

Nonspecific binding of IgE to allergens

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Nonspecific IgE binding to allergens was observed in testing myeloma IgEs, namely, IgE-VL and IgE-PS, chimeric IgE (IgE-JW8), and the recombinant IgE Fc_ε peptide CH1-CH4, in two different immunoassays. This binding was concentration-dependent but detectable only at higher IgE concentration. In RAST inhibition, IgE–allergen interactions could be reduced by using either matching or nonmatching allergens. In order to test whether the nonspecific binding of IgE to allergens was due to carbohydrate interaction, myeloma IgEs and the chimeric IgE were desialized with neuraminidase. Desialized samples were equally well recognized by xenogenic antibodies as native IgEs, but binding of IgE to Fc_ε receptors on basophils was affected by the treatment, as shown in the histamine-release assay. Desialization of IgE affected also its binding capacity to allergens in RAST: binding of chimeric IgE was reduced, but nonspecific binding of myeloma IgE-VL was enhanced. Hence, nonspecific allergen–IgE binding may be partly due to a lectin-like interaction, but may depend mostly on the tertiary structure of IgE. Thus, nonspecific IgE–allergen interactions might present a problem 1) at high IgE concentration, and 2) depend on the grade of sialization of IgE, which might affect its conformation. This may explain why patients with elevated total IgE levels often have multiple weak positive RASTs with non-cross-reactive allergens.

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Patients with high total IgE levels frequently exhibit a tendency to develop cross-reactions to various allergens (1, 2). This cross-reactivity is not confined to related allergens; thus, it may be explained by recognition of similar epitopes (3). Cross-reactivities between botanically related birch pollen and hazelnut (4) and nonrelated birch pollen and apple (5) have been observed and proven by Western and Northern blotting techniques. However, these authors stressed that clinical symptoms often occurred simultaneously with an increase of total IgE levels; e.g., during the pollen season or after commencement of immunotherapy. The observed cross-reactivities were explained by IgE binding to homologous sequences in closely related plants (4, 6), to highly conserved panallergens (7), or to cross-reactive carbohydrate determinants (8, 9 [review]). The phenolic contents of allergen preparations were also suspected of causing cross-reactivities (2).

On the other hand, it is known that IgE is heavily glycosylated (10). Therefore, one cannot exclude the possibility that binding of IgE to cross-reactive

determinants is due to sugar residues. It is speculated that functional heterogeneity of the IgE molecule may exist (11), due to differential glycosylation (12, 13 [review]). Glycosylation seems to be important for certain effector functions of human IgE if influenced by an epsilon-binding protein and related lectins (12, 14, 15). In the presence of IgE, plant lectins also were shown to elicit histamine release from human basophils, probably due to a lectin-like interaction (16, 17).

However, the possible interaction of carbohydrate side-chains of IgE with allergens has not been demonstrated until now, even when it might have lectin-binding abilities (18). We obtained myeloma IgE from a patient "VL" reported to suffer from allergic symptoms (19). We tested the myeloma IgE-VL in RAST and found reaction of purified myeloma IgE to all allergens tested. Surprisingly, another myeloma IgE and a recombinant Fc_ε peptide, consisting of the C_H domains only and used as a negative control, were also positive in RAST. However, the observed binding to allergens occurred only at high IgE concentrations.

Material and methods

Patients and sera

Patients allergic to house-dust-mite or grass-pollen allergens were selected according to positive case history, positive skin prick test, and high specific IgE to house-dust mite (*Dermatophagoides pteronyssinus* [d1]) or timothy grass pollen (*Phleum pratense* [g6]). RAST class values were equal to 4 (allergen disks for mite d1/d2, and for grass pollen g3/g4; Pharmacia, Uppsala, Sweden). Equal parts of sera from mite-allergic patients and from grass-pollen-allergic patients were pooled. This serum pool had a total IgE content of 1084 IU/ml (evaluated by PRIST, Pharmacia).

A normal human serum (NHS) pool was obtained by pooling equal parts of sera from four nonatopic rhinitis patients negative in multi-RAST disks coupled with mixtures of tree pollen (tx3), grass pollen (gx3), weed pollen (wx3), house-dust mites (hx2), epithelia (ex1), molds (mx6), and total IgE levels between 50 and 180 IU in PRIST (Pharmacia).

IgE preparations

Myeloma IgE-PS (IgE from a myeloma patient, P.S.; a kind gift from Drs T. and K. Ishizaka, La Jolla, CA, USA) and *myeloma IgE-VL* (a myeloma IgE from a female patient with noted but undocumented episodes of allergic symptoms) (19), purified by gel filtration on HPLC, were used in dot immunoassays and RAST. *Chimeric IgE*, consisting of a mouse variable region (V_H) and human C_ϵ constant region, was purified from culture supernatants of the mouse hybridoma line JW8/5/13 by affinity chromatography, as previously described (20).

