

Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy

Wolfgang Hemmer, PhD,^a Margarete Focke, PhD,^a Daniel Kolarich, PhD,^b
Iain B. H. Wilson, PhD,^b Friedrich Altmann, PhD,^b Stefan Wöhrle, MD,^a
Manfred Götz, MD,^a and Reinhart Jarisch, MD^a Vienna, Austria

Background: Up to 50% of patients with stinging-insect allergy have double-positive RAST results to honeybee and yellow jacket (YJ) venom. True double sensitization and cross-reactivity through venom hyaluronidases are considered main reasons for this multiple reactivity.

Objective: We investigated the role of antibodies against cross-reactive carbohydrate determinants in venom double positivity. **Methods:** CAP inhibition experiments were performed with crude oilseed rape (OSR) and timothy grass pollen extracts and a neoglycoprotein construct displaying a MUXF glycan, as present in pineapple-stem bromelain (MUXF-BSA). CAP to OSR was used as a rough measure for carbohydrate-specific IgE in individual sera.

Results: CAP results to OSR pollen were positive in 2 of 14 single-positive honeybee venom sera, 2 of 16 single-positive YJ venom sera, and 33 (80.5%) of 41 double-positive sera ($P < .00001$, χ^2 test). CAP inhibition was performed in 16 selected patients with a CAP class of 3 or higher to both venoms. In 9 of 11 patients with a highly positive CAP result to OSR (CAP score to OSR > CAP score to second venom), pollen extracts, MUXF-BSA, or both were able to completely inhibit IgE binding to one of the venoms, whereas this was not the case in 5 patients with a negative or weakly positive CAP result to OSR (CAP score to OSR < CAP score to second venom).

Conclusions: The data suggest that carbohydrate-specific IgE is a major cause for the double positivity to honeybee and YJ venom seen in patients with Hymenoptera allergy. Because these antibodies may have low clinical relevance, they may severely impede the correct diagnosis of Hymenoptera venom allergy. (J Allergy Clin Immunol 2001;108:1045-52.)

Key words: α 1-3-Fucose, carbohydrate epitopes, cross-reactive carbohydrate determinant, cross-reactive carbohydrate determinants, honeybee venom allergy, Hymenoptera venom allergy, N-linked glycans, oilseed rape, venom double positivity, yellow jacket venom allergy

Abbreviations used

CCD: Cross-reactive carbohydrate determinant
OSR: Oilseed rape
YJ: Yellow jacket

Skin testing and assessment of venom-specific IgE by means of RAST is a well-established measure to verify IgE-mediated sensitization in subjects with a history of Hymenoptera venom allergy and to distinguish between honeybee and vespid venom allergy. Although most patients report adverse events from only one insect venom, many react with both honeybee and yellow jacket (YJ) venom on routine allergy testing. Such double positivity is particularly evident with in vitro methods and may affect 30% to 50% of all patients with a positive history.¹⁻³ The immunologic basis for the multiple reactivity is understood insufficiently. True double sensitization may account for some of the results; however, taking into account the low prevalence of Hymenoptera allergy, sensitization to both insect venoms in one individual should be rare. Another explanation is cross-reactivity caused by sequence homologies between the venom hyaluronidases.^{4,5} Finally, many double-positive in vitro results may simply be artifacts caused by an oversensitivity of the CAP system compared with that of the conventional RAST.^{2,3,6}

In the present study we address another possible factor in double positivity: cross-reactivity through carbohydrate epitopes. Oligosaccharide side chains N-linked to asparagine residues of plant and animal glycoproteins, referred to as cross-reactive carbohydrate determinants (CCDs), are gaining attention as highly cross-reactive IgE-binding structures. Surprisingly, although several observations have favored the idea that CCDs are implicated in venom double positivity, their role has not yet been specifically addressed.

It was reported that polyreactive human sera binding through CCDs with a broad range of pollen and food extracts also bind with honeybee venom.⁷⁻¹¹ In fact, most honeybee allergens are known to be glycoproteins bearing N-glycosidically linked oligosaccharides,¹² and IgE

From ^athe FAZ-Floridsdorf Allergy Centre, Vienna; and ^bthe Institute of Chemistry, Universität für Bodenkultur, Vienna.

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Reprint requests: Wolfgang Hemmer, PhD, FAZ-Floridsdorf Allergy Centre, Franz-Jonas-Platz 8/6, A-1210 Vienna, Austria.

