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Food Allergy: Specific Binding of IgE Antibodies from Plant Food Sensitized Individuals to Carbohydrate Epitopes

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(Received for publication 21 March 1994)

The presence of anti-carbohydrate immunoglobulin E (IgE) antibodies was investigated in a group of 54 patients who were sensitive to at least two pollen species and at least five vegetable foods. Enzyme-linked immunosorbent assays (ELISAs) and ELISA inhibition assays were performed with immobilized food-related oligosaccharides (e.g. stachyose from legumes) and extracts of plant gums (e.g. gum arabic), as well as with allergen extracts from apples, celery and other fruits and vegetables. Furthermore, a combined procedure of immunoblotting and periodate treatment was applied to identify the epitope nature of glycans from vegetable food glycoproteins. The results suggested that 12/54 of the sera contained IgE antibodies against carbohydrate structures. The antibodies were also found to be highly cross-reactive. For six patients, galactose may be an important epitope. The clinical relevance of the phenomenon requires further investigation.

Keywords: Carbohydrate epitopes, cross-reaction, plant food allergy, IgE, ELISA, immunoblotting, periodate oxidation

INTRODUCTION

Allergy to fruits and vegetables in pollen-sensitive patients is a well-known phenomenon which is due to the cross-reactivities of allergen-specific human immunoglobulin E (IgE) antibodies with food and pollen allergens. It has been the subject of many recent studies. Calkhoven *et al.* (1987) were the first to identify three distinct cross-reactive structures in birch pollen and vegetable foods. Recently, our understanding of the nature of these structures has increased. The majority of the birch pollen-related food hypersensitivities is due to the existence of a family of plant proteins which share common epitopes with *Bet v I*, the major allergen of birch pollen. *Bet v I* is a glycoprotein of known amino acid sequence with a molecular mass of approximately 17 kDa. Individual allergens with

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cross-reactivity to *Bet v* I have been identified, for example in apples, hazelnuts, pears and celery roots (Ebner *et al.*, 1991; Hirschwehr *et al.*, 1992; Vieths *et al.*, 1993a, 1994a,b). A family of conserved plant proteins (profilins) with molecular masses of 12–15 kDa is present in several pollen species and a wide variety of vegetable foods. Profilins have been found to be highly cross-reactive plant pan-allergens. They are associated with actin, profilactin and prolyl hydroxylase, occur in many eukaryotic organisms and are involved in numerous physiological processes (e.g. signal transduction and the regulation of actin polymerization) (Valenta *et al.*, 1992; Vallier *et al.*, 1992; van Ree *et al.*, 1992). Furthermore, cross-reactions of allergen-specific IgE antibodies can be caused by "cross-reactive carbohydrate determinants" (CCD) of food and pollen glycoproteins (Aalberse *et al.*, 1981; Calkhoven *et al.*, 1987; Broichmann *et al.*, 1992). Until now, this phenomenon has been little investigated and its clinical relevance is unknown. In a minority of allergic patients, there is some evidence that CCD are responsible for allergenic cross-reactions with a wide variety of phylogenetically unrelated food plants, various pollen species and animal glycoproteins, such as phospholipase A₂ (PLA₂) from honey-bee venom (Aalberse *et al.*, 1981; Calkhoven *et al.*, 1987; Weber *et al.*, 1987). Recently, α -1,3-linked L-fucose of the N-glycan from PLA₂ has been identified as an important epitope for IgE from bee venom allergic patients, and antibodies directed against this structure cross-reacted with the glycan of the plant protease bromelain from pineapples (Tretter *et al.*, 1993).

The conclusion that CCD from food glycoproteins are cross-reactive structures has been drawn mainly from two kinds of experiments. The first are competitive RAST or enzyme-linked immunosorbent assay (ELISA) inhibition assays using native plant allergens coupled to the solid phase and periodate-oxidized, as well as untreated or control-treated, allergenic extracts to inhibit the antibody binding. As protein epitopes can be affected by the oxidation procedure, periodate treatment has to be carefully optimized and controlled by appropriate experiments. The second kinds of experiments are inhibition assays using food allergens at the solid phase and plant polysaccharides (e.g. gum traganth, galactomannan from locust bean gum, or guar, agarose and pectin) as inhibitors of antibody binding. There are two drawbacks to this method. Firstly, the aqueous plant gum extracts which were used in the above-mentioned studies usually contain protein impurities, and therefore antibody binding to CCD cannot be totally confirmed from this type of experiment. Secondly, in our own, unpublished experiments, strong non-specific inhibition effects of galactomannans from carob and guar on the binding of IgE from various human pool sera occurred with every immobilized plant allergen used (e.g. grass pollen, mugwort pollen, birch pollen and apple).

