

Lectins and the radioallergosorbent test

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An investigation into the possible role of lectin binding of IgE in RAST of legume and wheat extracts is reported. Lectins from pea, broad bean, lentil, jack bean, soybean, peanut, and wheat germ were coupled to RAST discs. The discs were pretreated with lectin-specific sugars in an attempt to inhibit RAST with sera from 11 sensitive patients. In all cases, RAST was almost unaffected by the inhibitory sugars, indicating that nonimmune binding of IgE by lectins in legume or wheat RAST was not significant when RAST was carried out with allergens bound to the usual paper discs. IgE contains binding sites for all the lectins examined, and five of the sera had high total IgE >300 IU/ml. It is suggested that competition by IgG and other serum glycoproteins may explain the lack of effect by the added sugars. All sera contained endogenous glucose at a level of about 3 mmol/L that may have accounted for some self-inhibition of the lectin binding by pea, broad bean, lentil, and jack bean lectins. There was, however, significant immune binding of some of the lectins by specific IgE, and it is concluded that these lectins may be important in expression of IgE-mediated allergic responses. (J ALLERGY CLIN IMMUNOL 1987;80:558-61.)

Immediate hypersensitivity (type I reaction) to foods is a relatively common phenomenon in atopic patients. Allergies to legumes form a significant fraction of these IgE-mediated reactions and can sometimes produce fatal or near-fatal anaphylactic reactions.¹ Diagnosis of legume allergy is usually made from the patients' history and the results of skin tests, but confirmation is often sought by performance of RAST. When RAST is used in this way or in researching the particular substances eliciting the reactions, it must be highly specific and not elicit false positive results.

It was suggested by Aalberse et al.² that some food extracts used for the diagnosis of food allergy could elicit positive RAST as a result of nonimmune reactions. Freed³ discussed the possibility of lectin interference in RAST of birch or grass pollens and reported the apparent role of lectins in the expression of immediate-type allergic reactions. Aalberse et al.² found that IgE binding to an extract of green pea was inhibited by 10 mmol/L of Me α -Man. This monosaccharide is the specific inhibitory sugar of a number of lectins, including that from the green pea. Lectins are polypeptides that have a strong affinity for certain

Abbreviations used

PSA:	Green pea lectin (<i>Pisum sativum</i>)
LEN:	Lentil lectin (<i>Lens culinaris</i>)
FAV:	Broad bean lectin (<i>Vicia fabia</i>)
WGA:	Wheat germ agglutinin (<i>Triticum vulgare</i>)
CON A:	Concanavalin A (<i>Canavalia ensiformis</i>)
SBA:	Soybean agglutinin (<i>Glycine max</i>)
PNA:	Peanut agglutinin (<i>Arachis hypogaea</i>)
Me α -Man:	α -Methyl mannoside
GlcNAc:	N-acetyl glucosamine
GalNAc:	N-acetyl galactosamine
Gal:	Galactose

monosaccharides. Many common food legumes, including soybean, contain lectins that may bind to the carbohydrate portion of IgE.⁴ Such nonimmune binding could interfere in RAST. Our earlier investigation of peanut allergens, including PNA, had not indicated any significant nonimmune binding of IgE.⁵ The present study was undertaken in order to examine the possible interference by lectin binding of IgE in RAST carried out with legume and other food extracts.

MATERIAL AND METHODS

Sera of patients

Serum samples were obtained from eight patients with a clinical history of allergy to peanut and confirmed as being IgE mediated by positive skin test and RAST. Sera were also obtained from three similarly diagnosed wheat-sensitive

