

Prevention of latex allergy by selection of low-allergen gloves

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Summary

Background In recent years the prevalence of type I allergy to latex has continuously increased, in particular among healthcare workers, to about 10%. While most forms of type I allergy caused by other environmental allergens can be treated by pharmacotherapy or specific immunotherapy, minimizing exposure to latex proteins may represent an effective preventive measure for latex allergy.

Objective To investigate whether it is possible to select by *in vitro* and *in vivo* testing low-allergen latex gloves for prevention of latex allergy.

Methods We obtained separate extracts by standard aqueous extraction from the inner and outer surfaces of 15 different commonly used (10 examination, five surgical) glove brands. The extracts were analysed by quantitative (bicinchoninic protein assay, immunoglobulin [Ig] E-ELISA, ELISA competition) and qualitative (SDS-PAGE, silver staining, IgE immunoblotting) methods for their protein and allergen contents. In addition, the glove extracts were analysed for their capacity to induce basophil histamine release and immediate skin reactions.

Results Extracts from different glove brands contained cross-reactive IgE epitopes. However, IgE binding studies, basophil histamine release and skin testing showed that different glove brands and their inner and outer surfaces contained widely varying protein and allergen contents. While the determination of total protein contents was not sufficient to identify low-allergen gloves, IgE measurements, basophil histamine release and skin testing were in good agreement and allowed to select low-allergen products.

Conclusion We suggest the use of low-allergen latex products identified by IgE binding, basophil histamine release assays and skin testing as a feasible preventive measure for latex allergy.

Keywords: allergen content, basophil histamine release, ELISA, immunoglobulin E immunoblotting, latex gloves, latex allergy, latex allergy prevention, skin testing

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Introduction

Latex products (e.g. medical products such as gloves, catheters, many office and household items) are manufactured from natural rubber obtained from the tropical tree

Hevea brasiliensis. Due to a more wide-spread use of latex products, in particular gloves, among healthcare workers, the prevalence of type I allergy to latex continuously increased in this risk group during the last few years [1,2]. Depending on the examined cohort, the prevalence of sensitization ranges from 3 to 12% in hospital workers [3]. However, natural rubber latex (NRL) allergy is also an important health issue beyond the known risk groups:

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latex-specific immunoglobulin (Ig) E antibodies were found in 6.4% of the sera of unselected healthy blood donors [4] and recently, a clinically relevant latex sensitization was found in 2.6% of 493 patients not belonging to known risk groups [5]. Sub-clinical sensitization (i.e. positive skin prick test or latex-specific IgE in the serum without clinical manifestations) was found in 24.4% of the same cohort.

In most symptomatic latex-allergic patients, localized immunological contact urticaria or rarely generalized weal-and-flare reactions with burning and itching predominate [6]. In addition to contact urticaria, the IgE-mediated allergic contact dermatitis represents a second cutaneous manifestation of immediate-type allergy to latex proteins [7].

The immediate type I skin manifestations (e.g. contact urticaria) result from the allergen-induced cross-linking of allergic effector cells (e.g. mast cells, basophils) [8] while the more chronic skin reactions (e.g. IgE-mediated contact dermatitis) require allergen-specific T-cell activation, a process which is greatly enhanced when allergens are presented by professional antigen-presenting cells (e.g. monocytes, dendritic cells) via specific IgE antibodies [9].

Affection of mucous membranes results in rhinitis, conjunctivitis, asthma or even life-threatening anaphylactic reactions (stages III and IV according to the von Krogh and Maibach classification [10]). Anaphylactic reactions and occupational impairment due to contact with latex products emphasize the need to develop therapeutic strategies for latex allergy.

So far more than a dozen different latex allergens have been characterized by immunochemical and molecular biological methods [11]. Many of them represent plant defence or structural proteins that share IgE epitopes with plant food as well as pollen allergens. Pharmacotherapy and specific immunotherapy may represent the most relevant form of therapy for manifestations of type I allergy induced by other environmental allergens (e.g. pollen, mite, animal, mould allergens). However, they are either less suited or unavailable for the treatment of latex allergy. Avoidance of latex products has therefore been suggested as an effective primary treatment measure of latex allergy [12]. However, latex gloves cannot be completely avoided since they provide better protection against blood-borne pathogens than non-latex glove alternatives [13].

