

Allergenic cross-reactions among legume foods—An in vitro study

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The specific IgE binding by protein extracts of 11 food legumes, including soybean, was examined by RAST and RAST inhibition. Sera from 15 peanut-sensitive patients were, with very few exceptions, positive in the RAST to all the legumes. RAST-inhibition testing of each extract against RAST discs of the other legumes indicated considerable cross-reactivity of IgE binding between the legumes. Cross-allergenicity was demonstrated to be most marked between the extracts of peanut, garden pea, chick pea, and soybean. The results have important implications for selection of effective hypoallergenic diets and for the diagnosis of patients hypersensitive to foods. (J ALLERGY CLIN IMMUNOL 1987;79:433-8.)

Immediate hypersensitivity reactions to legumes are relatively common in atopic patients who are sensitive to foods. Severe reactions to some of the legumes have been reported, in particular to peanut, garden pea, and garbanzo (chick pea).^{1,2} Peanut is sometimes grouped with the nuts but is, of course, a legume. In common with most food allergens, the identity and properties of the proteins responsible for the allergic reactions remain obscure. Multiple allergies to a number of the legumes are frequently observed. These allergies are often described as cross-reactions but are seldom precisely confirmed as such. Allergenic cross-reactivity may be defined in this context as the elicitation of an allergic response as a result of an allergen binding to IgE antibody that has been produced through sensitization to another allergen.

The structural characteristics of allergens that control cross-reaction are unknown.³ One possibility is that cross-reacting allergens have similar allergenic determinants. Allergenic cross-reactivity has been noted in studies of inhalant and pollen allergens⁴⁻⁶ in which multiple sensitization was suspected. However, investigation of this phenomenon in the area of food hypersensitivity has been very limited,^{7,8} although multiple food allergies are known to be common.^{9,10} Despite limited knowledge of such interrelationships, it is usual to specify exclusion from the diet of whole

Abbreviation used

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

groups of foods, based mainly on taxonomic data. Although this may prove effective, it is often difficult to execute, especially for staple foods such as cereals or legumes and indeed may be unnecessary in many cases. Definitive knowledge about allergenic cross-reactions would put such treatments on a more sound scientific basis and may enhance their efficacy.

This article describes an in vitro study undertaken to examine the possible occurrence and the extent of allergenic cross-reaction between commonly eaten food legumes.

MATERIAL AND METHODS

Patients' sera

Sera were collected from 40 patients with clinical reactions to peanut that included symptoms of urticaria, angioedema, asthma, and, in three cases, anaphylaxis. Diagnosis had been confirmed in all patients by history, skin prick testing, and RAST. Each of these sera was tested by RAST with discs of peanut, garden pea, soybean, haricot bean, and brown lentil extracts prepared as described below. Those sera (15) that elicited positive RAST to three or more discs were used for further testing. Five of these sera were selected for quantitative RAST-inhibition testing, and clinical details of these sera are recorded in Table 1. Serum for controls was obtained from two patients with severe allergies to pollens but not to foods.

Legumes and extracts

Samples of 11 legumes were purchased from retail sources and are listed in the legend to Fig. 1. All samples were examined for the presence of atypical or damaged

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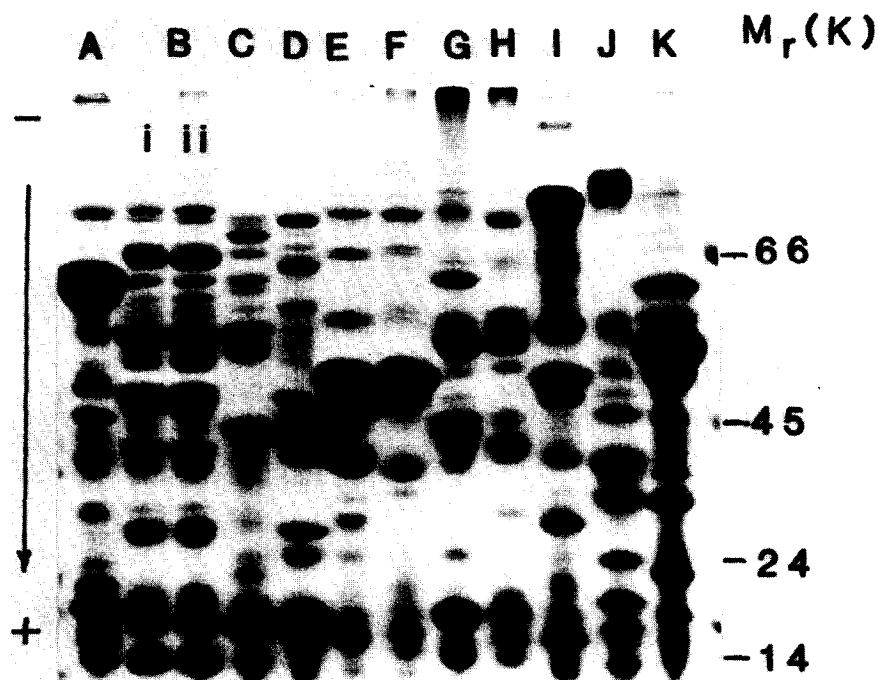