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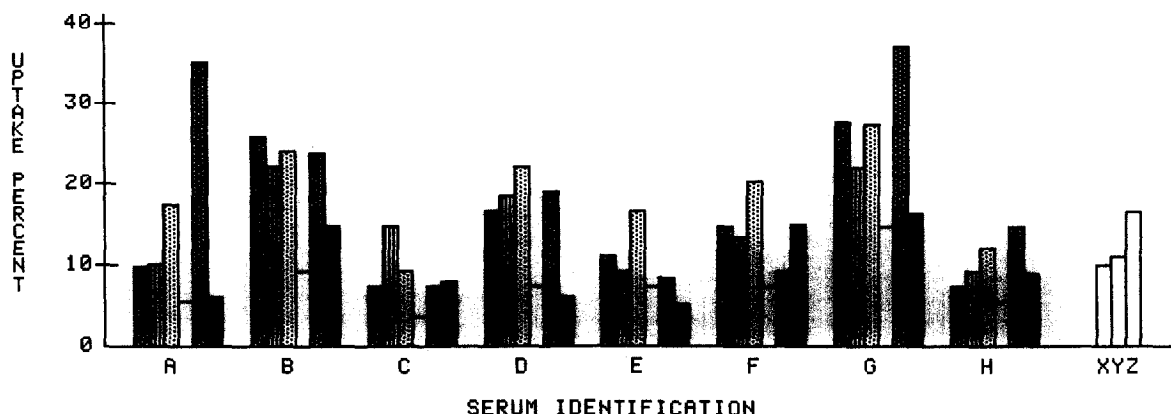


FIG. 1A. Results of RAST for each of the sera from peanut-sensitive patients A, B, C, D, E, F, G, H, and wheat-sensitive patients X, Y, and Z. From *left to right*, respectively, the bars represent the percent radioactive uptakes by discs coupled with PSA, FAV, LEN, CON A, PNA, and SBA. Sera from wheat-sensitive patients X, Y, and Z were tested only with WGA discs.

patients. Sera from two pollen-sensitive and one nonatopic patient were collected for control sera.

Lectins and sugars

PSA was prepared and purified by affinity chromatography on Sephadex G-75 as described by Higgins et al.⁶ LEN and FAV were prepared in a similar manner. WGA was prepared and purified by affinity chromatography on a column of ovomucoid Sepharose 4B according to the method of Marchesi.⁷ CON A, SBA, and PNA were purchased from E-Y Laboratories (San Mateo, Calif.). The lectins were checked for homogeneity by sodium dodecyl sulfate gradient gel electrophoresis, and each lectin elicited the expected one or two sharp bands. The lectin-inhibitory sugars, Me α -Man, GlcNAc, GalNAc, and Gal were obtained from Sigma Chemical Co. (St. Louis, Mo.).

RAST method

RAST discs from each of the lectins (exposed to 50 μ g per disc) were prepared on a CNBr-activated filter paper as described previously.⁵ To determine the possible contribution to RAST uptake from lectin-IgE nonimmune binding, lectin discs were first incubated individually with a 50 μ l of a 100 mmol/L solution of the appropriate sugar in RAST buffer for 1 hour before addition of serum. Specific inhibitory sugars for each of the lectin discs were selected from data of Baldo⁸ and were as follows: PSA, FAV, LEN, and CON A and were treated with Me α -Man. PNA was treated with Gal, SBA, with GalNAc, and WGA, with GlcNAc.

Untreated control sera were provided by similar incubation with RAST buffer alone. After this preincubation, 50 μ l of serum was added, and the discs were incubated, washed, and treated with anti-IgE as per the Phadebas (Pharmacia South Seas Pty., Ltd., Sydney, Australia) procedure. Determination of RAST uptake was made in triplicate for all treatments. The sera from wheat-sensitive patients were subjected to the WGA RAST only because of the small quantities available and the low probability of binding with the legume lectins.

Total IgE

The total IgE in the sera was determined by use of the Phadebas PRIST (Pharmacia) reagent kit according to the instructions of the manufacturer. All sera were diluted 10 times to elicit results within the range of the standard curve.