In the present study, the prevalence of IgE antibodies directed against CCD in a patient group with highly polyvalent sensitizations was evaluated using easily obtainable food-related oligosaccharides and polysaccharides, as well as allergenic extracts from plant foods. Therefore, appropriate immunoassays and confirmation experiments were developed to circumvent the problems described above.

MATERIALS AND METHODS

Patient Sera

All sera from allergic patients were collected at the Hospital Borkum Riff der BfA, Borkum, Germany (Dr H. Aulepp) and at the Technische Universität Berlin, Occupational Health Service, Berlin, Germany (Dr S. Berten). The sera from 54 patients who were sensitized to two or more pollen species and five or more vegetable foods were used. Sensitization was concluded from a positive RAST (class of ≥ 2) and/or positive skin tests and/or an undoubted anamnesis. The total IgE concentration of the 54 patients ranged from 40 U ml⁻¹ to > 20 000 U ml⁻¹ (1 U = 2.4 ng of IgE). The median value was 1320 U ml⁻¹. Only two of the patients were sensitive to honey-bee venom. Some patients (17/54) had an additional neurodermitis which was not directly related to the activity of food-specific IgE antibodies. Control sera with IgE against protein epitopes were obtained from patients

sensitized to apple and celery (individual sera MM, 216), birch pollen (serum pool Wn 13/32, $n = 7$) or grass pollen (serum pool = 18/100, $n = 11$). The sera had positive RASTs to the named plant allergens but showed no IgE binding to the immobilized carbohydrates used in the study. A negative control serum pool (Wn 13/26) from seven patients with no history of food and pollen allergy was also used.

Plant Food Extracts

Allergenic extracts from apples (Granny Smith), celery roots and other fruits and vegetables were prepared using a low-temperature method as previously described (Vieths *et al.*, 1992a) and stored freeze-dried at -20°C until used. Gum traganth, gum arabic, carob and xylan (from oat bran) were extracted with sodium carbonate buffer, pH 9.6, at 60°C for 1 h, followed by a 30 min treatment in an ultrasonic bath, and centrifugation at $3000 \times g$. The supernatants were then freeze-dried (extract concentration: 5 mg ml^{-1} dry weight of crude material).

Oligosaccharides

Saccharose, raffinose ($\alpha\text{-D-Gal-(1}\rightarrow\text{6)-}\alpha\text{-D-Glc-(1}\rightarrow\text{2)-}\beta\text{-D-Fru}$) and stachyose ($\alpha\text{-D-Gal-(1}\rightarrow\text{6)-}\alpha\text{-D-Gal-(1}\rightarrow\text{6)-}\alpha\text{-D-Glc-(1}\rightarrow\text{2)-}\beta\text{-D-Fru}$) were from Fluka (Neu-Ulm, Germany). The purity was checked by high-pressure liquid chromatography (HPLC) as described below, yielding one single peak. Lacto-*N*-tetraose ($\beta\text{-D-Gal-(1}\rightarrow\text{3)-}\beta\text{-D-GlcNAc-(1}\rightarrow\text{3)-}\beta\text{-D-Gal-(1}\rightarrow\text{4)-D-Glc}$), 6-O-(2-acetamido-2-desoxy- $\beta\text{-D-glucopyranosyl)-D-galactopyranose}$ ($\beta\text{-D-GlcNAc-(1}\rightarrow\text{6)-D-Gal}$), methyl-2-O- $\alpha\text{-D-mannopyranosyl-}\alpha\text{-D-mannopyranoside}$ ($\alpha\text{-D-Man-(1}\rightarrow\text{2)-}\alpha\text{-D-Man-1-O-methyl}$) and methyl-3-O- $\alpha\text{-D-mannopyranosyl-}\alpha\text{-D-mannopyranoside}$ ($\alpha\text{-D-Man-(1}\rightarrow\text{3)-}\alpha\text{-D-Man-1-O-methyl}$) were obtained from Sigma (Deisenhofen, Germany).

