

Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom

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IgE in some human sera reacted with an antigen present in a large number of unrelated foods: potato, spinach, wheat, buckwheat, peanut, honey, and others. The antigen, which was periodate-sensitive and heat-stable, was also found in pollen. Even more surprisingly, these antibodies often reacted in vitro with bee and vespid venom and were sometimes apparently induced by Hymenoptera stings. (J ALLERGY CLIN IMMUNOL 68:356, 1981.)

This investigation was undertaken after the observation that many sera that were positive in RAST¹ for one vegetable food were also positive for a variety of other vegetable products. We considered three explanations: (1) nonimmune binding of IgE to the vegetable material, (2) the serum donors were specifically sensitized to all these foods, possibly because of some defect of the gastrointestinal mucosa, and (3) the different assays detect the same crossreacting antibody.

Crossreactions between products of botanically unrelated species have been described before. Andersson et al.² described an association between ragweed pollinosis and melon- or banana-induced pruritus and a crossreactivity between ragweed and cantaloupe. They refer to a paper by Pasani et al. in an Argentinian journal, reporting crossreactivity between a large number of foods and grass pollen in guinea pig passive cutaneous anaphylaxis. Bahna et al.³ found crossreactions between rice or wheat and

buckwheat, whereas Kato and Sasaki⁴ found crossreactions between the thiol proteases papain, ficin, and bromelain. Baur⁵ extended these observations and showed by RAST inhibition that papain, bromelain, wheat flour, rye flour, grass pollen, and birch pollen mutually inhibited IgE antibodies to each antigen.

MATERIALS AND METHODS

Sera

Most investigations reported here were performed with serum P. This was obtained by plasmapheresis in 1974 from a 26-yr-old woman who had atopic eczema and who was hypersensitive to chicken egg white. Eating an egg caused an itching erythema around the mouth, itching of the throat and, within an hour, some hives, but no gastrointestinal complaints. Eating other foods did not induce symptoms. She had no hay fever. There was no history of an insect sting for at least 5 yr prior to the plasmapheresis, which was performed with her informed consent. In a serum sample obtained in 1981 from the same subject, RAST titers had decreased to 10% to 30% of the 1974 values.

Serum A was obtained from a girl born in 1964. In August 1979 she was stung on her foot by an insect, presumably a honeybee; this caused a large local reaction that lasted for 2 days. She complained of lack of appetite for about a week. Ten days after the sting, a rash developed on the sun-exposed skin and lasted for 2 days. In the subsequent 6 mo two more similar bouts occurred without an identifiable cause. Furthermore, several episodes of exercise-induced urticaria occurred, which lasted for only a few hours. No

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Abbreviations used

RAST:	Radioallergosorbent test
BWA:	Buckwheat antigen preparation
Con-A:	Concanavalin A
PBS:	Phosphate-buffered saline
HBV:	Honeybee venom
CFA:	Complete Freund's adjuvants
CCD:	Crossreacting carbohydrate determinant

relationship was found with the consumption or handling of a particular food product. She had no hay fever or atopic eczema. No definite differences were found between the first serum sample of November 1979 and the last sample from June 1980.

W. A. and G. U. were two female novice beekeepers from whom serial serum samples were obtained. W. A. had a history of mild atopy and a positive RAST for *Dermatophagoide pteronyssinus* and cat dander. G. U. had no anamnestic or serologic indications for atopy. Both developed large local reactions several times subsequent to honeybee stings.

Sera were also obtained from donors a few weeks after they had been stung by a honeybee or a paravespula species. Some of these sera were from persons without alarming reactions; serum W belongs in this category. Others were from hypersensitive patients.

We also selected sera from samples that were submitted for diagnostic IgE tests. The criteria for selection were either a total IgE level of more than 200 IU/ml, a grass pollen RAST of more than 10% binding of total counts, a positive bee venom RAST, or a positive RAST for chicken egg white and/or cow's milk.

Antigens

Extracts were prepared from uncooked foods; unless indicated otherwise, the raw material (or, in case of buckwheat and grains, the flour) was obtained from a local health-food store. Soybean flour was a gift from Unimills B. V., Zwijndrecht, The Netherlands. Peanut flour was a gift from Calvé B. V., Delft, The Netherlands. Tragacanth gum was obtained from a local pharmacy. Other gums were kindly provided by Dr. B. de Vries, Unilever B. V., Duiven, The Netherlands. Grass juice was prepared by homogenization of freshly cut grass in water at pH 8.0 (10% w/v); the grass was collected in spring before pollination.