The DNA fragment coding for the *CH1-CH4* peptide was obtained from the pC_ϵ -spl. plasmid kindly provided by Dr Honjo (Osaka University Medical School, Japan), as previously described in detail (21). The pC_ϵ -spl. plasmid was cut with the restriction enzyme Sac I and a 1-kb DNA fragment, coding for the CH1-CH4 domains, including the last three adjacent amino acids of the CH2 domain, was isolated. The purified fragment was then ligated into the Sac I digested expression vector pTrc 99A (22), which carries a tac promoter-operator. It was kindly provided by Dr E. Amann (Behringwerke AG, Hamburg, Germany). The recombinant DNA was used for transformation of *Escherichia coli* XL-Blue strain (Stratagene, San Diego, CA, USA). Transformants were selected on Luria broth (LB) plates containing 100 µg ampicillin per ml and then screened for CH1-CH4 peptide production. It was screened by the procedure of Helfman et al. (23)

with polyclonal and monoclonal anti-IgE antibodies (data not shown).

Expression of the CH1-CH4 peptide by one selected positive transformant was achieved as follows. A 10-ml culture in LB containing 50 µg ampicillin per ml was grown for 2 h at 37°C. When an OD of 0.5 at 600 nm was reached, 1 mM isopropyl-β-D-thiogalactoside was added, and the cells were further incubated for another 2 h. Then, from 1 ml of cell suspension, a whole-cell extract was prepared in lysate buffer (62 mM Tris-HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 700 mM 2-β-mercaptoethanol), dialyzed against distilled H₂O, and lyophilized. IgE preparations were stored lyophilized at -20°C and diluted short before usage. Protein quantities in IgE preparations were determined by the method of Bradford (24). After the appearance of single protein bands in non-reducing SDS-PAGE or in IgE immunoblot (using Le27 for detection), indicating the presence of monomeric IgE, preparations were used for subsequent immunoassays.

Deglycosylation

Neuraminidase was obtained from Boehringer Mannheim Biochemica. A volume of 50 µg of IgEs (IgE-PS, IgE-VL, IgE-JW8) and ovalbumin in 50 mmol/l sodium acetate, pH 5.5, was incubated with 1 mU neuraminidase at 37°C for 1, 6, and 20 h. Then, the reaction was stopped with 10 mM EDTA. For subsequent experiments, samples treated for 20 h with neuraminidase were used.

Immunodot assay

Detection of IgE by xenogenic antibodies. Native and desialized IgE antibodies were diluted 1 mg/ml in Tris-buffered saline (TBS; 2 M Tris, 1 M HCl, 2 M NaCl, pH 7.4) and were dotted in serial dilutions (1 µl dots) in duplicate onto nitrocellulose (Millipore Corp., Bedford, MA, USA): myeloma IgE-PS; myeloma IgE-VL; chimeric IgE-JW8, native and desialized; and recombinant IgE peptide CH1-CH4. Dots were air-dried. For IgE detection, strips were blocked with TBS containing 0.15% casein (Fluka Chemie AG, Buchs, Switzerland). Then, detection antibodies were applied for 2 h at room temperature: peroxidase-labeled monoclonal anti-IgE antibody Le27 (25) (1 : 1000 in TBS/casein) and peroxidase-labeled polyclonal anti-IgE antibody (Tago, Burlingame, CA, USA) 1 : 2000 in TBS/casein. Strips were then washed with TBS and developed for approximately 30 min in 16.6% (v/v) developing solution (stock: 4-chloro-1-naphthol 3 mg/ml methanol), 0.042% (v/v) hydrogen peroxide in TBS. The reaction was stopped by

washing with H₂O. Enzymatic reactions were analyzed with a hand-held densitometer (Gretag Ltd, Regensburg, Switzerland).

Detection of IgE binding to dotted allergens. Allergens (grass pollen mix, ryegrass pollen, mugwort pollen, plantain pollen, house-dust mite, storage mite, cat dander, dog dander, and house dust including house-dust mite) were dotted in triplicates at a concentration of 1 µg per dot, dried, and saturated as above. IgEs were diluted to 2400 ng per ml, and, together with buffer control, applied overnight at 4°C, and washed as above. Bound IgE was detected by peroxidase-labeled anti-IgE antibody Le27 (1 : 1000 for 2 h), and the reaction developed as above. Reactivities were compared to dotted standard IgE (IgE-PS) 50 ng per dot.

