

Allergens, IgE, mediators, inflammatory mechanisms

Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins

Maurits J. van der Veen, MD,^{a, b} Ronald van Ree, PhD,^b Rob C. Aalberse, PhD,^b Jaap Akkerdaas, MSc,^b Stef J. Koppelman, PhD,^c Henk M. Jansen, MD, PhD,^a and Jaring S. van der Zee, MD, PhD^a *Amsterdam and Zeist, The Netherlands*

Background: In our outpatient population, approximately one third of patients sensitized to grass pollen were found to have significant serum levels of anti-peanut IgE in the RAST, without positive peanut skin prick test (SPT) response and without peanut-related allergic symptoms. It was suggested earlier that poor biologic activity of IgE antibodies directed to cross-reactive carbohydrate determinants (CCD) of glycoproteins might explain these discrepancies.

Objective: In this study we investigated the biologic activity of IgE directed to CCD.

Methods: Sera of 32 patients allergic to grass pollen with significant levels of anti-peanut IgE, a negative response on peanut SPT, and no symptoms of peanut allergy were tested for the presence of anti-CCD IgE. Eleven of these patients with greater than 3.0 IU/ml anti-peanut IgE (patients 1 to 11) were selected together with four control patients allergic to peanut, on the basis of a positive response on peanut SPT and a history of peanut allergy (patients 12 to 15). Inhibition of the peanut RAST was performed by using proteinase K-treated grass pollen extract as a CCD source. Basophil histamine release assays (BHRAs) were performed with peanut extract and the isolated peanut major allergens Ara h 1 and Ara h 2. In addition, intracutaneous tests with peanut extract were performed.

Results: In 29 (91%) of 32 patients with discrepant peanut RAST and SPT responses, anti-CCD IgE (≥ 0.1 IU/ml) was detected. In patients 1 to 11 almost complete inhibition of the peanut RAST with CCD was found ($94.3\% \pm 5.5\%$; mean $\pm$ SD). In contrast, in the patients allergic to peanut only partial inhibition (59%) was found in one subject ($p = 0.002$, Mann-Whitney test). In the BHRAs and the intracutaneous tests of patients with discrepant peanut RAST and SPT results, reactivity was found only at high concentrations of peanut allergens. When related to specific IgE levels, reac-

tivity to peanut allergens in the BHRAs of these patients was found to be at least a factor of 1000 less when compared with reactivity to control inhalant allergens.

Conclusion: We conclude that cross-reactive IgE directed to carbohydrate determinants of glycoproteins, as found in grass pollen-sensitized patients, has poor biologic activity. It can therefore cause positive RAST results without apparent clinical significance. (*J Allergy Clin Immunol* 1997;100:327-34.)

Key words: Grass pollen allergy, peanut allergy, cross-reactivity, glycoproteins, carbohydrate determinants

The N-linked carbohydrate groups of glycoproteins induce IgE, leading to cross-reactivity between several foods and grass pollen allergens.¹⁻⁶ These so-called cross-reactive carbohydrate determinants (CCDs) are present on glycoproteins of vegetables such as peanut, celery, potato, and tomato.^{1-4, 6} Moreover, carbohydrate-specific IgE not only binds to plant-derived sugar groups but also to N-glycans in insect venoms (phospholipase A₂)^{1, 2, 7, 8} and seafood (e.g., crustaceans and mollusks).⁹ It is found both in subjects allergic to pollen and in beekeepers, indicating that sensitization can occur by inhalation of pollen or by insect stings.^{1, 2, 7, 8} Positive RAST results, selectively based on the presence of anti-CCD IgE, are estimated to occur in 10% to 15% of patients sensitized to grass pollen.⁴

IgE that is cross-reactive to food allergens can be found relatively frequently in patients allergic to pollen,¹⁰ leading to clinical symptoms as described for the apple-birch pollen syndrome¹¹ or melon- and banana-related complaints in ragweed pollinosis.^{12, 13} These biologically active cross-reactions in pollen allergy can often be explained by the presence of IgE directed to the birch major allergen (Bet v 1) or to profilin, which is found in all eukaryotic cells.^{4, 10, 14-16} However, although clinical data are scarce, it is suggested that patients with IgE cross-reactivity selectively based on CCD-specific IgE antibodies do not experience symptoms of food allergy.^{2, 4} Hence the biologic activity of anti-CCD IgE is questionable, and its presence seems to be clinically irrelevant. If the latter is true, distinction between anti-CCD IgE and IgE antibodies with other specificity

From ^athe Department of Pulmonology, Academic Medical Center, University of Amsterdam, Amsterdam; ^bthe Department of Allergy, Central Laboratory of the Netherlands Red Cross Bloodtransfusion Service, Amsterdam; and ^cthe Netherlands Organization for Applied Scientific Research Nutrition and Food Research Institute, Zeist.

Supported by the Netherlands Asthma Foundation grant 93.47.

Received for publication Dec. 31, 1996; revised Mar. 20, 1997; accepted for publication Mar. 24, 1997.

Reprint requests: J. S. van der Zee, MD, Academic Medical Center, University of Amsterdam, Department of Pulmonology, F4-208, P.O. box 22700, 1100 DE Amsterdam, The Netherlands.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/82144

Abbreviations used

CCDs:	Cross-reactive carbohydrate determinants
CI:	Confidence interval
BHRA:	Basophil histamine release assay
ICT:	Intracutaneous test
SDS-PAGE:	Sodium dodecylsulfate–polyacrylamide gel electrophoresis
SPT:	Skin prick test

will improve the predictive value of food allergy diagnostics *in vitro*.

