serum IgE; patient 8 is the patient without peanut sensitivity with normal serum IgE.

Food antigen specificity of monoclonal antibodies to *Ara h I*

To determine the specificity of the monoclonal antibodies prepared against *Ara h I* to detect peanut antigen, an ELISA was developed with the pooled peanut-specific IgE (from patients who had positive DBPCFC results to peanut). All seven monoclonal antibodies that were fully characterized bound only peanut antigen (Table II shows four of these monoclonal antibodies to *Ara h I*). There was minimal binding in the ELISA to peas and chick peas but none to soy, green beans, lima beans, or ovalbumin. When the normal serum pool was used in this ELISA, no peanut-specific IgE could be detected to either *Ara h I* or crude peanut extract.

TABLE II. IgE-specific binding to legumes captured by *Ara h I* monoclonal antibodies

Protein	<u>Capture antibody</u>			
	8D9	8F10	2E9	7B3
Pooled challenge-positive peanut serum				
63.d kd (<i>Ara h I</i>)	0.834	0.846	0.903	0.884
Crude peanut	0.854	0.868	0.875	0.883
Soy	0.125	0.132	0.133	0.122
Peas	0.254	0.231	0.256	0.233
Chick peas	0.238	0.196	0.198	0.244

Green beans	0.096	0.145	0.138	0.122
Lima beans	0.121	0.098	0.093	0.126
Ovalbumin	0.092	0.139	0.127	0.131
Normal serum				
Crude peanut	0.122	0.094	0.125	0.099
63.5 kd	0.131	0.096	0.092	0.126

Ara h I monoclonal antibodies used as capture antibodies in ELISA with various legumes as the antigen. Values are expressed as optical density units.

In the United States three varieties of peanuts are commonly consumed — Virginia, Spanish, and Runner. In a similar ELISA, we attempted to determine whether there were differences in monoclonal antibody binding to the three varieties of peanuts. There was only a minor variation in the ability of the peanut-specific IgE to bind to the captured peanut antigen (data not shown).

Site specificity of seven hybridoma antibodies

An ELISA inhibition assay was used to determine the site specificity of the seven monoclonal antibodies to *Ara h* I. As determined by ELISA inhibition analysis there are at least four different epitopes, which could be recognized by the various monoclonal antibodies (■Table III). ■Fig. 1 depicts a schematic that incorporates the ELISA inhibition data to suggest at least four different epitopes on this allergen.

FIG. 1. (a) Site specificity of seven *Ara h* I monoclonal antibodies as shown in ■Table III. A to D represent four distinct IgG epitopes on *Ara h* I. **(b)** Site specificity of peanut-positive IgE pool with seven *Ara h* I monoclonal antibodies as shown in ■Fig. 2. X - Z represent three distinct IgE epitopes on *Ara h* I.