We selected buckwheat flour as a practical source of the crossreacting antigen because buckwheat flour extract contains a high concentration of the antigen, whereas the concentrations of total nondialyzable solids and of total protein are relatively low.

Extraction of the allergen was most effective at alkaline pH; addition of 56 gm/L NaCl to the extraction fluid did not substantially improve the extraction efficacy. At pH 9 or above, considerable browning of the extract occurred; therefore we prepared the extract (10% w/v) in water at pH 8. The extract was then adjusted to pH 4 with HCl; this re-

sulted in the precipitation of a large amount of protein, whereas most of the activity remained in the supernatant. The supernatant was subsequently dialyzed against water, and no activity was found in ultrafiltrate. The precipitate formed was discarded, and the solution was lyophilized. The resulting preparation was coded BWA. The yield of BWA per gram of buckwheat was 5 to 10 mg nondialyzable solid, 30% of which was protein, when estimated according to the method of Bradford.⁶

BWA was fractionated by gel filtration and affinity chromatography over Con-A Sepharose. The gel filtration was performed at room temperature with PBS as a buffer. BWA (25 mg in 0.5 ml PBS) was fractionated over a column of 38 by 2.4 cm Ultrogel ACA 54. Most activity was found in the void volume. For affinity chromatography, BWA (250 mg in 10 ml PBS) was passed through a column of 9 by 2.7 cm Con-A Sepharose in PBS. More than 95% of the activity was adsorbed and 20% was eluted by 0.1M α -methylmannoside; we did not attempt to recover the remaining activity. The serologic activity of BWA was highly susceptible to periodate treatment. In contrast, there was a relative resistance to proteolytic enzymes: high concentrations of pepsin or pronase (10% w/w) were required for 90% destruction of the activity during 3 hr at 37° C. A diluted antigen preparation was boiled for 30 min without loss of activity.

Other food extracts were similarly prepared in water, dialyzed, and lyophilized. Tragacanth gum was suspended in boiling 0.01N NaOH (1 mg/ml) and the suspension was allowed to cool; the supernatant was then adjusted to pH 7.6 and used without further treatment. A 1:500 dilution caused 50% inhibition of the potato RAST. HBV was from Sigma Chemical Co., St. Louis, Mo. (grade IV). Before coupling to CNBr-activated Sepharose, HBV was first gel filtered to remove the bulk of the mellitin. Four hundred milligrams of venom in 2 ml of PBS were fractionated over 2.8 by 45 cm column of Sephadex G75. For coupling to Sepharose, all fractions with absorption at 280 nm were collected until approximately 10% of the mellitin peak was eluted; 60 ml of a pool of these fractions (66 ml in total) was coupled to 4 gm of CNBr-activated Sepharose. Vespid venom (yellow jacket, lot 216796 YJ) was generously given by Dr. A. Benton for use in RAST inhibition. For direct RASTs we prepared a venom-sac extract from *Paravespula germanica*; an extract made from 50 sacs was coupled to 200 mg of activated Sepharose.

RAST

Allergens were coupled to CNBr-activated Sepharose (Pharmacia) according to the manufacturer's instructions; unless indicated otherwise, 5 to 15 mg allergen were coupled to 300 mg of Sepharose. The test was performed by incubation of up to 50 μ l of serum with up to 1.5 mg of Sepharose-coupled allergen. The incubation was performed either on a shaking incubator or on a vertical rotator. In the former incubation, a final volume of 0.3 ml was used; in the latter incubation, the volume was 0.55 ml. Sepharose-bound IgE was determined with ¹²⁵I-labeled anti-IgE, prepared as described previously.⁷

TABLE I. Solid-phase absorption of serum P

	Allergen used for solid-phase absorption			
	HSA	Cat dander	Egg white	BWA
Total IgE	812 $\pm$ 12	750 $\pm$ 7	782 $\pm$ 9	751 $\pm$ 8
RAST cat	48	2	48	49
RAST egg white	33	30	1	35
RAST BWA	20	17	19	2
RAST potato	17	16	16	1
RAST HBV	15	17	15	1

HSA = human serum albumin.

Serum P (0.5 ml) was incubated overnight at 4° C with 300 mg of Sepharose to which the indicated materials were coupled (10 mg HSA, egg white, or BWA, and 1.2 ml of a 10% cat dander extract). The incubation volume was adjusted to 5 ml with PBS containing 10 mM EDTA, 3 mg/ml bovine serum albumin, 0.2% Tween-20, and 5 mM Na₂S₂O₃. In the four supernatants, total IgE and RAST activity were determined. The IgE values represent the mean of four tests $\pm$ standard error, and results are expressed in IU/ml. RASTs were performed in duplicate in a single assay using 200 μ l supernatant per test; the results are expressed as percentage of total radioactivity.

