

Identification of unique peanut and soy allergens in sera adsorbed with cross-reacting antibodies

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Background: Soybean and peanut are members of the legume family and share several common antigenic fractions. Patients allergic to one of these foods have serum IgE antibodies that immunologically cross-react with other legumes. Nevertheless, ingestion of other legumes generally does not induce an allergic reaction, suggesting that cross-reacting antibodies are not clinically relevant.

Objective: This study was designed to identify unique peanut or soybean antigenic fractions, with sera adsorbed to remove cross-reacting antibodies, thus resulting in sera with IgE antibodies unique to either soy or peanut.

Methods: Cross-reacting antibodies to soy were removed from the sera of two patients allergic to peanut and soy and three patients allergic to peanut by soy-affinity chromatography. Cross-reacting antibodies to peanut were adsorbed from the sera of a patient allergic to peanut and soy and a patient allergic to peanut by peanut-affinity chromatography. Adequate removal of cross-reacting antibodies was verified by ELISA after each adsorption step. Unadsorbed sera and sera adsorbed to remove cross-reacting antibodies (either to soy or to peanut) were assayed for specific IgE binding to peanut or soy immunoblots.

Results: Unique peanut-specific IgE antibodies (i.e., soy antibody-adsorbed) were found to bind to peanut fractions at 46, 29, 25, 19, 17, 14, and 5 kd on immunoblots of whole peanut protein. Similarly, unique soy-specific IgE (i.e., peanut antibody-adsorbed) were found to bind a fraction at 46 kd, and to a lesser extent, to a fraction at 21 kd on immunoblots of whole soy protein. The 73% reduction of IgE antibody binding to peanut by ELISA after adsorption of cross-reacting antibodies indicates extensive cross-reactivity between soy and peanut antigens.

Conclusions: Antigen-affinity chromatography is an effective method for removal of cross-reacting antibodies. We identified IgE antibody binding (with sera where cross-reacting antibodies were removed) to several unique antigenic fractions of peanut and soy. Further studies will determine the clinical significance of these fractions in IgE-mediated food hypersensitivity reactions. (*J Allergy Clin Immunol* 1996;98:969-78.)

Key words: Food hypersensitivity, peanut, soybean, legumes, cross-reactivity, affinity chromatography

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Received for publication Dec. 4, 1995; accepted for publication Apr. 23, 1996.

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0091-6749/96 \$5.00 + 0 1/174664

Abbreviations used

PBS:	Phosphate-buffered saline
PBS-T:	PBS + 0.05% Tween
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

Peanut and soybean are among the most prevalent allergens in children with food hypersensitivity.^{1,2} Proteins from both plants may be found in an increasing number of processed food products, resulting in a significant increase of possible early

exposure and sensitization in children. Although both plants belong to the same botanical family, differences in clinical reactions can be observed. Patients allergic to peanut usually have a life-long sensitivity and are more often at risk for fatal or near-fatal anaphylactic reactions.^{3,4} Reactions to soy may be non-IgE-mediated or IgE-mediated and are usually outgrown in a majority of patients.^{5,6}

Extensive *in vitro* cross-reactivity has been demonstrated among different legumes. In a previous study we reported that cross-reacting antibodies to other legumes could be found in sera of patients with a positive double-blind, placebo-controlled food challenge response to one member of the legume family.⁷ Common protein fractions were recognized by cross-reacting antibodies in several legumes (peanut, soybean, lima bean, pea, garbanzo bean, and green bean). More specifically, a prominent band at 20 kd was identified in all legumes except green bean. Nevertheless, major allergenic fractions have been identified in peanut and soybean. Peanut-I was isolated by chromatography and sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and was described as the first major peanut allergen.⁸ More recently, Ara h 1, a peanut protein fraction at 63.5 kd, was identified by ELISA and IgE immunoblots and was isolated and cloned. It is believed to be a major peanut allergen in children with food allergy and atopic dermatitis.⁹ Ara h 2 has also been classified as a major peanut allergen; it is composed of two closely migrating bands with a mean molecular weight of 17 kd.¹⁰ Other peanut fractions from different commercial peanut extracts have been identified¹¹; however, more relevant allergenic fractions of peanut may still be unrecognized. Although major allergens of soybeans have not been as well characterized as peanut allergens, two relevant fractions (7S and 11S) have been isolated by ultracentrifugation from crude soybean proteins. In one study serum IgE antibodies from children allergic to soy showed a similar affinity to both fractions,¹² whereas in another study IgE antibodies bound strongly to a unique 30 kd protein from the 7S-globulin fraction.¹³ Immunoblotting with sera from adults allergic to both peanut and soy showed binding to several protein fractions between 50 and 60 kd, whereas IgE from individuals allergic to soybeans strongly bound to a unique 20 kd allergenic fraction.¹⁴

The extensive cross-reactivity among the legumes raises some doubt about the clinical specificity of the major allergens. We hypothesized that

the removal of cross-reacting antibodies from the sera of patients allergic to soy and peanut by affinity chromatography may allow the identification of unique allergenic fractions of soy and peanut. Cross-reacting antibodies to soy were removed from the sera of children allergic to peanut and soy by soy-affinity chromatography. Similarly, cross-reacting antibodies to peanut were adsorbed by peanut-affinity chromatography. IgE antibodies from soy-adsorbed sera or peanut-adsorbed sera permitted us to identify antigens unique to peanut and soy on immunoblots electrotransferred from tricine-SDS polyacrylamide gels.