FIG. 1. SDS-PAGE (10% total polyacrylamide concentration, 4% cross-linked concentration) of legume extracts. Samples of the extracts were reduced with 2-mercaptoethanol. The gel was stained with Coomassie blue R-250. The samples consisted of 150 μ g of each of the following: A, peanut, *Arachis hypogaea*; B, garden pea, *Pisum sativum*; C, soybean, *Glycine max*; D, chick pea, *Cicer arietinum*; E, haricot bean, *Phaseolus vulgaris*; F, red kidney bean, *Phaseolus vulgaris*; G, broad bean, *Vicia faba*; H, brown lentil, *Lens culinaris (esculenta)*; I, great northern bean, *Phaseolus vulgaris*; J, lima bean, *Phaseolus lunatus*; and K, mung bean, *Phaseolus aureus*. Molecular weight standards used (10 μ g of each) were 66,000 bovine serum albumin, 44,000 ovalbumin, 24,000 trypsinogen (phenylmethanesulfonyl fluoride treated), and 14,000 lysozyme. Note: B, i and ii, represent a dried whole pea and split pea extract, respectively. These were found to be electrophoretically similar and indistinguishable in preliminary RAST. Extract B i was used in subsequent experiments.

TABLE I. Patient data for sera used in the final RAST inhibition (Table III)

Patient	Sex	Age (yr)	Known legume sensitivity*	Symptoms
V	M	13	Peanut, pea, soybean	Anaphylaxis, asthma, angioedema
W	F	19	Peanut, pea	Anaphylaxis, angioedema
X	M	11	Peanut, soybean, "beans"	Syncope, angioedema
Y	M	9	Peanut, soybean	Angioedema, urticaria
Z	F	6	Peanut	Angioedema, urticaria

*Believed to occur on ingestion. No challenges were made because of the possible severe result.

seeds; no mould growth was noted. The samples were ground and defatted as described previously for raw peanuts.² Protein extracts were made from each legume meal by stirring a 1/10 w/v suspension in 0.1 mol/L of Tris-HCl buffer (pH 8.5), 0.1 mol/L in NaCl, for 2 hours. The sus-

pensions were centrifuged 20,000 $\times$ g for 30 minutes to remove the insoluble debris, and the clear supernatants were dialyzed against 0.1 mol/L NH_4HCO_3 solution before freeze-drying. The extracts were stored dry at -20°C until they were used.

