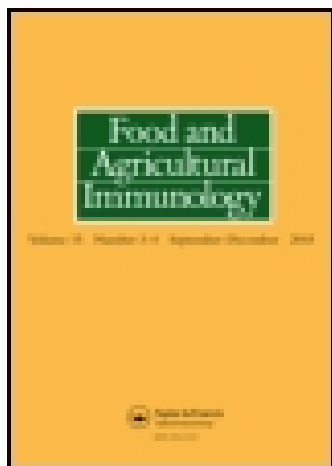




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Monoclonal Antibodies against Selected Peanut Allergens: Production and Use as Affinity Agents

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An extract of dry-roasted commercial peanut mix (CPE) was examined for allergenic activity in peanut-sensitive individuals, using skin tests and radioallergosorbent (RAST) assays. Proteins in the extract were characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. The proteins were electroeluted in three fractions in the ranges 15–25, 26–58 and 65 kDa. The 15–25 kDa molecular weight fraction produced the most reactive skin tests in peanut-sensitive subjects and was chosen for monoclonal antibody production. Six hybridoma cell lines secreting peanut-specific antibodies of the IgM isotype (kappa light chain) were produced. Immunopurified CPE proteins were then subjected to SDS-PAGE, resulting in five major bands with approximate molecular weights of 14, 25, 38, 40 and 44 kDa. Immunoblotting of these separated proteins revealed: (1) three bands with approximate molecular weights of 38, 44 and 65 kDa, which bound IgE from peanut-sensitive patients; and (2) that the monoclonal antibodies recognized epitopes in bands at approximate molecular weights of 12, 14, 23 and 25 kDa. RAST inhibition assays showed that the affinity-purified proteins were able to inhibit the binding of serum IgE from peanut-allergic individuals to solid-phase CPE.

Keywords: Peanut allergens, monoclonal antibodies, RAST assay, immunoblot

INTRODUCTION

Peanuts are sold as a vegetable, canned, frozen, or roasted in the shell, and are used in more than 50 confections and bakery products. They are a popular food item and a good source of dietary protein.

Despite the valuable nutritional and functional aspects of this legume, peanuts are one of the most allergenic foods known, and can cause severe, IgE-mediated type I hypersensitivity.

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ity reactions (Fries, 1982; Yunginger *et al.*, 1988; Sampson, 1990). The prevalence of peanut allergy in the general population is not known, but has been estimated to be between 0.3 and 25% of the population (Taylor, 1980). The degree of the allergic reaction in sensitive individuals varies from mild urticaria to severe, life-threatening anaphylactic episodes.

More than half of the peanuts produced in the US are made into peanut butter (Woodroof, 1983); as a result of increased use of peanut protein products as extenders and texture and flavor enhancers, especially in formulated foods (Cherry, 1983; McWatters, 1983), peanut-allergic people are finding it much more difficult to identify and avoid this legume. Sensitive individuals may be inadvertently exposed to foods contaminated with peanut proteins during processing. This can occur during processing from carryover due to inadequate cleaning of processing equipment used for non-peanut and peanut products, or in rework of products. Yunginger *et al.* (1983) found peanut butter cross-contamination in sunflower butter due to inadequate cleaning of common equipment; the contaminated sunflower butter elicited an allergic reaction in a peanut-sensitive patient. For many peanut-allergic individuals, the identification of peanut allergens in foods is important because the ingestion of minute amounts can trigger a severe allergic reaction (Sampson, 1990).

A multiplicity of peanut allergens has been demonstrated (Sachs *et al.*, 1981; Taylor *et al.*, 1982; Barnett *et al.*, 1983; Bush *et al.*, 1983; Barnett & Howden, 1986; Meier-Davis *et al.*, 1987; Burks *et al.*, 1991/2a,b), and although they have been found to be contained in the protein or glycoprotein fractions of the seed, they have yet to be fully characterized and identified. Part of the problem lies in the large number of peanut proteins; investigators have reported more than 30 (Barnett *et al.*, 1983; Bush *et al.*, 1983; Meier-Davis *et al.*, 1987).