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antibodies specifically binding with the glycan moiety of these allergens have been described in more than 20% of patients allergic to honeybee venom.^{13,14} The glycosylation of vespid venoms has not been studied in great detail, but at least some allergens are glycoproteins.¹⁵⁻¹⁷ Recently, the glycans of honeybee phospholipase A and hyaluronidase have been thoroughly analyzed and found to be α 1-3 fucosylated at the innermost core N-acetylglucosamine residue.^{18,19} By means of such α 1-3 fucosylation, which is currently believed to be a key structure of allergenic carbohydrate epitopes and which also occurs in many food and pollen glycoproteins,²⁰ honeybee and vespid venoms may cross-react with nonrelated plant glycoproteins and also with each other.

The present study focuses on the occurrence of carbohydrate-specific IgE antibodies in patients allergic to venom with double-positive RAST results to honeybee and YJ venom. Using pollen extracts rich in α 1-3-fucosylated glycoproteins and neoglycoprotein constructs with defined glycans, we were able to demonstrate that in a remarkably high percentage of these patients, IgE binding to one of the venoms is entirely caused by carbohydrate-specific antibodies.

METHODS

Patients

Between January 1998 and April 1999, 361 consecutive patients with suspected Hymenoptera allergy were routinely investigated for specific IgE against honeybee and YJ venom. Specific IgE was assessed in a random order either by means of RAST ($n = 157$, Kallestad) or by means of CAP ($n = 204$, Pharmacia) testing. Venom skin testing was done by means of skin prick testing (1, 10, or 100 μ g/mL), followed by intradermal testing with 0.1 μ g/mL.

CAP to oilseed rape (*Brassica napus*) pollen

CAP to oilseed rape (OSR; canola, *Brassica napus*) pollen was used as an indicator for carbohydrate-specific IgE antibodies in individual sera. We have previously shown²¹ that the major allergens in OSR pollen are high-molecular-weight glycoproteins between 30 and 70 kD binding serum IgE from atopic subjects through their carbohydrate moieties. Sera reacting with these glycoproteins usually also bind with extracts from many other pollens, rubber latex, and a variety of foods (unpublished observations).

CAP to OSR pollen was performed in 41 randomly selected patients with double-positive test results and in 14 and 16 patients with single-positive CAP results to honeybee or YJ venom, respectively, to explore the prevalence of carbohydrate-specific IgE in patients with venom allergy.

Characterization of N-linked oligosaccharides in OSR pollen

Asparagine-linked glycans from glycoproteins of the OSR pollen extract were purified and analyzed, as recently described.²² Briefly, the extract was mixed with formic acid to give a final concentration of 5% (vol/vol) and digested with pepsin. After enrichment of glycopeptides by means of cation exchange chromatography and gel filtration, N-glycans were released with N-glycosidase A (Roche) and purified by means of cation exchange, gel filtration, and passage through a reversed phase matrix. Finally, the glycans were analyzed by means of MALDI TOF mass spectrometry on a DYNAMO (ThermoBioAnalysis) by using 2,5-dihydroxybenzoic

acid as the matrix. Structures of N-glycans were deduced from molecular masses on the basis of previous studies on plant pollen N-glycan structures.^{22,23}

Hymenoptera venoms, pollen extracts, and neoglycoproteins

Lyophilized pure honeybee and YJ venom-sac extracts (mixture of *Vespula vulgaris*, *V. maculifrons*, *V. squamosa*, *V. germanica*, *V. flavopilosa*, and *V. pensylvanica*) were provided by Vespa Laboratories. Pollen from OSR and timothy grass (*Phleum pratense*) was purchased from Kabi-Pharmacia-Allergon and extracted in PBS, as described earlier.²¹

In RAST inhibition studies 2 neoglycoproteins were used. One conjugate, MUXF-BSA, contained a core α 1,3-fucosylated and xylosylated N-glycan (Man α 6Man β 4(Xyl β 2)GlcNAc β 4(Fuc α 3)GlcNAc β -Asn) from pineapple-stem bromelain, whereas the other, MM-BSA, is a nonfucosylated and nonxylosylated control consisting of the common core pentasaccharide (Man α 6(Man α 3)Man β 4GlcNAc β 4GlcNAc β -Asn; Fig 1). Preparation of these neoglycoproteins is described in detail elsewhere.²⁰ In brief, commercially available bromelain (Sigma) was digested with pepsin in 5% formic acid to obtain small MUXF glycopeptides. Fibrin (Sigma) was digested in the same way, and galactose and N-acetylglucosamine were removed enzymatically to generate MM glycopeptides. Subsequently, the glycopeptides were cross-linked to BSA. According to amino sugar analysis, the resulting neoglycoproteins had about 3 to 4 mol of glycopeptide per mole of protein.