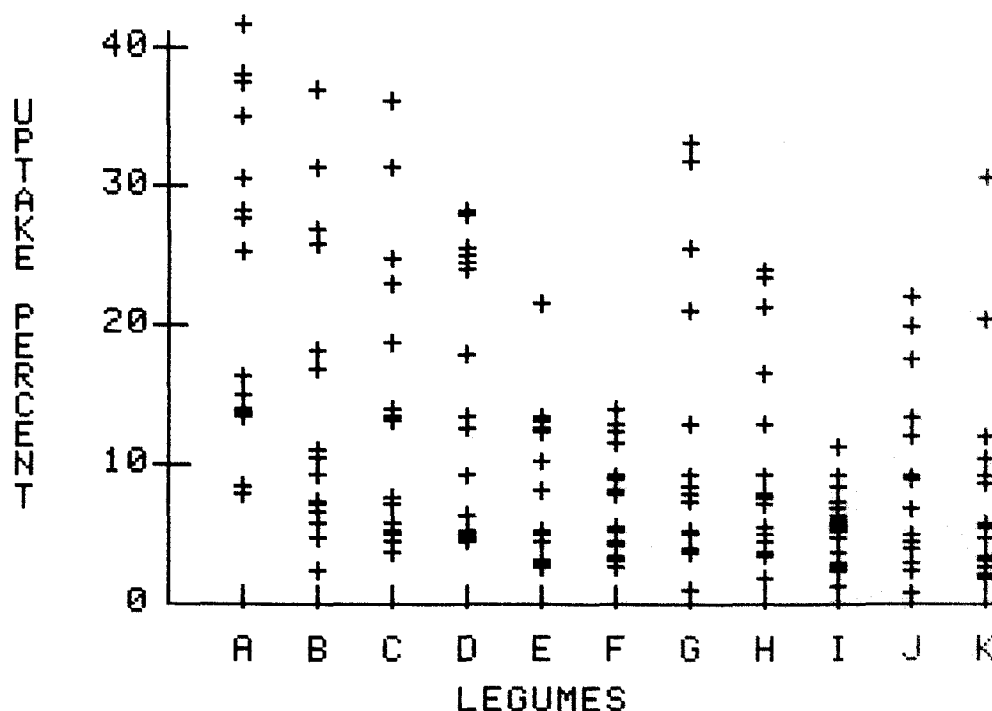


FIG. 2. The distribution of RAST results as found for sera from 15 peanut-sensitive patients with discs (100 μ g per disc) to which were bound each of the 11 legume extracts of Fig. 1.

RAST examination

The *in vitro* IgE binding of the legumes was measured by application of the RAST and RAST inhibition after procedures described previously.² RAST discs were made by exposing CNBr-activated discs to solutions containing 100 μ g per disc of each of the 11 legume protein extracts. RAST was carried out in duplicate for each of the sera and legume discs. Ten of the 15 sera from the peanut-sensitive patients demonstrated >5% radioactive uptake for most of the legumes and were tested further by RAST inhibition. These sera were diluted 1:5 and preincubated with 50 μ g of each of the legume protein extracts, and the RAST inhibition was determined. The five most generally reactive sera were selected, and the original, undiluted sera were pooled. Quantitative RAST inhibition, with the serum pool, used 0.1 to 100 μ g of extract from four apparently cross-reactive legumes, peanut, garden pea, soybean, and chick pea versus discs of the same legumes.

SDS-PAGE

SDS-PAGE was carried out on the protein extracts according to the method of Laemmli¹¹ modified for performance in a Gradiopore (Gradiopore, Ltd., Pyrmont, Australia) apparatus with gel slabs 7 by 7 by 0.3 cm. The legume extracts were reduced with 2-mercaptoethanol to reveal their subunit patterns.

RESULTS

SDS-PAGE

Fig. 1 illustrates the result of the examination of 150 μ g of each of the sample extracts. Each of the

legume extracts elicited a characteristic and complex pattern with bands of molecular weight ranging from about 10,000 to 100,000.

RAST for specific IgE antibodies

The results of RAST for the 11 legumes and with the 15 sera are presented in Fig. 2. The binding to specific IgE demonstrated great variation, but in no case was the RAST positive for only one of the extracts. IgE in the sera of patients sensitive to peanut was apparently able to bind to other legume protein extracts to a greater or lesser extent in almost all possible combinations of legume and serum.

Fig. 3 presents a specific RAST response histogram of the five sera selected for the quantitative RAST-inhibition tests. The controls (mean value illustrated at *right hand* end of the group of bars for each legume) elicited radioactive uptakes of <1.5% with all the legume discs, indicating no nonspecific binding of IgE by the discs. We had previously demonstrated that the presence of lectins, which are abundant in many legumes, does not lead to nonspecific binding to IgE in the RAST.*

The results of the RAST inhibition of legume discs by the extracts at a single concentration are presented in Table II. The raw data were expressed in the form of numbers of sera demonstrating >20% inhibition.

*Barnett D, Howden MEH. Unpublished results.