Sachs *et al.* (1981) isolated and partially purified a peanut allergen named 'Peanut-1'. From sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, 'Peanut-1' contained two major bands, with molecular weights of 20 and 30 kDa. However, this allergen was not responsible for all of the allergenic activity in the peanut extract.

Barnett and Howden (1986) identified and purified a 65 kDa Con A-reactive glycoprotein peanut allergen, based on their results obtained with radioallergosorbent (RAST) inhibition studies, which had a *pI* of 4.6. Burks *et al.* (1991) identified a 63.5 kDa molecular weight glycoprotein peanut allergen using immunoblotting and ELISA methods with sera from peanut-sensitive atopic dermatitis patients. This allergen was found to have a *pI* of 4.55, and has been named *Ara h 1*. Although it appears that the above two allergens described are perhaps the same protein, *Ara h 1* does not bind to Con A.

In a later report, Burks *et al.* (1992) described the identification and purification of another peanut allergen with a molecular weight of 17 kDa in SDS-PAGE and a *pI* of 5.2. This allergen has been named *Ara h 2*.

Despite these efforts, the majority of peanut allergens have not been fully characterized. The complex composition of peanuts, along with the lack of standard reagents for identifying and quantifying peanut allergens, prompted us to explore monoclonal antibody technology to increase the number of reagents available for study of this problem. In this study, monoclonal antibodies against peanut proteins were used as a reverse immunosorbent for the purification of allergenic components directly from a crude roasted peanut extract.

MATERIALS AND METHODS

Materials

Crude peanut extract (CPE). A dry-roasted commercial mix of peanuts (40% US split Florunner, 40% Chinese and 20% Virginia varieties) was generously donated by Kraft General Foods Ltd (Canada). For preparation of CPE, the peanuts were blended into a paste, then defatted with alternate washes of acetone and petroleum ether. After air-drying

overnight, the residue was ground to a fine powder, then extracted in 1 M-NaCl-20 mM-sodium phosphate, pH 7.0 (1:20, w:v) (Basha & Cherry, 1976). The extract (200 ml) was clarified by centrifugation at $20\,000 \times g$ for 20 min at 4°C, and the supernatant solution was dialyzed overnight against three changes of 0.01 M-phosphate-buffered saline, pH 7.5 (PBS) at 4°C. The CPE was concentrated using stirred cell ultrafiltration (Amicon Corporation, Danvers, MA, USA; membrane 2 kDa cut-off), and the protein content was determined using the bicinchoninic acid protein assay (Pierce Chemical Co., Rockford, IL, USA).

Peanut-sensitive subjects. Sera were obtained from adult patients of the University of Wisconsin Hospital Allergy Clinic (Madison, WI, USA). Peanut sensitivity was confirmed by positive skin test and RAST results. Control serum was obtained from six people with no known allergies, and also six individuals with mold (*Alternaria*) allergy, and consequent high mold-specific IgE serum levels.

Methods

Skin testing. Peanut extract (1:20, w:v) in 50% glycerin plus 0.4% phenol was purchased from Greer Laboratories, Inc. (Lenoir, NC, USA), or prepared from dry-roasted peanuts by the method described above. Individuals were tested at the University of Wisconsin Hospital Allergy Clinic. Positive controls for the tests contained 1 mg of histamine ml⁻¹ in 50% glycerin plus 0.4% phenol. Wheal size (average of length and width values, in mm) was recorded approximately 10–15 min after puncture of the skin.

Direct RAST assays. CPE (3.4 mg ml⁻¹) was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Biochemicals, Piscataway, NJ, USA) (5–10 mg peanut protein ml⁻¹ Sepharose 4B) according to the manufacturer's directions. A total of 0.5 ml of a 3% (w/v) solution of coupled Sepharose-CPE and 0.1 ml of serum diluted 1:5 (v:v) in RAST buffer from peanut-allergic subjects or controls were incubated in test tubes at room temperature for 18–24 h with rotation. The dilution buffer (RAST buffer) consisted of 0.05 M-sodium phosphate, 0.43 M-sodium chloride, 3% (w/v) bovine serum albumin (BSA), 0.05% (w/v) sodium azide and 0.05% Tween 20 (v/v). After incubation, the solid phase was washed three times with 3 ml of RAST buffer, and then with ¹²⁵I-labeled rabbit anti-human IgE (25 000–35 000 cpm) (Phadebas RAST test kit, Pharmacia Biochemicals) was added to each tube. The reagents were incubated again as described above. The solid phase was again washed, and the bound radioactivity measured (5 min tube⁻¹) using a gamma scintillation counter (Searle Laboratories, Des Plaines, IL, USA).

