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Natural rubber latex and hymenoptera venoms share ImmunoglobulinE-epitopes accounting for cross-reactive carbohydrate determinants

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Clinical and Experimental Allergy

Summary

Background Epidemiological data on the prevalence and risk factors of latex sensitization have suggested a significant association between latex sensitization and the presence of one or more positive skin prick test responses to aeroallergens, food allergens and to one or more insect venoms. Xylose and core 3-fucose are typical complex glycans in plants and are foreign to mammals. Plant N-glycans and insect N-glycans may cross-react in humans.

Objective The aim of our study was to investigate whether there are cross-reactive IgE-binding structures in natural rubber latex (NRL) and hymenoptera venoms and to examine their nature.

Methods Hundred and twenty-five consecutive patients with insect venom allergy were screened for coincidental latex-specific IgE. IgE-binding components in the venoms from *Apis mellifera* and/or vespula species and in NRL extracts were characterized by IgE-immunoblotting to the natural allergen sources and determination of specific IgE to recombinant allergens. Cross-reactive components were investigated by inhibition experiments. The involvement of carbohydrates in the constitution of cross-reactive IgE-epitopes was further examined by specific IgE-binding to cross-reactive carbohydrate determinants (CCD) in bromelain and horseradish peroxidase as well as by periodate treatment.

Results NRL glove extracts inhibited patients' serum IgE-binding to venom allergens. Vice versa, the IgE-binding to latex glove extracts could be inhibited by pre-incubation with the insect venoms. Specific IgE-binding to recombinant latex allergens was absent, whereas the cross-reactive IgE-epitopes were sensitive to periodate treatment and specific IgE to CCD (MMXF and MUXF type) could be detected.

Conclusion Insect venoms and NRL share IgE-binding CCD that may be responsible for positive serological test results to NRL in patients with insect venom allergy. This copositivity occurs frequently (13.6%) among venom-allergic individuals and did not elicit clinical symptoms upon contact to latex in the patients examined. In contrast, true cosensitization to insect venoms and NRL allergens can occur and may not be missed.

Keywords bee venom, carbohydrate epitopes, cross-reactive carbohydrate determinants, insect venom allergy, latex sensitization, MMXF, MUXF, N-glycans, yellow jacket venom

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Introduction

Proteins from natural rubber latex (*Hevea brasiliensis*) are a potent source for Type I allergic reactions (cutaneous, nasal, bronchial and systemic reactions) [1, 2]. Recently, epidemiological data obtained in an unselected

paediatric population have suggested an association between latex sensitization and the presence of one or more positive skin prick test responses to aeroallergens, food allergens and to one or more insect venoms [3]. These data correspond with our previous clinical observation of a high prevalence of specific IgE to natural rubber

latex (approximately 10%) in insect venom-allergic patients [4].

In contrast, to well-characterized cross-reactive allergens in pollen, tropical fruits, vegetables and natural rubber latex [1, 5, 6], information regarding the presence of common IgE-binding components in insect venoms and latex is scarce. In a recent report in this journal, venom allergy has been demonstrated to mimic allergy by the presence of clinically irrelevant IgE for natural rubber latex (NRL) in 5/40 (i.e. 12.5%) patients [7].

In our study, we screened consecutive insect venom-allergic patients ($n = 125$) for concomitant specific IgE to NRL ($n = 17$) to investigate whether there were cross-reactive IgE-binding moieties present in latex and hymenoptera venoms and to determine their nature and clinical relevance.

Materials and methods

Characterization of patients with hymenoptera venom allergy and concomitant latex-specific Immunoglobulin E

Patients (p) and controls (c) were characterized by case history, positive skin tests and examination for specific IgE (CAP FEIA) after written consent had been obtained. Approval had been given by the local ethics committee for this study. Diagnostic procedures including venom skin test procedures were performed according to the Guideline on Diagnosis and Therapy of honeybee venom and yellow jacket allergy provided by the German Society for Allergy and Clinical Immunology [8].

Consecutive patients ($n = 125$) with clinically relevant hymenoptera venom allergy (classified according to Mueller [9]) were screened for coincidental latex-specific IgE, which was found in 17 patients. Fifteen patients ($n = 15$) were accessible for further examinations. None of the patients reported previous symptoms upon contact to latex. Age, gender, serological and clinical characterizations of patients are summarized in Table 1. As controls, sera from individuals ($n = 30$) with a symptomatic latex allergy [10] without insect venom allergy and sera from non-atopic individuals ($n = 20$) were included.

Protein extracts and skin tests

For immunoblotting, extracts from the natural allergen sources were prepared as described [11]. For glove extracts, surgical latex gloves Reference OP Anatom were purchased from Meditrade (Kufstein, Austria) and extracts were prepared as described [11]. A lyophilized latex serum from low-ammoniated natural latex sap kindly provided by U. Koch (Department of Dermatology, Erlangen) was dissolved in sterile water to a final concentration of 1 mg/mL before use. Dried whole venom from honey bee (*Apis mellifera*) and a mixture of yellow jacket venoms (from *Vespula germanica*, *Vespula maculifrons*, *Vespula*

vulgaris, *Vespula pennsylvanica*, *Vespula flavopilosa* and *Vespula squamosa*) purchased from Sigma-Aldrich (Taufkirchen, Germany) were dissolved in sterile water to a final concentration of 1 mg/mL before use.

For skin prick testings (according to [12]) and intradermal skin testings, commercially available venom extracts (ALK Scherax, Hamburg, Germany), extracts from latex sap [Allergopharma, Reinbek, Germany; protein content (62 µg/mL)] and a latex fluid (Fa. Semperit, Vienna, Austria) prepared by the Frugoni's method [buffer (v/v): phosphate buffer (pH 7.5) 0.5/glycerin 0.5/0.9% NaCl ad 10.0; protein content (3.6 µg/mL)] [13] were used. The validation of the intradermal test for latex in our study was performed in latex-allergic and non-allergic individuals. In brief, 30 µL of the latex fluid prepared by the Frugoni's method was tested diluted 1 : 100, 1 : 10 and undiluted (end concentration 3.6 µg/mL) at the volar site of the lower arm in patients and non-atopic controls ($n = 20$). The diameters of weal reactions were measured. The readings, scoring and interpretation of the intradermal testing were performed as described in Table 1. Equal amounts of histamine hydrochloride [0.17 mg/mL] and 0.9% saline were used as positive and negative controls, respectively.