Partial Hydrolysis of the Polysaccharides

To avoid non-specific inhibition of the ELISA, the polysaccharide fractions (5 mg ml^{-1} initial dry weight of crude material) were partially hydrolyzed with either trifluoroacetic acid (TFA) (2 M, 80°C , 1 h) or hydrochloric acid (2 M, 80°C , 2 h). The resulting solutions were neutralized and analyzed by ion-exchange HPLC using a pulsed amperometric detector (Stumm & Baltes, 1992) and by thin-layer chromatography (TLC) (Matissek *et al.*, 1989). Fractions with a reduction of the polysaccharide peak area of at least 90% and an intensive oligosaccharide pattern were used for ELISA inhibition assays as described below. The loss of non-specific inhibition potency was checked with the birch pollen pool serum (1:5) and apple allergen discs at an inhibitor concentration of 5 mg ml^{-1} . No attempts were made to characterize further the structures of the oligosaccharides obtained.

Chemicals

All chemicals were of analytical grade unless described as otherwise.

ELISA and ELISA Inhibition

Protein extracts of vegetable foods were coupled to cyanogen bromide-activated filter discs as previously described (Vieths *et al.*, 1992a) using protein concentrations of $12 \mu\text{g ml}^{-1}$ in the coupling solution. The same method was used to immobilize the carbohydrates, with the exception that higher coupling concentrations (20 mg ml^{-1} for oligosaccharides and 5 mg ml^{-1} for plant gum extracts) were used. Control discs were deactivated with 1 M-ethanolamine.

Direct IgE binding to the allergen discs was determined as follows. Patient sera were diluted 1:5 (v/v) in incubation buffer (0.05 M-Tris, pH 7.4; 0.15 M-NaCl; 0.3% (w/v) bovine

serum albumin (BSA); 1% (v/v) Tween 20). Diluted serum (50 μ l) was incubated with an allergen disc overnight at room temperature. Immuno-detection of the bound IgE antibodies and enzymatic staining were performed as described (Vieths *et al.*, 1993b) using a goat affinity-purified anti-IgE alkaline phosphatase conjugate (1:10 000) and *p*-nitrophenyl-phosphate (PNPP) as the substrate. Absorbance was measured at 405 nm. Blank-corrected ELISA results were considered to be definitely positive only if the following three conditions were fulfilled: (i) when the absorbance, *X*, obtained with the allergenic patient's serum and a specific allergen disc was minimally twice as high as the signal, *N*, of the buffer control: $[(X - N):N] \geq 1$; (ii) when the absorbance, *X*, was at least double the signal, *D*, of the same serum with an deactivated disc: $[(X - D):D] \geq 1$; (iii) when the signal, *X*, was minimally two times higher than the result, *B*, obtained with the serum pool Wn 13/32 (from birch pollen allergic patients) and the same allergen disc: $[(X - B):B] \geq 1$ (in initial experiments there was no evidence that serum Wn 13/32 contained anti-carbohydrate antibodies, so that this measurement served as a control for the non-specific binding of IgE).

Competitive ELISA inhibition assays were carried out as described (Vieths *et al.*, 1992b, 1993b) using 50 μ l of patient serum dilution and 50 μ l of inhibitor solution. In standard confirmation experiments carried out to check the specificity of IgE binding to the carbohydrate solid phases, only one inhibitor concentration was used (0.25 or 0.5 mg ml⁻¹ of protein from vegetable food extracts, 2 mg ml⁻¹ of oligosaccharides, 5 mg ml⁻¹ of partially hydrolyzed polysaccharides). For negative control inhibitions 2 mg ml⁻¹ of saccharose were used. IgE binding was taken as being specific if more than 50% inhibition occurred and the non-specific inhibition by saccharose was less than 20%. All ELISA determinations were run in duplicate.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), Protein Transfer and Immunoblotting

Discontinuous SDS-PAGE was carried out as previously described (Vieths *et al.*, 1992c, 1993b). For silver staining, 4–6 μ g cm⁻¹ of total protein from apple extract or 150 μ g cm⁻¹ (initial dry weight) from plant gum extracts were loaded on the gels. For subsequent immunoblotting, 14 μ g cm⁻¹ of protein from vegetable food extracts or 400 μ g cm⁻¹ of plant gum extracts (corresponding to initial dry weight) were applied. Semi-dry electroblotting, human serum incubation and peroxidase immunostaining of the bound IgE antibodies were carried out as previously described. A total protein stain of one nitrocellulose (NC) strip with the separated allergens and molecular weight marker proteins was made with India ink.