In our outpatient department we routinely investigate new patients who display respiratory symptoms for occurrence of inhalant allergies using RAST, skin prick test (SPT), and a standard questionnaire. To study the role of carbohydrate-based cross-reactivity in this population we added, as a common CCD-containing food source, peanut (*Arachis hypogaea*) extract to the panel of allergens tested. In approximately one third of the patients allergic to grass pollen, positive results on peanut RAST were found without positive peanut SPT response and no obvious peanut-related allergic symptoms.

This study was performed to determine the prevalence of anti-CCD IgE in a sample of these patients and to study its biologic activity by using basophil histamine release assays (BHRAs) and intracutaneous skin tests (ICTs) with peanut allergens.¹⁷

METHODS

Patients

RASTs and SPTs with a series of allergens, including grass pollen and peanut, were routinely performed in 606 patients. These patients were new adult patients attending the outpatient departments of Pulmonology and Otorhinolaryngology at the Academic Medical Center who were first seen with symptoms of asthma or rhinitis. In addition, a questionnaire concerning allergy-related complaints was completed by an investigator during a standardized interview. After RAST was performed, sera were stored at -20°C .

Eleven grass pollen-sensitized patients with peanut RAST scores greater than or equal to 3+, a negative peanut SPT response, and no peanut-related allergic symptoms were selected for further analysis (patients 1 to 11) (Table I). Four patients allergic to peanut were selected as positive control patients (patients 12 to 15) (Table I) on the basis of positive peanut RAST scores greater than or equal to 3+, a positive peanut SPT response, and a positive history of peanut allergy (i.e., "oral allergy syndrome," angioedema, or anaphylactic shock). In addition, three atopic patients with positive house dust mite and/or grass pollen RAST scores greater than or equal to 4+ but negative peanut RAST results were selected as negative control patients.

CCD source

Proteinase K-treated grass pollen (*Lolium perenne*) extract, in which the peptide backbone of grass pollen glycoproteins is completely digested,¹⁸ was used as a CCD source for RAST and

RAST inhibition experiments. In the digested grass pollen extract, no intact protein was detected on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in conjunction with silver staining. This is in contrast to a control grass pollen extract that was subjected to the same treatment but in the absence of proteinase K. In addition, no remaining grass pollen (major) allergen activity was detected in the grass pollen extract after digestion with proteinase K by using Lol p 5,¹⁸ Lol p 11,¹⁹ and profilin¹⁴ assays. Moreover, the ability of the proteinase K-treated grass pollen extract to inhibit the grass pollen RAST results of grass pollen-sensitized patients was found to be decreased by at least a factor of 1000 when compared with the undigested control extract (results not shown).

Major allergens

The peanut (Ara h 1,²⁰ Ara h 2²⁰), house dust mite (Der p 1²¹), and grass pollen (Lol p 5¹⁸) major allergens used in this study were isolated as previously described.

RAST

RAST was performed as previously described.² Allergens were insolubilized by coupling to CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden). For allergen extracts and isolated major allergens, approximately 20 and 0.5 μg of protein per test was bound to solid phase, respectively. In the routine peanut RAST, an extract of unroasted peanuts was used (Calvé, Delft, The Netherlands). Grass pollen allergens were derived from a mix of *Dactylus glomerata* and *Phleum pratense* (ALK Benelux, Groningen, The Netherlands). In the CCD RAST, the proteinase K-digested grass pollen extract was coupled to solid phase. Peanut-profilin solid phase was obtained by overnight incubation of poly(L-proline) Sepharose with peanut skin test extract and washed four times before use.¹⁴ For selected patients, RAST was performed with the peanut skin test extract and the peanut major allergens Ara h 1 and Ara h 2.

For the intraindividual comparison of RAST with the peanut skin test preparation and a control inhalant allergen (i.e., *D. pteronyssinus* [ALK, Copenhagen, Denmark], Der p 1, or Lol p 5), a correction was made for possible differences in the amount of allergen bound to solid phase according to the method described earlier by Aalberse et al.²² Briefly, a dilution curve of solid phase was made in the presence of diluted serum, producing approximately half maximal binding of radioactivity.

RAST results were expressed in international units per milliliter using an in-house standard with mouse/human chimeric antibodies against Der p 2 and house dust mite solid phase, calibrated against the World Health Organization standard.²³ The detection level of this assay was 0.1 IU/ml specific IgE. The routine RAST results were expressed in RAST classes (0, 0.0 to 0.29 IU/ml; 1+, 0.3 to 1.00 IU/ml; 2+, 1.01 to 3.00 IU/ml; 3+, 3.01 to 9.00 IU/ml; 4+, 9.01 to 27.0 IU/ml; 5+, >27.0 IU/ml). A commercially available specific IgE anti-peanut assay was used according to the manufacturer's instructions (CAP system, Pharmacia).