The diagnostic procedures performed for diagnosis of honey bee venom and yellow jacket allergy according to Rueff et al. [8] included intradermal testings with venom concentrations starting from 0.00001 up to 1 µg/mL each read after 20 min. The relevant end-point titration for an allergic reaction is in general between 0.1 and 1 µg/mL. However, reactions up to 1 µg/mL could also be, at least partly, toxic reactions. The concentration diagnostic for allergy, also with insect venoms, is not absolutely fixed, but may vary from individual to individual.

Sodium dodecyl sulphate-poly acrylamide gel electrophoresis, immunoblotting and inhibition immunoblotting

SDS-PAGE and immunoblotting were performed as described in Mahler et al. [11]. In brief, 50 µg protein extract (latex extract, bee venom and vespula species venom) per centimetre slot of the preparative gel was separated by preparative 12.5% SDS-PAGE and blotted onto nitrocellulose (Schleicher and Schuell, Dassel, Germany). All blocking and washing steps were performed in buffer A (50 mM/L sodium phosphate, pH 7.5%, 0.5% v/v Tween-20%, 0.05% w/v Na₂N₃) as described by Mahler et al. [11]. Sera from patients and control subjects were diluted 1 : 10 in buffer A; bound IgE was detected with iodine¹²⁵-labelled anti-human IgE antibodies (Pharmacia RAST, Uppsala, Sweden) diluted 1 : 10 in buffer A. Autoradiographies were exposed to a Kodak XOMAT film with intensifying screens (Kodak, Heidelberg, Germany) at -70°C for 8 days.

Table 1. Demographic, serological and clinical characterization of patients and controls

Patient no.	Age (years)	Sex	Profession	Total IgE (kU/L)	Bee venom CAP (kU/L), CAP FEIA class in 0	Yellow jacket venom CAP (kU/L), CAP FEIA class in 0	Latex CAP (kU/L), CAP FEIA class in 0	Skin test (latex): positive: +, negative: -, not done: n.d. prick i.c.		Exposure to natural rubber latex: examination glove (EG), household glove (HG) contact	Clinical symptoms upon bee (b), yellow jacket venom (w) and latex (l) contact
								prick	i.c.		
1	49	F	Nurse	289	16.1 (cl. 3)	84.8 (cl. 5)	6.33 (cl. 3)	-	-	Unpowdered EG, previously powdered EG	Mueller III (w); none (l)
2	30	M	Banker	492	35.2 (cl. 4)	> 101 (cl. 6)	9.3 (cl. 3)	-	1+	Frequently HG	Mueller IV (w); none (l)
3	42	F	Housewife	188	6.33 (cl. 3)	5.61 (cl. 3)	1.12 (cl. 2)	-	2+	Frequently HG	Mueller III (b); none (l)
4	41	M	Transport worker	65.5	1.65 (cl. 2)	7.11 (cl. 3)	0.97 (cl. 2)	-	2+	Infrequently HG, condoms	Mueller II-III (w); none (l)
5	58	M	Forest worker	275	4.27 (cl. 3)	9.73 (cl. 3)	2.52 (cl. 2)	-	3+	Infrequently HG, condoms	Mueller IV (w); none (l)
6	45	M	Secretary	193	6.62 (cl. 3)	3.88 (cl. 3)	3.14 (cl. 2)	-	2+	Frequently HG, condoms	Mueller II (w); none (l)
7	47	M	Train conductor	161	9.89 (cl. 3)	0.74 (cl. 1)	1.31 (cl. 2)	-	1+	Condoms	Mueller I (b); none (l)
8	28	F	Housewife	60.7	7.66 (cl. 3)	1.27 (cl. 2)	2.2 (cl. 2)	-	2+	Frequently HG	Mueller I-II (b); none (l)
9	44	F	Banker	142	1.56 (cl. 2)	12.9 (cl. 3)	0.93 (cl. 2)	-	2+	Infrequently HG	Mueller III (w); none (l)
10	49	F	Office worker	50.8	1.9 (cl. 3)	0.84 (cl. 2)	0.9 (cl. 2)	-	2+	Infrequently HG	Mueller III (b); none (l)
11	29	F	Housewife	756	5.32 (cl. 3)	5.30 (cl. 3)	5.36 (cl. 3)	-	1+	Frequently HG	Mueller I (w & b); none (l)
12	33	F	Nurse	96.9	57.1 (cl. 4)	2.89 (cl. 2)	1.97 (cl. 2)	-	2+	Unpowdered EG, previously powdered EG	Mueller III (b); none (l)
13	75	F	Pensioner	525	7.24 (cl. 3)	27.7 (cl. 4)	2.4 (cl. 2)	-	1+	Frequently HG	Mueller II (w); none (l)
14	41	M	Bee-keeper	73.4	2.51 (cl. 2)	1.70 (cl. 2)	1.42 (cl. 2)	-	1+	Frequently HG	Mueller I (b); none (l)
15	22	F	Merchant	89.6	2.47 (cl. 2)	4.41 (cl. 3)	2.11 (cl. 2)	-	1+	Frequently HG, condoms	Mueller II (w); none (l)
c1	29	F	Laboratory assistant	561	< 0.35 (cl. 0)	< 0.35 (cl. 0)	1.49 (cl. 2)	3+	n.d.	Previously powdered EG, condoms	shock, angioedema (l)
c2	37	M	Scientist	37.8	< 0.35 (cl. 0)	< 0.35 (cl. 0)	< 0.35 (cl. 0)	-	-	Unpowdered EG	none

CAP FEIA classes and kilounits per litre (kU/L) are related as follows: < 0.35 kU/L, class 0; 0.36–0.7 kU/L, class 1; 0.8–3.6 kU/L, class 2; 3.7–17 kU/L, class 3; 18 to 50 kU/L, class 4; 51–99 kU/L, class 5; > 100 kU/L, class 6. Severity levels according to Mueller [9] are defined as level 0, local reaction; level I, mild systemic reaction; level II, moderate systemic reactions; level III, severe systemic reactions; level IV, shock reaction.

