Adverse reaction to lupine-fortified pasta

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A 5-year-old girl with peanut sensitivity experienced urticaria and angioedema after ingesting a spaghetti-like pasta fortified with sweet lupine seed flour. The pasta was extracted and used in immunologic studies in patients with peanut sensitivity to determine whether such individuals are at similar risk. Results of skin prick tests with the lupine pasta extract were positive in five of seven subjects; these patients also reported a history of adverse reactions to green peas. In direct RAST studies IgE binding from pooled sera from patients with peanut sensitivity to the lupine pasta extract was 7 times that of a nonallergic control serum, and individual serum samples demonstrated binding from 1 to 6 times that of the negative control. Direct RAST studies of lupine seed flour with serum samples from patients with peanut allergy demonstrated IgE binding 1 to 11 times that of the negative control. Immunoblotting studies of electrophoretically separated pasta extract and lupine seed flour proteins showed IgE-binding protein bands at approximately 21 kd and in the range of 35 to 55 kd molecular weight. We conclude that some peanut-sensitive patients may be at risk for adverse reactions to lupine. (J ALLERGY CLIN IMMUNOL 1994;94:167-72.)

Key words: *Legume, hypersensitivity, cross-reaction, lupine*

Legumes are the edible dicotyledons of plants in the family Leguminosae, the second largest family of seed plants. They are economical sources of protein and calories and are considered to be one of the cheapest and most convenient high-protein materials for offsetting the amino acid deficiency of cereal proteins.[1, 2] Although economical and of high nutritive value, legumes can also cause severe, IgE-mediated hypersensitivity reactions.

Lupine *(Lupinus albus),* a member of the legume family, is a pealike plant cultivated all over the world, primarily for use as a feed, or to be plowed under for its nutrients.[3] However, this legume has also been evaluated over the years for use in foods for human consumption. Years of selective breeding have resulted in a lupine strain ("sweet lupine") that is pleasing to the palate and contains fewer alkaloids than previous strains.[4] Sweet lupine protein or flour has been suggested for use in bread,[5] cookies,[6] and milk substitutes.[7] Pasta enriched with lupine seed flour is available in retail stores. The manufacturers advertise that these products have increased fiber content and high protein values.

Abbreviations used

BSA:	Bovine serum albumin
CPE:	Crude peanut extract
PBS:	Phosphate-buffered saline
SDS-PAGE:	Sodium dodecylsulfate–polyacrylamide gel electrophoresis

METHODS

Subjects with peanut allergy

Serum samples were obtained from patients at the University of Wisconsin Hospital Allergy Clinic (Madison, Wis.). Adult patients (age range, 27 to 48 years) with histories of anaphylactic episodes implicating peanuts as the causative agent and positive skin test and RAST results to commercial peanut extracts were used. None of the patients had ever undergone oral challenge with peanuts because of their history of anaphylaxis. Control serum was obtained from six individuals who were not allergic to peanuts. All subjects gave written informed consent to the procedures, which had the approval of the Human Subjects Committee, University of Wisconsin–Madison.

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TABLE I. Skin prick test and direct RAST results for lupine in subjects with peanut allergy

	Skin test (Σ orthgonal diameters)				RAST scores*		
Patient	Histamine	Peanut†	Lupine pasta	Dil. (μg/ml)	Lupine pasta	Lupine pasta‡	Lupine seed
1	62	81	38	3600	5.7	6.1	11.1
2	49	54	NR		1.1	1.3	1.2
3	42	31	17	3600	3.0	3.2	6.5
4	42	83	NR		1.1	1.5	1.0
5	35	76	27	3600	2.6	3.6	4.4
6	27	37	30	3600	2.3	2.8	3.2
			23	360			
			19	36			
7	10	37	18	3600	1.1	1.6	1.5
Pool§					6.5		

Dil., Dilution; *NR*, no reaction.
*RAST score = cpm patient ÷ cpm control.
†Commercial peanut extract, 30 mg/ml.
‡Precipitated with ammonium sulfate before RAST.
§Pooled serum sample of patients 1 to 7.