CAP inhibition

Inhibition of honeybee and YJ venom CAP results was carried out in 16 selected patients with double-positive test results and CAP results of at least class 3 (≥ 3.5 kU/L) to both venoms. A demographic characterization is included in Table I. None of these patients had a double-positive history. The patients were subdivided into 2 groups according to their reactivity with OSR pollen. Group A included 5 patients (patients 1-5) with a low or negative OSR pollen CAP result (CAP score to OSR pollen < CAP score to second venom), suggesting low titers of carbohydrate-specific IgE in these sera. Group B included 11 patients (patients 6-16) with a high CAP score to OSR pollen (CAP score to OSR pollen > CAP score to second venom), suggesting high titers of carbohydrate-specific IgE in these sera.

Twenty-five microliters of serum was mixed with 25 μ L of the PBS-buffered inhibitor protein solution to give a final protein concentration of 100 μ g/mL. After overnight incubation at 4°C, CAP results were assessed as usual. In some patients CAP inhibition was performed with increasing protein doses (4, 20, and 100 μ g/mL) to obtain dose-response curves. The following extracts were tested in the inhibition assays: honeybee and YJ venom (homologous and heterologous inhibition, respectively), OSR pollen, timothy grass pollen, and MUXF-BSA. BSA and MM-BSA were run as negative controls. All inhibition experiments were done with the Pharmacia CAP system.

Competition assays with rabbit anti-Bra n antiserum

A carbohydrate-specific polyclonal rabbit antiserum against the high-molecular-weight glycoprotein fraction of OSR pollen (anti-Bra n), which also strongly binds with honeybee and YJ venom in Western blots (unpublished results), was used to compete with human IgE for antibody binding to venom allergens. Prewashed honeybee and YJ allergen discs (CAP) were preincubated with serial dilutions of anti-Bra n (1:200, 1:60, and 1:20) for 3 hours at room temperature and, after washing, were tested with selected sera

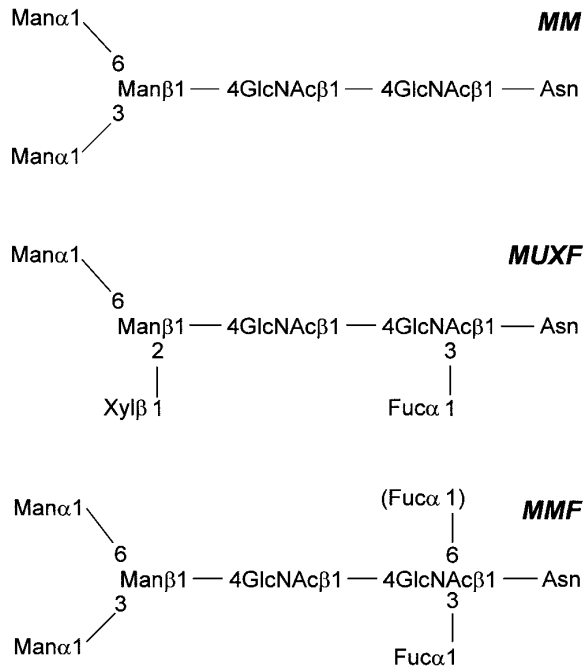


FIG 1. Structure of N-linked glycans displayed by the neoglycoproteins MM-BSA (top) and MUXF-BSA (middle). MMF oligosaccharides (bottom) are typical for honeybee phospholipase A and hyaluronidase. *Man*, Mannose; *GlcNAc*, N-acetylglucosamine; *Fuc*, fucose; *Xyl*, xylose.

(patients 1, 3, 7, 9, 10, 13, and 14). Alternatively, patient sera were coincubated with anti-Bra n at corresponding final concentrations, and CAP was performed as usual. All sera were diluted to achieve a CAP score of between 5 and 10 kU/L for both venoms in the positive control (PBS).

RESULTS

Routine assessment of venom-specific IgE with RAST and CAP

Choosing 0.7 kU/L as the positive cut-off point for both systems, CAP was more sensitive to detect isolated YJ venom allergy (CAP, 22%; RAST, 11%; $P < .005$, χ^2 test). The overall number of positive test results to YJ venom (CAP, 46%; RAST, 36%) and the overall number of positive test results to any venom (CAP, 55%; RAST, 46%) was higher with the CAP, without achieving significance ($P = .09$ and $P = .06$, respectively). There was no difference in the frequency of double-positive test results (CAP, 25%; RAST, 24%).

N-linked oligosaccharides in OSR pollen

The glycan composition of crude OSR pollen extracts, as assessed with mass spectroscopy, is shown in Table II, together with published data for rye, birch, and olive pollen and honeybee phospholipase A.