RAST inhibition assays. RAST inhibition assays were performed as described above, except that various amounts of CPE or affinity column eluate were present in the reaction mixture.

SDS-PAGE. Vertical slab SDS-PAGE was carried out using the method of Laemmli (Laemmli, 1970). Apparatus, molecular weight standards and chemicals were from BioRad Laboratories (Richmond, CA, USA). Approximately 50 µg of sample were loaded into each lane. Gels were stained with 0.1% Coomassie brilliant blue R250 in 10% acetic acid: 20% methanol.

Electro-elution was done in a model 1750 electrophoretic sample concentrator (Isco, Lincoln, NE, USA), according to the manufacturer's instructions. Three eluted fractions, in the ranges of 15–25, 26–58 and 65 kDa were eluted from SDS-PAGE gels, assayed for protein content and concentrated by stirred-cell ultrafiltration (Amicon; membrane 2 kDa cut-off).

Immunoblotting of peanut proteins. CPE or affinity-purified peanut proteins separated by SDS-PAGE as described previously were electrophoretically transferred to an Immobilon P (0.45 μm ; Millipore Corporation, Bedford, MA, USA) membrane according to the method of Towbin *et al.* (1979), except that 1 mM-dithiothreitol was present in the blotting buffer. The procedure was performed in a Transblot apparatus (BioRad Laboratories) for 1 h at 100 V (constant voltage). An India ink stain (Hancock & Tsang, 1983) of the blotted membrane was performed to assure adequate transfer of the proteins.

After removal from the Transblot apparatus, the membrane was blocked with a solution of 0.2% (w/v) non-fat dry milk diluent (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, USA) in PBS containing 0.05% (v/v) Tween 20 (PBS-T) for 1 h at 25°C. The membrane was washed three times with PBS-T, and then cut into strips approximately 0.5 cm wide. For analysis of IgE binding, the strips were then incubated with peanut-sensitive patient sera diluted 1:5 (v:v) in PBS-T containing 3% (w/v) BSA for 18–24 h at 25°C. The membrane strips were washed again, and then incubated overnight with rabbit anti-human IgE labeled with ^{125}I (40 000 cpm strip $^{-1}$, Phadebas RAST kit, Pharmacia). Autoradiographic detection of IgE binding was done by exposing X-OMAT AR X-ray film (Eastman Kodak, Rochester NY, USA) to the membrane at -70°C . Exposure times ranged from 1 to 3 days.

For analysis of monoclonal antibody binding, the strips were incubated with stationary-phase cell growth (greater than 2×10^6 cells ml^{-1}) culture supernatant solution, diluted serially in PBS-T, for 2 h at 25°C. The strips were then incubated with goat anti-mouse IgM labeled with biotin (Boehringer-Mannheim Biochemicals, Indianapolis, IN, USA) which was diluted 1:5000 (v:v) in PBS-T, for 1 h at room temperature. A solution of streptavidin peroxidase diluted 1:3000 (v:v) in PBS-T was added and allowed to incubate for 45 min at room temperature. Until this point, the membrane was washed with PBS-T between each incubation step. At this step, the washing reagent was changed to 0.05 M-Tris-HCl, pH 7.4, and the strips were washed four times, 5 min each. Monoclonal antibody binding was detected by the addition of 4-chloro-1-naphthol (Sigma Chemical Co., St Louis, MO, USA) substrate solution containing 50 μl of 30% (v/v) H_2O_2 .