METHODS

Patients

Sera were obtained from five children with allergy (5 to 17 years of age) with a history of anaphylactic reactions to peanuts and with high levels of peanut-specific serum IgE antibodies (CAP-RAST FEIA; Pharmacia Diagnostics, Evansville, Ind.). Two patients (BP and BM) also were first seen with symptoms of IgE-mediated soy allergy. None of the patients were reactive to any other legume. In addition to food allergies, two patients had atopic dermatitis, asthma, and allergic rhinitis; one patient had atopic dermatitis and asthma; and one patient had asthma.

Preparation of soy and peanut extracts

Thirty grams of soy flour were incubated in 150 ml of phosphate-buffered saline (PBS) overnight on a rotating platform in a cold room at 4° C. The soy mixture was then clarified by centrifugation at 1000 g, and the supernatant was removed and lyophilized. The protein content was determined by Coomassie Plus Protein Assay Reagent (Pierce Chemical Co., Rockford, Ill.) The peanut extract was obtained from three commercial lots of Florunner peanuts and processed as previously described.⁹

Antigen-specific serum antibody adsorption

Forty milligrams of soy protein was added to 10 ml of active ester agarose gel (Affi-gel 10; Bio-Rad, Richmond, Calif.) and placed on a rocking platform for 4 hours at 4° C. One milliliter of 0.1 mol/L ethanolamine HCl (pH 8.0) was then added to the gel and mixed by rotation for 1 hour. A 1.0 × 10 cm chromatography column (Bio-Rad) was packed with the affinity gel. Three milliliters of patient serum was added to the soy-affinity column for removal of antibodies with affinity to soy protein. After 1 to 2 hours of exposure time, the column was rinsed with 30 ml of PBS. The first 6 ml of the adsorbed serum was collected and concentrated to the initial volume of 3 ml on a Centriprep 30 concentrator (Amicon, Beverly, Mass.) at 1200 g for 30 minutes. The column was then rinsed with 30 ml of 0.01 mol/L sodium phosphate (pH 12.5) to elute soy-bound antibodies.

TABLE I. Concentrations of serum IgE antibodies determined by CAP-RAST to peanut and soy for the five patients studied

Concentration of IgE antibodies	BP	BM	DH	AT	DT
Peanut (IU/ml)	2405	1995	357	1560	2225
Soy (IU/ml)	68	77	6.6	27	88

Normal values are less than 0.35 IU/ml. Patients BP and BM are allergic to peanut and soy; patients DH, AT, and DT react to peanut exclusively.

ies. The soy-adsorbed serum was again run over the soy-affinity column, and the rinsing and eluting were repeated until no soy-binding activity was detectable by ELISA. The complete procedure was repeated with sera from each of the five patients. In addition, one patient's serum sample (DT) was run over an Affi-gel column containing human serum albumin to control for nonspecific binding to the column or possible denaturation of the protein during the procedure.

Similarly, an affinity column was generated with 40 mg of peanut antigen and active ester agarose gel. The same adsorption and elution procedures were repeated with sera from a patient allergic to peanut and soy (BP), and a soy-tolerant patient with peanut allergy (DT) by peanut-affinity chromatography.

ELISA for IgE

After each passage of sera over the soy- or peanut-affinity chromatography columns, the presence of soy- and peanut-specific IgE was monitored by ELISA and by CAP-RAST FEIA. Furthermore, specific IgE antibodies to Ara h 1 and Ara h 2 were determined in unadsorbed sera and sera from which soy-specific antibodies had been removed. Two rows of 96-well microtiter plates (Dynatech Laboratories, Chantilly, Va.) were filled with 50 μ l of a solution of soy or peanut antigen (10 μ g/ml) in coating buffer (0.1 mol/L sodium bicarbonate, pH 9.6) and incubated overnight at 4° C. Fifty microliters of sera and adsorbed sera (at 1:5 dilution) in antibody buffer (PBS + 0.05% Tween [PBS-T] + 2% bovine serum albumin) were incubated for 2 hours at room temperature after the plates were washed with PBS-T buffer. After incubation, plates were washed, and 50 μ l of a solution of biotin-conjugated goat anti-human IgE (0.625 μ g/ml; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) in antibody buffer was added and incubated for 2 hours. After washing the plates, streptavidin-peroxidase (Sigma Chemical Co., St. Louis, Mo.) in avidin buffer (PBS-T + 2% bovine serum albumin + 0.5% gelatin) was incubated in each well for 30 minutes, the plates were washed again and developed with Sigma FAST OPD (Sigma Chemical Co.). Optical densities were measured at 490 nm and 650 nm with an automated ELISA plate reader (Molecular Device Corporation, Menlo Park, Calif.).