CAP IgE binding to OSR pollen

Only 2 (14.3%) of 14 patients with single-positive results to honeybee venom had IgE to OSR pollen (mean score, 0.54 ± 0.34 kU/L; median, 0.34 kU/L; highest,

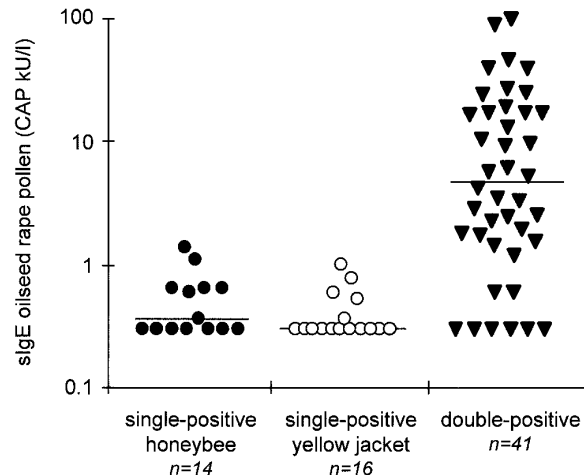


FIG 2. IgE binding to OSR pollen extracts in patients with stinging-insect allergy. Fourteen patients with a single-positive CAP result to honeybee venom, 16 patients with a single-positive CAP result to YJ venom, and 41 patients with double-positive CAP results were randomly selected, and their reactivity with OSR pollen glycoproteins was assessed by means of CAP. Median CAP scores for each group are indicated.

1.38 kU/L), and only 2 of 16 YJ venom single-positive sera had IgE to OSR pollen (12.5%; mean, 0.41 ± 0.22 kU/L; median, 0.30 kU/L; highest, 1.03 kU/L). In the double-positive group 33 of 41 sera had positive results to OSR pollen (80.5%; $P < .00001$, χ^2 test), and CAP scores were significantly higher compared with those in the single-positive groups ($P < .0001$, Mann-Whitney test; mean, 13.99 ± 22.01 kU/L; median, 4.18 kU/L; highest, >100 kU/L; Fig 2).

CAP inhibition

In 4 of 5 patients from group A (patients 1-5), pollen allergens and MUXF-BSA did not inhibit IgE binding to any venom to a significant degree (Table I). There was moderate inhibition in patient 5. Specific reciprocal inhibition between honeybee and YJ venom was seen in patients 4 and 5, whereas in patients 1, 2, and 3 CAP was inhibited only by the homologous venom.

In 9 of 11 patients from group B (patients 6-14), OSR pollen extracts were able to inhibit IgE binding to one of the venoms as effectively as the homologous venom, suggesting that IgE binding to this venom was due to carbohydrate epitopes (Table I). Inhibition with the heterologous venom and with grass pollen and MUXF-BSA was equally effective in most cases, although not in all. Dose-response curves are shown for patients 7 and 8 in Fig 3. In 2 patients (patients 13 and 14) the plant glycoproteins abrogated IgE binding to both venoms, suggesting that carbohydrates were the only antigenic determinants recognized by these patients in either venom.

Patients 15 and 16 from this group appeared to be truly double sensitized because no or only moderate inhibition could be achieved by the heterologous venom and by pollen allergens (Table I). Both patients had pollen allergy, indicating the possibility that the high OSR CAP

TABLE I. Demographic data and RAST inhibition results in 16 patients with venom double-positive test results

Patient No.	Age (y)	Sex	Culprit insect	Mueller grade	CAP (kU/L)			Total IgE	Inhibition of HB CAP (%)			
					HBV	YJV	OSR		HBV	YJV	OSR	GR
1	75	F	YJ	IV	15.9	11.6	0.6	140	94	8	0	0
2	13	M	YJ	LLR	9.9	12.9	4.2	620	73	0	2	20
3	13	M	HB	II	17.0	12.3	1.9	1420	90	11	18	6
4	18	F	HB	I	39.0	7.7	2.4	140	82	19	7	0
5	42	F	HB	LLR	29.3	19.7	4.6	360	95	82	56	50
6	11	M	HB	I	69.2	7.8	24.7	680	70	0	0	0
7	55	M	YJ	I	8.5	22.4	39.3	40	88	89	81	44
8	59	M	YJ	IV	7.7	81.4	9.5	990	69	41	61	62
9	68	F	?	III	11.9	10.2	17.2	210	80	68	70	68
10	56	F	YJ	III	7.5	37.4	17.1	580	70	64	80	96
11	56	F	YJ	III	4.3	25.4	8.7	630	44	26	50	72
12	23	F	HB	IV	85.4	56.5	67.5	1840	64	0	46	59
13	37	M	YJ	II	21.0	7.6	17.2	100	66	67	64	75
14	52	F	YJ	II	27.2	14.3	21.9	1360	82	46	77	76
15	13	M	YJ	I	66.8	16.7	46.6	2000	96	39	30	12
16	17	M	?	I	4.4	12.8	6.2	1150	73	18	1	7