Serum glucose levels

The enzyme-linked ultraviolet method of Boehringer-Mannheim (Boehringer-Mannheim GmbH, Penzburg, West Germany, catalog No. 139106) was used for the estimation of serum glucose levels.

RESULTS

RAST profile

RAST uptake profiles of the sera under control conditions (i.e., no sugar pretreatment) are illustrated in Fig. 1A. The control sera (from pollen-sensitive and nonatopic subjects) were tested with the lectins, and all sera elicited RAST uptakes of <2.5% of total radioactivity (Fig. 1B). WGA discs were tested with wheat-sensitive patients' sera only.

Total IgE and glucose concentration

The results of the total IgE and serum glucose measurements are presented in Table I.

Effect of preincubation

A comparison of RAST results from treated and untreated discs is presented in Table II. The results for the three control sera are omitted, since the uptakes were very low (Fig. 1B) and variable. (Untreated mean uptake percent was 1.10% with a standard deviation of 0.38%.) The near-background figures were almost unaffected by the addition of sugars. (After treatment mean uptake percent was 1.21% with a standard deviation of 0.51%.) The concentration of added sugar used in the experiments (final, 50 mmol/L) was

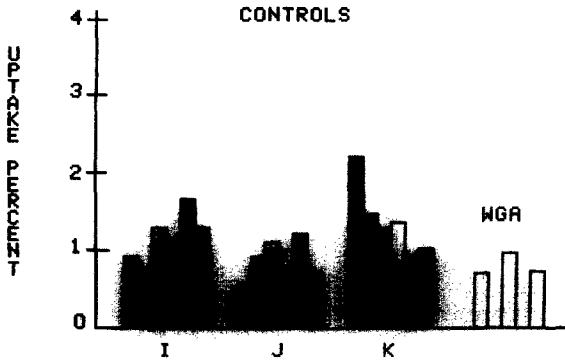


FIG. 1B. Percent radioactive uptakes in RAST by control sera I, J, and K. The bars correspond to discs coupled with lectins as in Fig. 1A. Sera I and J were from pollen-sensitive patients, and K was from the nonatopic subject.

chosen to be well above that observed as being effective by Aalberse et al.²

DISCUSSION

RAST results of Table II indicate that the effect of the addition of lectin-inhibitory sugars was almost negligible. The three control sera demonstrated almost no lectin binding, either specific or nonimmune. When the apparent ineffectiveness of the preincubation with 100 mmol/L of sugars was observed, experiments with a mixed serum (A to H) and PSA discs only were undertaken to determine the optimum inhibitory sugar concentration. The results indicated virtually no change to the nil effect when concentrations ranging from 1 to 200 mmol/L were used. Five of the 14 sera tested, including one of the pollen-sensitive control sera, had total IgE >300 IU/ml but were not distinguishable as being inhibited in RAST by the sugars. This is in contrast to the observations of Aalberse et al.² with Sepharose-coupled pea extract. However, our RAST was carried out with the paper disc system. It was also expected that the results would indicate the significance, if there was any, of nonimmune IgE binding of lectins in the frequently observed, apparent cross-reactivity of legume allergens.⁹ In view of the results of this study, it must be concluded that any cross-reaction is due to other factors, such as taxonomic relatedness.

IgE is a glycoprotein in which the carbohydrate moieties are located on the heavy chain of the molecule. Mannose, GlcNAc, Gal, sialic acid, and fucose are present in the amount of 16, 12, 7, 5, and 4 mol, respectively, per mole of heavy chain.¹⁰ The terminal sugars have been demonstrated to be mannose together with either Gal or sialic acid.^{10,11} Therefore, there are potential lectin-binding sites available on the antibody molecule for all of the lectins tested in this investi-

TABLE I. Results of measurement of serum glucose levels and total IgE

Serum	Glucose (mg/ml)*	Total IgE (IU/ml)
A	0.69	163
B	0.45	682
C	0.51	125
D	0.56	500
E	0.40	260
F	0.48	292
G	0.85	810
H	0.65	195
X	0.64	536
Y	0.82	195
Z	0.63	183
Control		
Pollen I	0.58	510
Pollen J	0.80	190
Nonatopic K	0.62	120

*1 mg/ml approximately equals 5 mmol/L of glucose.

gation. Why then was considerable lectin binding of IgE not observed under the conditions in this study?