(a)	A	B	C	D
	8F10	8D9 2E9 1B6 7B3	7B3	6F9
(b)	X	Y		Z
	8F10	8D9 6B5		6F9

TABLE III. ELISA inhibition for seven monoclonal antibodies to *Ara h I*

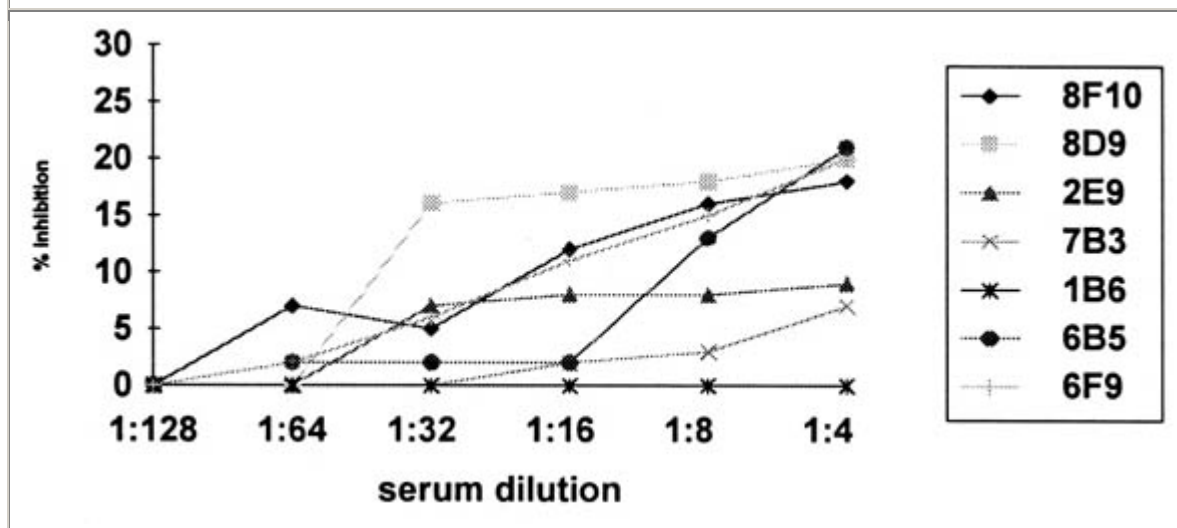
	Inhibiting antibody (%)							Alt 1
	8F10	8D9	2E9	7B3	1B6	6B5	6F9	
	Biotinylated monoclonal antibody							
8F10	71	10	11	11	2	5	5	0
8D9	31	82	34	0	28	26	5	0
2E9	26	35	53	15	29	27	10	0
7B3	22	4	0	50	16	13	10	0
1B6	0	43	39	0	55	34	6	0
6B5	22	52	35	18	52	75	8	0
6F9	20	20	12	12	35	27	54	0

Site specificity of seven *Ara h I* monoclonal antibodies as determined by ELISA inhibition analysis. Values are expressed as percent inhibition.

Site specificity of human IgE

Results of inhibition assays with monoclonal antibodies to inhibit IgE binding to *Ara h I* are shown in Fig. 2. Monoclonal antibodies 8F10, 8D9, 6B5, and 6F9 showed significant inhibition to IgE binding. These three inhibition sites correspond with three of the four different IgG epitopes recognized by the monoclonal antibodies in the inhibition experiments (Fig. 1).

FIG. 2. Site specificity of seven *Ara h I* monoclonal antibodies inhibiting anti-peanut-specific IgE binding to *Ara h I*. Values are expressed as percent of anti-peanut-specific IgE binding to *Ara h I* without inhibiting monoclonal antibody.