RAST

RAST allergen disks, standard disks, and standard IgE were purchased from Pharmacia. RAST experiments were performed according to the manufacturer's instructions, with slight modifications. In brief, IgE samples were diluted (1000 IU/ml) in Dulbecco's PBS, or PBS containing 50% heat inactivated fetal calf serum (FCS), or PBS containing 50% normal human serum (NHS) (see above). Samples of 100 µl were then applied to multi-RAST allergen disks (Pharmacia, Uppsala, Sweden), or to disks coupled with extracts from house-dust mite (*D. pteronyssinus*, d1) and from timothy grass pollen (*P. pratense*, g6), for 3 h at room temperature. Disks were then washed in

0.9% NaCl solution/0.05% Tween 20. Then polyclonal ¹²⁵I-labeled rabbit anti-IgE antibody (50 µl, approximately 60 000 cpm/disk) was applied overnight at RT. RAST disks were then washed with 0.9% NaCl solution/0.05% Tween 20 (30 min, three changes), and bound activity was determined in a gamma-counter (Gammamatic, Kontron Instruments AG, Zurich, Switzerland). Values were compared to the RAST standard curve (Pharmacia), obtained by testing the supplied standard disks with 50 µl undiluted standard serum (Pharmacia).

RAST inhibition

A serum pool (50 µl) from allergic patients (see above) and 100 µl of the IgE preparations (1000 IU/ml) were incubated with 0, 5, 50, or 500 µg house-dust mite (*D. pteronyssinus*) (d1) or timothy grass pollen (*P. pratense*) (g6) extract overnight at 4°C. RAST disks coupled with house-dust mite (*D. pteronyssinus*) (d1) or timothy grass pollen (*P. pratense*) (g6) were added, and then the RAST experiment was continued as described above.

Histamine-release assay using PBL passively sensitized to IgE

The blood samples were collected from a volunteer known to be a good releaser in histamine-release tests. Mononuclear cells (PBL) were isolated from 20 ml blood by Ficoll-Hypaque density sedimentation. PBL were diluted in RPMI 1640, containing 0.25% bovine serum albumin. One part of the cells

Table 1. Binding of myeloma IgEs and recombinant IgE peptide (CH1-CH4) to various allergens in immunodot

Binding of various IgEs to allergens (%)*					
Outdoor allergens ^a	Grass pollen mix	Ryegrass pollen	Birch pollen	Mugwort pollen	Plantain pollen
IgE-PS ^b	13.1	8.7	10.9	30.6	24.0 ^c
IgE-VL	100.0	10.7	12.8	38.3	29.7
IgE (CH1-CH4)	6.5	6.5	4.4	37.1	15.3
Buffer	0	0	0	0	0
Indoor allergens ^a	House-dust mite	Storage mite	Cat dander	Dog dander	House dust and house-dust mite
IgE-PS ^b	23.1	28.9	23.0	17.3	34.6
IgE-VL	27.9	36.2	30.6	27.9	44.6
IgE (CH1-CH4)	15.0	15.0	12.0	15.0	20.9
Buffer	0	0	0	0	0

^a In immunodot assay, allergen extracts were dotted at concentration of 1 µg per dot, and standard IgE-PS at 0.5 ng/dot (see c).
^b 1000 IU of myeloma IgEs PS and VL and recombinant IgE peptide (CH1-CH4) in PBS 50% FCS were applied to dotted strips. Alternately, PBS 50% FCS as control was used. Dots were developed with peroxidase-labeled monoclonal anti-IgE antibody (Le27).
^c Signal obtained by revealing antibody Le27 against standard IgE was set equal to 100%. IgE that adsorbed to dotted allergen was detected by same revealing antibody and expressed as percent reactivity to dotted IgE directly recognized.

Table 2. Myeloma IgE, chimeric IgE, and recombinant IgE in RAST

	IgE-PS	IgE-VL	IgE-JW8	IgE (CH1-CH4) ^a
Tree pollen (tx9)	2 ^b	2	2	2
Grass pollen (gx3)	2	1	2	1
Weed pollen (wx3)	2	2	3	1
House dust (hx2)	2	2	2	1
Molds (mx1)	2	1	2	1
Epithelia (ex1)	2	2	3	1
Bee venom	2	2	2	2
Penicillin G	0	0	0	0

^a Purified myeloma IgEs PS and VL, chimeric IgE JW8, and recombinant IgE peptide (CH1-CH4) were diluted in PBS; 2400 ng (1000 IU) per RAST disk was used.

^b "Specific IgE" binding was detected with multi-RAST disks and bee venom disks from Pharmacia (Uppsala, Sweden). Values indicate detected RAST classes.

was used directly in the histamine-release assay. An aliquot of cells was first treated with low pH and then passively sensitized with purified myeloma

IgE (IgE-PS, IgE-VL), chimeric IgE (IgE-JW8), or recombinant IgE peptide CH1-CH4. In brief, 3×10^6 PBL per ml were incubated with 5% (v/v) 0.5 N HCl, at a final pH of 3.5 for 10 min at room temperature. pH was then neutralized (pH 7.5) with 3.5% (v/v) 0.5% NaOH. Stripped cells were immediately washed in 10 ml RPMI containing bovine serum albumin and then incubated with IgE preparations, 1 μ g/ml RPMI, for 30 min at room temperature. Cells were then washed 3×10 min in RPMI, and after the last washing they were resuspended in histamine-release buffer (see below).