Lectin Affinity Detection

Cut and blocked NC strips with electrophoretically separated allergens were incubated with 1 ml of biotinylated *Canavalia ensiformis* lectin (Con A; Medac, Hamburg, Germany) in 0.01 M-phosphate-buffered saline (PBS), 0.05% (v/v) Tween 20, 0.1% (w/v) BSA, at a concentration of 1 μ g ml⁻¹ for 1 h. The blots were washed three times with PBS–Tween and incubated with streptavidin–horseradish peroxidase conjugate (Medac, Hamburg, Germany) (1:20 000 (v/v)) in incubation buffer for 20 min. Enzymatic staining with 3,3',5,5'-tetramethylbenzidine(TMB)/dioctylsodium sulphosuccinate (DONS) was performed as described (Vieths *et al.*, 1992c).

Periodate Oxidation

Protein (1 mg) from apple extract in 0.5 ml of 0.01 M-potassium phosphate buffer, pH 7.4 (PB), was treated with 0.5 ml of 0.05 M-NaIO₄ in 0.02 M-sodium acetate buffer, pH 5.0, for 2 h in the dark. The reaction was stopped with ethylene glycol (50 μ l). Subsequently, 12.5 mg of NaBH₄ were added, the mixture was incubated for 1 h at room temperature, 50 μ l of acetic acid were added, and the solution was brought to pH 7 with NaOH. Another

sample of apple extract was control-treated in the same manner, but with the inactivated oxidation reagent. The extracts were washed twice with PB using disposable microconcentrators (Centricon 3, Amicon, Witten, Germany) and diluted to the original volume with PB. Before their use in ELISA inhibition assays, the extracts were analyzed by SDS-PAGE and the gels were silver stained. The control-treated and oxidized apple extracts had nearly identical protein patterns, and both contained an intense 18 kDa apple allergen band.

Cut NC strips loaded with the electrophoretically separated extracts from apples, celery roots, gum arabic and gum traganth were oxidized and control-treated in the same way, except that 0.1 M-NaIO₄ was used. The treatment was stopped with sorbitol and the following steps (NaBH₄ treatment) were omitted.

RESULTS AND DISCUSSION

SDS-PAGE of Plant Gum Extracts

The crude extracts of gum traganth, gum arabic, locust bean gum (carob), xylan and the products obtained by partial acid hydrolysis were analyzed by SDS-PAGE followed by silver staining (results not shown). Two to four weak and indistinct protein bands were seen with all crude extracts. From the band intensity, a maximum of 0.3% soluble protein impurities of the initial dry weight was estimated. No protein bands were detected in the partial hydrolyzates.

ELISA Determination of IgE Directed against Carbohydrate Epitopes

The 54 sera from patients with polyvalent sensitizations to pollen and vegetable foods (selected on the basis of the criteria initially described), the non-allergic control serum and the birch pollen pool serum were tested for direct IgE binding to allergen discs loaded with stachyose, saccharose, gum arabic, gum traganth, locust bean gum, xylan and the deactivated solid phase. Positive ELISA results were confirmed by ELISA inhibition using stachyose, saccharose or partially hydrolyzed preparations of the polysaccharides as the inhibitors. A positive result in the non-competitive ELISA was decided using the criteria described in Materials and Methods. The IgE binding to an allergen disc was evaluated as being specific only if over 50% inhibition was obtained with 2–5 mg ml⁻¹ of the specific carbohydrate. The ELISA results were positive for 9/54 sera (17%) (Table 1). Six sera bound to stachyose discs. Antibodies from serum Bo7 also reacted with gum arabic and xylan, IgE from patient Bo13 bound to gum arabic and traganth, and Bo50 revealed exclusive IgE binding to traganth. The data obtained with the three additional sera listed in Table 1 were questionable, but prompted their inclusion in further investigations. Extended ELISA measurements were carried out with nine of the sera from Table 1, using allergen discs of lacto-*N*-tetraose, 6-O-(2-acetamido-2-desoxy- β -D-glucopyranosyl)-D-galactopyranose, methyl-2-O- α -D-mannopyranosyl- α -D-mannopyranoside and methyl-3-O- α -D-mannopyranosyl- α -D-mannopyranoside. The ELISA was positive only with lacto-*N*-tetraose and four sera (Bo7, Bo13, Bo50 and Bo64). Bo64 was not definitely positive with all other allergen discs used.

Immunoblot Study

Immunoblot analyses with separated apple and celery allergens on NC membranes were carried out with 11 of the sera listed in Table 1, and with the control sera MM and 216 (the patients were sensitive to celery and apples, but had negative ELISA results with the carbohydrate solid phases). Eight of the sera revealed intense IgE antibody reactions, with many allergen bands of molecular masses above 30 kDa. IgE binding was strongly reduced by periodate oxidation of the NC strips loaded with the allergens, indicating the existence of periodate-sensitive determinants. In contrast, the 'protein-specific' control sera MM and 216 retained their reactivity completely. Since the amount of serum was limited, serum

TABLE 1. ELISA and immunoblot results for 12 human sera with suggested IgE against carbohydrate structures.