Patients 1 to 5 showed low reactivity with OSR pollen (CAP score to OSR < CAP score to second venom), and patients 6 to 16 showed strong binding with OSR pollen (CAP score to OSR > CAP score to second venom). Figures for CAP inhibition indicate the maximum percentage inhibition obtained with 100 µg/mL inhibitor protein. Skin prick test results indicate the allergen concentration (in micrograms per milliliter) eliciting a positive reaction. HBV, Honeybee venom; YJV, yellow jacket venom; GR, timothy grass pollen; MUXF, MUXF-BSA neoglycoprotein; ND, not determined; LLR, large local reaction.

TABLE II. Composition of N-linked glycans of crude OSR pollen extracts according to mass spectroscopy

Glycan structure	OSR	Rye	Birch	Olive	HB PLA ₂
MUF	2.0	2.2	—	—	8.1
MMX	24.7	—	—	—	—
MMF	7.9	—	—	—	48.2*
MUXF	1.4	47.0	3.0	2.5	—
MMXF	39.9	14.1	8.4	9.1	—
MGnXF/GnMXF	2.0	5.7	8.1	11.1	—
GnGnXF	1.5	4.2	59.4	22.7	—

Figures for single glycans represent relative proportions in total glycans (in percentages). Figures do not add up to 100% because results are given only for the major fucosylated-xylosylated glycans. Data for rye, birch, and olive pollen are from Wilson et al.²³ and data for honeybee phospholipase A₂ (HB PLA₂) are from Kubelka et al.¹⁹ For structure of glycans, see Fig 1.

*Including MMF glycans with additional α1-6 fucosylation.

score in these patients was due to minor OSR allergens, such as profilin or Ca-binding proteins.²¹

BSA and MM-BSA never significantly inhibited IgE binding to honeybee or YJ venom (data for MM-BSA not shown in Table I).

In 4 patients (patients 7, 9, 12, and 14) inhibition experiments were also performed for OSR pollen CAP (Fig 4). Both venoms strongly inhibited binding to OSR pollen allergens. Inhibition by honeybee venom was always equivalent to that of OSR, and the capacity of YJ venom was variable; in cases in which YJ venom was a very potent inhibitor, MUXF-BSA was a very poor inhibitor, and vice versa (see also dose-response curves in Fig 3).

Competition assays with rabbit anti-Bra n antiserum

The rabbit anti-Bra n antiserum reduced IgE binding to honeybee and YJ venom in a patient-specific pattern consistent with the results from RAST inhibition. No difference was found between anti-Bra n preincubation and coincubation (results shown for preincubation in Fig 5). Anti-Bra n did not significantly inhibit YJ venom RAST results in truly double-sensitized patients (patients 1 and 3) or in patients with YJ allergy cross-reacting with honeybee venom through CCDs (patients 7, 9, and 10), whereas it did reduce RAST results by 80% in patients having only anti-CCD IgE (patients 13 and 14). Honeybee venom RAST results were inhibited by anti-Bra n by 60% to 85% (mean, 75%) in all patients reacting with honeybee carbohydrates (patients 7, 9, 10, 13, and 14), starting at a concentration of 1:60. Interestingly, IgE binding to honeybee venom was also significantly inhibited by anti-Bra n in truly double-sensitized patients (patients 1 and 3), but inhibition was weaker, more linear, and already evident with an anti-Bra n concentration of 1:200.

DISCUSSION

Cross-reactivity through shared hyaluronidase epitopes and true double sensitization have been assumed to be the main causes for coexisting sensitization to honeybee and YJ venom.^{4,5,17} Here we provide evidence that CCDs represent an important and thus far unrecognized source of this double positivity, presumably explaining 50% or more of the double-positive results. This estimation would be consistent with data by Reisman et al,^{24,25} who