RAST inhibition

To obtain comparable initial anti-peanut IgE levels before RAST inhibition (approximately 2.0 IU of anti-peanut IgE per ml), sera were first tested in twofold dilution steps (0.01 mol/L ethylenediaminetetraacetic acid, 0.3% bovine serum albumin, phosphate-buffered saline) in the RAST with the peanut skin test preparation. Next, the diluted serum samples were retested in duplicate after preincubation for 2 hours with the CCD

TABLE 1. Characteristics of patients 1 to 15

Patient No.	Age (yr)	Sex	RAST score (0-5)		SPT (wheal diameter; mm)		Peanut-related allergic symptoms
			Grass pollen	Peanut	Grass pollen	Peanut*	
1	27	M	5	4	9	3	—
2	21	M	5	3	11	<3	—
3	25	M	4	4	10	<3	—
4	21	F	5	4	13	3	—
5	28	M	4	3	7	<3	—
6	21	F	4	3	9	<3	—
7	34	M	4	3	13	<3	—
8	44	M	4	3	17	<3	—
9	33	F	4	4	ND	ND	—
10	65	F	3	3	6	<3	—
11	61	M	3	4	7	<3	—
12	23	M	5	5	11	11	Angioedema
13	18	F	0	4	<3	13	Angioedema, anaphylactic shock
14	26	F	4	4	7	7	Oral allergy syndrome
15	32	F	1	4	<3	11	Angioedema, anaphylactic shock

*Mean of repeated SPT results in duplicate.

ND, Not done.

source (equivalent to 50 µg [wt/vol] grass pollen extract per test).

In addition, peanut RAST inhibition experiments were performed with a series of twofold dilutions of the CCD source, untreated grass pollen extract (range, equivalent to 100 to 0.4 µg [wt/vol] in water grass pollen extract per test), and the peanut skin test preparation (range, 100 to 0.4 µg per test).

Skin tests

SPTs were performed and documented in a standardized way.²⁴ For the routine SPT, a series of allergens was used including peanut (5 mg/ml; ALK Benelux) and grass pollen mix (10,000 BU/ml; ALK). A positive SPT result was defined as a skin reaction with a wheal of greater than or equal to 3 mm with surrounding erythema in combination with a negative SPT response to the control fluid (phosphate-buffered saline, 50% glycerol, 0.5% phenol).

The ICT was performed with three 10-fold dilutions of the peanut skin test preparation (range, 5 to 500 µg/ml) as described previously²¹ by using 0.03 ml of each allergen concentration. The results are expressed as the mean of the maximal and orthogonal diameter of the wheal-and-flare skin reaction. For safety reasons peanut ICTs were not performed in patients with a history of peanut allergy.

BHRA

Incubation of isolated leukocytes with stimulants was performed with a method modified from that described by Lichtenstein and Osler.^{21, 25} Stimuli used were: series of 10-fold dilutions of the peanut skin test extract (range, 20 to 2.0 × 10⁶ ng/ml), isolated peanut major allergens Ara h 1 and Ara h 2 (range, 0.5 to 5.0 × 10⁴ ng/ml), and a control allergen, such as *D. pteronyssinus* (range, 0.5 to 5.0 × 10⁴ ng/ml), Lol p 5 (range, 0.5 to 5.0 × 10⁴ ng/ml), or Der p 1 (range, 0.06 to 6.0 × 10³ ng/ml). In addition, polyclonal sheep anti-IgE (1000, 100, 10 ng/ml; CLB no. SH25P01; Amsterdam, The Netherlands) and phorbol myristate acetate (600 ng/ml) were used as controls. Histamine content in the supernatant of the samples was

measured by an automated fluorometric method.²⁶ After subtraction of the spontaneous release, histamine release was calculated as the percentage of total cellular histamine content. Histamine release greater than or equal to 10% was regarded as a positive response, and histamine release threshold concentrations were calculated by intrapolation with log-linear regression.²¹ When threshold concentrations exceeded maximum or minimum concentrations, twice or half the concentration was used for statistical and graphical analysis, respectively. For comparison of the results found with allergen extracts and isolated major allergens, the allergen content of the former was estimated at 10% of total protein content.^{18, 20, 27-29}

RESULTS

Routine diagnostic data

Of the 606 routinely screened patients, 173 (29%) were sensitized to grass pollen as indicated by a positive grass pollen RAST score (≥1+) and a positive grass pollen SPT response. Of these patients allergic to grass pollen, 50 (29%) were found to have significant levels of anti-peanut IgE (RAST score ≥1+) in combination with a negative peanut SPT response and no peanut-related allergic symptoms.

RAST

Sera of 32 patients with peanut RAST scores of 2+ or greater and negative peanut SPT responses were available for further serologic analysis. A median level of 3.0 IU/ml IgE anti-peanut (range, 0.9 to 18.0 IU/ml) was found when these sera were retested in the routine peanut RAST. These results were confirmed with a commercially available specific IgE assay (CAP, Pharmacia). A good correlation was found between the outcomes of the two anti-peanut IgE assays ($r = 0.94$;

TABLE II. Results of RAST, ICT, and BHRAs in patients 1 to 15

Patient No.	RAST (IU specific IgE/ml)				Peanut ICT (wheal/flare diameter; mm)		BHRA (threshold concentration; µg/ml)		
	Peanut*	CCD	Ara h 1	Ara h 2	0.5 mg/ml	0.05 mg/ml	Peanut	Ara h 1	Ara h 2
1	3.7	3.0	<0.1	<0.1	10/32	8/26	9.1	1.8	>50
2	2.0	6.3	<0.1	<0.1	10/32	7/—	1024	>50	>50
3	3.4	3.5	<0.1	<0.1	11/38	9/—	>2000	>50	>50
4	6.5	9.2	<0.1	<0.1	ND	8/29	106	ND	ND
5	2.3	1.5	<0.1	<0.1	11/32	7/—	542	ND	ND
6	1.5	2.7	<0.1	<0.1	7/22	—/—	>2000	>50	1.3
7	1.8	0.5	0.8	<0.1	10/42	9/30	157	18.2	>50
8	1.5	0.9	<0.1	<0.1	10/41	7/—	465	>50	14.2
9	12.2	10.4	<0.1	<0.1	ND	ND	5.0	1.2	>50
10	2.2	1.3	<0.1	<0.1	ND	ND	ND	ND	ND
11	4.1	1.9	<0.1	0.4	ND	ND	ND	ND	ND
12	59.9	0.7	22.7	54.2	ND	ND	<0.02	0.04	<0.0005
13	61.5	<0.1	17.2	30.6	ND	ND	<0.02	0.009	<0.0005
14	2.0	0.4	0.2	0.9	ND	ND	0.2	>50	0.003
15	26.7	<0.1	11.6	38.1	ND	ND	—**	—**	—**

*Peanut skin test preparation.