Tricine-SDS polyacrylamide gels and immunoblotting

Peanut or soy protein extract (2 mg/ml) was mixed with an equal volume of SDS sample buffer (50 mmol/L Tris HCl, pH 6.8), containing 4% SDS, 2% β -mercaptoethanol, 12% glycerol bromphenol blue, and pyronin Y, and boiled 10 minutes for denaturation. Separation was performed by tricine-SDS polyacrylamide gel to obtain adequate resolution of low-molecular-weight proteins, modified from the method of Schagger and von Jagow.¹⁵ The running gel was prepared from a stock of 49.5% wt/vol acrylamide (Sigma Chemical Co.) and 1.5% wt/vol bisacrylamide (Bio-Rad: 49.5% T, 3% C) in 3.0 mol/L Tris (pH 8.45) with 0.3% SDS gel buffer solution and glycerol (Fisher Biotech, Fair Lawn, N.J.). A 4.5% stacking gel and 15% running gel were prepared from the stock 49.5% T, 3% C, in gel buffer solution. Both gels were polymerized with 10% ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine. The electron tank (Hoefer Scientific Instruments, San Francisco, Calif.) was loaded with 0.1 mol/L Tris (pH 8.25), 0.1 mol/L tricine, and 0.1% SDS in the upper tank and 0.2 mol/L Tris (pH 8.9) in the lower tank. Electrophoresis was performed at 30 V through the stacking gel and at 80 V overnight through the running gel. Glycine-SDS polyacrylamide gels (or SDS-PAGE gels) with resolution for protein fractions between 66 and 14 kd prepared according to the method of Dreyfuss et al.¹⁶ and modified as previously described,⁷ were performed for further immunoblotting with the unadsorbed serum and the soy-adsorbed serum of one patient (BP).

The soy and peanut proteins were electrotransferred from the polyacrylamide gel to nitrocellulose paper at 0.15 A for 6 hours in 50 mmol/L of Tris-glycine buffer (pH 9.1) containing 20% methanol. After transfer, the nitrocellulose blots were blocked overnight in PBS-T with 0.5% gelatin. Protein staining with 0.1% amido black was obtained for each gel to confirm proper electrophoresis and protein transfer on nitrocellulose paper. The blots were then incubated with nonadsorbed serum and adsorbed serum (5:1 vol/vol dilution) for 2 hours at room temperature on a rocking platform. The blots were washed five times for 5 minutes with PBS-T and incubated for 2 hours with biotin-conjugated goat anti-human IgE in antibody buffer (1:1600). After five