For readings of the intradermal tests, weal diameters were measured and interpreted (< 3 mm: negative; Positive: 3–5 mm: 1+; 6–10 mm: 2+; 11–15 mm 3+; > 15 mm: 4+).

Immunoblot inhibition experiments with natural rubber latex, *A. mellifera* and *Vespula* species venoms were performed under conditions established for these complex protein mixtures as described [5]. In brief, 1 mL of serum diluted 1 : 10 in buffer A was pre-incubated overnight at 4 °C with 50 µg of bee venom, yellow jacket venom, latex glove extract or bovine serum albumin as control. Pre-adsorbed sera were then used as described for immunoblotting.

Quantitative CAP FEIA inhibition studies

To quantify the degree of inhibition visible in the inhibition immunoblots, CAP FEIA (Pharmacia, Freiburg, Germany) inhibition studies were performed. Representative sera were pre-adsorbed (overnight at 4 °C) with either 50 µg of bovine serum albumin (BSA), bee venom, yellow jacket venom or latex glove extract before determination of specific IgE-binding in the CAP FEIA system. Inhibition can be determined in percentage of natural IgE-binding (as determined by pre-incubation with BSA alone).

Periodate treatment

To determine whether carbohydrates may play a role in the constitution of common IgE-epitopes of bee, yellow jacket and latex allergens, blotted extracts were treated with sodium m-periodate (Sigma, St. Louis, MO, USA) [14]. In brief, nitrocellulose strips containing blotted antigen were first incubated in buffer A, and then in 10 mM sodium m-periodate/50 mM sodium acetate buffer pH 4.5, or, respectively, without sodium m-periodate in the dark, each at room temperature for 30 min. After blocking in buffer A for 10 min twice and for 40 min, the nitrocellulose strips were then exposed to serum IgE as described for immunoblotting.

In vitro examination of specific immunoglobulinE-binding to recombinant latex allergens and cross-reactive carbohydrate determinants

To further elucidate the nature of serum IgE-binding structures, the specific IgE binding to recombinant latex allergens (rHev b 1, rHev b 2, rHev b 3, rHev b 5, rHev b 6.01, rHev b 6.02, rHev b 8, rHev b 9, rHev b 11 expressed as recombinant MBP (maltose binding protein)-fusion proteins was determined by CAP FEIA (Pharmacia). As a negative control, to exclude non-specific binding, the MBP was used. Specific IgE binding to rHev b 7 was examined by allergen microarray [15] (kindly provided by Jens-Malte Baron, Department of Dermatology, University of Aachen, Germany).

As a marker for CCDs, IgE-binding to bromelain and horseradish peroxidase (HRP) was investigated (CAP FEIA; Pharmacia).

Results

Demographic, serological and clinical characterization of patients and controls

In $n = 17$ individuals out of 125 consecutively screened insect-allergic patients, coincidental latex-specific IgE was found *in vitro*, of which $n = 15$ were accessible for further examinations. The demographic, serological and clinical characterization of patients is summarized in Table 1.

For control purposes, vice versa, in individuals with symptomatic NRL ($n = 30$) without a history of anaphylaxis to insect venoms or multiple pollen sensitization specific IgE-binding to the insect venoms was examined *in vitro*, which was found to be positive (CAP FEIA classes 1–4) in 21/30 unselected patients with symptomatic allergy to natural NRL (data not shown).

Six of the insect venom-allergic patients had experienced clinical symptoms to bee, eight patients to yellow jacket and one patient (11) to bee and yellow jacket stings. The severity of symptoms did not correlate with the amount of specific serum IgE or total IgE.

None of the 15 insect-allergic patients had experienced symptoms upon contact to latex. The sources of latex exposure are given in Table 1. Latex-specific IgE-antibodies in the insect-allergic patients ($n = 15$) account for CAP FEIA classes 2 and 3. A contamination of the latex CAP with hymenoptera venoms was ruled out by the manufacturer. Skin prick tests to latex were negative in these patients.

As intradermal testing is the most sensitive method of skin testing and should be preferred to skin prick-puncture tests for the detection of patients with low-grade sensitization and for evaluation of allergenic extracts of low potency [16] consecutively, intradermal testing with a standardized latex fluid was performed in the $n = 15$ insect-allergic patients with concomitant latex-specific IgE. Intradermal skin testings to latex were weakly positive (1+ to 2+) in patients 2–15 (Table 1). Control data for the intradermal tests with the same NRL fluid were obtained in non-atopic subjects ($n = 20$) and were found to be negative. Irritative reactions were excluded.

Serum immunoglobulinE from insect-allergic patients detects distinct immunoglobulinE-binding moieties in natural rubber latex

The serum IgE of the insect-allergic patients with coincidental IgE to latex binds specifically to bee venom allergens (Fig. 1), yellow jacket venom allergens (Fig. 2) and to six distinct components between 24 and 61 kDa in the IgE-immunoblot with nitrocellulose-blotted NRL fluid (data not shown). The IgE-binding to components contained in the extract of the finished latex product is