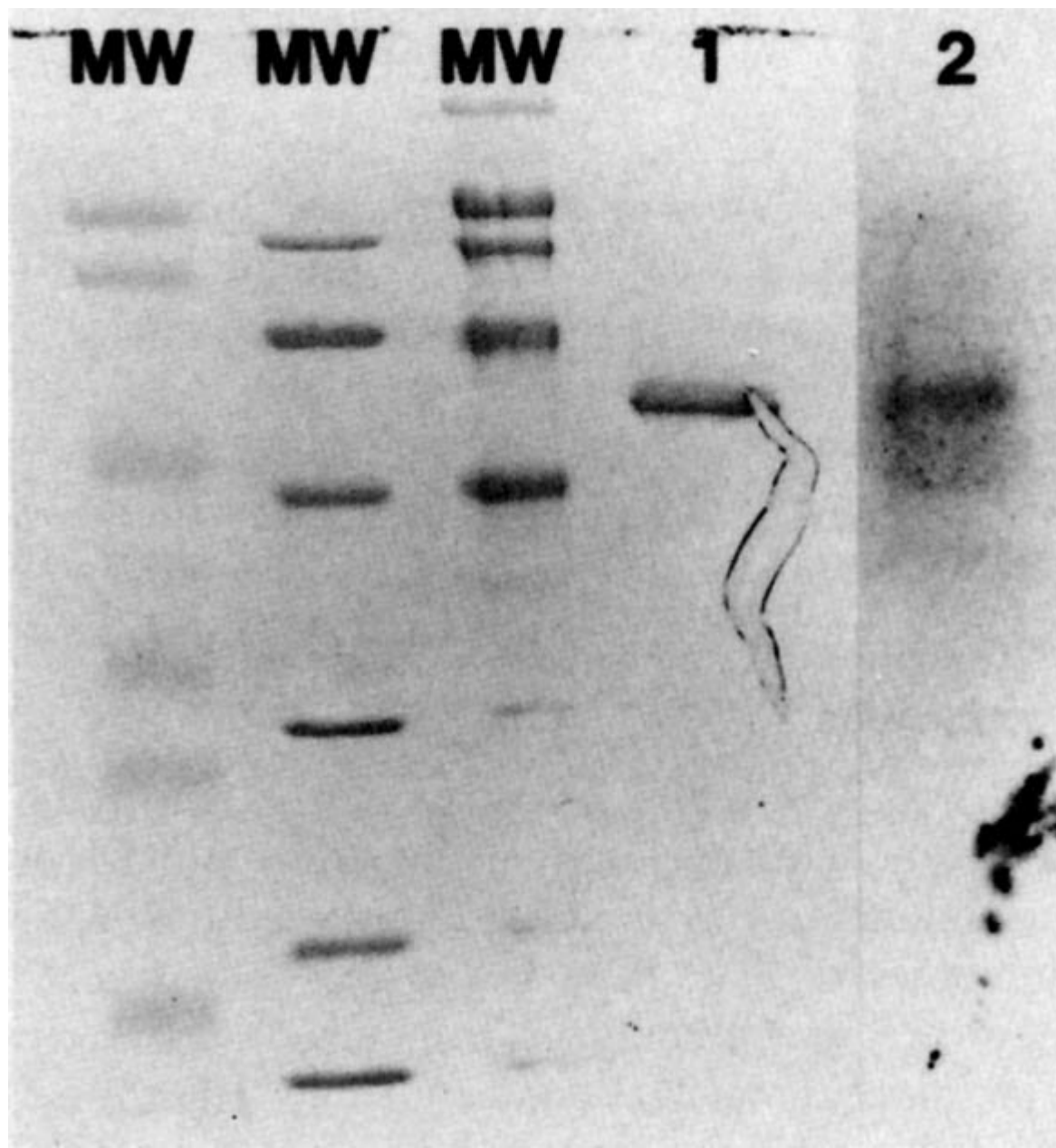


Immunoaffinity purification of *Ara h I*

A crude Florunner peanut extract was passed through an affinity column with monoclonal antibody 8D9 coupled as the immunoabsorbent. After extensive washing, the bound allergen was eluted with 100 mmol/L triethylamine, pH 11.5. This single-step purification resulted in an allergen of more than 90% purity on SDS-PAGE (Fig. 3). The eluted fraction had one major band on SDS-PAGE at a molecular weight of 63.5 kd. This eluted allergen was comparable in molecular weight to the original fast protein liquid chromatography–

purified *Ara h* allergen. An IgE-specific immunoblot was done to ensure that this eluted allergen bound IgE from the pooled peanut-specific IgE serum (■Fig. 3). The eluted allergen bound IgE from the peanut-positive pool in an IgE immunoblot. IgE from patients with elevated serum IgE values who were not sensitive to peanuts and from patients with normal serum IgE values did not bind to this allergen (picture not shown).

FIG. 3. SDS-PAGE of eluted *Ara h* I allergen from immunoaffinity column (*lane 1*) and IgE immunoblot of same allergen with challenge-positive peanut serum pool (*lane 2*). MW, Molecular weight markers (*left lane, top to bottom*) - 106,000 d; 80,000 d; 49,500 d; 32,500 d; 27,500 d; 18,500 d.



Anti-peanut-specific IgE ELISA with eluted allergen

An IgE-specific ELISA with the individual peanut-specific IgE serum was developed to examine the eluted allergen from the monoclonal antibody column. The six patients who had positive DBPCFC results to peanut had measurable amounts of anti-peanut-specific IgE in their serum, whereas two patients, who had an elevated serum IgE level but were not sensitive to peanut, had no detectable levels of anti-peanut IgE (■ Table IV).

TABLE IV. Individual anti-peanut-specific IgE binding to eluted *Ara h I*

Patient No.	Percent of peanut-positive pool
1	250
2	537
3	375
4	859
5	188
6	176
7	ND
8	ND

Individual anti-peanut-specific IgE values to eluted *Ara h I* allergen expressed as a percent of binding compared with the challenge-positive peanut serum pool. Patients 1 to 6 had positive DBPCFC results to peanut; patient 7 is the patient without peanut sensitivity with elevated serum IgE; patient 8 is the patient without peanut sensitivity with normal serum IgE.