Monoclonal antibody production. Six BALB/c mice (6–8 weeks old), certified as free of pathogens (Harlan Sprague Dawley, Inc., Madison, WI, USA) were immunized intra-peritoneally with 25 μg of electro-eluted (range 15–25 kDa) CPE. The protein was dissolved in PBS, and emulsified in a ratio of 1:1 (v:v) with Freund's complete adjuvant (Difco Laboratories, Detroit, MI, USA). Booster injections (20 μg each, given intra-peritoneally) were given monthly, without adjuvant. Four days before fusion, the mouse exhibiting the highest polyclonal titre (the dilution of serum which gave a 50% maximum absorbance reading in enzyme-linked immunosorbent assay (ELISA), approximately 1:500) was given an intravenous injection of 20 μg of 15–25 kDa protein fraction, followed by intra-peritoneal injections 3 and 2 days before fusion.

The murine myeloma cell line P3/NS-1/1-AG4-1 was obtained from American Type Culture Collection (Rockville, MD, USA). All tissue culture work was done at the Hybridoma Facility of the Biotechnology Center at the University of Wisconsin-Madison. Polyethylene glycol 1500 was obtained from Boehringer-Mannheim Biochemicals and all other cell culture reagents were obtained from Gibco Laboratories (Grand Island, NY, USA). Cell fusion and culture were done according to the method of Galfre and Milstein (1981), with minor modifications. Growth was scored 5 days post-fusion, and a Poisson distribution was calculated to determine fusion efficiency. Positive colonies were expanded and cloned by the limiting dilution method (Henry *et al.*, 1980).

Indirect ELISA for screening hybridoma colonies. Microtitre plates were coated with CPE or 15–25 kDa electro-eluted protein fraction in PBS at a concentration of 4 $\mu\text{g ml}^{-1}$ (100 μl

well⁻¹) at 4°C overnight. The unoccupied sites on the plate were blocked with 1% (w/v) BSA in PBS for 1 h at 37°C before further steps were carried out. Goat anti-mouse IgM plus IgG labeled with biotin (Boehringer Mannheim Biochemicals) 1:10 000 (v:v) diluted in PBS-T was added and the mixture was incubated for another hour at 37°C. Streptavidin peroxidase (Boehringer Mannheim Biochemicals) conjugate diluted 1:15 000 (v:v) in PBS-T was then added and incubated at room temperature for 25 min. Bound streptavidin peroxidase was detected by addition of *o*-phenylenediamine (10–15 min at room temperature in the dark); the enzyme reaction was stopped by addition of 1 M-HCl. After each addition, the plates were washed four times with PBS-T. The absorbance at 490 nm was read in a ThermoMax Kinetic microtitre plate reader (Molecular Devices, Menlo Park, CA, USA).

Isotyping. A commercially available mouse immunoglobulin isotype ELISA kit (Boehringer Mannheim Biochemicals) was used according to the manufacturer's directions.

Purification of monoclonal antibodies. Either cell culture supernatant solution or ascites fluid from each clone was precipitated by dropwise addition of a saturated solution of ammonium sulfate to 50% saturation. The solution was left at 4°C overnight, then centrifuged at 8000 × *g* for 20 min at 4°C. The pellet was resuspended in an adequate volume of distilled water, and the procedure repeated. The resulting pellet was resuspended in distilled water, dialyzed against 0.1 M-Tris-HCl containing 0.5 M-NaCl, pH 7.4, and concentrated using a Centriprep-30 filtration device (Amicon, 2 kDa membrane cut-off).

The concentrated antibody solution was partially purified on a Sephacryl HR-300 column (2.8 × 90 cm; Pharmacia Biochemicals). The void volume was collected, concentrated by membrane ultrafiltration (Centriprep-30, Amicon) and then dialyzed. The antibodies were then purified using ion-exchange chromatography on an ABx column (1.7 × 15 cm; J. T. Baker, Phillipsburg, NJ, USA).

Affinity columns. Purified immunoglobulin was bound to commercially available (Amino-link; Pierce Chemical Co.) activated agarose. Approximately 4–8 mg of antibody were bound to each 2 ml column; after coupling, protein assays showed that 80–90% of the antibody was retained on the columns (data not shown). The columns were stored in azide at 4°C and allowed to reach room temperature before use. Crude peanut extract (6800 µg) was diluted to a volume of 2 ml with 0.01 M-PBS, pH 7.4, then applied to the columns. After incubation for 1 h at room temperature, the column was washed with PBS until the absorbance (at 280 nm) of the eluate reached zero. Bound protein was eluted with 0.1 M-glycine-HCl, pH 2.8.