Inhibition of YJ CAP (%)						Skin prick test (HBV/YJV) allergies Other		
MUXF	BSA	YJV	HBV	OSR	GR	MUXF	BSA	
0	0	90	0	3	0	4	8	ND
17	0	55	0	0	0	6	5	NEG/100
13	27	92	11	0	4	13	10	100/100 Mite
ND	0	90	74	25	9	0	0	100?/neg
51	0	98	54	40	20	29	0	100/100 Birch, plantain
0	3	63	47	46	51	51	6	ND
23	0	97	22	17	22	15	0	neg/10
73	13	96	20	4	0	6	0	neg?/10
71	4	82	0	0	0	0	11	ND
96	22	93	7	8	11	7	2	ND
93	0	87	0	2	ND	0	2	ND
54	16	64	6	22	23	13	1	neg/10 Grass
78	0	80	60	71	73	79	16	100/100
86	0	66	40	66	70	72	3	100/100 Grass, mite
27	11	88	63	57	39	42	0	100/100 Grass
13	0	93	0	0	0	0	0	neg/10 Grass, birch, cat, mite

found extensive cross-inhibition between honeybee and vespid venom in about 70% of double-positive sera, but conflicts with more recent studies reporting full cross-inhibition in less than 20%.^{26,27} Specific cross-reactivity between the venoms not related to carbohydrates was seen in only 2 of 16 patients, suggesting that shared amino acid epitopes are less important in coexisting honeybee and vespid venom allergy than thus far believed.

We consider our findings important for 2 reasons. First, they provide a new and reasonable explanation for the surprisingly high proportion of double-positive sera. Our data do not support the view that oversensitivity of the CAP system is a major cause for double positivity. As a second point, our findings may contribute to the understanding of the clinical significance of double positivity inasmuch as the clinical relevance of carbohydrate-specific IgE antibodies may be low. Although a clinical role has been proposed in some studies,^{28,29} many investigators believe that these antibodies do not implicate clinical hypersensitivity because they lack significant biologic activity in terms of effective cross-linking and histamine release.^{7,11,30,31} If this holds true also in Hymenoptera allergy, the double positivity would turn out in many patients to be a negligible in vitro phenomenon.

Consistent with this view, none of our patients had a double-positive history. Nevertheless, some observations in this study should caution against entirely neglecting carbohydrates as functioning allergens in stinging-insect allergy unless more data are available. For example, sera from patients 13 and 14, reporting Mueller grade II reactions, were found to bind only with carbohydrate epitopes in either venom. Furthermore, these patients had positive skin test responses to both venoms, whereas this was not the

case in other patients. Correlating in vitro data with skin test reactivity might thus be helpful in discriminating between relevant and nonrelevant double positivity, but this has to be analyzed in a larger cohort of patients. No conclusions can be drawn at this point from this small sample.

With respect to the origin of CCD-specific IgE in patients allergic to venom, Aalberse et al⁷ suspected honeybee stings to be potent inducers of anticarbohydrate IgE. Our data suggest that vespid stings may also induce anticarbohydrate IgE because we found more patients with YJ allergy coreacting through CCDs with honeybee venom than vice versa. It is also possible, however, that anti-CCD antibodies in some sera do not originate from Hymenoptera stings at all but from respiratory sensitization to pollen glycoproteins, which are well-known targets of IgE antibodies.^{11,21,32} Five of our patients had pollinosis, and 4 of them did have carbohydrate-specific IgE. Diagnosis of stinging-insect hypersensitivity by means of in vitro techniques may thus be limited because it can not be ruled out that a positive venom RAST result could be encountered in atopic subjects never before exposed to Hymenoptera stings. Likewise, carbohydrate-specific IgE might partly explain the described linkage of venom allergy and atopy.³³⁻³⁵

α 1-3 fucosylation of oligosaccharides is assumed to be a key event determining the wide immunologic cross-reactivity among unrelated glycoproteins.²⁰ By using 2 neoglycoprotein constructs displaying defined oligosaccharides (ie, fucosylated MUXF-BSA and nonfucosylated MM-BSA), we could confirm that α 1-3-linked fucose is important also in the context of Hymenoptera double positivity. However, our data also indicate differences in the glycan repertoire between honeybee and YJ venom.