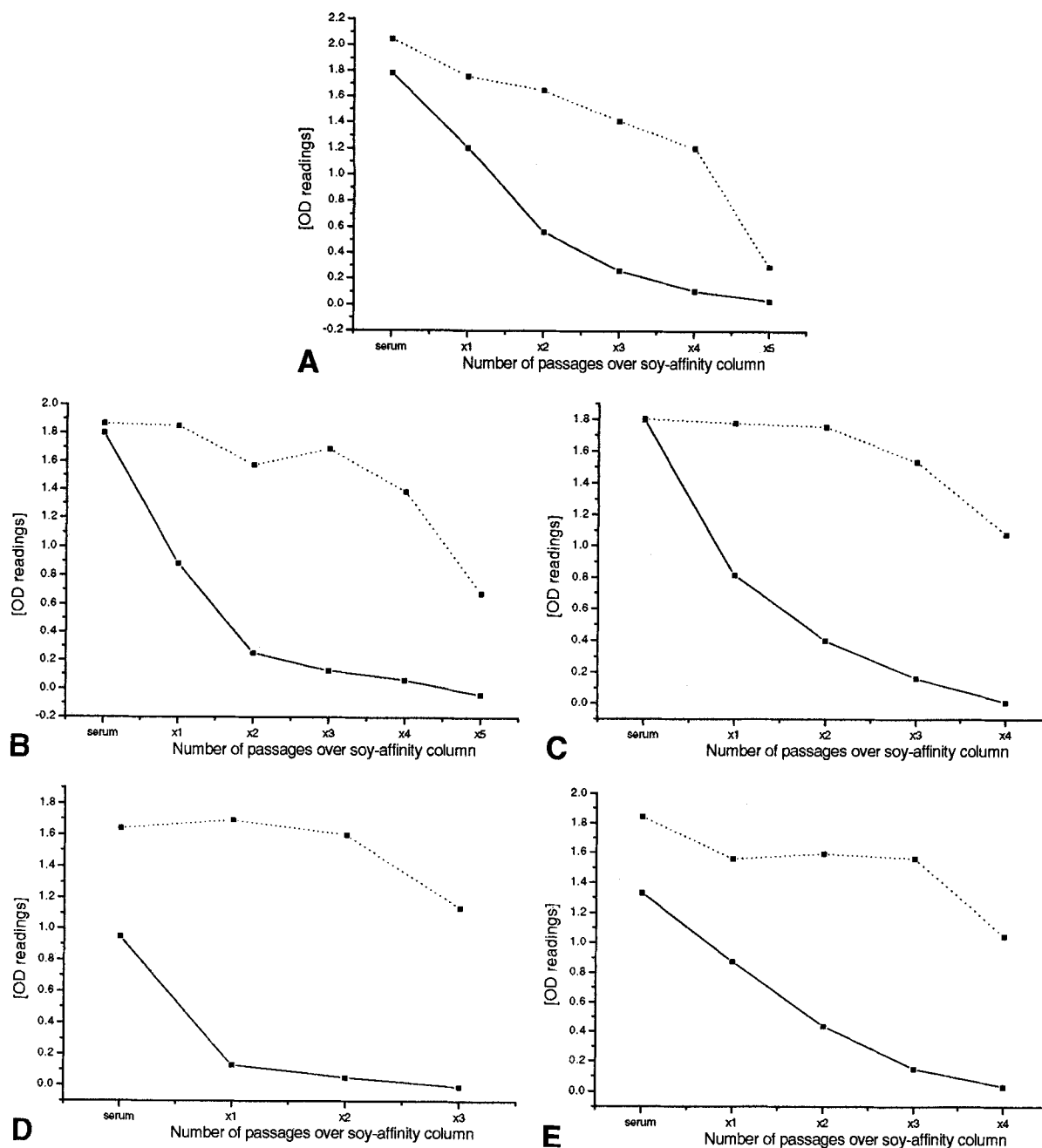


FIG. 1. Results of IgE binding to soy and peanut by ELISA from nonabsorbed sera and after successive passes over the soy-affinity chromatography column. *Dotted line* represents IgE binding to peanut antigen; *solid line* represents IgE binding to soy antigen. **A**, Patient BP. **B**, Patient BM. **C**, Patient DH. **D**, Patient AT. **E**, Patient DT.

washes, the blots were incubated with streptavidin-peroxidase in avidin buffer (1:1000) for 30 minutes, washed, and developed with Sigma FAST DAB (Sigma Chemical Co.). The reaction was stopped by rinsing the blots several times in distilled water. The molecular weights of protein fractions with IgE binding were determined by scanning densitometry (Ultrosan; LKB, Broma, Sweden) and compared with molecular weight markers.

RESULTS

Antigen-specific serum antibody adsorption

Soy-binding antibodies were removed from the sera of five patients allergic to peanut by soy-affinity chromatography. All five patients were initially selected because they had high levels of IgE antibodies to peanut (Table I). The adsorption

of soy-binding IgE antibodies was monitored after each adsorption procedure of 1 hour by ELISA (Fig. 1). Repeated adsorption steps by soy-affinity chromatography progressively diminished soy-specific IgE antibody titers to background optical density readings. A total of three to five passes over the affinity column were necessary to remove all soy-specific IgE antibodies, with sera from patients allergic to soy requiring the most extensive adsorption. Progressive diminution of specific IgE binding to peanut by ELISA confirms that cross-reacting antibodies were adsorbed onto the soy-affinity column (Fig. 1). On the other hand, the serum sample run over the human serum albumin column showed no significant decrease in peanut-specific IgE antibody with about 95% recovery of the specific antibody: before adsorption, 2225 IU/ml; after adsorption, 2114 IU/ml.

To determine the fraction of non-cross-reacting peanut-specific IgE, we compared the amount of specific IgE with crude peanut antigen, as well as with Ara h 1 and Ara h 2, before and after adsorption of cross-reacting antibodies. Because high concentrations of peanut-specific IgE in the sera might have saturated antigen-binding sites, several fivefold serial dilutions (from 1:5 to 1:625) were performed. ELISAs were then run at optimal serial dilutions to determine specific IgE binding to crude peanut antigen, Ara h 1 and Ara h 2 in nonadsorbed and soy-adsorbed sera. Fig. 2 shows that the average IgE antibody adsorption onto soy-affinity chromatography was 73% for crude peanut extract, 79% for Ara h 1, and 76% for Ara h 2. Specific IgE antibodies were less depleted in patient DH, possibly related to the low soy-specific serum IgE antibody titer (Table II).

Tricine-SDS polyacrylamide gels and immunoblotting

Crude peanut extract and soy flour extract were separated by electrophoresis on a tricine-SDS polyacrylamide gel (Fig. 3). Various bands were evident after migration of both proteins; and major peanut fractions were found at 39, 29, 27, 19, 17, 14, and 12 kd. Major soy fractions were found at 46, 40, 33, 19, and 9 kd. Immunoblots were incubated with nonadsorbed sera and soy-adsorbed sera for IgE binding. The following peanut protein fractions were bound by specific IgE in all five nonadsorbed sera: 46, 29, 24, 19, 17, and 12 kd (Fig. 4). A double band at 4.5 and 5.5 kd was found in all but one patient. The band at 17 kd probably

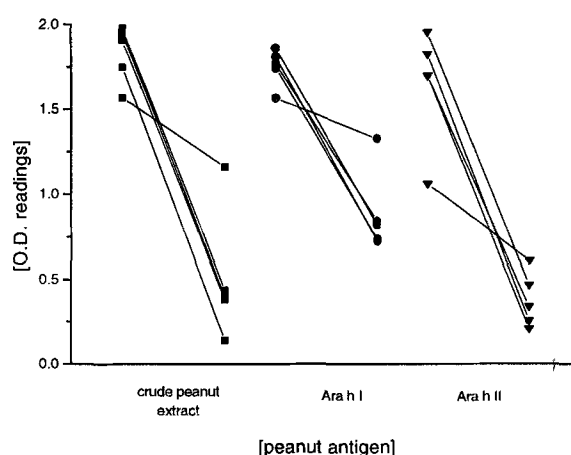


FIG. 2. IgE antibody binding to whole peanut extract, purified Ara h 1, and Ara h 2 by ELISA before and after soy-specific antibody adsorption over the soy-affinity chromatography column. Each square represents optical density (O.D.) readings for a specific patient.