Histamine release was assayed with a histamine radioimmunoassay (RIA) kit (Immunotech SA, Marseille, France) according to the manufacturer's instructions. In short, untreated and stripped/passively sensitized mononuclear cells were used at cell concentrations of 0.5×10^6 /ml in histamine-release buffer (supplied in the histamine RIA kit).

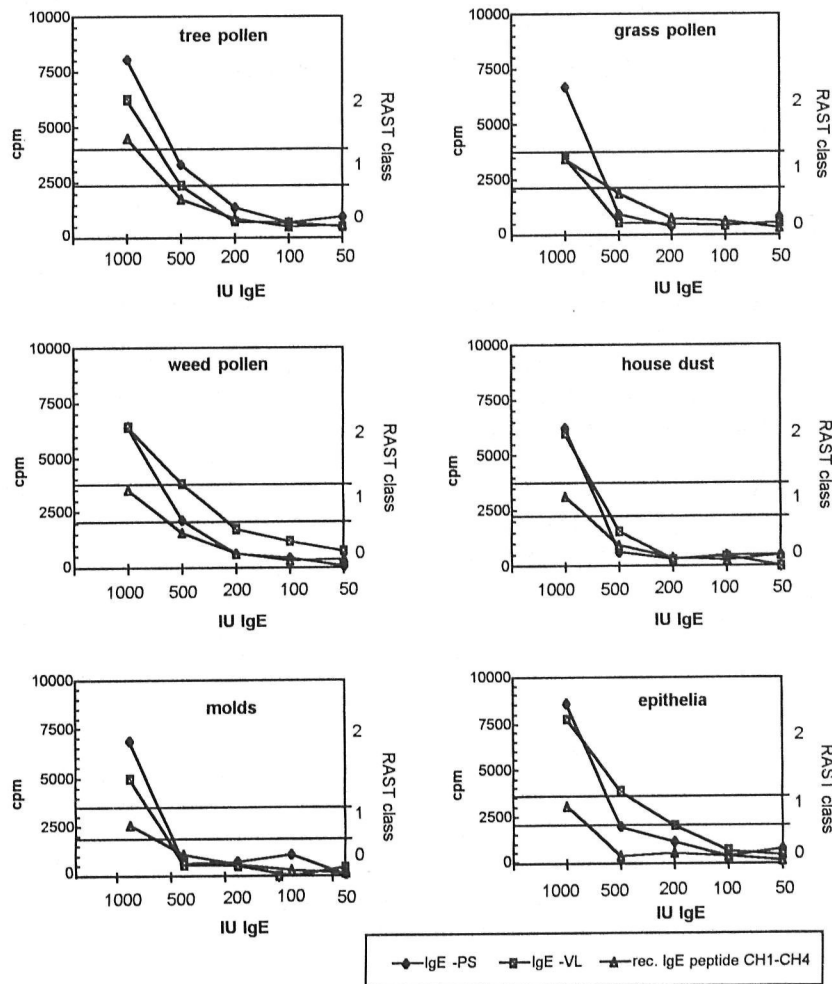


Fig. 1. Evaluation of nonspecific IgE binding. Commercial multi-RAST disks coupled with tree, grass, weed pollen allergens, house dust, molds, and epithelia extracts, and penicillin G were used. Myeloma IgEs (IgE-PS and IgE-VL) and recombinant IgE peptide (CH1-CH4) were diluted in PBS, and serial dilutions from 2400 ng/ml (corresponding to 1000 IU) to 120 ng/ml (corresponding to 50 IU/ml) were applied. IgE was detected with supplied ^{125} I-labeled rabbit antihuman IgE antibody. Reactivity was compared to supplied standard serum, and RAST classes were calculated.