**No response.

ND, Not done.

regression coefficient, 1.09 [95% confidence interval (CI), 0.94 to 1.24]). In 29 (91%) of the 32 sera, anti-CCD IgE was detected with a median level of 0.8 IU/ml (range, 0.1 to 10.8 IU/ml). Anti-peanut IgE profilin was found in six (19%) of 32 patients (median, 0.4 IU/ml; range, 0.1 to 1.7 IU/ml).

RASTs with the peanut skin test preparation and the peanut major allergens Ara h 1 and Ara h 2 were performed with sera from patients 1 to 15 (Table II). In accordance with the results of the routine peanut RAST, levels of serum IgE directed to the peanut skin test preparation were found to be lower in patients 1 to 11 compared with the patients allergic to peanut (12 to 15). The RAST results of patients 1 to 11 were further characterized by significant levels of anti-CCD IgE and low or undetectable levels of anti-Ara h 1 IgE and anti-Ara h 2 IgE. In contrast, low levels of anti-CCD IgE and significant levels of anti-Ara h 1 IgE and anti-Ara h 2 IgE were found in the patients allergic to peanut (12 to 15).

RAST inhibition

The peanut RAST results of patients 1 to 11 were found to be almost completely inhibited by preincubation of the diluted serum samples with proteinase K-digested grass pollen extract ($94.3\% \pm 5.5\%$, mean $\pm$ SD) (Fig. 1). In contrast, in the patients allergic to peanut (12 to 15) only partial inhibition was found in one subject (59%), with no significant inhibition in the others (Fig. 1). This difference in RAST inhibition between the two groups of patients was found to be statistically significant ($p = 0.002$, Mann-Whitney U test).

In patients 3, 4, 9, 12, and 13, inhibition of the peanut RAST was also studied with series of dilutions of

untreated and proteinase K-digested grass pollen extract and peanut extract. Again, inhibition with CCD was established in patients with discrepant peanut RAST and SPT results but not in patients allergic to peanut, whereas clear RAST inhibition with peanut extract was found in both groups of patients. In patients 3, 4, and 9, the results with digested and undigested grass pollen extract were similar (results not shown). In Fig. 2, representative examples of the RAST inhibition curves as found in each group with CCD and peanut extract are shown (patients 3 and 13). Depicted is the percentage of the uninhibited value at each concentration of the inhibiting reagent. The affinity of IgE antibodies directed to peanut allergens and CCD was found to be similar as indicated by the parallel running slopes of the RAST inhibition curves found with CCD in patient 3 and with peanut extract in patient 13 (Fig. 2).

Skin tests

The results of the SPTs and ICTs with peanut extract are shown in Tables I and II, respectively. In patients 1 to 8 and 12 to 15, the peanut SPT was repeated in duplicate and had results similar to those in the routine peanut SPT with negative or borderline test results in patients 1 to 8 and clear positive test results in patients 12 to 15.

In the peanut ICTs of patients 1 to 8, low-grade skin reactivity was found in all patients with 0.5 mg/ml peanut extract (Table II). With 0.05 mg/ml peanut extract, skin reactions including erythema were found in three of eight patients tested (Table II), whereas no skin reactivity was found with 0.005 mg/ml peanut extract (results not shown). In the three atopic control patients without anti-peanut IgE, no skin reactivity was found in the

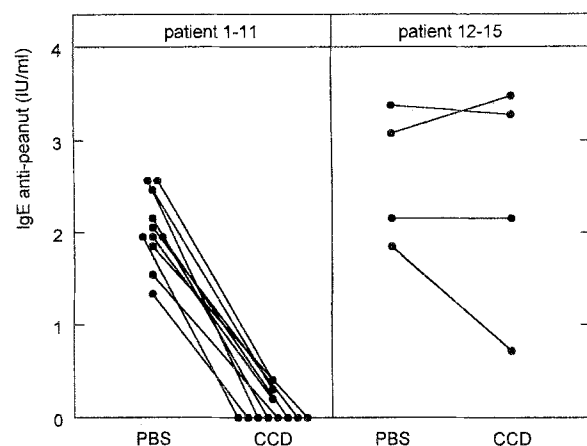


FIG. 1. Results of peanut RAST inhibition in patients with discrepant peanut RAST and SPT results (patients 1 to 11) and peanut allergic patients (patients 12 to 15). *PBS*, Phosphate-buffered saline, 0.01 mol/L ethylenediamine tetraacetic acid, 0.3% bovine serum albumin; *CCD*, proteinase K-digested grass pollen extract.

peanut ICTs with any of the concentrations tested (results not shown).