Some experiments were also performed with allergen-coated discs and ¹²⁵I-anti-IgE, both from Pharmacia. Essentially the same results were obtained. RAST results are expressed as percentage of total radioactivity after blank correction; this blank consisted of 6% bovine serum albumin or cord serum, and amounted to 0.4% to 1.5% of the total radioactivity. We consider a RAST score of more than 2% of total radioactivity a positive test result because 50 μ l of serum of a high IgE-negative control serum (from a filaria-infected patient) that was diluted to contain 5000 IU/ml had a RAST score of up to 2%. This was assumed to reflect nonspecific uptake of IgE by the allergosorbent. All serum samples were tested at a total IgE concentration of less than 5000 IU/ml.

RAST inhibition assays for the determination of cross-reacting antigen were performed in duplicate with serum P and with Sepharose-coupled potato extract, unless indicated otherwise. The reason for choosing potato Sepharose rather than buckwheat Sepharose was to ensure that in the buckwheat fractionation experiments only crossreacting allergen was detected. Antigen solution was incubated with an equal volume of serum P for 2 to 4 hr at room temperature; 50 μ l of the mixture were then incubated overnight with Sepharose-coupled allergen extract, and Sepharose-bound IgE was determined with ¹²⁵I-anti-IgE. The amount of allergosorbent was adjusted to give 10% binding of radioactivity in the absence of inhibitor. The specificity of the inhibitors was tested by analogous experiments with cat dander-positive serum and cat dander Sepharose. No nonspecific inhibition was detected.

Total IgE was determined with a competitive immunoradiometric assay.⁸

Periodate treatment was performed with 0.01M NaIO₄ in acetate buffer (pH 5.0) at room temperature in the dark. After 2 hr the reaction was stopped with sorbitol. In some experiments the reaction product was then treated with NaBH₄ for 1 hr; the excess of NaBH₄ was oxidized with acetone, and no effect of the borohydride reduction was found. As a control for the reagents, sorbitol was preincu-

bated with the periodate and the allergen was added to this mixture; no effect of this treatment on the allergen activity was then found.

Gel filtration of serum P was performed at room temperature with PBS as a buffer; 0.5 ml of serum were fractionated over a column of 33 by 2.4 cm Ultrogel ACA 44.

Immunizations of rabbits and guinea pigs were performed with CFA. BWA (0.5 mg in 0.5 ml of PBS) or HBV (0.5 ml of the void-volume fraction obtained by gel filtration of 400 mg of HBV over Sephadex G75, OD280 = 1.1) was mixed with an equal volume of CFA and injected intramuscularly. After 5 wk a second injection was given with the same material in incomplete Freund's adjuvant. Sera were obtained 7 to 14 days after this (or a subsequent) injection, again with antigen in incomplete adjuvant.

RESULTS

Specificity of RAST with serum P

Serum P was positive in a remarkable variety of RASTs; a positive RAST was found for pollen from various grasses, weeds, and trees, *D. pteronyssinus*, dander from cats, dogs and rabbits, cow's milk, chicken egg white, mussel, rice, wheat, buckwheat flour, peanut, soybean, hazelnut, walnut, banana, strawberry, apple, pineapple, orange, ginger, potato, spinach, tobacco leaves, grass juice, buckwheat honey, honeybee venom and vespid venom; negative RASTs were found for control Sepharose (CNBr-activated Sepharose inactivated as usual with glycine), guinea pig dander, and *Aspergillus*.

Because serum P had a high total IgE level (8100 IU/ml), we considered the possibility that what we found in the RAST was nonimmune binding of IgE.

For some allergen extracts, nonimmune binding of IgE to the allergosorbent was indeed observed; this was especially marked for the green pea, soybean,