Serum	ELISA	ELISA inhibition	Immunoblot binding > 30 kDa ^a	Allergen bands quenched by periodate treatment
Bo7	+ ^b S, L, X, GA ^c	+	+	+
88/91	+	+	+	n
64/91	S	+	(+)	(+)
65/91	S	+	n ^d	n
Bo49	+	+	—	—
Bo13	S	+	+	+
158/91	L, GA, T ^c	+	—	—
Lev	+	+	—	—
Bo50	S	+	+	+
Bo64	L, T	n	+	+
28/Wü	+	n	+	+
29/Wü	—	n	+	+

^a To allergens from apple and celery extract.

^b (+) Positive; (—) negative. Inhibitions were designated (+) if an oligosaccharide or partially hydrolyzed polysaccharide at a concentration of 2–5 mg ml⁻¹ caused over 50% inhibition of IgE binding to the allergen disc used.

^c S = stachyose; L = lacto-N-tetraose; X = xylan; GA = gum arabic; T = gum traganth.

^d n = not done.

88/91 could not be tested with periodate-treated blot strips. Interestingly, the three sera with questionable ELISA results (Bo64, 28/Wü and 29/Wü) were clearly positive in this experiment. Three sera with positive ELISA results (Bo49, 158/91 and Lev) revealed only weak binding to the plant allergens on immunoblots. The immunoblot results are summarized in Table 1. As an example, immunoblot analyses with the sera Bo7, Bo13 and the two control sera are presented in Figure 1. In contrast to the observations made with bands of higher molecular masses, the reactivity of sera Bo7, Bo13, Bo50 and Bo64 with the major protein allergen from apple (at approximately 18 kDa) was not influenced by the oxidation procedure. This result probably indicates co-sensitization of the patients to protein epitopes of the allergen.

Furthermore, the sera Bo7, Bo13, control serum 216 and the biotinylated lectin Con A were used to probe blots of gum arabic (Figure 2). The first two sera, but not the control serum 216, showed strong, but diffuse, IgE binding in the mass region above 30 kDa, which drastically decreased after periodate treatment. The same behaviour was observed with Con A, indicating the destruction of Con A-reactive carbohydrate structures by the oxidation process.

Summarizing, the immunoblot results suggest that the IgE antibodies of 8/11 sera recognized periodate-sensitive epitopes in a wide mass range higher than 30 kDa. This is in accordance with the results of other authors who have reported diffuse IgE and IgG antibody binding in regions of higher masses for mannans or mannoproteins from bakers'

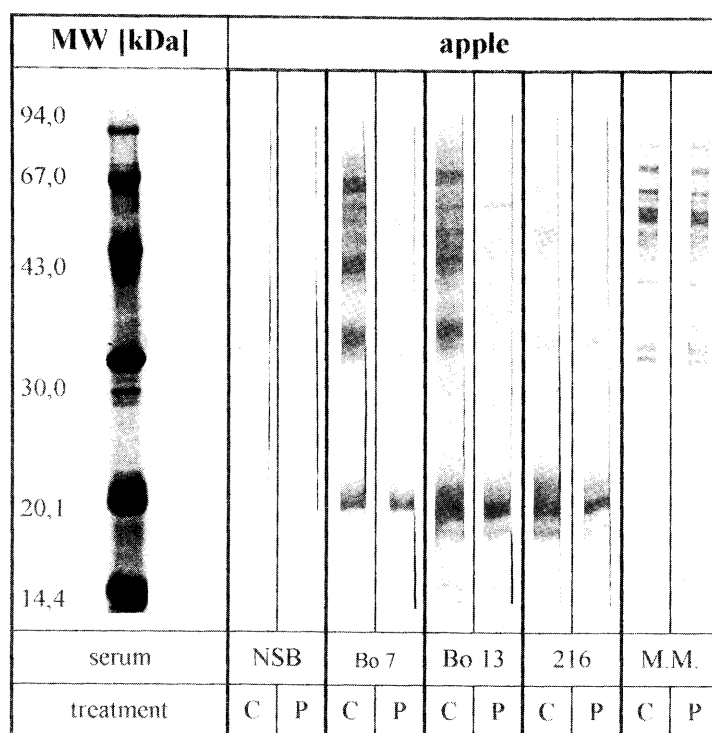


FIG. 1. Binding of IgE from human sera to apple allergens separated by SDS-PAGE and transferred to NC. Sera as indicated. NSB, buffer control; C, control-treated NC strips; P, NC strips treated with sodium periodate (0.1 M). Sera MM and 216 were from patients sensitized to apple and celery but with no evidence of IgE binding to carbohydrate epitopes (positive controls).

yeast (Kortekangas-Savolainen *et al.*, 1993) and *Aspergillus umbrosus* carbohydrate antigens (Kaukonen *et al.*, 1993).