BHRA

The threshold concentrations for basophil histamine release are in Table II. In the group of patients with discrepant peanut SPT and RAST results, the threshold concentrations for histamine release on stimulation with peanut extract were found to be high (median, 465 $\mu\text{g/ml}$; range, 5 to $>2000 \mu\text{g/ml}$; $n = 9$). In addition, high threshold concentrations for the peanut major allergens Ara h 1 and Ara h 2 were found (Ara h 1: range, 1.2 to $>50 \mu\text{g/ml}$; Ara h 2: range, 1.3 to $>50 \mu\text{g/ml}$; $n = 7$). In contrast, in the BHRA of patients allergic to peanut (12 to 14), strong reactivity to peanut allergens was found with low histamine release threshold concentrations for peanut extract and the peanut major allergens Ara h 1 and Ara h 2 (peanut extract: range, <0.02 to $0.2 \mu\text{g/ml}$; Ara h 1: range, 0.009 to $>50 \mu\text{g/ml}$; Ara h 2: range, <0.0005 to $0.003 \mu\text{g/ml}$). Patient 15 was found to be a non-responder in this assay³⁰ with negative results on all stimuli (anti-IgE, allergens).

The results of the BHRA with peanut extract in two patients with discrepant peanut RAST and SPT results (patients 2 and 7) and one patient allergic to peanut (patient 14) are depicted in Fig. 3. Although the level of IgE directed to the peanut extract was similar in these patients (approximately 2.0 IU/ml), the reactivity to the peanut skin test preparation was found to be significantly lower for patients 2 and 7 than for patient 14. This is indicated by the differences in threshold values and the histamine release curves of at least a factor of 1000 between patient 14 and patients 2 and 7 (Fig. 3).

In Fig. 4, the relation is shown between histamine release threshold concentrations and specific IgE levels, as found with peanut extract and a control inhalant allergen in patients 1 to 4 and 6 to 9. Patient 5 was

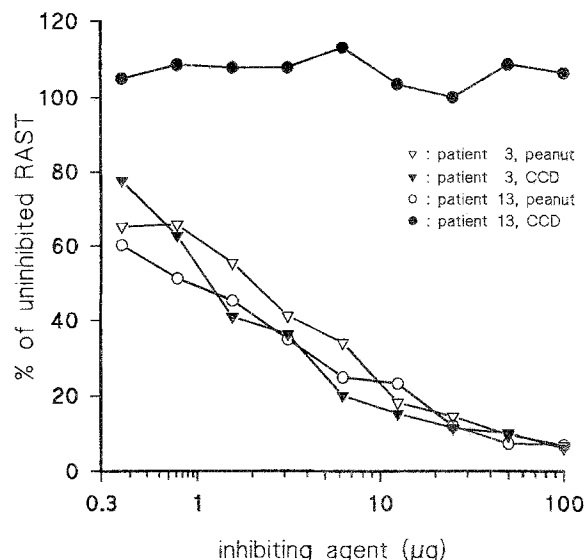


FIG. 2. Peanut RAST inhibition with peanut extract and CCD (proteinase K-digested grass pollen extract) in one patient with discrepant peanut RAST and SPT results (patient 3) and one patient allergic to peanut (patient 13). Depicted is the percentage of the uninhibited value at each concentration of the inhibiting reagent.

excluded because no threshold concentration for the control allergen could be calculated as a result of high-level histamine release with the lowest concentration of control allergen used (peanut threshold, 542 $\mu\text{g/ml}$). Fig. 4 shows the poor reactivity on stimulation with peanut allergens in patients with positive peanut RAST results selectively based on anti-CCD IgE. In relation to specific IgE levels, higher threshold concentrations of approximately a factor of 1000 were found with peanut extract compared with the control allergen.

The slopes of the fitted regression curves of peanut extract and control allergen-related data points were found to be similar (-2.43 [95% CI -0.40 to -4.47], $r = -0.78$, $p = 0.03$ and -2.59 [95% CI -0.63 to -4.56], $r = -0.80$, respectively; $p = 0.02$). Refitting of the logarithmic transformed data sets with the mean of the two slopes (i.e., -2.51) resulted in a difference in the y intercept of 1492 ($p < 0.0001$, t -test), indicating the difference in biologic activity of anti-CCD IgE and IgE directed to inhalant control allergen.

In the patients allergic to peanut (12 and 13), a similar comparison of reactivity to peanut extract and a control inhalant allergen could not be made because of extreme differences in specific IgE levels. In patient 14, however, reactivity to peanut extract and Lol p 5 was found to be similar (results not shown). In the three atopic control patients who did not have anti-peanut IgE, no histamine release was found after stimulation with concentrations of peanut extract up to $2000 \mu\text{g/ml}$.

DISCUSSION

In one third of grass pollen-sensitized patients in an outpatient population, IgE directed to peanut was detected without a positive peanut SPT response and with

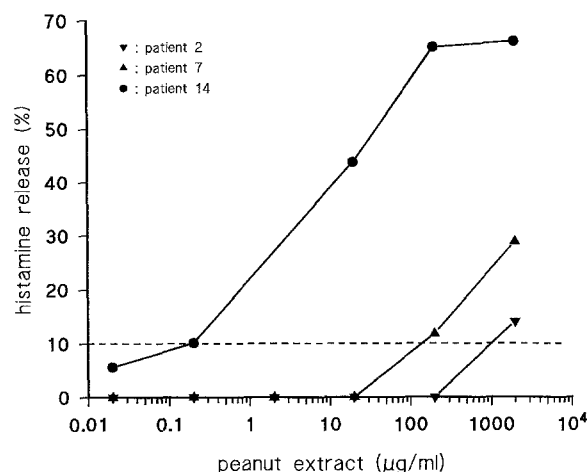


FIG. 3. Results of BHRA with peanut extract in two patients with discrepant peanut RAST and SPT results (patients 2 and 7) and one patient allergic to peanut (patient 14) with similar level of IgE directed to peanut extract (i.e., 2.0 IU IgE/ml).