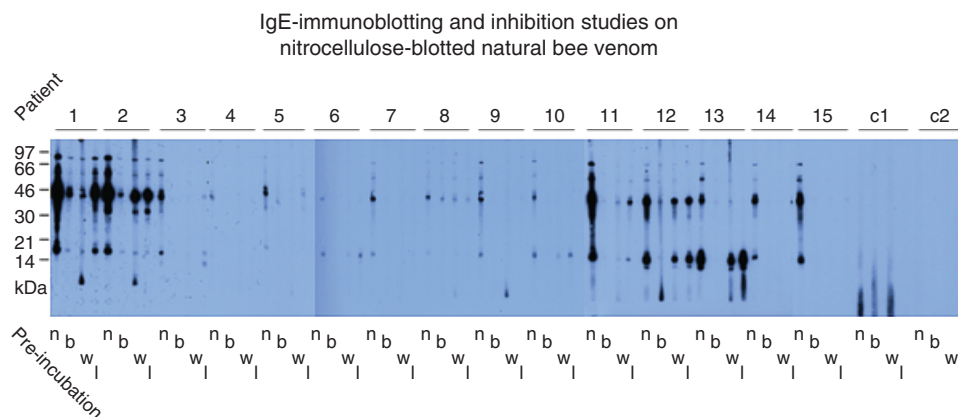


Fig. 1. IgE-immunoblotting and inhibition studies on nitrocellulose-blotted bee venom with sera from patients 1–15, control c1 (symptomatic latex allergic), and c2 (non-allergic). Preadsorption is indicated below the respective lane: pre-adsorption with bovine serum albumin (n), bee venom (b), yellow jacket venom (w) or latex-extract (l). Bee venom allergens of approximately 10, 16, 36, 43, 50 and 80 kDa (lanes 1–15 n) are detected. All sera from insect-allergic patients ($n = 15$) recognize the 43 kDa component, $n = 13$ the 16 kDa component, $n = 12$ the moieties of 50 and 80 kDa, $n = 5$ the 36 kDa compound and $n = 3$ the 10 kDa component.

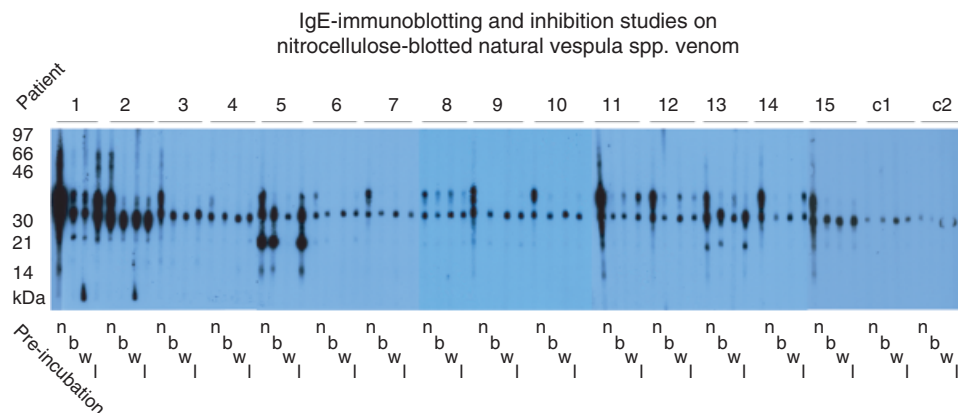


Fig. 2. IgE-immunoblotting and inhibition studies on nitrocellulose-blotted yellow jacket venom with sera from patients 1–15, control c1 (symptomatic latex allergic), and c2 (non-allergic). Preadsorption is indicated below the respective lane: preadsorption with bovine serum albumin (n), bee venom (b), yellow jacket venom (w) or latex-extract (l). IgE-binding to allergens of approximately 14, 23, 34, 38, 50 and 66 kDa is detected (lanes 1–15 n). All sera bind to the 34 and the 38 kDa allergens; high molecular moieties (50 kDa and above) are detected by $n = 7$ (lanes 1–3 n, 11 n, 13–15 n), the 23 kDa component by $n = 6$, and the 14 kDa component by $n = 4$.

demonstrated in Fig. 3. Owing to the rather harsh manufacturing process of gloves, extracts from the finished products may consist of allergens and truncated allergens that result in smears of IgE-detection in Western blot analysis. Serum IgE from 8/15 insect-allergic patients bind to approximately 35 kDa, 13 to approximately 46 kDa and/or moieties of higher molecular weight as a continuous smear in the NRL glove extract (Fig. 3, lanes 1–5 n, 7 n, 9–15 n). In sera from patients 6 and 8 (CAP FEIA class 2 to latex), specific IgE-binding was exclusively detectable in the IgE-immunoblot with the natural latex serum made from low-ammoniated latex fluid (data not shown). The latex-allergic control (c1) exhibited pronounced IgE-binding at about 20 kDa and from 35 to 100 kDa (Fig. 3, lane c1 n).

In the control sera (c1, c2), no specific IgE-binding to insect venom allergens exceeding the buffer control was observed (Fig. 1, lanes c1 n and c2 n).

Insect venoms and natural rubber latex share immunoglobulinE-epitopes

The presence of latex-specific IgE-antibodies in insect venom-allergic patients led us to investigate by specific immunoblot inhibition whether insect venom and latex glove extract contain similar IgE-epitopes. The inhibition of specific IgE-binding to nitrocellulose-blotted allergens of bee venom (Fig. 1), yellow jacket venom (Fig. 2) and latex glove extract (Fig. 3) due to specific pre-adsorption of sera is shown. The IgE-binding of the non-specifically

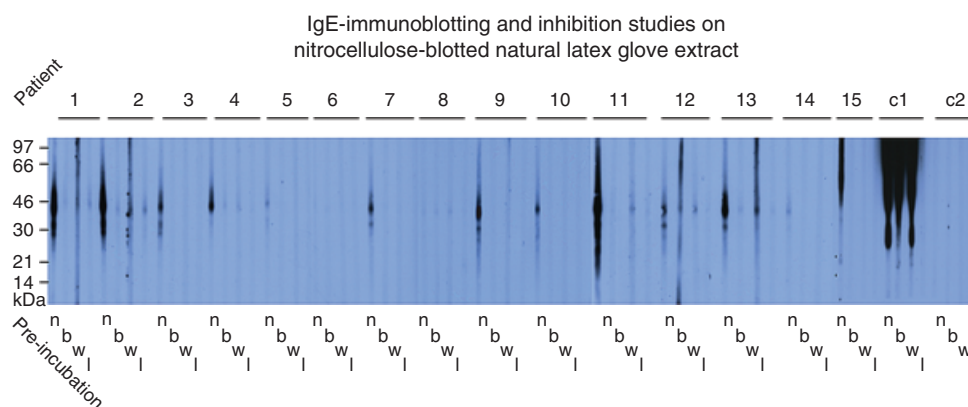


Fig. 3. IgE-immunoblotting and inhibition studies on nitrocellulose-blotted latex glove extract with sera from patient 1–15, control c1 (symptomatic latex allergic), and c2 (non-allergic). Pre-adsorption is indicated below the respective lane: pre-adsorption with bovine serum albumin (n), bee venom (b), yellow jacket venom (w) or latex-extract (l).