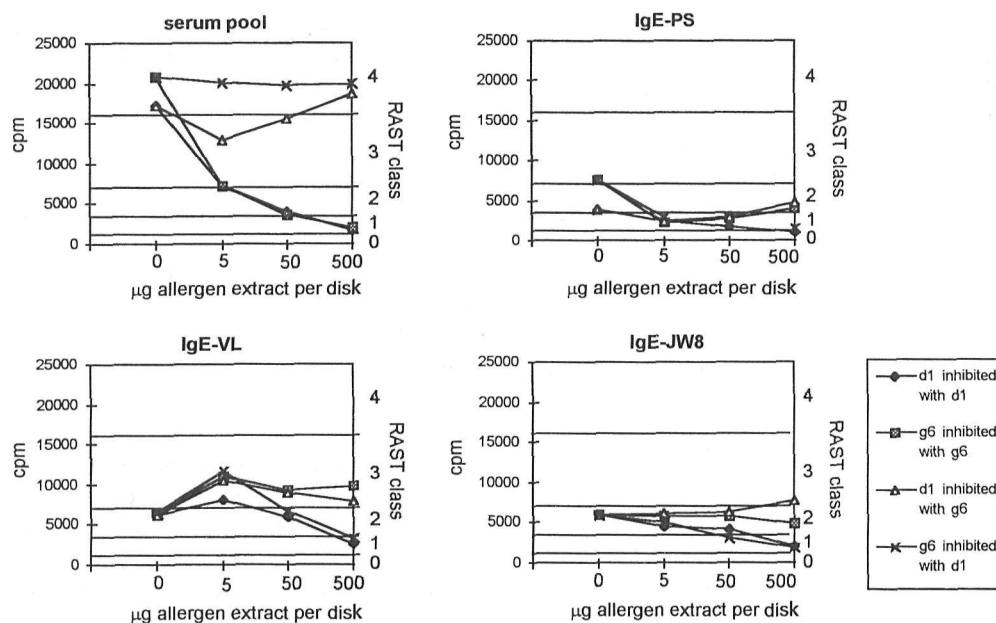


Fig. 2. Inhibition of IgE binding to *D. pteronyssinus* (d1) and *P. pratense* pollen (g6) on RAST disks. Myeloma IgEs (IgE-PS and IgE-VL) and chimeric IgE-JW8 were tested at 2400 ng (1000 IU) per ml PBS, and reactivity was compared to serum pool from allergic patients. Samples were tested either uninhibited or inhibited by 5–500 µg d1 and g6 extract per disk. Bound IgE was detected with supplied radiolabeled anti-IgE antibody from RAST kit (Pharmacia), and detected cpm levels were expressed as RAST classes 1–4.

A volume of 100 µl of cell suspensions per well was pipetted into microtiter plates and incubated in duplicate with 50 µl of monoclonal anti-IgE Le27 (0.1 µg/ml), with H₂O (total histamine content), or with histamine release buffer only (spontaneous histamine release), for 30 min at 37°C. Plates were then centrifuged at 4°C (300 g) and transferred on ice. Supernatants were collected and azylyzed. A volume of 50 ml of azylyzed samples and 50 ml of azylyzed histamine standards (0.5–150 mmol/l) were transferred into tubes coated with monoclonal antihistamine antibodies. Then, 500 µl azylyzed ¹²⁵I-labeled histamine was added to each tube and the mixture incubated for 18 h at 4°C. Bound ¹²⁵I-labeled histamine was counted in a gamma-counter.

Results

Myeloma IgE, chimeric IgE, and recombinant IgE react with allergens in immunodot

IgE antibody was isolated from the serum of a patient (V.L.) suffering from IgE myeloma and allergic symptoms. An immunodot assay was performed to test whether the myeloma IgE-VL binds specifically to allergens. Series of indoor and outdoor allergens were dotted on nitrocellulose strips which were incubated with the myeloma IgE-VL and, as a control, with another myeloma IgE-PS and with a recombinant Fc_ε peptide CH1-CH4. The

IgE preparations were used at concentrations of 1000 IU/ml. As shown in Table 1, myeloma IgE-VL binds to all allergens tested, and the reactivity was especially strong to grass and mugwort pollen extracts, as well as mite allergens. IgE-PS and the recombinant Fc_ε peptide CH1-CH4 also bound to the allergens, indicating nonspecific binding of IgE to allergens. The fact that even the recombinant Fc_ε peptide lacking the variable Ig domains bound to allergens suggests that allergens can bind non-specifically to the constant domains.

Myeloma and chimeric and recombinant IgE CH1-CH4 in RAST

To confirm IgE binding to allergens, we used the myeloma IgEs, the chimeric IgE-JW8, and the recombinant IgE peptide CH1-CH4 in a commercial RAST with indoor and outdoor allergens (Table 2). The results obtained were similar to those obtained in the above immunodot assay. Both IgE myelomas and the chimeric IgE (IgE-JW8) bound to all indoor and outdoor allergens in the range of RAST classes 1–3. The recombinant IgE peptide showed allergen-binding activity of RAST classes 1–2.

To improve quantification of the nonspecific IgE/allergen interactions, we used several dilutions of IgEs in the RAST assay. Fig. 1 shows that below a concentration of 500 IU the binding of IgEs to the different allergens was usually nonsignificant.