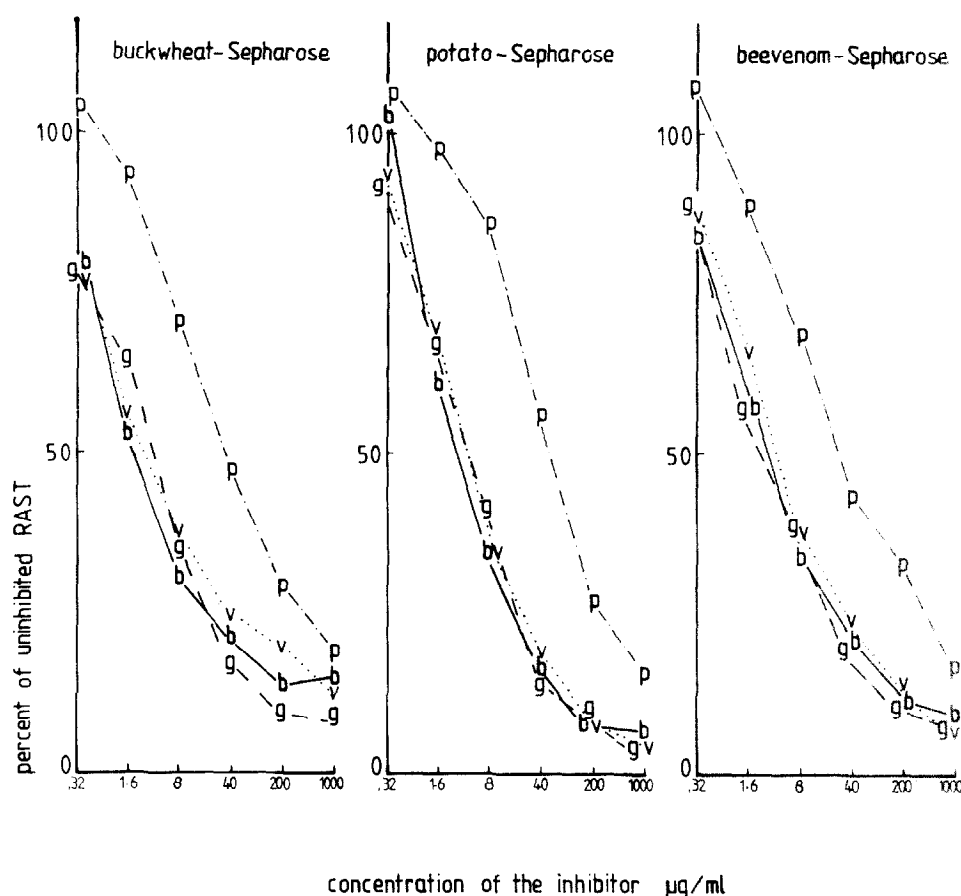


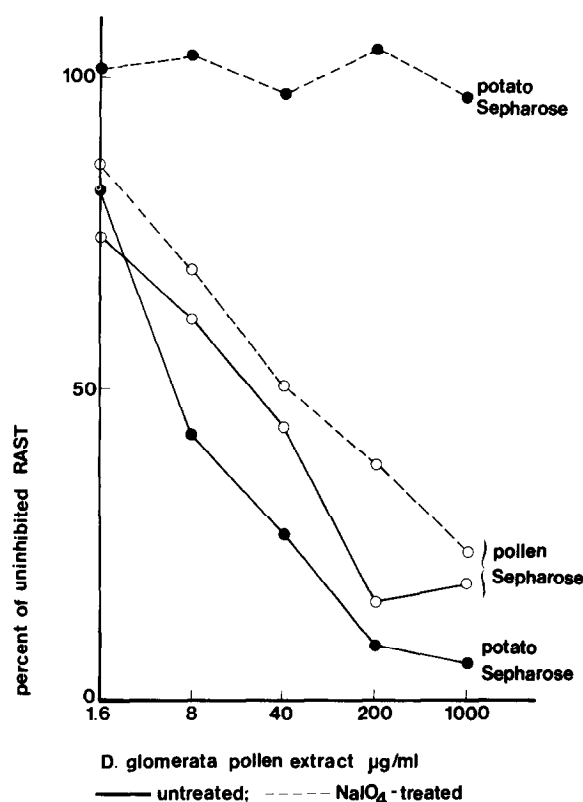
FIG. 1. RAST inhibition test with serum P. Two hundred microliters of serum P were mixed with 200 μ l of inhibiting extract. After 2 hr of preincubation, 50- μ l aliquots of the mixture were added to Sepharose-coupled buckwheat (left), potato (middle), or HBV (right). The results are expressed as percentage of the uninhibited RAST score. *b*, Buckwheat flour preparation BWA; *p*, dialyzed potato tuber extract; *v*, dried HBV; *g*, dialyzed grass pollen (*Dactylis glomerata*) extract.

and grape. This nonimmune binding is partly caused by lectins that bind to the carbohydrate part of IgE. Such lectins, e.g. Con-A, are nonselective in the sense that IgE is bound from every serum that has a sufficiently high total IgE level. In the case of green pea this effect was clearly demonstrable with sera with a total IgE level of more than 300 IU/ml. This type of IgE-binding was inhibited by α -methylmannoside in the case of green pea, or by Con-A. In contrast to the above-mentioned nonimmune binding, the buckwheat RAST was selective; out of a group of serum samples with the same IgE level only a few were positive. To demonstrate this, we selected (from serum samples submitted for diagnostic RAST investigations) sera with an IgE level of about 400 IU/ml (range 350 to 450) and sera with 1200 IU/ml (range 1000 to 1400). Of the 23 samples with 400 IU/ml, 19 were negative; the RAST scores of the positive sera were 3%, 7%, 10%, and 12%. From the 19 samples with 1200 IU/ml, 13 were negative; the RAST scores

of the positive samples were 3%, 9%, 10%, 15%, 16%, and 25%. From these results we concluded that most sera do not contain IgE that is bound by buckwheat Sepharose.