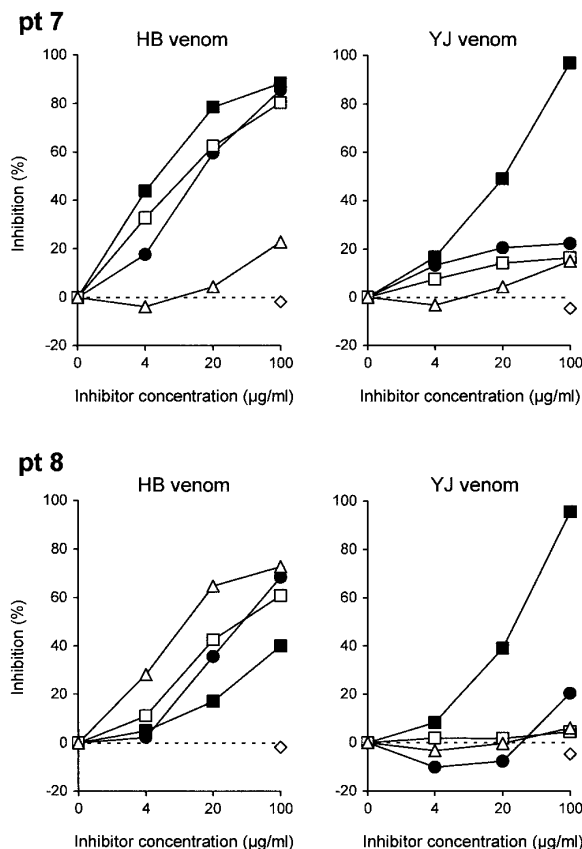


FIG 3. Dose-dependent inhibition of honeybee (HB) and YJ venom CAP results in 2 selected patients with double-positive results and a history of YJ venom allergy and positive CAP results to OSR pollen (patients 7 and 8). Patient sera were incubated overnight at 4°C with doses of 4, 20, or 100 µg/mL inhibitor protein, and nonbound IgE levels were measured by means of CAP. Inhibition by BSA (negative control) was done only with 100 µg/mL. IgE binding to honeybee venom was equivalently inhibited by OSR pollen extracts in both patients. MUXF-BSA was highly effective in patient 8 but not in patient 7, being inversely correlated with the inhibitory capacity of YJ venom. *Filled circles*, Honeybee venom; *filled squares*, YJ venom; *open squares*, OSR pollen; *open triangles*, MUXF-BSA; *open diamonds*, BSA.

For example, the inhibitory capacity of MUXF-BSA was often inversely correlated with that of YJ venom, whereas such a reciprocity was not seen with honeybee venom, signifying higher amounts of MUXF equivalents in honeybee venom. Furthermore, the observation that in honeybee venom higher concentrations of the rabbit anti-Bra n antiserum were needed to obtain effective blockade of carbohydrate-specific IgE than in YJ venom might indicate a generally higher quantity of CCDs in honeybee venom. This could also explain the unexpected finding that anti-Bra n moderately inhibits antibody binding in patients not having anti-CCD antibodies. We speculate that in the latter patients IgE binding is sterically hampered by the rabbit IgG in a strictly dose-dependent manner if amino acid epitopes are close to glycosylation sites. It remains to be elucidated, however, to what extent the observed differences between the venoms are due to inadvertent chemical modification of allergen glycans during extraction and storage.

The intermediate inhibitory capacity of the 2 pollen extracts fits well into this picture because they contain many different glycans covering a broad range of anti-

body responses. It is noteworthy that OSR pollen was a much more consistent inhibitor than timothy grass pollen. One reasonable explanation for this could be that OSR pollen, but not Gramineae pollens, contains MMF glycans, which are characteristic for venom glycoproteins.¹⁹ This would also suggest that not only fucose but also the terminal α 1-3-linked mannose residue (Figure 1) might be involved in antibody binding in some sera. Interestingly, OSR pollen apart from MMF glycans also has high amounts of other glycans displaying a terminal dimannose antenna, such as MMX and MMXF, which is not the case in other pollens.

In summary, we demonstrated that IgE against CCDs is a major cause for Hymenoptera venom double positivity. RAST results to OSR pollen appears to be a simple and practicable measure to detect sugar-specific IgE in individual sera. This might be helpful in discriminating between patients cross-reacting through CCDs and truly double-sensitized patients who may require immunotherapy with 2 venoms.

We thank ALK-Abellø Vespa Laboratories for providing honeybee and YJ venom.