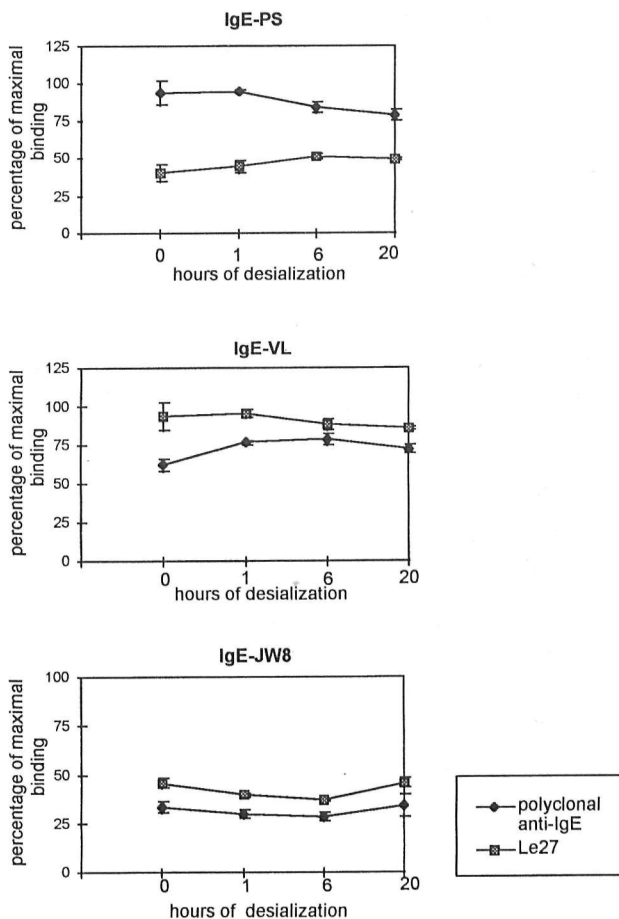


Fig. 3. Effects of neuraminidase treatment of myeloma and chimeric IgEs on binding of anti-IgE antibodies. Amount of 1 mg of each IgE preparation, native and desialized, was dotted onto nitrocellulose strips and incubated with peroxidase-labeled monoclonal anti-IgE antibody Le27 (1 : 1000), and peroxidase-labeled polyclonal anti-IgE antibody (1 : 2000). y-axis indicates percentage of binding of respective antibodies.

Inhibition of IgE binding in RAST

For further investigation of the nonspecific binding of the IgEs to the allergens, RAST inhibition was performed with myeloma IgEs (IgE-PS, IgE-VL), chimeric IgE (IgE-JW8), and, as control, a serum pool from allergic patients. RAST disks coupled with extract from *D. pteronyssinus* (d1) or with extract from *P. pratense* (g6) were incubated with the preformed complexes of the different preparations of IgEs with extract from either house-dust mite (d1) or timothy-grass pollen (g6) at different concentrations. Fig. 2 shows that the binding of serum pool IgE to d1 disks was inhibited by increasing concentrations of d1 extract, and the binding to g6 disks by g6 extract, as expected. No inhibition was observed when the nonmatching allergens were used.

Preincubation of myeloma IgEs and chimeric IgE-JW8 with d1 extracts (but not with g6) reduced

the nonspecific IgE-binding to d1 disks in the presence of high allergen concentration, mimicking specific inhibition. With g6 disks, inhibition was achieved with the nonmatching, but not with the matching allergen, showing nonspecificity of the IgE/allergen interaction.

Deglycosylation of IgEs

The recombinant epsilon domains are not glycosylated and were less potent in nonspecific binding. For investigation of whether nonspecific IgE-allergen interactions may be due to glycosylation, myeloma and chimeric IgEs were desialized with neuraminidase. Deglycosylation was controlled by SDS-PAGE (data not shown). In an immunodot assay, a monoclonal antibody (Le27) and a polyclonal anti-IgE antibody were used to test the integrity of the desialized IgE preparations. Fig. 3 shows that the deglycosylated IgE preparations could still be recognized by the anti-IgE antibodies. To determine whether the desialized IgEs can also bind to basophils, we tested IgE-PS, IgE-VL, IgE-JW8, and recombinant Fc_ε peptide CH1-CH4 in a histamine-release assay (Table 3). PBL were isolated, stripped for residual IgE, and sensitized to native or desialized IgEs. The anaphylactogenic monoclonal antibody Le27 (26) released histamine equally well from PBL sensitized to native or desialized IgE-PS. Desialized IgE-VL bound significantly less to basophils than the native, and the opposite effect was observed with chimeric IgE: more histamine was released with the desialized than the normal IgE-JW8, reflecting better binding to Fc_ε receptors of the desialized IgE-JW8. The monoclonal anti-IgE antibody BSW17, a nonanaphylactogenic antibody (26), did not trigger histamine release from basophils (data not shown).

Table 3. Histamine release from PBL sensitized to desialized IgE

Histamine release induced by anti-IgE Le27		
	nmol ^a	% ^b
Control PBL	7.4	100
Stripped PBL	1.8	24.5
+ IgE-PS	3.6	48.8
+ Deglycosylated IgE-PS	3.1	41.7
+ IgE-VL	4.2	57.1
+ Deglycosylated IgE-VL	0.7	9.4
+ IgE-JW8	0.6	8.2
+ Deglycosylated IgE-JW8	5.2	69.7
+ IgE (CH1-CH4)	2.0	27.3

^a Values indicate net histamine release obtained from 5×10^6 PBL per ml.