no obvious symptoms of peanut allergy. These positive peanut RAST results were confirmed with a commercially available specific IgE assay. In a group of these patients with significant levels of anti-peanut IgE, IgE directed to CCD of glycoproteins was detected in 91% of cases. In contrast, anti-peanut IgE profilin was found in only 6%, indicating that profilin will be of minor importance in these cross-reactions. From these results and in accordance with earlier reports,^{1,2,4} we postulated poor biologic activity of anti-CCD IgE as an explanation for these discrepancies between findings *in vitro* and *in vivo*. This could also explain the high prevalence of positive food RAST results in patients allergic to grass pollen reported by Bircher et al.³¹ or the recently described high prevalence of anti-Hymenoptera venom IgE found in the general population.³² In this study the biologic activity of anti-CCD IgE was investigated with BHRAs and ICTs with peanut allergens.¹⁷

The presence of IgE directed to the carbohydrate moieties of glycoproteins was investigated in RAST and RAST inhibition experiments with proteinase K-digested grass pollen extract. After the proteinase K treatment, no intact protein was found in this preparation when analyzed by SDS-PAGE in conjunction with silver staining. Moreover, in contrast to the untreated grass pollen extract, loss of grass pollen allergenic activity was found in major allergen assays and grass pollen RAST inhibition experiments. Recently, Fischer et al.³³ reported that the major timothy grass pollen allergen Phl p 4 is trypsin-resistant, thereby suggesting that protease digestion might leave some important allergens unaffected. Our experiments, however, indicated complete digestion of the protein backbone of glycoproteins. This contradiction can be explained by the difference between the proteases used in both studies, with trypsin being a more specific proteolytic enzyme than proteinase K. The monosaccharide composition of IgE-binding carbohy-

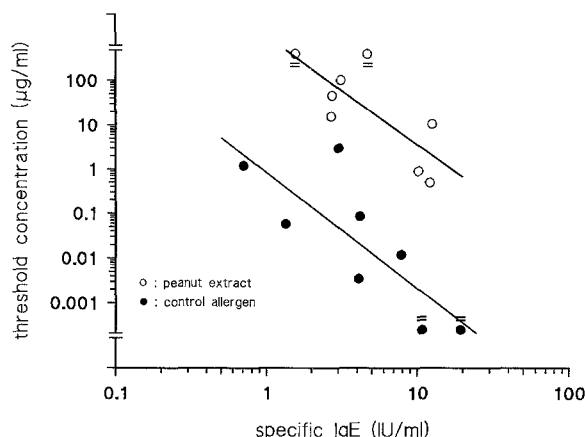


FIG. 4. The relation between specific IgE and threshold concentrations for histamine release in BHRA with peanut extract and control inhalant allergen in patients with discrepant peanut RAST and SPT results (peanut extract: $r = -0.78$, $p = 0.03$; control allergen: $r = -0.80$, $p = 0.02$; $n = 8$). Threshold values exceeding measurement limits are indicated by equals signs.

drate structures in grass pollen was previously determined for a representative allergenic glycoprotein, Lol p 11.¹⁹ This major grass pollen allergen was shown to contain all monosaccharides usually found in plant-derived complex glycans. Among these were xylose and fucose, which have been indicated as pivotal in IgE and IgG binding.^{8,34}

In this study levels of IgE directed to peanut were found to be higher in patients allergic to peanut than in patients allergic to grass pollen having discrepant peanut RAST and SPT results. This difference hampered the direct quantitative comparison of the two groups of patients in their reactivity to peanut allergens. In the histamine release assay of patients with discrepant peanut RAST and SPT results, the reactivity to peanut allergens was therefore intraindividually compared with the reactivity to control allergens. This was done by comparing the relation of corresponding threshold concentrations with specific IgE levels.²¹ For RAST inhibition, a correction for differences in anti-peanut IgE between the two groups was made by diluting sera, resulting in similar initial levels of anti-peanut IgE.

In the 11 patients with discrepant peanut RAST and SPT results who were studied, the positive RAST results were demonstrated to be the consequence of serum anti-CCD IgE. Almost complete inhibition of the peanut RAST was found after preincubation of the serum samples with a CCD source. In contrast, partial inhibition was found in only one of four patients allergic to peanut, indicating that CCD is not a prominent allergenic structure in these patients. Comparison of the inhibition curves of the peanut RAST with CCD and peanut extract in both groups of patients showed similar affinity of IgE antibodies directed to CCD as those directed to peanut allergens.

The peanut major allergens Ara h 1 and Ara h 2 are both glycoproteins, yet low binding of IgE to Ara h 1 and

Ara h 2 was found in sera with significant levels of IgE anti-CCD in only 1 of 11 cases for each major allergen. These results suggest that not all glycoproteins will induce IgE anti-CCD and might be limited to certain carbohydrate determinants. Further studies into the biochemical structure³⁵⁻³⁸ of the glycosyl portion of immunogenic glycoproteins that induce IgE must be performed to clarify this phenomenon. In contrast, high levels of IgE directed to Ara h 1 and Ara h 2 in combination with low or undetectable levels of IgE anti-CCD were found in patients allergic to peanut. These findings could be characteristic for patients allergic to peanut. The use of purified peanut major allergens might therefore improve the specificity of peanut allergy diagnostics in vitro. However, this will only be feasible when all major peanut allergens and their role in allergic diseases are characterized.