Fractions of 1 ml were collected and immediately neutralized by addition of 1.0 M-Tris-HCl, pH 9.2. Fractions were pooled and, for SDS-PAGE, precipitated by the addition of five volumes of methanol at –20°C, and incubated overnight at –20°C. The proteins were recovered by centrifugation at 5000 × *g* for 15 min. For RAST and ELISA testing, the pooled fractions were dialyzed against PBS (membrane 2 kDa cut-off) overnight at 4°C, then concentrated by placing the bags in crystalline polyethylene glycol (molecular weight 20 kDa; J. T. Baker). The concentrated pooled fractions were then stored at –20°C.

RESULTS

Isolation of Allergenic Peanut Protein Fraction

The CPE preparation was shown to possess allergenic activity *in vivo* by skin test results in five peanut-sensitive subjects (Table 1). A wheal diameter of greater than 3 mm was considered indicative of a positive response; the CPE gave a positive response in four out

TABLE 1. Response to CPE in peanut-allergic patients

Patient	Diameter of wheal (mm)			
	Histamine	Commercial	CPE	RAST ratio ^a
1	5	3	4	11
2	4	9	11	43
3	4	5	7	11
4	5	9	10	32
5	5	4	3	4
6	ND ^b	ND	ND	40
7	ND	ND	ND	30
8	ND	ND	ND	65
9	ND	ND	ND	2
10	ND	ND	ND	9
11	ND	ND	ND	38

^aRAST Ratio = cpm peanut-allergic serum/cpm control serum.

^bND = not done.

of five patients tested. The CPE also exceeded the commercial peanut extract response in four out of five individuals. A RAST assay for the CPE was also done with a different group of peanut-sensitive subjects. As determined by IgE binding in the RAST assay, the CPE possessed *in vitro* allergenic activity, also shown in Table 1. A RAST ratio greater than 2 indicates a positive response; only one patient out of 11 registered a negative IgE binding response to the CPE, and several serum samples exhibited very high specific IgE binding to the extract.

The extract was further characterized by SDS-PAGE analysis (Figure 1), followed by immunoblotting using peanut-sensitive patient sera. IgE bound to one protein band with molecular weight 65 kDa, five proteins of between 29 and 47 kDa and approximately six proteins of between 15 and 25 kDa. The control serum did not display any IgE binding to the peanut proteins.

Electro-elution was used to purify partially the proteins in these molecular weight ranges. The 15–25 kDa fraction provoked the most reactive skin tests in the peanut-allergic individuals studied (Hefle, 1991). These results, and previous work in our laboratory which had identified two major peanut allergens at 15 and 20 kDa (Bush *et al.*, 1983), led to the choice of the 15–25 kDa fraction for monoclonal antibody studies.

Hybridoma Cell Lines and Monoclonal Antibodies

Immunoblotting of the CPE results showed that all the monoclonal antibodies bound to a major band at 25 kDa and to minor bands at 12, 14, 16, 18, 40 and 55 kDa. Figure 2 shows the blotting patterns of two of the six antibodies.

Affinity Purification of Peanut Proteins

On average, approximately 4% (300/6800 μ g) of the protein applied was retained on the columns. Loading more than 6800 μ g on to the columns did not increase the yield of affinity-purified protein.

The affinity column eluates were characterized using SDS-PAGE. All these eluates gave a pattern of five major bands with approximate molecular weights of 14, 25, 38, 40 and 44 kDa; two are shown in Figure 3. All the bands were shown to be glycoproteins by positive periodic acid-Schiff staining (Sigma Chemical Co.) of the SDS-PAGE gels.