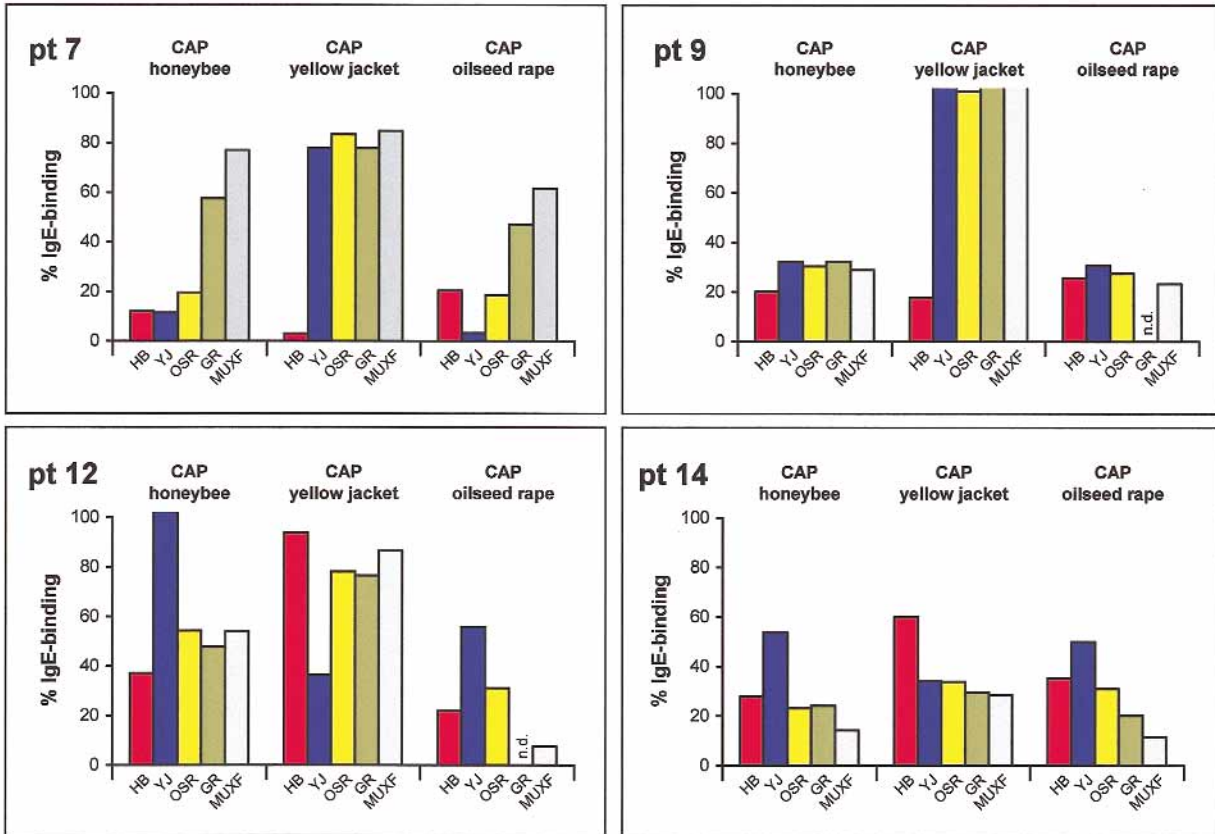


FIG 4. Inhibition of IgE binding to honeybee venom, YJ venom, and OSR pollen by Hymenoptera venoms, pollen allergens, and MUXF-BSA (all 100 μ g/mL) in 4 patients with double-positive test results with anti-CCD IgE antibodies (patients 7, 9, 12, and 14). Bars represent the percentage of IgE binding in the inhibited serum in comparison with that in the noninhibited serum. IgE binding to honeybee venom was inhibited by OSR pollen proteins and was comparable with autologous inhibition in all patients. The efficacy of YJ venom in inhibiting honeybee and OSR CAP results was reciprocal to that of MUXF-BSA. HB, Honeybee venom; GR, timothy grass pollen; MUXF, MUXF-BSA; n.d., not determined.

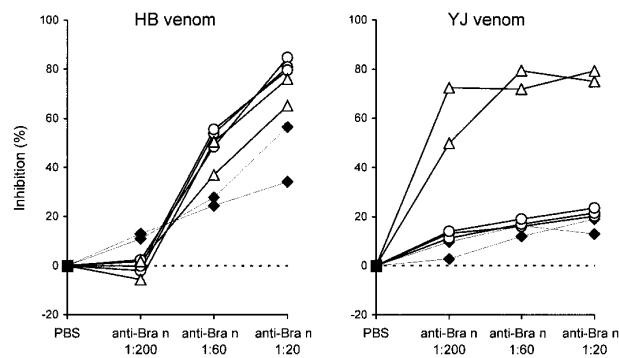


FIG 5. Inhibition of IgE binding to honeybee and YJ venom in 7 patients with venom double-positive test results by different concentrations of a carbohydrate-specific rabbit antiserum (anti-Bra n). Open circles, Patients with YJ venom allergy cross-reacting with honeybee glycans (patients 7, 9, and 10); open triangles, patients reacting only with venom carbohydrates (patients 13 and 14); filled diamonds, truly double-sensitized patients without carbohydrate-specific IgE (patients 1 and 3).

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