Pasta

Spaghetti-type pasta fortified with sweet lupine seed flour (Lupini Pasta; International Nutrition and Genetics Corp., Eden Prairie, Minn.) was purchased at a local grocery store.

Sweet lupine seed

Sweet White Lupin Seeds were obtained from Wolf River Valley Seeds (White Lake, Wis. [lot no. WRV-MOB-91-1]).

Extraction

Pasta and peanuts. The pasta was boiled in water according to the manufacturer's directions, rinsed, and then ground to a paste with a mortar and pestle. The paste was extracted in a solution of 1 mol/L NaCl and 20 mmol/L Na_2HPO_4 (pH 7.0) at a 1:10 wt/vol ratio, by stirring, overnight at 4° C. The extract was clarified by centrifugation at 10,000*g* for 20 minutes at 4° C, and the supernatant solution was dialyzed (membrane cut-off = 1.0 kd) against 0.01 mol/L phosphate-buffered saline (PBS), pH 7.4, overnight (three changes) at 4° C. The protein content was determined with a tannin-based assay.[8] The extract was stored at −20° C.

The peanuts were extracted as described above, yielding a crude peanut extract (CPE).

Seeds. The seed coats were removed manually, and the remaining seed material was ground to a dry paste with a mortar and pestle. The paste was extracted as described above.

Skin tests

The protein concentrations of the CPE and the lupine extract were both 3600 μg/ml. Serial 10-fold dilutions of extracts were prepared with PBS as the diluent. The diluent served as the negative control. Positive controls for the tests contained 1 mg histamine per milliliter in 50% glycerin plus 0.4% phenol. Wheal and flare sizes (sum of the orthogonal diameters in millimeters) were recorded approximately 10 to 15 minutes after puncture of the skin.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis

Vertical slab gel electrophoresis was carried out by the method of Laemmli[9] in a Mini-Protean II gel apparatus (apparatus, molecular weight standards, and chemicals from Bio-Rad Laboratories, Richmond, Calif.). Resolving gels (8 × 7.3 cm) were 12.5% wt/vol polyacrylamide, with 4% stacking gels. Samples were boiled for 5 minutes in sample buffer containing 40 mmol/L dithiolthreitol. Fifty micrograms of sample were loaded for each lane. Gels were stained with FAST Stain (Zoion Research, Allston, Mass.).

Immunoblotting

The extracts were subjected to sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) as described above. Proteins were transferred to an Immobilon P (0.45 μm; Millipore, Bedford, Mass.) membrane in a Transblot apparatus (Bio-Rad Laboratories) for 2 hours at 50 V (constant voltage), according to the method of Towbin et al.[10] Part of the membrane was stained with India ink to assure transfer of the proteins (100 μl India ink/100 ml 0.01 mol/L PBS, pH 7.5, containing 0.05% Tween 20).

The membrane was blocked with 0.01 mol/L PBS containing 3% bovine serum albumin (PBS-BSA) for 1 hour at 25° C. After washing three times with PBS containing 0.05% Tween 20, the membrane was cut into

strips with a scalpel. The strips were incubated with peanut-sensitive patient sera (diluted 1:10 in PBS-BSA) for 18 to 24 hours at 4° C. The strips were washed again, and then incubated overnight with 2 ml rabbit anti-human IgE labeled with iodine 125 (diluted in PBS-BSA to 70,000 cpm/ml) (Phadebas RAST kit; Pharmacia Biochemicals, Piscataway, N.J.). After washing again, autoradiographic detection of IgE binding was done by exposing X-OMAT AR x-ray film (Eastman Kodak, Rochester, N.Y.) to the strips at −70° C. Exposure time was 3 days.