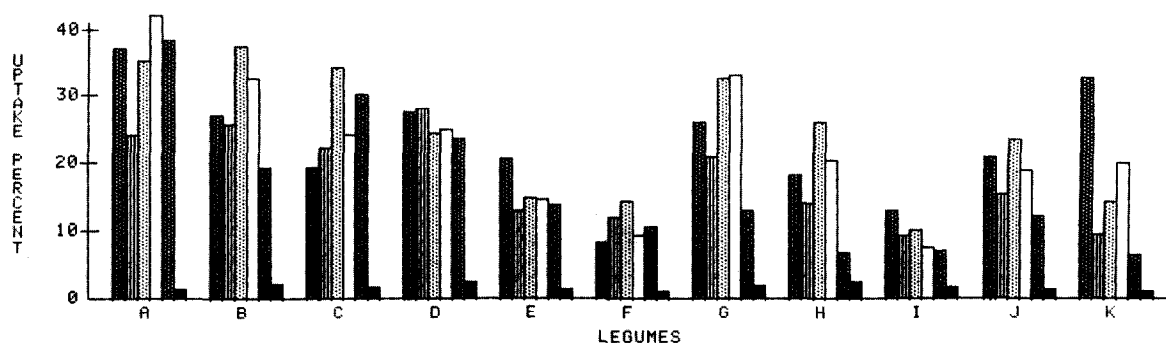


FIG. 3. RAST radioactive uptakes for the five sera chosen from the results of RAST inhibition testing (Table I) for further inhibition examination. The legume letter code corresponds to that of Fig. 1. The results are presented for each legume and corresponding serum V to Z of Table I in the shaded bars from left to right. The solid bar on the right of each block represents the mean uptake for the two control sera with the respective legume extract.

TABLE II. Results of RAST inhibition of legume RAST discs (100 µg per disc) with legume extracts (50 µg)*

Legume extract	Legume RAST disc										
	A	B	C	D	E	F	G	H	I	J	K
A	10	9	9	10	7	5	8	6	4	6	6
B	8	10	6	8	4	7	4	4	5	3	6
C	8	6	10	5	2	4	3	2	1	3	2
D	9	8	9	10	6	4	5	5	2	3	4
E	8	7	8	7	10	5	4	3	2	2	2
F	2	3	3	3	6	10	3	3	3	1	2
G	8	6	4	5	4	3	10	4	2	2	2
H	7	5	2	4	3	3	4	10	3	2	3
I	4	6	3	2	2	2	2	3	10	2	4
J	4	3	4	3	2	3	4	3	2	10	2
K	6	5	2	3	4	2	2	3	4	3	10

Ten sera, diluted 1:5, were tested. The legume reference letters are the same as those in the legend to Fig. 1.

*The figures in the table represent the number of sera demonstrating inhibition of 20% or greater in duplicate tests where inhibition percent is defined as

$$\left(1 - \frac{\text{Radioactive count after inhibition}}{\text{Radioactive count uninhibited}}\right) \times 100$$

This was done for the purpose of comparing the extent of apparent cross-reaction between the different extracts.

The result of more restricted and quantitative RAST inhibition of a pooled serum is presented in Table III. This data present some indication of the extent of cross-allergenicity between the four legumes eliciting highest RAST inhibitions.

DISCUSSION

It would appear from the results of this study that multiple food allergy among the legumes is likely to be common. In the preliminary screening of 40 sera

from peanut-sensitive patients, 15 of the sera were positive in the RAST to at least two other legumes. All these sera demonstrated specific IgE binding, in later tests, to extracts of almost every one of the 11 legumes.

The results of RAST-inhibition tests with the 10 more reactive sera indicated extensive cross-allergenicity between the extracts (Table II). The most significant cross-reactions, for the sera from this group of patients, were between peanut, garden pea, soybean, and chick pea. The first three of these legumes are common dietary items, but chick pea is comparatively unusual in Western countries except in some

TABLE III. Inhibitor (microgram) needed to produce 50% inhibition of RAST for four legumes

Extract	Disc			
	Peanut	Garden pea	Soybean	Chick pea
Peanut	1.5	2.0	1.9	5.0
Garden pea	19.0	2.0	42.0	21.0
Soybean	6.0	19.5	2.5	32.0
Chick pea	12.0	22.0	38.0	2.2

The results were derived from interpolation of semilog plots of the inhibition obtained by varying amounts of extract between 0.1 and 100 μ g with the discs from the four legumes and a pool of the five sera of Table I and Fig. 3.

ethnic foods. The strong RAST reactivity (Fig. 3) and cross-reaction of chick pea is thus suggestive of cross-sensitization through exposure to one of the other foods.