(BSA) pre-adsorbed sera is exhibited for comparison in lane n (Figs 1–3, lanes 1–15 n, c1 n, c2 n).

Pre-adsorption with bee and yellow jacket venom causes cross-inhibition. Preadsorption of sera with natural yellow jacket venom led to reduced IgE-binding to nitrocellulose-blotted bee venom allergens (Fig. 1, lanes 1–15 w); however, the extent of inhibition varied in different sera for the 43 kDa allergen (Fig. 1, lane 1 w, 2 w, 12 w) and the 16 kDa allergen (Fig. 1 lane 12–13 w).

Vice versa, pre-adsorption with bee venom in most sera led to a remarkable inhibition of IgE-binding to yellow jacket venom allergens of 38 kDa (Fig. 2, lanes 1–15 b), 23 kDa (Fig. 2, lane 1 b, 11 b, 15 b) and high molecular components (50 kDa and higher; Fig. 2, lanes 1–3 b, 11 b, 13–15 b). The IgE-binding to the 34 kDa yellow jacket venom allergen was hardly inhibited.

This marked cross-inhibition of bee and yellow jacket venom accounts for common IgE-epitopes in both venoms.

Pre-adsorption with natural rubber latex inhibits immunoglobulinE-binding to nitrocellulose-blotted insect venoms. Pre-adsorption with an NRL glove extract (exhibited in lane l) reduced the IgE-binding to nitrocellulose-blotted bee venom allergens in most sera (Fig. 1, lanes 2–15 l) including high-molecular-weight allergens (50–80 kDa) (Fig. 1, lanes 1–3 l, 5 l, 7 l, 9–15 l) and to the 38 kDa component and high molecular components of the nitrocellulose-blotted yellow jacket venom (Fig. 2, lanes 2–14 l). These results indicate shared IgE-epitopes in latex glove extract and the insect venoms. However, certain epitopes appeared to be present in lower amounts or not at all in the latex glove extract, as inhibition of IgE-binding to the 16 kDa and to the 43 kDa components of bee venom was scarce in some cases (Fig. 1, lanes 1 l, 2 l, 12 l, 13 l) and no inhibition of IgE-binding to the 34 kDa allergen of yellow jacket venom could be achieved with the NRL extract (Fig. 2, lanes 1–15 l).

Pre-adsorption with insect venoms inhibits immunoglobulinE-binding to nitrocellulose-blotted natural rubber latex extract. Vice versa, pre-adsorption of sera with bee venom (Fig. 3, lanes 1–15 b) and with yellow jacket venom (Fig. 3, lanes 1–15 w) inhibited IgE-binding to nitrocellulose-blotted latex glove extract in most of the insect-allergic patients. In the latex-allergic control (c1), except for inhibition at 20 kDa by pre-adsorption with bee venom (Fig. 3, lane c1 b), no further inhibition of IgE-binding to latex allergens could be achieved by the insect venoms, indicating latex-specific epitopes uniquely present in latex.

Quantitative in vitro immunoglobulinE-inhibition studies confirm the qualitative findings

The quantitative IgE-inhibition studies (Table 2) confirm the qualitative findings of the inhibition IgE-immunoblots. Pre-adsorption with the primary sensitizing venom led to 83–100% auto inhibition, 47–100% inhibition of IgE-binding to the other insect venom and 74–100% inhibition of IgE-binding to the latex extract.

The latex extract inhibited IgE-binding to yellow jacket venom to a degree of approximately 3–17% in the examined yellow jacket-allergic patients and 29% of the IgE-binding to bee-venom in bee venom-allergic patients (Table 2).

The finding of complete inhibition of IgE-binding to latex allergens by pre-adsorption with insect venoms confirms insect venoms as being the primary sensitizing agents in the respective patients.

Periodate-sensitive immunoglobulinE-epitopes in natural latex and natural insect venoms

To investigate the effects of deglycosylation on IgE-binding to latex glove allergens and insect venoms, nitrocellulose-blotted extracts were exposed to periodate.

Table 2. Quantitative IgE-binding and inhibition (in percent) to allergens (given in the left margin) by pre-adsorption with allergen preparations (see upper margins) in the CAP FEIA system

	Patient 1: allergic to Vespula spp. Venom				Patient 9: allergic to Vespula spp. venom				Patient 14: allergic to bee venom				Patient 15: allergic to Vespula spp. venom			
	Control (pre-incubation with BSA)	Pre-incubation with bee venom	Pre-incubation with Vespula spp. venom	Pre-incubation with latex glove extract	Control (pre-incubation with BSA)	Control (pre-incubation with latex glove extract)	Pre-incubation with bee venom	Pre-incubation with Vespula spp. venom	Control (pre-incubation with BSA)	Control (pre-incubation with latex glove extract)	Pre-incubation with bee venom	Pre-incubation with Vespula spp. venom	Control (pre-incubation with BSA)	Control (pre-incubation with latex glove extract)	Pre-incubation with bee venom	Pre-incubation with Vespula spp. venom
↓ CAP FEIA																
Specific IgE to bee venom (kU/L)	20.80	4.40	5.45	14.40	0.71	0.00	0.00	0.38	0.36	0.97	0.00	0.44	0.69	0.81	0.00	0.00
Percentage inhibition	0.0	78.8	73.8	30.8	0.0	100	100	46.5	49.3	0.0	100	54.6	28.9	0.0	100	100
Specific IgE to Vespula spp. venom (kU/L)	51.10	67.4	4.07	46.70	6.83	5.91	0.0	0.0	6.64	0.40	0.0	0.00	0.0	2.12	1.42	1.76
Percentage inhibition	0.0	0.0	92.0	8.6	0.0	13.5	100	100	2.8	0.0	100	100	100	0.0	33.0	83.0
Specific IgE to latex (kU/L)	8.82	0.76	2.34	1.85	0.48	0.0	0.0	0.0	0.0	0.50	0.0	0.00	0.0	0.88	0.0	0.0
Percentage inhibition	0.0	91.4	73.5	79.0	0.0	100	100	100	100	0.0	100	100	100	0.0	100	100