The eluates were also analyzed by immunoblotting with the monoclonal antibodies in order to indicate in which bands the antibodies recognized epitopes. The antibodies reacted

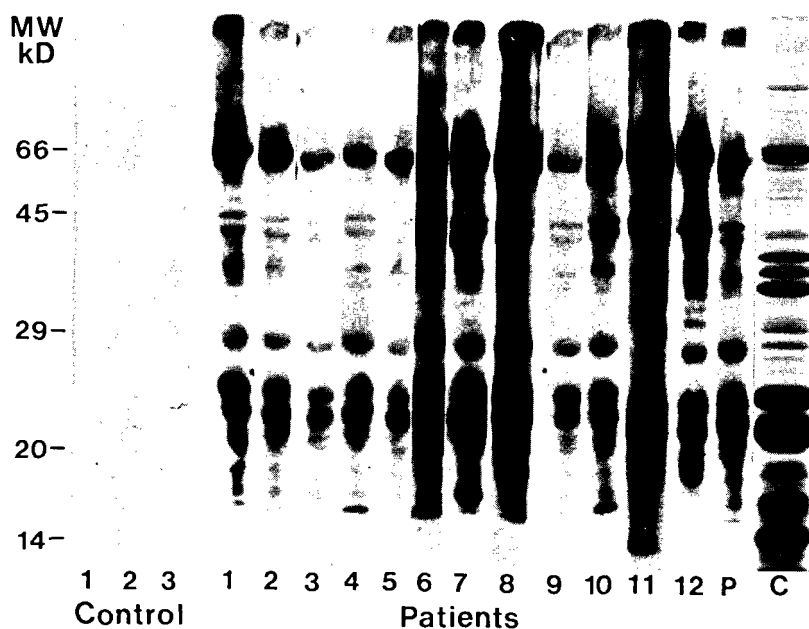


FIG. 1. Autoradiogram of an immunoblot of CPE with peanut-allergic patient sera. Bound IgE was detected with rabbit anti-human IgE labeled with ^{125}I . The exposure time was 36 h. Approximately 50 μg of protein were loaded into each lane. Approximate molecular weights are indicated on the left of the figure. Lane numbers denote different serum samples; the strip marked 'P' was incubated with a pooled sample of all peanut-sensitive sera, and the control strips were incubated with sera from three different negative control individuals. The lane marked 'C' is a Coomassie blue-stained gel lane of the SDS-PAGE-separated CPE proteins.

with four bands of approximate molecular weight 12, 14, 23 and 25 kDa (Figure 4). No non-specific binding of secondary antibody or streptavidin peroxidase was observed.

Correlation Between Peanut Proteins Recognized by the Monoclonal Antibodies and by Human IgE

A pool of peanut-sensitive patient serum was used to analyze the affinity-purified proteins by immunoblotting. Immunoglobulin E from the serum pool bound to three bands, at approximate molecular weights of 38, 44 and 65 kDa (Figure 5). The control lane, which was treated with serum from individuals with high IgE serum levels (but not peanut-specific IgE), shows the absence of non-specific binding.

A RAST inhibition assay was carried out with the affinity-purified proteins and CPE. All column eluates produced similar RAST inhibition curves; inhibition by eluate from columns 870C2F12 and 878D12G9 is shown in Figure 6. The affinity-purified fractions inhibited binding of peanut-specific IgE, but not as well as did CPE. Therefore, not all the allergenic potential of the peanut is represented by the affinity-purified proteins, as shown by the high level of protein required to provoke inhibition in the assay as compared with the CPE. Control experiments showed that other food proteins did not show inhibition (data not shown).

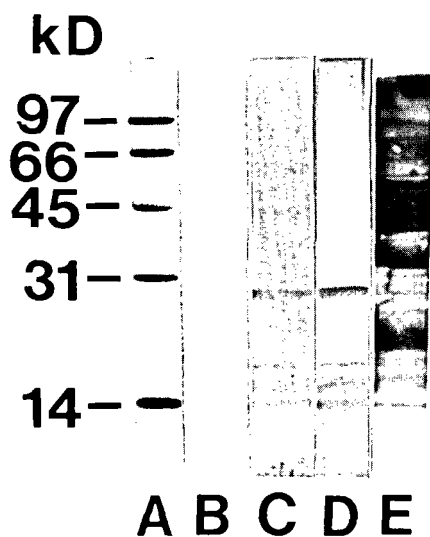


FIG. 2. Immunoblots of crude peanut extract with the monoclonal antibody. Lane A shows the molecular weight standards from the SDS-PAGE gel and lane B shows a control strip (no primary antibody). Lane C = monoclonal antibody 870B2C1; lane D = monoclonal antibody 878D12G9; and lane E = India ink protein stain. Approximately 50 μ g of protein were loaded into each lane.