^b Release obtained by triggering with Le27 was set equal to 100%.

These experiments showed that PS-IgE retained its biologic activity after desialization, in agreement with studies showing that glycosylation is not necessary for Fc ϵ RI binding (27). However, IgE-VL lost its binding ability, and IgE-JW8 gained activity.

Desialized IgE in RAST

IgE-PS, IgE-VL, and IgE-JW8 were tested again by RAST to compare the reactivity against allergens after neuraminidase treatment (Fig. 4). Binding of IgE-PS to all allergens was unaffected by desialization. In contrast, desialized IgE-JW8 reacted weaker with all allergens than the native preparation. The opposite result was obtained for IgE-VL where the reactivity was stronger with desialized IgE than with native nondesialized IgE. As for the sensitization of basophils, all three IgEs behaved differently after deglycosylation.

IgE binding in serum

To simulate serum conditions, we diluted IgE samples in NHS or FCS, and a new RAST assay was performed (Fig. 5). Binding of the native IgE preparations IgE-VL, IgE-JW8, and CH1-CH4 showed reactivity patterns similar to those obtained when diluting in PBS. Only IgE-PS reactivity with allergens decreased when diluting in serum.

These results indicate that nonspecific binding of IgE to allergens may also occur under "physiologic" conditions.

Discussion

There is evidence that clinical cross-reactivities between allergens are based on different immunologic mechanisms (3–9 [review]). However, patients with elevated IgE levels often have multiple weakly positive RASTs against non-cross-reactive allergens, pointing to a possible nonspecific IgE–allergen interaction. Our data show that two myeloma IgE proteins of unknown specificity, a chimeric IgE with a known specificity (NIP) and a recombinant IgE peptide composed of the four heavy chain domains, interact nonspecifically with a large variety of allergens. Of course, our study cannot clarify the actual extent of allergen-binding via the variable immunoglobulin chain, or nonspecifically via the constant domain of the IgE molecules. It may also be difficult to assess allergen binding precisely, because some of the myeloma IgEs seem to undergo a conformational change after deglycosylation and bind even better to allergens. Similarly, we have recently shown that the binding of antigen to chimeric IgE changes its conformation (28). However, nonspecific IgE binding to allergens in RAST occurred at higher

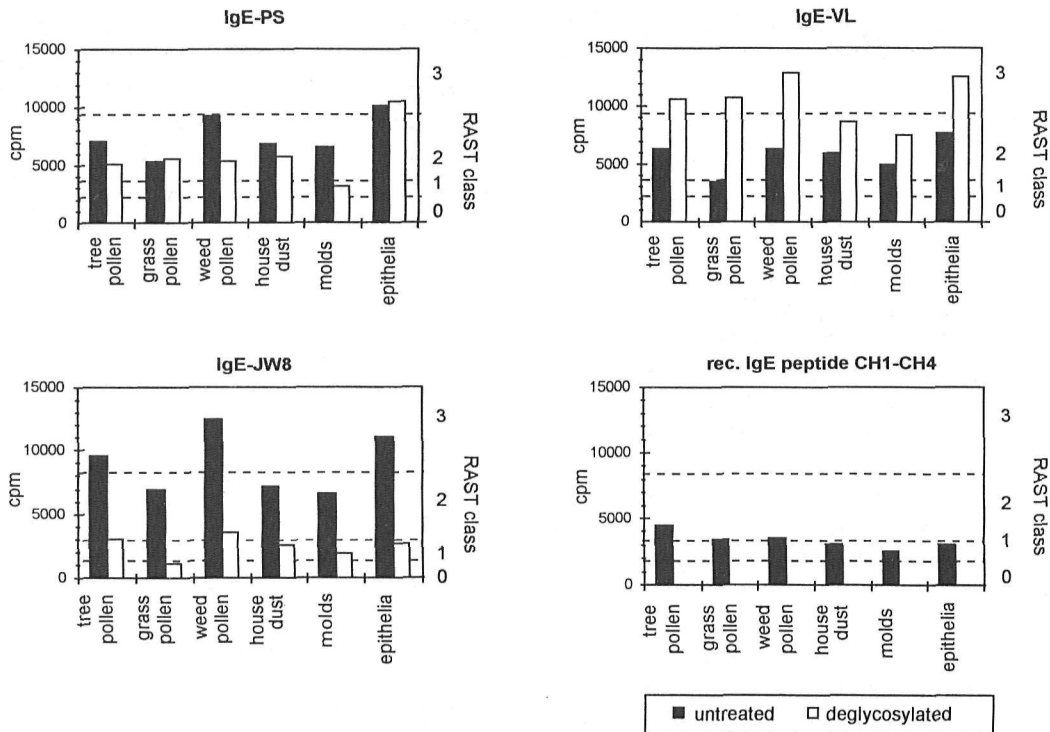


Fig. 4. Desialized and native IgE in RAST: 2400 ng/ml of native myeloma IgE-PS and IgE-VL, chimeric IgE-JW8, and untreated recombinant IgE peptide CH1-CH4 (black columns) were tested for binding to six different allergen families on multi-RAST disks (Pharmacia). Their reactivity was compared to desialized IgEs (white columns). Values indicate cpm.