A theoretical explanation of this observation is that in the negative sera an inhibitor is present that interferes with the binding of IgE to buckwheat Sepharose. This was tested as follows: Five sera with an IgE level of about 1500 IU/ml and negative in the buckwheat RAST were added to a sample that was positive in the buckwheat RAST. No inhibition of the buckwheat RAST was found.

Aggregated IgE might be responsible for this IgE binding. However, no difference was found between fresh and frozen sera. Furthermore, tests on fractions obtained by gel filtration of serum over Ultrogel ACA 44 did not indicate preferential binding of the ascending part of the IgE peak; IgE in the descending part bound equally well, or perhaps even slightly better. Absorption of serum P with Sepharose-coupled BWA



FIGS. 2A and 2B. The differential effect of periodate on grass pollen, tested by RAST inhibition and by direct RAST.

FIG. 2A. RAST inhibition. Grass pollen extract was dialyzed, then exposed to NaIO_4 (see Materials and Methods). The activity of the treated material was compared with that of the untreated material. In the grass pollen RAST, only a slight loss of inhibitory activity was found; in contrast, the inhibiting capacity in the potato RAST was completely destroyed by the periodate treatment.

removed IgE that was responsible for the buckwheat RAST. This absorption was selective as judged by the remaining total IgE and by the cat RAST or egg white RAST (Table I). From this result we concluded that only a small fraction of the IgE in serum P has affinity for Sepharose-coupled buckwheat.

These results strongly suggest that the binding observed in the buckwheat RAST is caused by IgE antibodies that react with a component in buckwheat. These IgE antibodies were not specific for buckwheat, since the absorption also removed anti-potato activity and even anti-HBV activity (Table I). The RAST inhibition results presented in Fig. 1 indicate a complete immunologic identity of an allergen in grass pollen, buckwheat flour, potato tubers, and HBV. Upon gel filtration over Sephadex G75, the inhibitory activity of HBV eluted in the void volume.

Other extracts were similarly tested for inhibitory

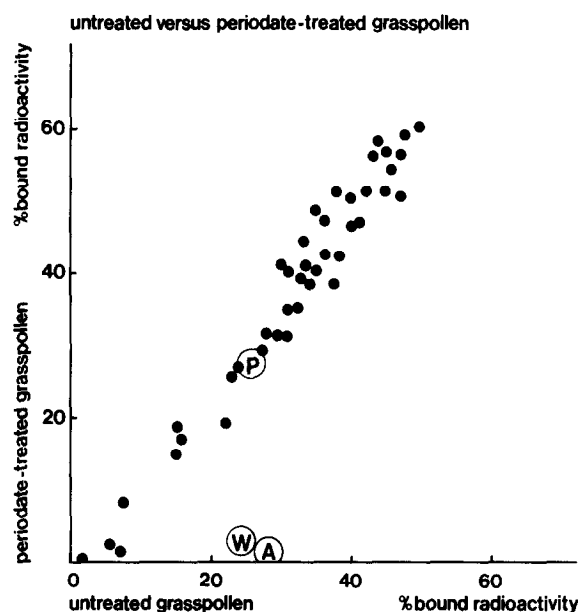


FIG. 2B. Direct RAST; correlation between the RAST with Sepharose-coupled untreated grass pollen extract (abscissa) and the RAST with Sepharose-coupled periodate treated grass pollen extract (ordinate). Forty-five sera with a positive grass pollen RAST were tested in duplicate in a single assay; 25 of these sera had a buckwheat RAST score of 5% binding or more, and the remaining 20 sera were negative in the buckwheat RAST. Three of the buckwheat-positive sera are marked by A, W, and P. These sera were further tested (see Fig. 3).

activity in the potato RAST; at 1 mg/ml concentration, more than 90% inhibition was found with dialyzed buckwheat, honey, gum tragacanth, gum arabic, pectin, vespid venom, and extracts of mussel, oyster, green coffee, tobacco, ginger, celery, spinach, oats, wheat, corn, barley, rice, rye, peanut, soybean, green pea, and tomato. Some inhibitory activity was found in *D. pteronyssinus* culture extract and in guar and carob gum, whereas no inhibitory activity was found with soluble carboxymethylcellulose, dextran, xanthan gum, carrageenan, sodium alginate, chicken egg white, or extracts of animal danders.