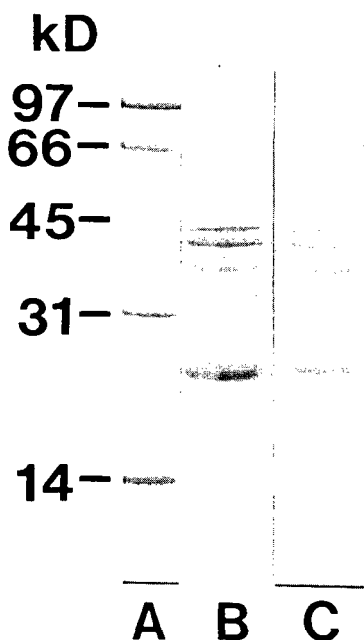


FIG. 3. Coomassie blue-stained SDS-PAGE gel of affinity-purified peanut proteins. Approximately 30 μ g of protein were loaded into each lane. Lane A contains molecular weight markers. Lane B = monoclonal antibody column 870B2C1 and lane C = monoclonal antibody column 878D12G9.

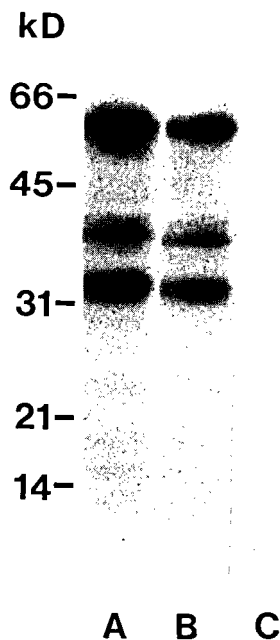


FIG. 4. Autoradiogram of an immunoblot of affinity-purified peanut proteins with peanut-allergic IgE. Bound IgE was detected with rabbit anti-human IgE labelled with ^{125}I . Exposure time was 36 h. A total of 20–30 μg of protein were loaded into each lane. Lane A = monoclonal antibody column 870B2C1 and lane B = monoclonal antibody column 878D12G9. Lane C was treated with control serum from patients with high serum levels of IgE (*Alternaria* allergy).

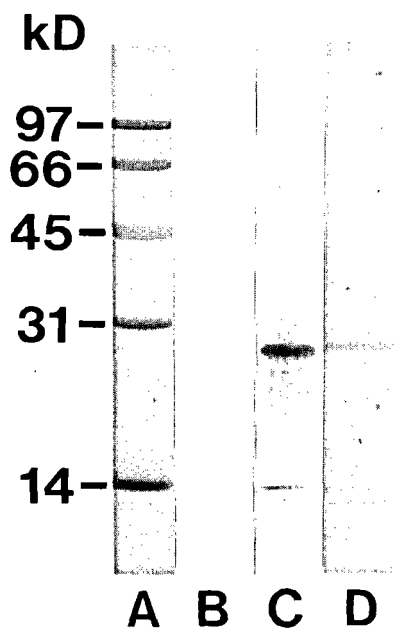


FIG. 5. Immunoblots of affinity-purified peanut proteins with monoclonal antibodies. Lane A shows the molecular weight markers from the SDS-PAGE gel and lane B shows a control strip (no primary antibody). Lane C = monoclonal antibody column 870B2C1 and lane D = monoclonal antibody column 878D12G9. Approximately 30 μg of protein were loaded into each lane.