corresponds to Ara h 2, described as a major peanut allergen. Several minor bands disappeared after adsorption of soy-specific antibody, as well as a stronger band at 12 kd. This band may correspond to a relevant antigenic fraction of soybean protein. Serum binding at 5 kd became more prominent and is particularly evident in patients BM and AT. To study peanut-specific IgE antibody binding at higher molecular weights, peanut and soy extracts were run over a standard glycine SDS-PAGE gel with resolution between 14 and 66 kd, and immunoblots were assayed for IgE binding with soy-adsorbed serum of one patient allergic to peanut and soy (BP). Several peanut fractions could be isolated with major bands at 63, 41, 23, and 15 kd (Fig. 5). Immunoblotting revealed serum IgE binding at 63, 41, 23, 20, 17, and 14 kd. Binding to these peanut fractions remained after adsorption of soy-specific antibodies; however, most minor bands disappeared. IgE binding to a band at 63 kd (Ara h 1) could be found with both the nonadsorbed and soy-adsorbed serum, supporting the clinical relevance of Ara h 1 as a major peanut allergen.

Nonadsorbed sera from the two patients allergic to soy (BP and BM) were reacted with the soy immunoblots (Fig. 6). IgE antibodies bound to protein fractions of 45 and 17 kd and to a large band at approximately 21 kd, whereas IgE antibodies from the soy-tolerant patients bound fractions at 45 kd, and to a lesser degree, at 21 kd. Control blots without patient sera revealed some nonspecific binding to the soy fraction at 21 kd (Fig. 7). After soy-specific IgE antibodies were adsorbed

TABLE II. Peanut- and soy-specific IgE antibody concentrations (IU/ml) by CAP-RAST FEIA after successive passes over a soy-affinity column (A) and a peanut-affinity column (B)

Patient	Nonadsorbed	Pass 1	Pass 2	Pass 3	Pass 4	Pass 5
A: Soy-adsorbed						
DT						
P	2225	238	117	63	66	
S	88	11	6	5	1	
BP						
P	2405	120	116	56	43	22
S	75	32	11	6	3	2
BM						
P	1995	244	—	168	111	73
S	110	30	—	6	4	2
DH						
P	357	119	104	81		
S	8	2	1	ND		
AT						
P	1560	236	206	93	85	
S	15	7	4	2	1	
B: Peanut-adsorbed						
DT						
P	2225	17	4	2		
S	88	6	2	1		
BP						
P	2405	61	8	6	4	
S	75	48	23	13	6	

P, Peanut; S, soy; ND, not detected.

from the sera, there was no IgE-specific binding to any soy protein fraction.

Peanut-specific serum antibody adsorption and immunoblotting

Serum from one patient allergic to soy and peanut (BP) and serum from one soy-tolerant patient with peanut allergy (DT) were depleted of peanut-specific antibody by peanut-affinity chromatography. Sera were monitored by ELISA to ensure complete removal of peanut-specific IgE antibodies (Fig. 8). All cross-reacting antibodies from patient allergic to peanut (DT) were removed by the peanut-affinity column (Fig. 8, B), whereas unique antibodies to soy remained in the serum from the patient with peanut and soy allergy (BP, Fig. 8, A). IgE antibodies in peanut-adsorbed serum from patient BP (allergic to soy and peanut) bound to soy fractions at 45, 26, and 21 kd (Fig. 9). Antibody binding to the fraction at 45 kd, and to a lesser degree, to the fraction at 21 kd (although some nonspecific binding to this fraction can be observed) persisted after peanut-antibody adsorption, suggesting that these fractions may be unique soy proteins. IgE antibodies binding to the fraction

at 26 kd were removed by the peanut-affinity column and are therefore unlikely to be clinically relevant to soy allergy.

DISCUSSION

Immunologic cross-reactivity is common among members of the same botanical family. Previous reports have shown that serum IgE antibodies from patients allergic to foods belonging to the legume family frequently recognized shared protein fractions.⁷ Although these patients have IgE antibodies to several legumes, they rarely react to more than one member of the legume family.¹⁷ On the basis of this observation, we hypothesized that antibodies cross-reacting with other legumes in patients allergic to peanut were not responsible for clinical reactivity to peanut. Furthermore, isolation of peanut-specific antibodies might enable us to determine which peanut protein fractions (allergenic epitopes) were responsible for clinical reactivity to peanuts. To address these hypotheses, we used soy protein-specific and peanut protein-specific affinity chromatography columns to remove cross-reacting antibodies from the sera of patients allergic to peanut and soy. Five patients allergic to