Antibodies to buckwheat in other sera

Sera from 43 unselected laboratory workers were tested for IgE antibodies to buckwheat. No serum with a RAST score of more than 5% binding was found; a RAST score between 2% and 5% was found in three. From serum samples submitted for diagnostic RAST investigations we selected four groups of sera:

(1) Sera with a total IgE level of 200 IU/ml or more; for 13 out of 98 sera a buckwheat RAST score of more than 5% binding was found.

(2) Sera with IgE antibodies to chicken egg white

and/or cow's milk; in this group of 48 sera we found 18 with a buckwheat RAST score of more than 5% binding.

(3) Sera with a grass pollen RAST of more than 10% binding; in this group of 223 sera we found 80 with a buckwheat RAST score of 5% binding or more.

(4) Sera with IgE antibodies to HBV; in this group of 16 sera, which did not include sera from beekeepers, we found 10 with a RAST score of more than 5% binding.

Sixteen of the buckwheat-positive sera of group 3 were tested with other Sepharose-coupled allergens; 15 were positive with potato, 12 with vespid venom, eight with honeybee venom, seven with mussel, and seven with tragacanth gum. Therefore not all sera that are positive in the buckwheat RAST have the same broad specificity as serum P; serum P is, however, certainly not unique. In Table II the reactivity pattern of another serum, serum W, is shown for comparison; virtually identical results were obtained with serum A.

The buckwheat RAST was often inhibited by grass pollen extract. With a few exceptions, the grass pollen RAST was not significantly inhibited by BWA. A dialyzed grass pollen extract was treated with 0.01M periodate and had less than 0.1% of the activity as an inhibitor of the potato-RAST relative to the untreated extract (Fig. 2A). The reactivity of the periodate-treated allergen was then compared with the native allergen in a direct RAST assay (Fig. 2B). The periodate-treated pollen did not react with IgE in serum A or serum W; the grass pollen RAST of these two sera was significantly inhibited by BWA (Fig. 3). The results reported in Fig. 3 were obtained with Pharmacia reagents. Since neither buckwheat discs nor potato discs were available, we used peanut discs as positive controls for the inhibition by BWA.

Table III shows serologic data of two novice beekeepers. These results indicate that bee stings can apparently induce IgE antibodies to vegetable foods such as buckwheat; the IgE antibody titer of serum from subject W. A. toward buckwheat was considerably lower than that to HBV.

Crossreacting antibodies in rabbit and guinea pig antisera

Four anti-BWA sera and two anti-HBV sera were tested for crossreacting antibodies. In agar diffusion tests we found a strong reaction with the immunizing antigen but no reaction with potato extract. Cross-reacting antibodies were, however, detected in RAST-type assays with a sheep anti-rabbit IgG reagent or a rabbit anti-guinea pig IgG reagent. The crossreactivity pattern of these six antisera was simi-

TABLE II. Comparison between the crossreactivity pattern of sera from two patients (P and W)*

	Serum P	Serum W
Grass pollen	33	28
Buckwheat honey	29	35
Peanut	28	34
Oats	27	32
Coffee	27	33
Potato	25	36
Spinach	25	33
Mussel	22	30
Wheat	21	20
HBV	20	44
Pectin	19	16
Tragacanth	19	18
Vespid venom	16	26
Carob	7	1

*A direct RAST assay was used (25 μ l of serum per test). The results, which were obtained in a single assay, are expressed as percentage bound radioactivity after blank correction.

lar and mimicked in general the pattern found with sera P, W, and A, with the exception of vespid venom; examples are shown in Table IV. The cross-reacting antigen was periodate sensitive.

DISCUSSION

Is the IgE binding caused by IgE antibodies to an antigen?

The IgE binding seems to lack specificity. Moreover, nonimmune binding of IgE in extracts of some vegetable materials is easily demonstrable. Might not the IgE binding, e.g., to Sepharose-coupled potato or buckwheat extracts, be caused by lectins in these extracts that bind IgE via its carbohydrate side chains? In support of this lectin hypothesis is the finding that some gums, notably tragacanth gum, are potent inhibitors of the buckwheat or potato RAST. Moreover, the inhibitory activity of grass pollen extract, for example, is destroyed by periodate treatment, a procedure that results in the breakdown of carbohydrates (Fig. 2A). These RAST inhibition experiments do not discriminate between inhibitors with affinity for the allergosorbent (i.e., sugars that block the putative lectin) and inhibitors with affinity for IgE (i.e., allergens that block the allergen binding site of IgE antibodies).