The IgE-binding bee venom components of approximately 16, 39, 43 kDa and high molecular allergens (50–80 kDa) contained IgE-epitopes sensitive to periodate treatment (Fig. 4, lanes 1–15 p), indicating the possible involvement of carbohydrates in the constitution of cross-reacting IgE-epitopes. Sera 2, 12 and 13 contain IgE-antibodies binding to periodate-resistant IgE epitopes at 43 and/or 16 kDa (Fig. 4, lanes 2 p, 12 p, 13 p), which is in parallel to scarce cross-inhibition by pre-adsorption with yellow jacket venom or latex extract as demonstrated (Fig. 1, lanes 2, 12, 13 w and l). The IgE-binding yellow jacket venom component of 34 kDa proved to contain periodate-resistant epitopes (Fig. 5, lanes 1–15 p), whereas the 38 kDa component contained periodate-sensitive epitopes (Fig. 5, lane 2–15 p). The IgE-epitopes of high molecular yellow jacket allergens (50 kDa and higher) were sensitive to periodate treatment, indicating the possible involvement of carbohydrates in the high molecular IgE-binding. The IgE-binding to 23 kDa in patient 5 and 13 and to the 16 kDa allergen in patients 5 proved to be resistant to periodate treatment (Fig. 5, lane 5p and 13p) corresponding to a weak inhibition in the pre-adsorption experiments with bee venom and latex extract (Fig. 2, lanes 1, 5 and 13 b and l).

The IgE-binding epitopes in nitrocellulose-blotted latex glove extract recognized by the serum IgE from insect-allergic patients with coincidental latex-specific IgE were all periodate-sensitive (Fig. 6, lanes 1–15 p). The IgE-epitopes in the natural rubber glove extract recognized at 35–100 kDa by the Hev b 5 positive serum-IgE of a latex-allergic control (Table 3, c1) were not periodate-sensitive, indicating different subsets of latex-specific IgE-antibodies in the insect-allergic patients and the symptomatic latex allergic control. Hev b 5 was isolated by cloning the cDNA [17]. The protein has a predicted mass of 16 kDa but runs as has been described [17] at 36–100 kDa in gels (Figs 3 and 6, c1).

Specific in vitro serum immunoglobulinE-binding to recombinant latex allergens and cross-reactive carbohydrate determinants

The examination for specific IgE binding to recombinant latex allergens [rHev b 1, rHev b 2, rHev b 3, rHev b 5, rHev b 6.01, rHev b 6.02, rHev b 7, rHev b 8, rHev b 9, rHev b 11 expressed as recombinant MBP (Maltose binding protein)-fusion proteins] was negative in the patients examined with insect venom allergy and concomitant positive CAP FEIA to latex (whole extract). The control serum c1 exhibited specific IgE to rHev b 5.

In contrast to control sera c1 and c2, most sera mounted specific IgE to bromelain and HRP as markers for (CCD) (Table 3).

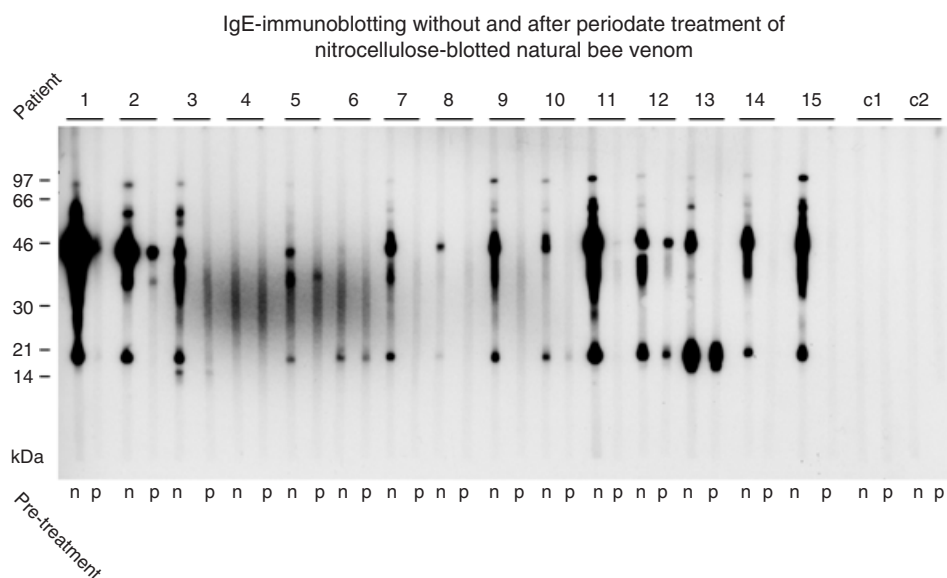


Fig. 4. IgE-binding of patients' and controls' sera (indicated above immunoblotting stripes) to nitrocellulose-blotted bee venom after periodate treatment (indicated below stripes as p) compared with untreated peers (indicated below as n).