For the ELISA-negative sera 28/Wü and 29/Wü, binding to carbohydrate structures cannot be firmly concluded from the blot experiments as sensitive conformational protein epitopes may be affected by the oxidation process. Differences between immunoblot and ELISA may be due to different sensitivities of both methods and/or differences in the composition of the protein-bound glycan structures and the carbohydrates used in the ELISA study.

Investigation of the Cross-reactive Nature of the IgE Antibodies from Serum Bo7

The cross-reactive nature of the carbohydrate determinants was further investigated with serum from patient Bo7. This female patient had a total IgE concentration of 1065 U ml^{-1} and was serologically sensitized to every plant food allergen studied (RAST classes ≥ 2 to apple, pear, strawberry, orange kiwi, lychee, potato, tomato, zucchini, Brussels sprouts, onion, garlic, celery and coriander seed, but no IgE binding to the deactivated solid phase). ELISA inhibition assays of apple, celery, orange and onion allergen discs, an 1:2.5 serum dilution and 0.5 mg ml^{-1} of protein from 10 plant food extracts as the inhibitors revealed significant inhibition of the IgE binding ($\geq 20\%$) with most of the foods, but not with ovalbumin (negative control). Inhibition results below 20% only occurred with apple allergen discs and onion, zucchini and Brussels sprouts as the inhibitors (Table 2). These results clearly indicated that the IgE antibodies of the patient were highly cross-reactive.

With stachyose discs, a strong self-inhibition with stachyose occurred (result not shown).

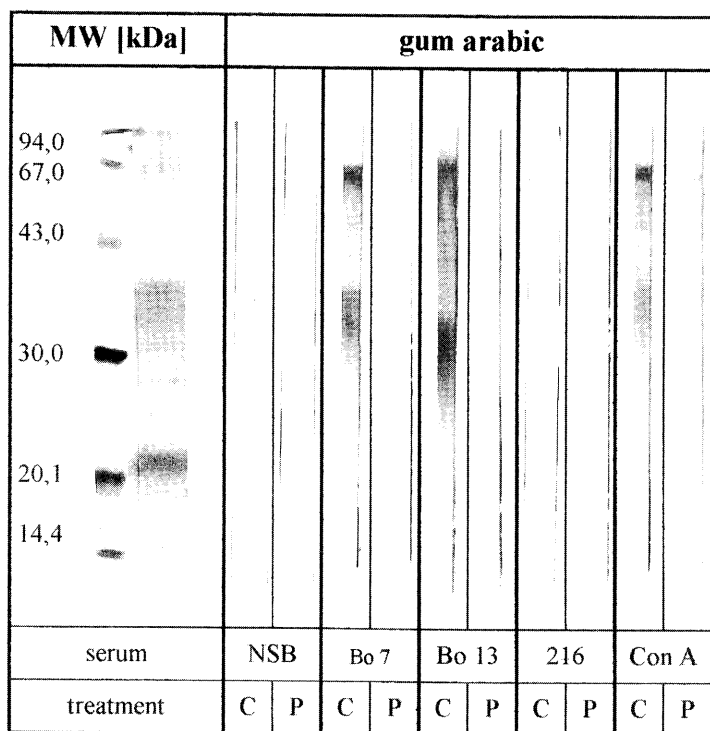


FIG. 2. Binding of IgE from human sera and of the lectin Con A to extract of gum arabic separated by SDS-PAGE and transferred to NC. Sera as indicated. NSB, buffer control; C, control-treated NC strips; P, NC strips treated with sodium periodate (0.1 M). Serum 216 contained IgE against the allergen *Bet v 1* from birch pollen but no evidence for IgE binding to carbohydrate epitopes was found.