ND, Not determined

Discussion

Individuals are genetically prone to produce IgE to specific antigens and to have allergic disease. No known features, which distinguish allergens as unique antigens, have been identified.¹⁸ The route of allergen administration, the dosage, the frequency of exposure, and genetic factors all determine the type and severity of an individual's allergic response. Three distinct foods (milk, eggs, and peanuts) account for approximately 80% of positive food challenge results in children.¹⁹⁻²¹

Two major allergens, *Ara h I* and *Ara h II* have recently been identified and purified from peanuts.^{11, 22} The first allergen, *Ara h I*, has two major bands, as determined by SDS-PAGE, with a mean molecular weight of 63.5 kd and an isoelectric point of 4.55. The second allergen, *Ara h II*, has a mean molecular weight of 17 kd and an isoelectric point of 5.2.

In this study seven monoclonal antibodies to *Ara h I* were extensively characterized. All seven monoclonal antibodies produced to *Ara h I*, when used as capture antibodies in an ELISA, presented antigens that bound IgE from patients with positive challenge results to peanut. There were no significant differences in the binding of IgE from any one patient to the allergen presented by any monoclonal antibody. When used in separate ELISA experiments, these monoclonal antibodies did not bind to other legume allergens, except for minimal binding to peas and chick peas (e.g., soy, green beans). This IgE binding to the pea and chick pea allergens presented by the peanut monoclonal antibodies may give us some important information about the IgE-binding sites in future experiments. Also, the monoclonal antibodies did not bind to one variety of peanuts preferentially over another.

To determine the site specificity of these monoclonal antibodies ELISA inhibition assays were done. At least four different and distinct IgG epitopes could be identified in the experiments with the allergen *Ara h I*. Similarly, in the IgE inhibition experiments there were three recognizable and distinct IgE epitopes. Monoclonal antibody 8F10 appeared to inhibit IgE binding significantly more than the other monoclonal antibodies, but there was still considerable inhibition at the other two IgE epitope sites. The results of this study are not unlike those of a study on *Der f I* in which at least three different epitopes were identified by monoclonal antibody analysis.²³ Chapman et al.²³ defined five nonoverlapping antigenic sites on *Der p I* by using monoclonal antibody technology. In similar studies monoclonal antibodies have been shown to bind to closely related epitopes on *Dac g I* and *Lol p I*.^{10, 24} Because of the small amounts of IgE antibody in sera

from allergic patients and the polyclonal nature of the response, the use monoclonal antibodies as probes to investigate epitopes recognized by IgE antibodies has increased.¹ A variety of solid-phase inhibition assays has been used; however, the interpretation of what level of inhibition should be regarded as significant has varied from 15% to 80%.¹ Because of the state of technology it has been difficult to assess how binding of the monoclonal antibody influences IgE binding and to what extent steric hindrance is involved. Consequently, these studies have some value in identifying monoclonal antibodies, which may bind at or near human antibody binding sites.¹

To further define the allergen *Ara h I* and to determine methods that would allow faster purification, an immunoaffinity column was prepared with monoclonal antibody 8D9. This immunoaffinity column eluted the 63.5 kd peanut allergen, which then bound IgE from our pooled peanut-positive serum. Several allergens, including *Fel d I* and *Der f III*, have been isolated with immunoaffinity columns.¹ Monoclonal antibody affinity chromatography has been used to purify milligram amounts of dust mite, cat, and pollen allergens in an essentially single-step procedure, without loss of antigenic reactivity.¹ The development of this technique has made purification of allergens from heterogenous source material with low allergen content easier. For some investigators the production of monoclonal antibodies to a crude allergen has been the first step in purification of the allergens from that mixture. Cockroach was one of the first allergens to be purified by this process.¹

Future studies on the antigenic and allergenic structure of allergens will encompass not only monoclonal antibody techniques but recombinant DNA technology. The cloning of allergens will allow us to determine structure-function relationships by various techniques including the production of overlapping peptides. Monoclonal antibody production to the allergens will be a significant part of this process. Recombinant DNA technology and monoclonal antibody production in combination will be used to examine the role of the T cell in the induction and regulation of the allergic response.²⁵

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Elide A. Pastorello, MD^a, Amedeo Conti, BSc^c, Valerio Pravettoni, MD^a, Laura Farioli, BSc^a, Federica Rivolta, MD^a, Raffaella Ansaloni, MD^b, Marco Spano, MD^b, Cristoforo Incorvaia, MD^a, Maria Gabriella Giuffrida, BSc^c, Claudio Ortolani, MD^b

Milan and Torino, Italy