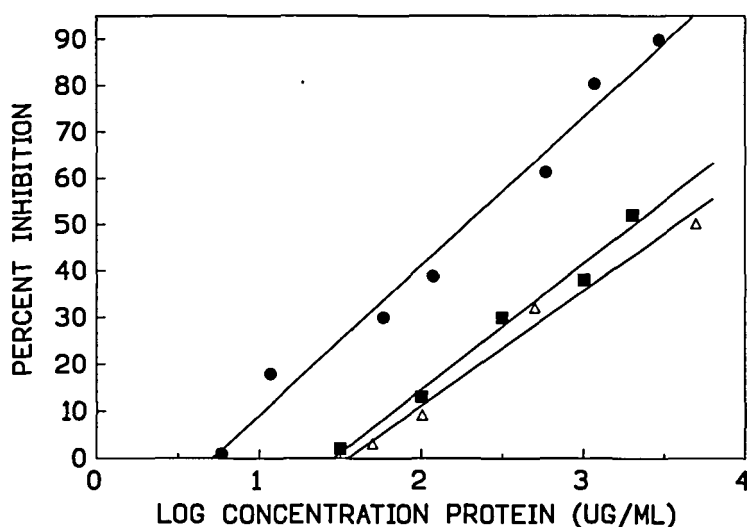


FIG. 6. RAST assay inhibition by the CPE and affinity-purified peanut proteins. (●) CPE; (■) eluate from monoclonal antibody column 878D12G9; and (△) eluate from monoclonal antibody column 870C2F12. The points are the means of nine trials; standard deviations for all values were below 4%.

DISCUSSION

Peanuts contain multiple allergenic proteins. In examining the immunoblotting data, it was noteworthy that the antibodies bound to one major band and a number of minor bands in the SDS-PAGE-separated CPE; immunoblotting of the proteins with the monoclonal antibodies showed recognition of epitopes on bands at 12, 14, 23 and 25 kDa. Monoclonal antibodies usually bind to a single, unique site; however, reactivity of monoclonal antibodies to more than one protein band in an allergenic extract has been noted in other studies (Shen *et al.*, 1988; Westphal *et al.*, 1988; Jarolim *et al.*, 1989; Walsh *et al.*, 1990; Wu *et al.*, 1990). Some of the bands could have been proteolysis products, or subunits of the other larger molecular weight proteins. Since the allergenicity of peanuts has been found to be spread throughout the conarachin and arachin fractions (Taylor *et al.*, 1982; Barnett *et al.*, 1983), another possibility is that certain peanut proteins that differ in other physicochemical aspects possess similar determinant groups.

Affinity-purified fractions from all monoclonal antibody columns consisted of five major bands in SDS-PAGE with approximate molecular weights of 14, 25, 38, 40 and 44 kDa, and some minor bands. Sachs *et al.* (1981) also found multiple bands (at 20 and 30 kDa, and several others whose molecular weights were reported to be above and below these two, but were not identified) in their 'Peanut-1' allergen. Immunoblotting of the affinity-purified proteins with peanut-sensitive patient sera showed IgE binding to bands with approximate molecular weights of 38, 44 and 66 kDa. The 14, 25 and 40 kDa bands seen in the SDS-PAGE did not bind IgE.

RAST inhibition assays showed that the affinity-purified proteins had less allergen content than the reference crude peanut extract. Previous studies using RAST inhibition (Sachs *et al.*, 1981; Barnett & Howden, 1986) and IgE ELISA (Burks *et al.*, 1992) have also shown similar results, indicating that one protein is not responsible for all of the allergenicity in the peanut. As not all of the proteins purified in the present study bind IgE, this is expected.

The source material used in this study was roasted peanuts, and raw peanuts might not contain these particular proteins; however, various researchers have concluded that the allergens of the peanut are heat stable, and the choice of raw versus roasted source material

is therefore not particularly important (Lemanske & Taylor, 1987). In RAST inhibition studies, Nordlee *et al.* (1981) found that dry-roasted and oil-roasted peanuts had approximately the same degree of allergenicity as a standard extract made from raw peanuts. Humans rarely consume raw peanuts, so study of heat-labile allergens might be of small value from a diagnostic perspective.

The isolation and identification of allergenic components of peanuts described in this study will contribute to the overall knowledge of the multiple peanut allergens. Amino acid sequencing of some of the IgE binding protein bands is currently underway in our laboratory. Determination of amino acid sequences of peanut allergens is important to help in answering questions regarding the structure and characterization of epitopes recognized by subsets of cells responsible for the production of allergen-specific IgE.

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