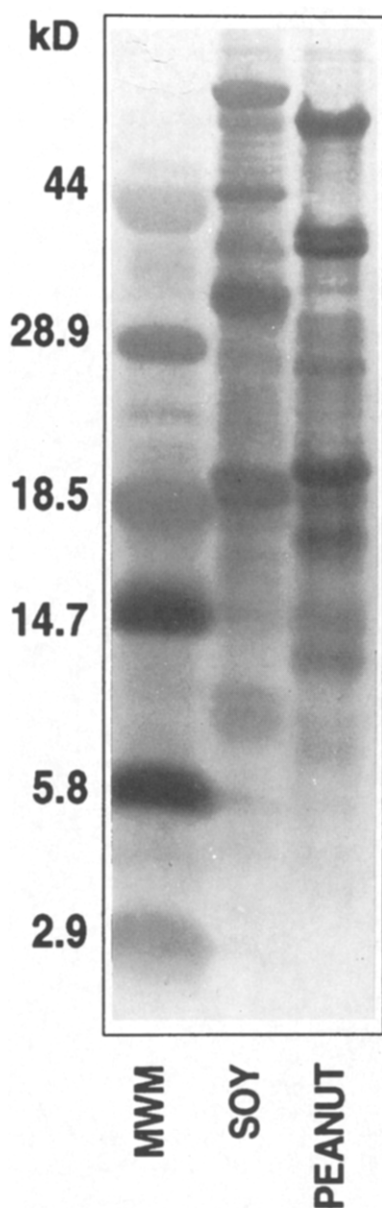


FIG. 3. Tricine-SDS polyacrylamide gel. Molecular weight marker (MWM), soy, and peanut fractions are stained with amido black 0.1%.

peanut with very high levels of specific IgE antibodies to peanut were studied. Two of these patients also experienced clinical reactions to soy and had elevated levels of specific IgE antibodies to soy; one patient who was soy-tolerant also had very high specific IgE titers to soy (Table I). After two sequential passes over the soy-affinity column, serum IgE antibodies to soy rapidly diminished (Fig. 1). However, further passes (up to a total of 5) with increased contact time were necessary to completely remove soy-binding antibodies. The first passes most likely removed antibodies with

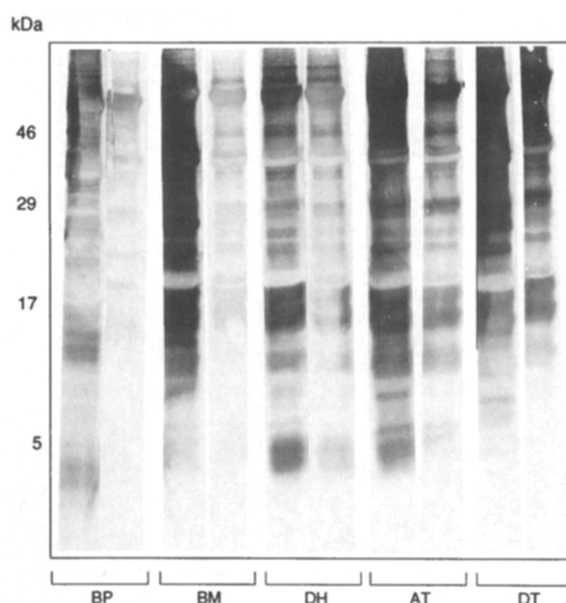


FIG. 4. IgE binding to peanut fractions isolated on tricine-SDS polyacrylamide immunoblots for two patients allergic to peanut and soy (BP and BM), and three soy-tolerant patients with peanut allergy (DH, AT, and DT). The first lane for each patient was reacted with nonadsorbed serum, and the second lane was reacted with soy protein-adsorbed serum.

high affinity to soy, whereas later passes with prolonged exposure time were necessary to remove antibodies with lower affinity. The concomitant decrease of IgE antibody binding to crude peanut extract confirms the presence of significant quantities of cross-reacting antibodies in the sera of patients allergic to peanut.

To determine the magnitude of cross-reactivity, IgE antibody binding to whole peanut extract, to Ara h 1, and to Ara h 2 were determined before and after adsorption. Binding to whole peanut antigen was decreased by 73% after the removal of soy cross-reacting antibodies, and binding to Ara h 1 was decreased by 79% and Ara h 2 by 76%. Ara h 1 belongs to the family of vicilin proteins, which are also found in soybeans and other legumes. Studies indicate that vicilins of various legumes share greater than 60% sequence identity,¹⁸ which may explain the extensive antibody cross-reactivity to this protein.

Food proteins are comprised of several fractions of various sizes, which can be separated by various gel electrophoretic methods. Because most of the potential antigens can be found between 15 and 50 kD, food proteins are usually separated well on SDS-PAGE. However, relevant allergenic fractions of lower molecular weight may not be iden-

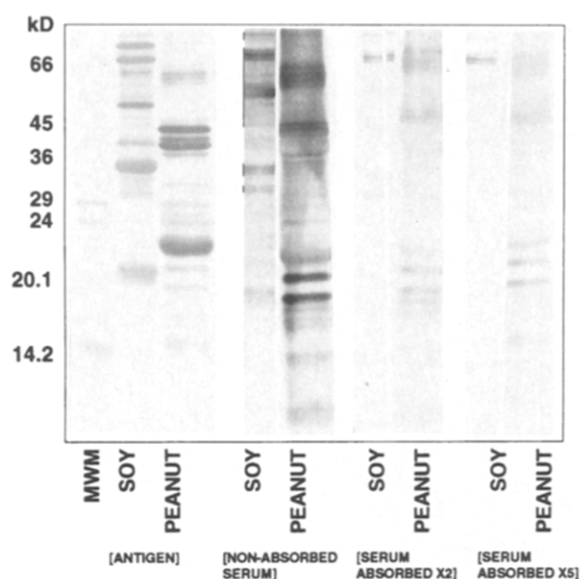


FIG. 5. Glycine-SDS-PAGE of soy and peanut. The first three lanes represent molecular weight marker (MWM), soy, and peanut stained with amido black 0.1%. The next three sets of lanes show IgE antibody binding to soy and peanut protein fractions with nonadsorbed serum from patient BP, serum passed twice over the soy-affinity chromatography column, and serum adsorbed five times over the column.