Furthermore, raffinose, but not saccharose, strongly inhibited IgE binding to stachyose discs (Figure 3(a)), suggesting that the galactose residues of raffinose and stachyose may be an important part of the epitope. Stachyose inhibited the ELISA with immobilized gum arabic (Figure 3(b)), and the stachyose ELISA was totally inhibited with control-treated, but not with periodate-treated, apple extract (Figure 3(c)). When apple allergen discs were used, a

TABLE 2. Results of ELISA inhibition assays with serum Bo7 and 0.5 mg ml⁻¹ of protein from various vegetable food extracts (% inhibition of IgE binding to the solid phase)

Inhibitor extract	Allergen disc			
	Celery	Apple	Orange	Onion
Celery	102	46	45	66
Apple	79	94	37	75
Strawberry	83	54	44	72
Orange	53	31	89	80
Lychee	47	30	60	50
Onion	44	9	38	77
Zucchini	43	10	54	60
Brussels sprouts	88	6	50	72
Aniseed	112	77	49	81
Basilicum	66	53	21	20
Ovalbumin	9	1	0	0

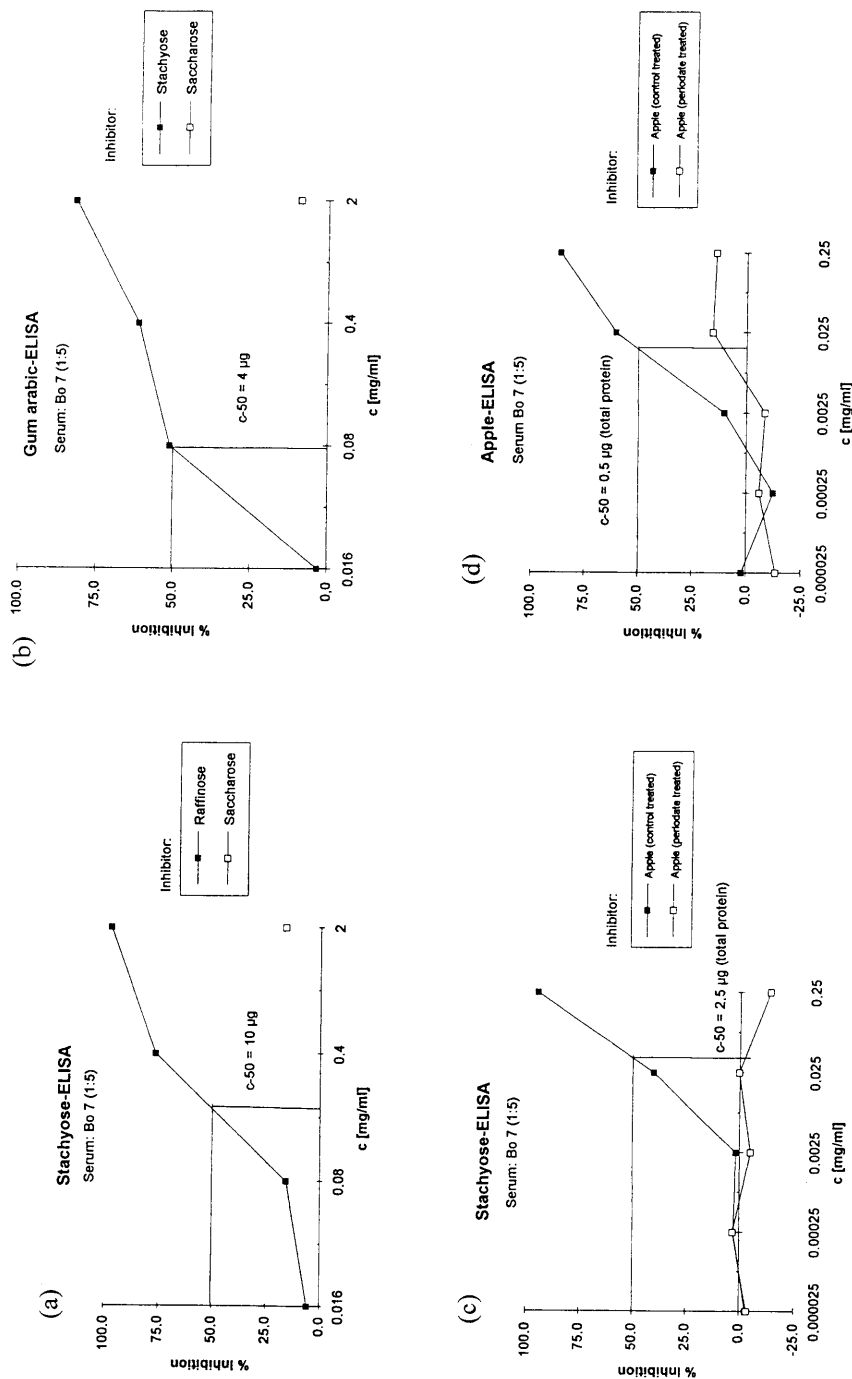


FIG. 3. ELISA inhibition experiments with serum of patient Bo7 (1:5). Inhibitor concentrations as indicated. C_{50} values are the total amount of inhibitor saccharide or inhibitor protein (from allergen extract) responsible for a 50% inhibition of the IgE binding to the solid phase. (a) Allergen disc: stachyose; inhibitor: (■) raffinose, (□) saccharose. (b) Allergen disc: gum arabic; inhibitor: (■) stachyose, (□) saccharose. (c) Allergen disc: stachyose; inhibitors: (■) control-treated apple extract, (□) periodate-treated apple extract. (d) Allergen disc: apple; inhibitors: (■) control-treated apple extract, (□) periodate-treated apple extract. The control experiment was performed with serum 216 which contained IgE against *Bet v 1* from birch pollen and the 18 kDa major allergen from apple. With this serum, the inhibition potency of the apple extract was 20% reduced (relative) by the periodate treatment (result not shown).

strong self-inhibition occurred with control-treated apple extract, but the inhibition potency was eliminated by the periodate treatment (Figure 3(d)). A control inhibition with serum 216 (which reacted to *Bet v* I from birch pollen and to the major protein allergen of 18 kDa in apple extract) a 20% relative decrease of the inhibition potency of the apple extract was caused by the oxidation process (result not shown).

From the experiments with the serum Bo7, it can be concluded that the CCDs are indeed highly cross-reactive, and that stachyose, raffinose, gum arabic and the glycoproteins from apples share common epitopes for human IgE. Furthermore, there is some evidence that galactose (or GalNAc) may be involved in the epitope structure.

Additional Data of the Patient Group with IgE against CCD