Direct RAST

The extracts (pasta extract = 3.6 mg/ml and seed extract = 18 mg/ml) were coupled to cyanogen-bromide–activated Sepharose 4B (Pharmacia Biochemicals) (5 to 10 mg protein per milliliter of Sepharose 4B), according to the manufacturer's directions. Coupled Sepharose-extract and sera from peanut-sensitive patients were diluted in "RAST buffer" (0.05 mol/L $NaHPO_4$, 0.43 mol/L NaCl containing 3% BSA, 0.05% sodium azide, and 0.05% Tween 20), to 3% wt/vol and 1:5, respectively. One half milliliter of the diluted Sepharose-extract and 0.1 ml of the sera were incubated together for 18 to 24 hours at 25° C with rotation. The solid phase was then washed three times with 3 ml of RAST buffer, after which ^{125}I-labeled rabbit anti-human IgE (50,000 to 85,000 cpm) (Phadebas RAST kit) was added to each tube. After incubation overnight at 25° C, the tubes were again washed, and bound radioactivity was determined (5 min/tube) with a gamma scintillation counter (Searle Laboratories, Des Plaines, Ill.).

RAST inhibition assays

RAST inhibition assays were done as described above, except that competitive inhibitors (peanut, lupine seed, or lupine-fortified pasta extract) at various concentrations were present in the reaction mixture.

RESULTS

Skin tests

The orthogonal diameters of the flare (largest length and largest perpendicular diameter) were used for comparison. Skin test results for five of seven patients with peanut allergy were positive to the pasta extract (Table I); these five individuals also reported a history of green pea sensitivity; however, this sensitivity was not substantiated by oral challenge or skin test results in this study. In one individual the skin test produced a positive response to a low concentration of lupine pasta extract (36 μg/ml). Because the primary component of pasta is semolina, one would expect that the response to lupine protein alone would be greater.

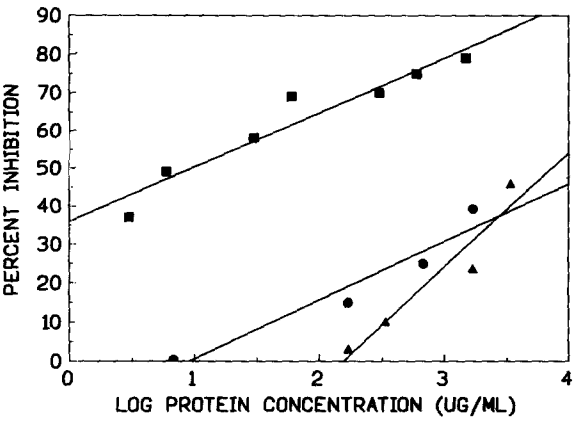

FIG. 1. Lupine RAST assay inhibition by the various lupine extracts and CPE. *Diamonds,* Lupine seed extract; *squares,* lupine pasta extract; *circles,* CPE. Standard deviations for all values were below 4%.

RAST

Direct RAST results showed IgE binding in four of seven peanut-sensitive serum samples to the lupine-fortified pasta extract. Direct RAST scores for these samples increased with further purification of the seed proteins by ammonium sulfate precipitation and use of the seed flour alone (Table I).

RAST inhibition studies showed that amounts of seed extract and CPE needed to inhibit 50% of the binding of peanut-sensitive IgE to lupine-fortified pasta extract coupled to Sepharose were 6300 and 1600 μg/ml, respectively (Fig. 1). The pasta extract presented problems in the RAST inhibition studies, probably because of the large amount of carbohydrate in the extract. The inhibition of binding in the latter experiment never dropped below 30%, indicating absence or hindrance of detector antibody binding at low levels of protein.

Immunoblotting

The extracts were further characterized with SDS-PAGE (Fig. 2), followed by immunoblotting with peanut-sensitive sera. Serum IgE from adults with peanut allergy bound to a band at 21 kd and to several bands with molecular weight in the range of 35 to 55 kd (Fig. 3). Sera from patient 7 did not demonstrate any IgE binding in these studies (data omitted). The control serum samples displayed negligible IgE binding to the lupine proteins.