BHRAs and ICTs demonstrated poor biologic activity to peanut allergens in patients having positive peanut RAST results predominantly based on IgE anti-CCD. High threshold concentrations for a positive test result were found for peanut extract or the isolated major peanut allergens Ara h 1 and Ara h 2. When related to specific IgE levels, the reactivity to peanut extract in the BHRAs was found to be approximately a factor of 1000 less when compared with the reactivity to a control inhalant allergen. This difference could even be more pronounced because the allergen content of the allergen extracts was conservatively estimated at 10%.^{18, 20, 27-29} A blocking activity by antibodies of other isotypes as an explanation for the low biologic activity³⁹ has been ruled out because histamine release was performed with washed basophils in serum-free medium. In contrast, in the BHRAs of patients allergic to peanut, low threshold concentrations of peanut extract and the major allergens Ara h 1 and Ara h 2 were found.

In the BHRAs and ICTs of atopic patients without IgE anti-peanut, no reactivity was found after challenge with high concentrations of peanut extract. The low-grade allergic reactivity to high concentrations of peanut allergens found in patients having IgE anti-CCD can therefore be considered to be nontoxic, specific, and IgE-dependent. The reactivity found might be the result of glycoprotein aggregate formation in the concentrated peanut extracts. It is our hypothesis that the glycosylation site of glycoproteins will function as a hapten when inducing IgE anti-CCD. As a consequence, monovalent binding of IgE anti-CCD to glycan epitopes of isolated glycoproteins will be detected in the RAST but cannot result in cross-linking of surface IgE on basophils and mast cells. In contrast, glycoprotein aggregates will have multiple carbohydrate antigenic sites with the potential to cross-link surface IgE anti-CCD, resulting in mediator release.

In summary, in approximately one third of patients allergic to grass pollen, significant levels of IgE directed to peanut were found in the absence of positive peanut SPT responses or complaints of peanut allergy. In the majority of these patients, cross-reactive IgE directed to

the glycan moiety of glycoproteins was detected. In these patients, only poor allergic reactivity was found after challenge with high concentrations of peanut allergens in ICTs or BHRAs. Moreover, when related to specific IgE levels, the reactivity to peanut allergens was found to be low compared with control inhalant allergens.

From this study we conclude that cross-reactive IgE directed to the glycosyl portion of glycoproteins has poor biologic activity. This can explain the discrepant peanut RAST and SPT results in a significant proportion of patients allergic to grass pollen as described in this study. Identification of IgE anti-CCD will improve allergy diagnostics in vitro by discriminating positive RAST results without apparent clinical significance in patients sensitized to grass pollen.

We thank Jan A. Weber for performing peanut CAP-RAST experiments and Marcel Mulder for histamine measurements.

REFERENCES

1. Aalberse RC, Koshte V, Clemens JG. Cross-reactions between vegetable foods, pollen and bee venom due to IgE antibodies to a ubiquitous carbohydrate determinant. *Int Arch Allergy Appl Immunol* 1981;66:259-60.
2. Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that cross-react with vegetable foods, pollen and Hymenoptera venom. *J Allergy Clin Immunol* 1981;68:356-64.
3. Faye L, Chrispeels MJ. Common antigenic determinants in the glycoproteins of plants, molluscs and insects. *Glycoconj J* 1988;5: 245-56.
4. Van Ree R, Aalberse RC. Pollen-vegetable food crossreactivity: serological and clinical relevance of crossreactive IgE. *J Clin Immunol* 1993;16:124-30.
5. Batanero E, Villalba M, Monsalve RI, Rodriguez R. Cross-reactivity between the major allergen from olive pollen and unrelated glycoproteins: evidence of an epitope in the glycan moiety of the allergen. *J Allergy Clin Immunol* 1996;97:1264-71.
6. Petersen A, Vieths S, Aulepp H, Schlaak M, Becker W-M. Ubiquitous structures responsible for IgE cross-reactivity between tomato fruit and grass pollen allergens. *J Allergy Clin Immunol* 1996;98:805-15.
7. Weber A, Schroder H, Thalberg K, März L. Specific interaction of IgE antibodies with a carbohydrate epitope of honey bee venom phospholipase A2. *Allergy* 1987;42:464-70.
8. Tretter V, Altmann F, Kubelka V, März L, Becker WM. Fucose alpha 1,3-linked to the core region of glycoprotein N-glycans creates an important epitope for IgE from honeybee venom allergic individuals. *Int Arch Allergy Appl Immunol* 1993;102:259-66.
9. Koshte VL, Kagen SL, Aalberse RC. Cross-reactivity of IgE antibodies to caddis fly with arthropoda and mollusca. *J Allergy Clin Immunol* 1989;84:174-83.
10. Valenta R, Kraft D. Type I allergic reactions to plant-derived food: a consequence of primary sensitization to pollen allergens. *J Allergy Clin Immunol* 1996;97:893-5.
11. Loewenstein H, Eriksson NE. Hypersensitivity to food among birch pollen allergic patients. *Allergy* 1983;38:577-87.
12. Anderson LB, Dreyfuss EM, Logan J, Johnstone DE, Glaser J. Melon and banana sensitivity coincident with ragweed pollinosis. *J Allergy* 1970;45:310-9.
13. Enberg RN, Leickly FE, McCullough J, Bailey J, Ownby DR. Watermelon and ragweed share allergens. *J Allergy Clin Immunol* 1987;79:867-75.
14. Van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable food. *Int Arch Allergy Immunol* 1992;98:97-104.