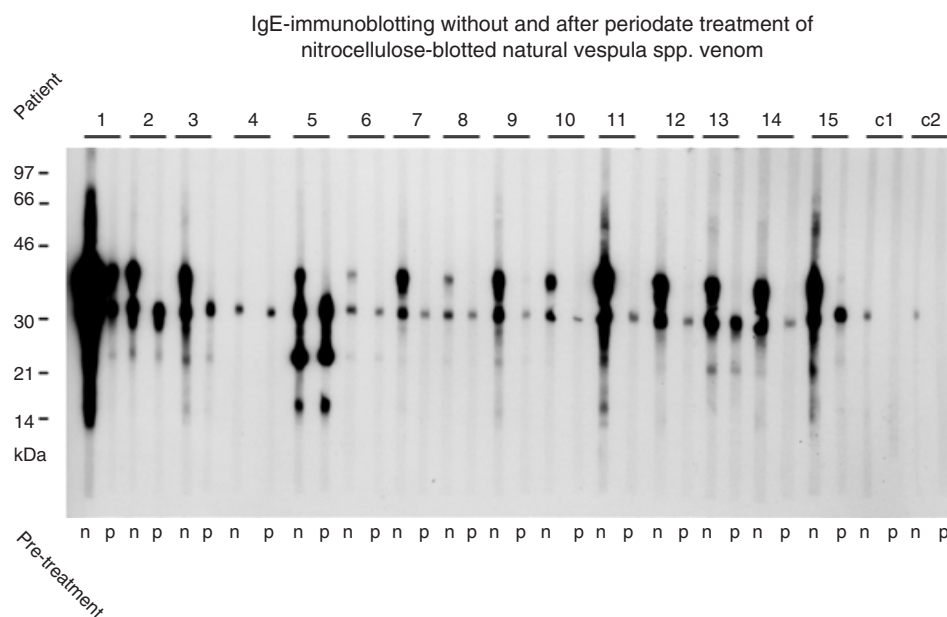


Fig. 5. IgE-binding of patients' and controls' sera (indicated above immunoblotting stripes) to nitrocellulose-blotted yellow jacket venom after periodate treatment (indicated below stripes as p) compared with untreated peers (indicated below as n).

Discussion

IgE cross-reactivities between latex, plant-derived food and pollen as typical manifestations of atopic disease have been extensively studied [1, 5, 6, 18, 19]. In contrast to aeroallergens and plant-derived foods, Type-I allergy to stinging insect venom does not belong to the typical cluster of atopic allergies and is not routinely looked for in latex-allergic individuals [20–24].

The aim of our study was to investigate whether there is a clear IgE-antibody cross-reactivity for *Hevea latex* and Hymenoptera venoms and to determine its nature.

Probably the most important reason for irrelevant *in vitro* results are protein-linked carbohydrates. In plants, the *N*-glycosylation of proteins starts by the transfer of the oligosaccharide $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ in the endoplasmic reticulum. This structure can subsequently be modified by glycosidases and glycosyltransferases. Depending

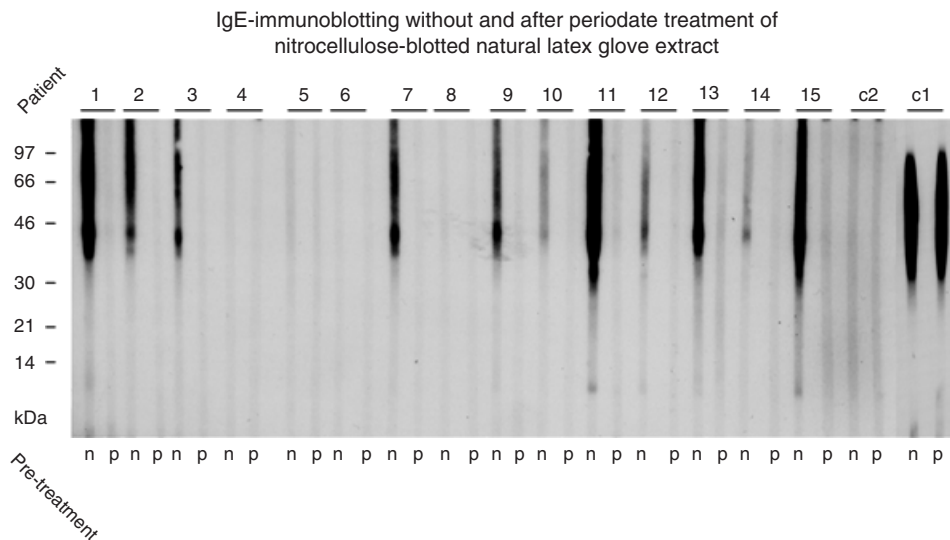


Fig. 6. IgE-binding of patients' and controls' sera (indicated above immunoblotting stripes) to nitrocellulose-blotted latex-extract after periodate treatment (indicated below stripes as p) compared with untreated peers (indicated below as n).

Table 3. Specific IgE against recombinant latex allergens and cross-reactive carbohydrate determinants (CAP FEIA)

Patient no.	rHev b 2 (kUA/L)	rHev b 3 (kUA/L)	rHev b 5 (kUA/L)	rHev b 6.01 (kUA/L)	rHev b 6.02 (kUA/L)	rHev b 8 (kUA/L)	rHev b 9 (kUA/L)	rHev b 11 (kUA/L)	Maltose binding protein (kUA/L)	Bromelain (kUA/L)	Horseradish peroxidase (kUA/L)
4	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35
9	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	0.43	0.40
10	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	0.41
13	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	1.34	2.41
14	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	0.75	1.00
15	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	0.68	1.09
c1	< 0.35	< 0.35	14.20	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35
c2	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35

on the accessibility of the glycan side chain, these enzymes can convert the precursor into high mannose-type *N*-glycans and then to complex *N*-glycans having an $\alpha(1,3)$ -fucose attached to the proximal glucosamine residue and/or a $\beta(1,2)$ -xylose residue attached to the mannose [25]. Xylose and core 3-fucose are typical for complex glycans from plants and are foreign to mammals exhibiting immunogenic properties in humans [25].