DISCUSSION

Many individuals with peanut allergy have IgE antibodies that cross-react with other legumes,[11, 12]

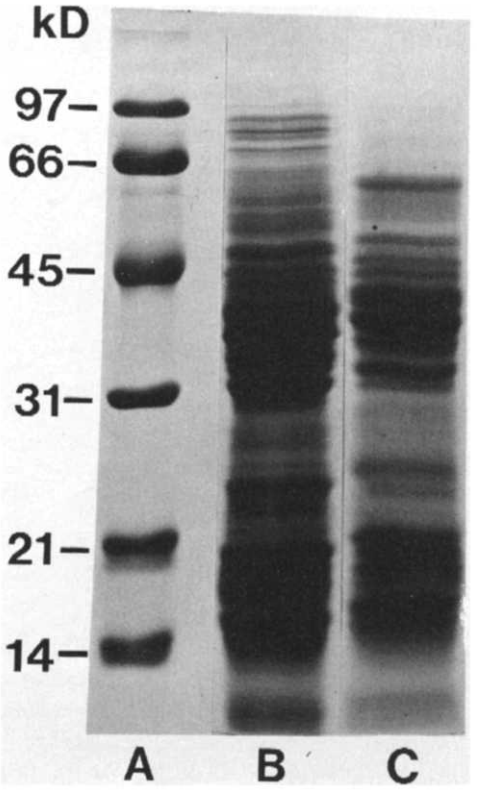

FIG. 2. FAST-stained (Zoion Research) SDS-PAGE gel of affinity-purified peanut proteins. Approximately 50 μg of protein was loaded per lane. *Lane A* contains molecular weight markers. *Lane B* contains lupine-fortified pasta extract. *Lane C* contains lupine seed flour extract.

although some researchers observe that clinically significant reactions to multiple legumes in their pediatric populations are not prevalent.[13] In contrast, many patients in our peanut-sensitive adult population report adverse reactions to several legumes. All of the patients with positive RAST and skin prick test results to lupine report allergic sensitivity to green pea, a botanical relative of lupine, but not to all legumes. In a report similar to this one, it was found that individuals with peanut allergy can have severe IgE-mediated reactions to taugeh (sprouted small green beans), a typical component of egg rolls.[14] Therefore although it appears from the literature that clinical sensitivity to one legume does not warrant elimination of all legumes from the diet in most cases, it should be evaluated on an individual basis.

The data show that the cross-reactivity between peanut and lupine is only partial because the RAST scores for peanut and lupine are not equivalent, the skin prick tests differ in severity, and the peanut extract inhibits the IgE binding more readily than the lupine seed extract does, based on protein concentration of extract. The RAST and skin test data obtained show that a substantial percentage of our patients who have IgE antibodies against peanut have IgE antibodies that cross-react with lupine and can experience an IgE-mediated hypersensitivity reaction to it. Therefore some patients with peanut allergy, particularly those with a history of adverse reactions to green pea, might also experience adverse reactions to lupine after ingestion.

The IgE-binding proteins of the lupine extract appear to have an approximate molecular weight of 21 kd and 35 to 55 kd as determined by SDS-PAGE, and are heat-stable. Three of the six sera bound only weakly to the 21 kd band, whereas this band appeared to be a major IgE-binding protein for the other three individual sera. The subjects who had a positive skin test reaction to lupine extract also reported a history of adverse reactions to green pea. In a study of 10 green pea–sensitive subjects, only crude pea and pea albumin extracts produced positive skin test results, whereas legumin (11S) and vicilin (7S), major pea storage globulins, did not produce positive skin test results; the albumin fraction retained all of its allergenic activity when heated or boiled.[15]

A green pea allergen with an approximate molecular weight of 1.8 kd, as determined by SDS-PAGE, was later purified from pea dialysate (produced by dialyzing pea extract against distilled water). The allergen contained approximately 30% carbohydrate.[16]

In contrast, the major allergens of another legume, the soybean, are found in the 2S, 7S, and 11S soybean globulin fractions.[17, 18] In another study by Ogawa et al.[19] in subjects with atopic dermatitis, most IgE-binding bands were assigned to protein components of the 7S globulin fraction, and very little reactivity was found in the 11S fraction. Most (65%) of these subjects reacted to a 30 kd protein band, named *Gly m* Bd 30 k, which was a minor protein of the 7S globulin fraction. However, this study was done with patients who did not experience severe or anaphylactic reactions to soy.