The 12 positive patients listed in Table 1 had total IgE levels ranging from 350 to $\geq 20\,000\text{ U ml}^{-1}$ (median: 1870 U ml^{-1}). Three patients (Bo13, Bo49 and Bo50) had a total IgE concentration of more than $10\,000\text{ U ml}^{-1}$. Nine patients showed symptoms after ingestion of more than one vegetable food. One patient tolerated all RAST-positive fruits and vegetables orally but suffered from contact urticaria of the hands after handling strawberries or raw potatoes. In two patients, no symptoms were elicited by RAST-positive foods. Six of the participants had an additional neurodermitis. Only one was sensitized to honey-bee venom.

CONCLUSIONS

The results of this study suggest that 12/54 (22%) of the participants possessed IgE antibodies directed against carbohydrate epitopes. In our opinion, this was conclusive for the 10/54 (19%) patients who were positive in the ELISA study, but there remained some doubt about two sera which revealed only positive immunoblot results.

Since the occurrence of anti-carbohydrate IgE antibodies in food allergic patients has rarely been described, the prevalence of 22% in our patient group seems unexpectedly high. However, only highly sensitized allergic patients took part in our study. Tretter *et al.* (1993) found that α -1,3-linked L-fucose was an important epitope for patients allergic to honey-bee venom and that the epitope is cross-reactive to the plant protease bromelain. Since only two of the 54 patients were sensitized to bee-venom, α -1,3-L-fucose cannot be responsible for the apparent sensitizations determined in our study. The ELISA and ELISA inhibition results further support the view that the sensitization pattern observed is independent of fucose, and that galactose or GalNAc may have epitope nature for our patients. In accordance with Tretter *et al.* (1993), no serological reactions with structures containing mannose were determined.

Aalberse *et al.* (1981) and Calkhoven *et al.* (1987) have stated that IgE directed against CCD are clinically insignificant. Broichmann *et al.* (1992) reported both symptomatic and asymptomatic patients from their study group. The same was true for our 12 positive patients, but most of them reacted clinically to more than one vegetable food. Since the symptoms may be caused by co-occurring sensitizations to protein epitopes, further evaluation of the clinical relevance of anti-CCD antibodies in plant food allergic patients under controlled conditions is required.

ACKNOWLEDGEMENTS

We are grateful to Mrs R. Tschirnich for excellent technical assistance. We also wish to thank Dr H. Aulepp, Hospital Borkum Riff of the BfA, Borkum Germany, for his cooperation and Mr R. Hatton from our Institute for his help in the preparation of the manuscript.

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