However, the results of direct RAST experiments, as opposed to the above-mentioned RAST inhibition experiments, indicate that the component responsible for the IgE binding is periodate sensitive. This was found for grass pollen extract with serum A and serum W (Fig. 2B) and for buckwheat and peanut with serum

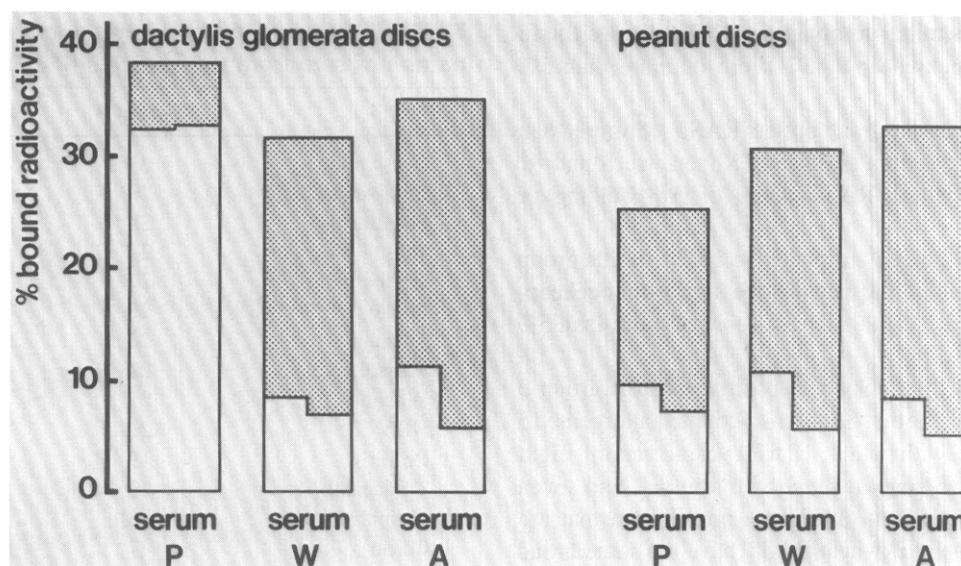


FIG. 3. The inhibitory activity of buckwheat extract on the RAST activity of sera A, W, and P (see Fig. 2B) was tested in the grass pollen RAST and in the peanut RAST, with allergen-coated discs and ^{125}I -anti-IgE both from Pharmacia. For each test we used 25 μl of serum and 25 μl of buffer containing BWA at 0, 200, or 1000 $\mu\text{g}/\text{ml}$. The total column height indicates the RAST result without BWA. Clear areas, RAST result found with 200 μg (left) or 1000 μg (right) BWA per ml. RASTs were performed in duplicate in a single assay; the results are expressed as percentage of total radioactivity.

P, serum A, and serum W. Moreover, all substances tested that were inhibitory in the buckwheat or potato RAST (including tragacanth gum and pectin) were able, when coupled to CNBr-activated Sepharose, to bind IgE from serum P in the direct RAST (Table II).

Lectin-IgE interaction is unlikely to be responsible for the IgE binding for several other reasons. (1) The results presented in Table I demonstrate that only a small fraction of the total IgE in serum P will bind to Sepharose-coupled buckwheat extract. (2) Most sera with elevated total IgE levels were negative; these negative reactions were not caused by an inhibitor. (3) The induction by bee stings (Table III) is difficult to reconcile with the lectin hypothesis. (4) Immunization of rabbits or guinea pigs with a buckwheat preparation or an HBV fraction induced antibodies with a cross-reactivity pattern closely similar to that of the IgE antibodies (Table IV). (5) Heidelberger⁹ found similar unexpected crossreactivities with antisera to pneumococcal carbohydrates.

Is the polyspecificity of serum P caused by multiple sensitizations or by crossreacting IgE antibodies?

The large number of positive RASTs found with serum P proved to be caused by a combination of multiple sensitizations and crossreacting antibodies.

No crossreactions were found between the antibodies to cat dander, egg white, and buckwheat (Table II). This indicates that donor P has made several independent IgE responses against noncrossreacting allergens. This points towards a hyperreactivity of the IgE-producing system, reflected also by the high total IgE level.

However, not all the positive RASTs of serum P reflect an independent IgE immune response, since the RASTs for the different vegetable products all recognize the same IgE antibodies; this is evident from the crossreactivity data.