One possibility could be that the lectin-immunoglobulin complexes were very weakly bound and dissociated during the RAST washing steps. However, this would appear unlikely because lectin-glycoprotein complexes will commonly form precipitates in agarose gel diffusion experiments that are stable to saline washing. Furthermore, the stability of lectin-glycoprotein complexes is evident by the extensive use of lectins for affinity-chromatography.⁸

A further explanation could be that the sugars used were not the ideal inhibitors. WGA is inhibited most effectively by oligomers of GlcNAc⁸ and can also bind to sialic acid.¹² Thus, for this lectin there may be some justification for this view. However, in the cases of the mannose-specific lectins PSA, FAV, LEN, and CON A, Me α -Man is acknowledged to be the most effective specific sugar.⁸ Moreover, CON A has a higher binding constant for Me α -Man than the other lectins of the mannose group,⁴ and yet, in the RAST profile (Fig. 1A), it is observed to be relatively poorly reactive. This would appear to indicate that the observed RAST uptakes were due to lectin-IgE immune binding.

The presence of endogenous lectin-inhibitory factors in the sera should be considered. Glucose is an inhibitor of the mannose-binding lectins and was found in all the sera at levels up to almost 5 mmol/L (Table I). It could account for some inhibition. There remains the apparent lack of reaction of the Gal-

TABLE II. Comparison of RAST results for lectins from legumes and wheat when RAST is carried out with and without preincubation of the discs with 50 µl of a 100 mmol/L solution of the appropriate inhibitory sugar*

Lectin (50 µg per disc)						
PSA	FAV	LEN	CON A	PNA	SBA	WGA
Peanut-sensitive patients						
A	97.7	84.1	100.9	116.4	93.4	104.4
B	95.0	103.7	100.4	83.1	94.4	98.5
C	89.1	97.2	95.6	99.3	96.8	99.3
D	89.1	94.2	103.4	102.9	104.7	94.6
E	83.5	97.4	100.7	100.2	86.8	100.7
F	93.5	103.6	102.0	106.6	94.5	102.1
G	101.8	92.8	114.0	90.9	99.7	99.2
H	97.3	82.2	89.5	112.9	87.1	97.5
Wheat-sensitive patients						
X						103.6
Y						96.9
Z						101.4

*Results expressed as $\left(\frac{\text{RAST-sugar inhibited}}{\text{RAST untreated}} \right) \times 100$ where RAST values in this ratio corresponded to percent of total radioactive uptakes. The collective mean of the ratios was 97.58 with a standard deviation of 7.35.

specific PNA and the GalNAc-binding SBA. Both of these lectins could conceivably bind to the IgE carbohydrate. An explanation of most of our results could be competition for lectin by relatively abundant serum glycoproteins, particularly IgG. IgG is normally present in sera at about 15 to 20 mg/ml, whereas the concentration of IgE, even in atopic patients, is several orders of magnitude lower at <0.5 µg/ml. IgG has been demonstrated to contain similar carbohydrate structures to those of IgE¹³ and thus could be a very effective competitor for the available lectin.

We conclude that lectins do not appear to interfere in the RAST of legume- or wheat-allergen extracts when RAST is carried out with the usual paper discs. Our RAST results demonstrated, however, that there was significant immune binding of some of the lectins by specific IgE. These lectins may therefore be important in the expression of IgE-based allergy (Fig. 1A). It may also be concluded that RAST, when it is performed under the usual conditions with paper discs, can be used as an indicator of binding of immune-specific IgE to legume- or other food-allergen extracts that contain lectins.

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