15. Valenta R, Ferreira F, Grote M, Swoboda I, Vtrala S, Duchene M, et al. Identification of profilin as an actin binding protein in higher plants. *J Biol Chem* 1993;268:22777-81.
16. Van Ree R, Fernández-Rivas M, Cuevas M, van Wijngaarden M, Aalberse RC. Pollen-related allergy to peach and apple: an important role for profilin. *J Allergy Clin Immunol* 1995;95:726-34.
17. Van der Zee JS, de Groot H, van Swieten P, Jansen HM, Aalberse RC. Discrepancies between the skin test and IgE antibody assays: study of histamine release, complement activation in vitro and occurrence of allergen-specific IgG. *J Allergy Clin Immunol* 1988;82:270-81.
18. Van Ree R, Clemens JGJ, Aalbers M, Stapel SO, Aalberse RC. Characterization with monoclonal and polyclonal antibodies of a new major allergen from grass pollen in the group I molecular weight range. *J Allergy Clin Immunol* 1989;83:144-51.
19. Van Ree R, Hoffmann DR, Van Dijk W, Brodard V, Mahieu K, Koelman CA, et al. Lol p XI, a new grass pollen allergen is a member of a family of soybean trypsin inhibitor related proteins. *J Allergy Clin Immunol* 1995;95:970-8.
20. De Jong EC, Van Zijverden M, Spanhaak S, Koppelman SJ, Pellegrom H, Penninks AH. Identification and partial characterization of multiple allergens in peanut protein. *Clin Exp Allergy* (submitted).
21. Witteman A, Stapel SO, Perdok GJ, Sjamsoedin DH, Jansen HM, Aalberse RC, et al. The relationship between RAST and skin test results in patients with asthma and rhinitis: a quantitative study with purified major allergens. *J Allergy Clin Immunol* 1996;97:16-25.
22. Aalberse RC, Reerink-Brongers EE, Hoorweg E. Methods for the assay of allergen-neutralizing antibodies based on competition with IgE antibodies for allergen. In: Peeters H, editor. *Protides of the biological fluids*. 24th Colloquium. Oxford: Pergamon Press; 1976. p. 725-9.
23. Schuurman J, Perdok GJ, Lourens TE, Parren PWHI, Chapman MD, Aalberse RC. The production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen Der p 2 and its use for the absolute quantification of allergen specific IgE. *J Allergy Clin Immunol* 1997;99:545-50.
24. Dreborg S, Frew A, editor. Allergen standardisation and skin tests. EAACI position paper. *Allergy* 1993;48(suppl14):49-82.
25. Lichtenstein LM, Osler AG. Histamine release from human leucocytes by ragweed pollen antigen. *J Exp Med* 1964;120:507-30.
26. Siraganian RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975;7:283-90.
27. Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P 1. *J Immunol* 1980;125:587-92.
28. Ford AW, Rawle FC, Lind P, Spiekma FT, Lowenstein H, Platts-Mills TAE. Standardization of *Dermatophagoides pteronyssinus*: assessment of potency and allergen content in ten coded extracts. *Int Arch Allergy Immunol* 1985;76:58-67.
29. Cottam GP, Moran DM, Standring R. Physicochemical and immunochemical characterization of allergenic proteins from rye-grass (*Lolium perenne*) pollen prepared by a rapid and efficient purification method. *Biochem J* 1986;234:305-10.
30. Nguyen K-L, Gillis S, MacGlashan DW Jr. A comparative study of releasing and nonreleasing human basophils: non-releasing basophils lack of an early component of the signal transduction pathway that follows IgE cross-linking. *J Allergy Clin Immunol* 1990;85:1020-9.
31. Bircher AJ, van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. *Clin Exp Allergy* 1994;24:367-74.
32. Schäfer T, Przybilla B. IgE antibodies to Hymenoptera venoms in the serum are common in the general population and are related to indications of atopy. *Allergy* 1996;51:372-7.
33. Fischer S, Grote M, Fahlbusch B, Müller WD, Kraft D, Valenta R. Characterization of Phl p 4, a major timothy grass (*Phleum pratense*) pollen allergen. *J Allergy Clin Immunol* 1996;98:189-98.
34. Faye L, Gomord V, Fitchette-Lainé AC, Chrispeels MJ. Affinity purification of antibodies specific for Asn-linked glycans containing $\alpha 1 \rightarrow 3$ fucose or $\beta 1 \rightarrow 2$ xylose. *Anal Biochem* 1993;209:104-8.
35. Petersen A, Becker WM, Moll H, Blümke M, Schlaak M. Studies on the carbohydrate moieties of the timothy grass pollen allergen Phl p. 1. *Electrophoresis* 1995;16:869-75.
36. Hino K, Yamamoto S, Sano O, Taniguchi Y, Kohno K, Usui M, et al. Carbohydrate structures of the glycoprotein allergen Cry j 1 from Japanese cedar (*Cryptomeria japonica*) pollen. *J Biochem* 1995;117:289-95.
37. Lerouge P, Faye L. Recent developments in structural analysis of N-glycans from plant glycoproteins. *Plant Physiol Biochem* 1996;34:263-71.
38. Ohsuga H, Su S-N, Takahashi N, Yang S-Y, Nakagawa H, Shimada I, et al. The carbohydrate moiety of the Bermuda grass antigen BG60. *J Biol Chem* 1996;271:26653-8.
39. Witteman AM, Stapel SO, Sjamsoedin DHS, Jansen HM, Aalberse RC, Van der Zee JS. Fel d 1-specific IgG antibodies induced by natural exposure have blocking activity in skin tests. *Int Arch Allergy Immunol* 1996;109:369-75.