We therefore conclude that the IgE-binding to potato or buckwheat is most likely caused by IgE antibodies that specifically bind to some ubiquitous antigen. The periodate susceptibility and the affinity for Con-A lead us to believe that this antigen or determinant is most likely a carbohydrate. We provisionally refer to this material as a CCD. This determinant is not chlorogenic acid¹⁰ because no dialyzable inhibitory activity was found. Maillard-type reaction products¹¹ are possible candidates; however, neither a ribose-albumin conjugate nor a lactose-albumin conjugate was inhibitory in the potato RAST. More likely candidates are the structurally related hydroxyproline-rich plant glycoproteins.¹² Because of the differences in the reactivity pattern that we found between

TABLE III. Appearance of IgE antibodies to buckwheat in two novice beekeepers

	Date	Total IgE (IU/ml)	HBV RAST score	Buckwheat RAST score
Serum W. A.	05-05-1979	2500	2	1
	05-26-1979	3000	20	28
	09-25-1979	2400	23	13
	08-20-1980	2700	17	5
Serum G. U.	05-03-1980	90	0	0
	07-03-1980	90	2	2
	12-23-1980	150	22	17

Novice beekeeper W. A. was first stung on 04-21-1979 (three stings), then on 05-05-1979 (two stings, after blood sampling) and on 05-12-1979 (one sting); after 05-26-1979, she was regularly stung during the subsequent 1979 and 1980 seasons. Novice beekeeper G. U. was first stung in May 1980 and then regularly during the subsequent 1980 season. All RASTs were performed with 50 μ l of serum except for the HBV RAST of serum W. A., which was performed with 5 μ l of serum. All RASTs of each donor were tested in a single assay. The buckwheat RAST of G. U. was inhibitable by HBV; 78% inhibition was found with 5 mg/ml HBV.

TABLE IV. Comparison between the crossreactivity pattern of patient serum A (50 μ l per test) and two rabbit antisera (0.02 μ l per test)*

	Serum A	Anti-HBV	Anti-BWA	Anti-CFA
HBV	30	31	9	2
Rice	29	23	n.t.	1
<i>Dactylis glomerata</i>	29	25	14	1
Potato	28	24	14	1
Spinach	26	18	14	1
Buckwheat	24	15	17	1
Buckwheat honey	24	16	12	1
Mussel	23	16	8	1
Gum tragacanth	22	16	12	1
Peanut	22	18	14	1
Vespid venom	19	13	4†	1
Soybean	18	16	13	1
Arabic gum	18	9	13	1
Apple	15	8	n.t.	1
Pectin	12	6	11	1
<i>D. pteronyssinus</i>	3	1	n.t.	2
Cat dander	0	1	n.t.	1
Chicken egg white	0	0	n.t.	1
Guinea pig dander	0	0	n.t.	1

*A direct RAST-type assay was used with either ^{125} I-labeled anti-rabbit IgG. The results, which were obtained per serum in a single assay, are expressed as percentage of bound radioactivity after blank correction. As negative controls, preimmunization samples from the same rabbits were simultaneously tested. The RAST score was less than 3% binding of radioactivity, with the exception of the test marked with a dagger, in which the preimmunization RAST score was equal to the postimmunization score. Another negative control serum was from a rabbit injected with CFA (anti-CFA) only.

sera, we assume that the anti-CCD antibodies in serum P recognize a family of closely related but not identical structures.

Are these IgE antibodies clinically relevant?

The clinical relevance of the IgE antibodies described is currently under investigation. Seven serum donors with a buckwheat RAST score of more than 20% have been questioned; none of these noticed

symptoms after eating vegetable foods. The absence of symptoms cannot be explained by inactivation of the allergen during cooking because raw fruits such as banana and tomato were also tolerated; moreover, the allergen is heat stable, and we were able to extract considerable amounts of RAST inhibitory activity from cooked potatoes.

Our hypothesis is that these IgE antibodies react with an antigenic determinant consisting of some car-

bohydrate side-chain present in a number of different glycoproteins found mainly in plants. For some reason—possibly continuous desensitization via oral exposure—this IgE antigen system will rarely, if ever, trigger mast cells or basophils. If further investigation should substantiate this hypothesis, it would be logical to disregard antibodies to this “allergen” for diagnostic purposes. In the RAST, this can be accomplished in principle by preabsorption of sera with BWA or a similar preparation, but complete absorption may be difficult to achieve (Fig. 3). Alternatively, if the relevant allergen is periodate resistant, the sera might be tested with periodate-treated allergens.

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