The quantitative comparison of cross-reactions presented in Table III illustrate an apparent dominance by the peanut extract. Fifty percent inhibition of the other extract discs was obtained with $<5 \mu$ g of peanut extract, whereas some of the other extracts required $>30 \mu$ g for similar inhibition. Since the sera used were from patients presenting mainly with peanut sensitivity, perhaps this would not be unexpected. However, it appears possible from the overall result of this study that, in many cases, patient's IgE is binding to proteins from the different legumes and presumably recognizing some common feature of these molecules.

In speculating on the molecular basis of the apparent cross-reactions, three possibilities may be considered. First, the legume storage proteins are believed to be at least partially homologous across a range of species.¹² Second, protease inhibitors are widely distributed in the Leguminosae and demonstrate extensive amino acid sequence homologies.^{13, 14} Last, lectins (i.e., proteins with affinity for particular carbohydrates) are found in many legumes. Lectins also demonstrate sequence homology and may be involved in the IgE response.^{15*} Any more precise explanation of the observed cross-allergenicity awaits greater knowledge of the molecular rationale of this phenomenon and requires research into the nature of the allergenic determinants involved.

The clinical significance of in vitro studies may be questioned because RAST is not always considered to correlate well with clinical food hypersensitivity.¹⁶ However, skin prick tests and RAST were found to be reliable for the diagnosis of immediate type I allergy to codfish, peas, nuts, peanut, and egg white in a study by Aas.¹⁷ Recently, excellent correlations were

obtained between skin prick tests, RAST, and clinical symptoms in an examination of peanut sensitivity in 100 children.¹⁸ If it is presumed that these relationships for peanut and pea may be extended to other legumes, then the results of this article suggest that exclusion from the diet of the whole legume group is indicated where strongly positive diagnosis indicates hypersensitivity to one of the highly cross-reactive legumes. Legumes other than these may be reintroduced later with less risk of relapse. Second, where hyposensitization is contemplated, the results of this study suggest that use of one of the four very cross-reactive legume extracts may possibly be protective, at least against the other three legumes of this group. This proposition requires further examination in view of the different inhibitory capacities demonstrated by the four extracts that may be due to the presence of different allergenic determinants.

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The determination of the eosinophil count: Comparison of two techniques

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The total eosinophil count obtained by use of the standard hemacytometer is considered by several investigators to be the most reliable method for the determination of the circulating eosinophil levels in the blood because it is believed to be less subject to error than the calculated eosinophil count obtained with Wright stained blood smears from the differential count. We compared the two techniques in outpatients as well as inpatients with and without allergies (total of 514 patients). Our results demonstrate that the eosinophil count by the two methods are similar. (J ALLERGY CLIN IMMUNOL 1987;79:438-41.)

The diagnosis and treatment of many diseases characterized by the presence of eosinophilia¹⁻⁴ depend on an accurate determination of the circulating eosinophil levels in the blood. There are several studies in the literature that favor the use of the TEC obtained with the standard hemacytometer,⁵ over the CEC, determined from the differential count.⁶⁻⁸

This study was carried out to compare the two techniques used to count eosinophils in the blood. Our findings suggest that there is no statistical difference between the TEC and the CEC, and that both methods are acceptable for the determination of the eosinophil count.

Abbreviations used

TEC: Total eosinophil count

CEC: Calculated eosinophil count

MATERIAL AND METHODS

The eosinophil count was done on paired samples in 514 subjects (230 inpatients and 284 outpatients) at Milwaukee Children's Hospital. The population studied included male and female allergic patients as well as nonallergic patients who ranged in age from 20 months to 18 years (mean 6.25 ± 7.0 SD).

The CEC was determined, with venous blood containing EDTA, from the differential count with Wright stained blood smears in which 200 leukocytes were examined and the percentage of eosinophils was recorded. The formula used was: eosinophil per cubic millimeter equals the percentage of eosinophils in the differential multiplied by the white cell count.⁵ The TEC was carried out as follows: venous blood anticoagulated with EDTA was drawn to the 1.0 line in two

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