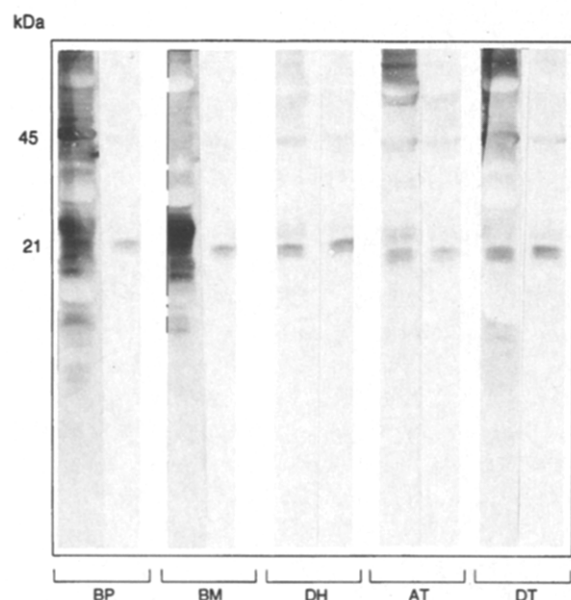


FIG. 6. IgE antibody from sera of two patients allergic to peanut and soy (BP and BM) and three soy-tolerant patients with peanut allergy (DH, AT, and DT) binding to tricine-SDS polyacrylamide soy immunoblots. The first lane for each patient was reacted with nonadsorbed serum; the second lane was blotted with soy-adsorbed serum.

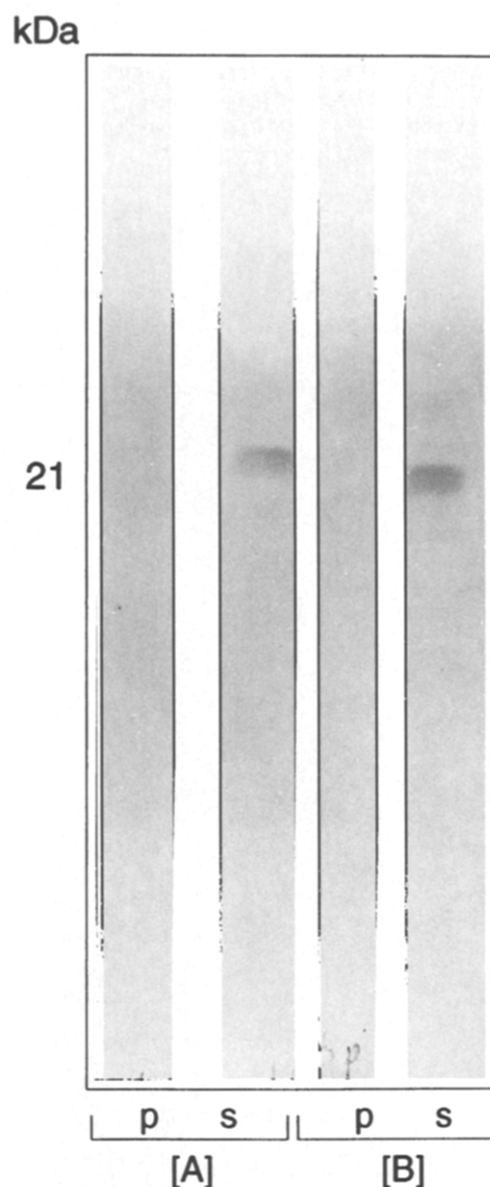


FIG. 7. Control immunoblots of soy (s) and peanut (p). **A** Lanes developed without serum. **B**, Lanes assayed with serum from a patient lacking antibody to both soy and peanut.

tified by this method. To evaluate the low-molecular-weight fractions of peanut and soy, we used tricine-SDS polyacrylamide gels, which efficiently separates low-molecular-weight protein fractions.¹⁵ This method is useful for separating protein fractions with molecular weights as low as 3 to 5 kD and therefore is useful for identifying low-molecular-weight allergenic fractions not seen on standard SDS-PAGE.

Soy and peanut tricine-SDS gel strips transferred to nitrocellulose were probed with nonad-