In our group of consecutively screened patients ($n = 125$) with insect venom allergy 17 patients (13.6%) exhibited clinically irrelevant IgE for NRL *in vitro*, which could be confirmed by immunoblot analysis. This frequency of copositivity for specific IgE to insect venoms and NRL confirms a recent report by Ebo et al. [7] in which 7/40 patients with venom allergy were sensitized for CCD and five (i.e. 12.5%) of them had clinically irrelevant IgE for latex [7]. In our control group ($n = 30$) of unselected patients with symptomatic allergy to NRL, 21/30 (70%) exhibited positive CAP FEIA results (classes 1–4) to bee and/or vespula venoms without a history of anaphylaxis to insect venoms or multiple pollen sensitization, which

points to an association of IgE-reactivity to latex and insect venoms rather than an underlying atopic status.

Whereas in unselected insect-allergic patients, double positivity occurs in 30–50% [26, 27], all of the insect-allergic patients in our study exhibited specific IgE to both venoms, although most had a history of allergic symptoms to only one of the venoms. This feature is inherent to the patient selection and is in concordance with the recent report of a significantly higher frequency of double positivity for honey bee and yellow jacket in patients sensitized to venom CCD compared with patients without sensitization for venom CCD [7].

NRL as well as venoms of *A. mellifera* (bee) and *Vespula* spp. (yellow jackets) consist of complex protein mixtures that vary significantly depending on the source containing peptides and glycoproteins [1, 28, 29]. In addition to sequence characterization of venom allergens from honey bee and vespids [30–34], recently, the *N*-linked carbohydrate chains from honeybee venom phospholipase A2 were characterized by resolution of primary structures [35] and further major venom glycoallergens binding with

carbohydrate-specific IgE were detected at 42 kDa (hyaluronidase), 46, 65 and 95 kDa in venom from honeybee, and 38 and 43 kDa in venom from yellow jackets [36]. A major component of plant glycoproteins frequently found in plant-derived allergens as well as HRP type is usually of the MMXF type ($\text{Man}_3\text{XylFucGycNAc}_2$). By truncation, $\text{Man}_2\text{XylFucGycNAc}_2$ (MUXF-, bromelain type) can be deduced, which is rarely present in plants (e.g. bromelain). In insect N-glycans, characteristically xylose is absent, whereas a core 3-fucose is present [37], which may cross-react with plant N-glycans.

Inhibitions achieved by pre-adsorption of IgE-immunoblotting in our study indicate that latex glove extract and insect venoms share IgE-epitopes. The pre-dominant inhibition of IgE-binding to latex glove extract after pre-adsorption with the insect venoms confirms that insect venoms were the primary sensitizer in the examined patients. These qualitative findings of common epitopes in insect venoms and NRL were confirmed by the quantitative findings in CAP FEIA inhibition. The insect venoms achieved a 74–100% inhibition of the IgE-binding to the latex allergens and vice versa, the latex pre-adsorption resulted in a 29% inhibition of bee venom-specific IgE-binding and 3–17% inhibition of *Vespula* spp.-specific IgE binding in the patients primarily sensitized to the respective venom.

Periodate-sensitive IgE epitopes in the insect venoms and the latex glove extract indicated the possible involvement of carbohydrates in the constitution of cross-reactive epitopes. In contrast, the epitopes recognized by a symptomatic latex-allergic control were not periodate-sensitive, indicating different subsets of latex-specific IgE antibodies. The absence of IgE binding to relevant recombinant latex allergens and the detectable IgE binding to bromelain (MUXF-type) and HRP(MMXF-type) (Table 3) in our patients further confirms the carbohydrate nature of the IgE-binding structures. Anti-carbohydrate antibodies were assumed to be most often of low clinical relevance; however, no general conclusion can be drawn, as certain carbohydrate components were shown to be responsible for histamine release from blood cells in hypersensitive patients [27, 38–40]. Recently, the specific nature of IgE binding to the glycans could be shown that, for a given individual, may be relevant whereas irrelevant for another [25, 41]. Recently, the possible participation of the carbohydrate epitopes of Hev b 2 isoenzymes in their IgE-binding capacity and cross-reactivity was investigated [42]. IgE antibodies in latex-positive sera chiefly recognized the glycosylated isoforms; however, the non-glycosylated isoform as well as the glycosylated isoforms produced positive results in a skin prick test. Carbohydrate epitopes in Hev b 2 were assumed to be relevant to an *in vitro* diagnosis of latex allergy and accompanying cross-reactivity; however, as carbohydrate epitopes do not necessarily provoke allergic symptoms, additional

in vivo tests were recommended [42]. Hev b 2-specific IgE binding was not detected in our patients.

In concordance with the data described by Ebo et al. [7]. All of our patients with clinically irrelevant IgE for latex exhibited a negative skin prick test to latex. In contrast, under the highly sensitive conditions of intradermal testings recommended for the detection of patients with low-grade sensitization and for evaluation of allergenic extracts of low potency [16], faint skin reactions were observed, reflecting a poor biologic activity of these antibodies. Intradermal testing is the most sensitive method of skin testing, but it also induces more false positive reactions than the skin prick test [16]. Irritative reactions were excluded by intradermal testing of non-allergic controls that were negative. The authors assume a sub-clinical sensitization in these individuals, as none of the individuals exhibited clinical symptoms of a Type-I allergy to NRL, at the time of examination. The role of the weakly positive intradermal test results is unclear at the given time. Condoms and/or protection gloves (used at work or in a household setting) made from NRL did not elicit clinical symptoms in the respective individuals. A follow-up of these insect-allergic individuals without symptoms but with positive antibodies and intradermal test for latex is pre-planned for the following years to find out whether the clinical findings will change.

In conclusion, phylogenetically unrelated natural rubber latex and insect venom share CCD epitopes (particularly $\alpha(1,3)$ -fucose containing ones as venoms do not contain $\beta(1,2)$ -xylose residues) that might interfere with correct serological diagnosis [7, 25, 37, 41]. However, cases with independent cosensitization to insect venom and natural rubber allergens may eventually occur and require concise preventive measures to latex.

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