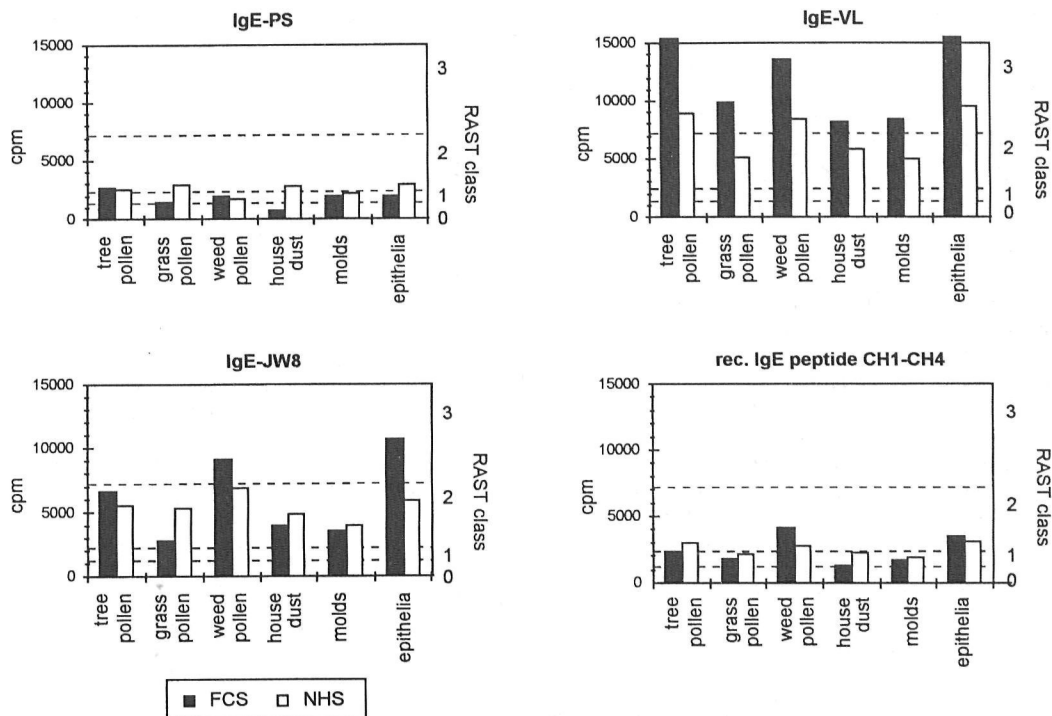


Fig. 5. Binding activity of IgEs diluted in fetal calf serum (FCS) (black columns) or normal human serum (NHS) (white columns) in RAST. Native myeloma IgE-PS and IgE-VL, chimeric IgE-JW8, and recombinant IgE peptide CH1-CH4 diluted in FCS or NHS were tested with allergen extracts coupled to multi-RAST disks.

total IgE values and even under “physiologic serum” conditions. Thus, our data indicate that in patient sera with elevated IgE levels, low RAST classes should be recorded with caution. Our data do not imply that this nonspecific binding to IgE has a clinical significance.

We have also shown here that the usual specificity testing by RAST inhibition may be an additional source of pitfalls as long as it cannot be decided whether and how much of the binding activity was mediated by the variable Ig domain. Of course, our data do not contradict findings of specific RAST inhibition (RAST class II or greater) if total IgE levels are below 100 units.

To investigate whether the observed nonspecific binding to allergens may be additionally influenced by the carbohydrate moieties on IgE, we deglycosylated IgE, with neuraminidase. Previous work has shown that the presence of N-linked sugars is not necessary for $Fc\epsilon RI$ binding (27). Thus, the recombinant IgE peptide CH1-CH4 was shown to bind to basophils, and could also be effectively triggered by a monoclonal anti-IgE antibody (Le27). However, in the present study, we demonstrated by histamine-release experiments that glycosylation may play a role in $Fc\epsilon$ receptor binding of IgE. Dramatic changes of histamine release were observed when native or desialized IgEs were used for sensitization of basophils. Desialization affected

also IgE binding to allergens. However, both effects were most likely due to conformational changes of IgE after desialization, indicating that glycosylation may play a crucial role in the conformation of IgE (15). Therefore, interactions between IgE and the cross-linking agent depend on the molecular structure of the cross-linking agent as well as that of IgE (16, 17).

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