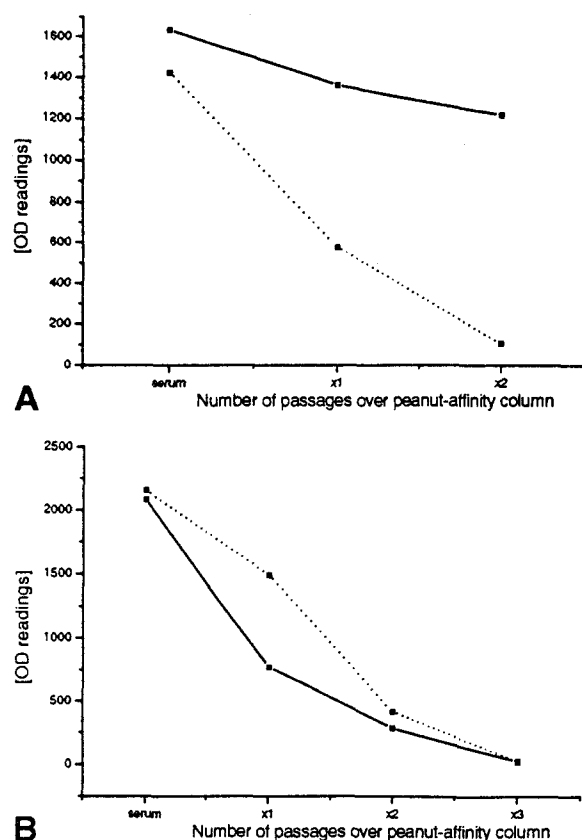


FIG. 8. Relative quantities of IgE binding to whole soy and peanut proteins by ELISA after successive passes over the peanut-affinity column. Dotted line represents IgE binding to peanut antigen; solid line represents IgE binding to soy antigen. **A**, Patient BP. **B**, Patient DT. OD, Optical density.

sorbed and soy-adsorbed sera from the three patients allergic to peanut and the two patients allergic to both soy and peanut. Significant peanut-specific IgE antibody binding on the peanut immunoblot was found at 46, 29, 24, 19, 17, and 14 kd; and a doublet was found at 5 kd. Sera from one patient (DT) did not bind to the 5 kd fractions, but otherwise, the other four patients demonstrated essentially identical patterns of IgE antibody binding. Previous studies have characterized several antigenic fractions in peanuts; Ara h 1 (63.5 kd) and Ara h 2 (17 kd) were determined to be major allergenic fractions.^{9,10} A minor fraction has been found at 31 kd,² and further fractions have been identified at 17 to 25, 34, 55, and 65 kd.¹¹ Tricine-SDS polyacrylamide gels demonstrated IgE antibody binding to unique peanut fractions at 5 and 13 kd. These fractions and their relevance in clinical manifestations of peanut allergy will be determined in future studies.

In the second part of the study, peanut-specific

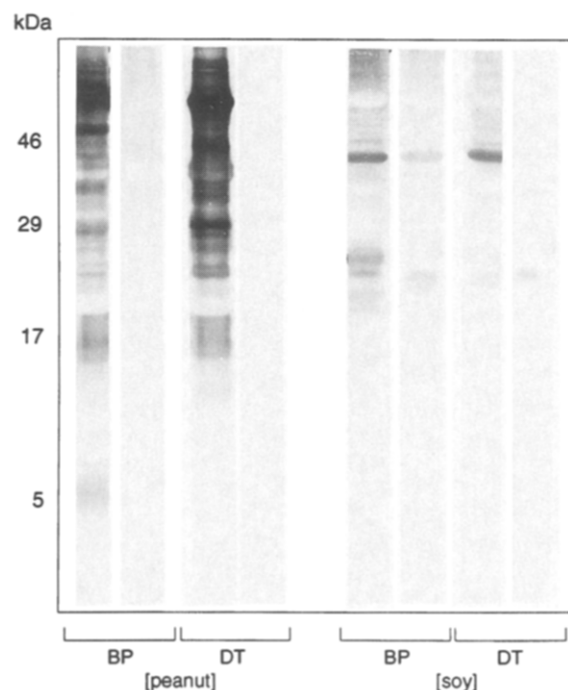


FIG. 9. IgE binding to tricine-SDS polyacrylamide peanut and soy immunoblots for one patient allergic to peanut and soy (BP) and one soy-tolerant patient with peanut allergy (DT). The first lane for each patient was reacted with non-adsorbed serum; the second lane was reacted with peanut-adsorbed serum.

antibodies were removed from the sera of two patients (one with clinical reactivity to peanut and soy and one with reactivity to peanut but with high levels of soy-specific IgE antibodies) by peanut-affinity column chromatography. No IgE antibody binding to the peanut blots could be detected after adequate adsorption of both sera. However, with the peanut-adsorbed sera from the patient allergic to peanut and soy (BP), one band on the soy blot with strong IgE antibody binding became prominent at 46 kd, and a weaker band remained at 21 kd. The clinical relevance of these two fractions unique to soy requires further study. To date, only partial characterization of soy antigens has been achieved, with relevant protein fractions in the 7S portion isolated by ultracentrifugation,¹² as well as a minor allergen at 20 kd and several different fractions between 15 and 55 kd.¹⁹ Furthermore, immunoblotting showed IgE binding in sera from soy-sensitive patients with atopic dermatitis to a band at 30 kd.¹³ The IgE antibody binding to a 21 kd fraction by tricine-SDS-PAGE in our experiments may correspond to the antigenic fraction previously described at 20 kd; the fraction at 46 kd has not been described previously. Further studies

are necessary to determine the clinical relevance of these antigenic fractions.

In conclusion, removal of cross-reacting antibodies from the sera of patients allergic to peanut and soy has enabled us to identify unique allergenic fractions of peanut and soy. Six unique allergenic fractions of peanut and two fractions of soybean were identified. Future studies will isolate and characterize these unique proteins and determine whether they represent the allergenic components of these legumes. Use of these unique peanut and soy fractions in diagnostic tests may provide more specific indicators of clinical reactivity to these two members of the legume family.

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