The Kunitz soybean trypsin inhibitor, a major component of the 2S globulin fraction with a molecular weight of approximately 20 to 21 kd, was found to be a major allergen by skin testing and RAST studies.[20]

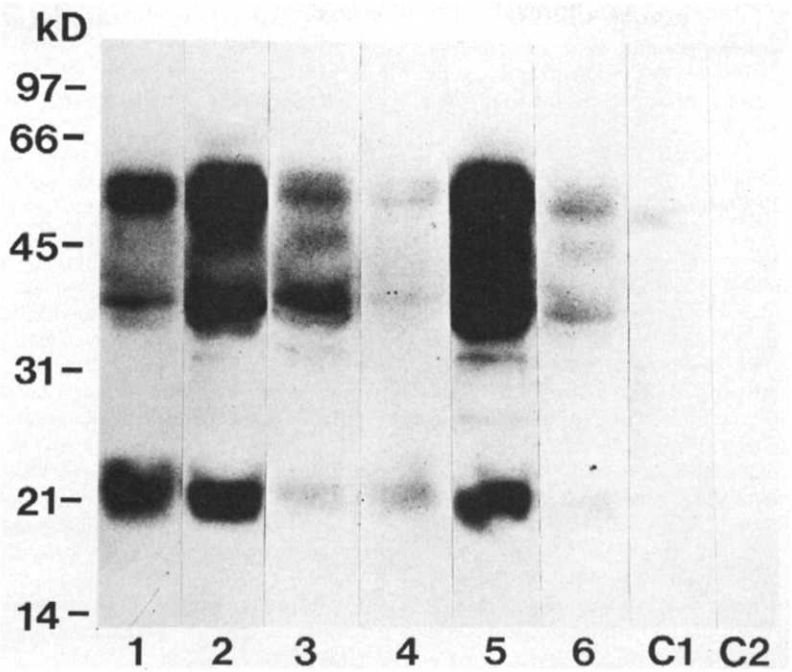

FIG. 3. Autoradiograph of an immunoblot of SDS-PAGE–separated lupine proteins with peanut-sensitive IgE. Bound IgE was detected with rabbit-anti-human IgE labeled with ^{125}I. Exposure time was 72 hours. Approximately 25 μg of protein was loaded per lane. Approximate molecular weights are indicated on the left. Lane numbers denote different serum samples; the strips marked *C1* and *C2* were incubated with sera from control (nonallergic) serum.

Herian et al.[21] described a 20 kd protein, which is a major allergen in patients who are allergic to soybean, but which is not the Kunitz soybean trypsin inhibitor. Other subjects in the study were sensitive to both peanut and soybean, and their sera demonstrated IgE binding to several bands in the 50 to 70 kd range. These allergens were not characterized further.

Bush et al.[22] found that green pea extract inhibited IgE binding in the serum of a patient with occupational soy flour–induced asthma to a soybean protein with an approximate molecular weight of 17 kd, indicating the presence of some cross-reactivity. The patient's IgE did not bind to peanut protein, and peanut extract did not inhibit IgE binding to the soy extract proteins.

Peanut allergens that have been identified and characterized include a 65 kd concanavalin A–reactive glycoprotein, which constitutes approximately 1% of the total peanut protein[23]; a 66 kd allergen, named *Ara h* I, which does not react to concanavalin A but has other similar characteristics to concanavalin A–reactive glycoprotein[24]; *Ara h* II, a 17 kd allergen[25]; and a 14 kd allergen.[26] None of these allergens appear to have the observed molecular weight of the lupine allergens.

The interrelationships of these proteins in each legume, as subunits or degradation products of one another, or as a result of influence of post-translational glycosylation on molecular weight, are not known at present. The cross-reacting sequences/epitopes in these legumes, which cause clinical and in vitro reactions to more than one legume, are also not known. More research is needed to obtain more precise information about the allergenic components of legumes. Structural studies of legume allergens and studies of the relative importance of certain residues in terms of allergenicity of the molecules, leading to definite knowledge of cross-reactivities, will give substitution/elimination diet therapy a better scientific foundation and may make progress in immunotherapy for legume sensitivity a safer possibility.

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