In this study we used different *in vitro* (protein determination, IgE binding, basophil histamine release) and *in vivo* (skin prick testing) methods for the identification of low-allergen latex glove brands. We obtained separate aqueous extracts from the inner and outer surface of 15 different latex glove brands for determination of their protein and allergen content. Sera from 33 latex-allergic individuals were used to analyse latex allergens in the extracts obtained by IgE immunoblotting and ELISA. The presence of

common IgE epitopes in the different glove brands was determined by IgE ELISA inhibition assays. Four extracts from gloves containing varying amounts of IgE-reactive moieties were further evaluated for their allergenic potential by basophil histamine release and skin prick testing to compare serological and biological test results. Our finding that IgE measurements, basophil histamine release and skin prick testing allowed the identification of low-allergen latex products, suggests the selection and use of low-allergen gloves as a feasible preventive strategy against latex allergy.

Materials and methods

Characterization of latex-allergic patients

Patients with latex allergy ($n = 33$) were characterized by a case history indicative of latex allergy, positive skin prick test results, and/or positive RAST results. Serum from a healthy nonallergic individual was included as negative control. A pollen-allergic individual not allergic to latex served as negative control in the skin prick test experiments. Table 1 describes the demographic, serological (total IgE and RAST values) and clinical characterization of the latex-allergic patients and the control individual in detail. The 33 patients with latex allergy (22 women, 11 men) were included in the study in the order of their appearance in the clinics as evidenced by positive case history and/or skin prick results. The average age of patients was 35 years (<20 years: four patients; 21–30 years: 10 patients; 31–40 years: eight patients; 41–60 years: 10 patients; >60 years: one patient). Fifteen patients were healthcare workers, two patients suffered from urogenital or neurological disorders (e.g. spina bifida) and the remaining 16 patients had professions which frequently are not associated with sensitization to latex.

Protein extracts

Five surgical and 10 examination glove brands (Table 2) currently used in the General Hospital of Vienna were collected. Brand A was polymer-coated at the inner surface. Eight brands were labelled to be 'hypoallergenic'. This term was developed to describe a reduced potential for developing type IV allergies to rubber additives included during the production of the product. To prevent confusion with regard to the capacity of natural rubber products to induce type I allergies this term should not be used any longer [14]. Aqueous extracts were separately obtained from the outer and inner glove surfaces according to the recommendations of the American Society for Testing and Materials (ASTM D 5712–95, 1995) [15]. In brief, two medium-sized pairs of each brand were sealed with a string at the proximal wrist

end of each glove, covered with exactly 200 mL of distilled water alone for skin prick testing, or with 5 mmol/L phenyl-methylsulphonyl fluoride (PMSF, Sigma, St Louis, MO, USA) for *in vitro* experiments, in a 1 L Erlenmeyer flask under agitation (260 r.p.m.) at 37°C for 2 h. Extracts of the inner glove surface were prepared in the same way after turning the inner surface out. Glove extracts were centrifuged at 4000 g for 30 min to remove insoluble particles, lyophilized and each redissolved in exactly 2 mL of phosphate-buffered saline (PBS) to yield the 'stock solution'.

Aliquots of 0.5 mL of ammoniated latex milk were extracted in an equal volume of sodium dodecyl sulphate (SDS) sample buffer [16].

Determination of protein content: micro BCA protein assay, SDS-PAGE and silver staining

Total protein concentrations were measured with the micro BCA (bicinchoninic) protein assay (Pierce, Rockford, IL, USA) in the glove extracts. Dilutions of bovine serum albumin (BSA) were used as protein standard solutions. The absorbance corresponding with the protein concentration was determined at 562 nm by spectrophotometry (Hitachi U-2000 spectrophotometer, Tokyo, Japan). The protein concentration is expressed in grams of protein per litre of glove extract and thus corresponds with the amount of protein extracted from the outer or inner surface of one pair of gloves (Table 2).

Thirty-microlitre aliquots of each glove extract stock solution were separated by analytical 12.5% SDS-PAGE [17]. Extracts from ammoniated latex milk were loaded on each gel as controls (data not shown). A Rainbow Marker (Amersham, UK) was used as a molecular weight standard. Proteins were visualized by silver-staining [18].

IgE immunoblotting

Three hundred-microlitre aliquots of each glove extract stock solution were separated by preparative 12.5% SDS-PAGE [17]. Proteins were blotted onto nitrocellulose (Schleicher & Schuell, Dassel, Germany) [19]. Nitrocellulose strips of 4-mm width were blocked twice for 5 min and once for 30 min in buffer A (50 mmol/L sodium phosphate, pH 7.5, 0.5% v/v TWEEN-20, 0.5% w/v BSA, 0.05% w/v NaN_3). Nitrocellulose strips containing blotted latex extracts were incubated with 1:10 in buffer A-diluted sera or with buffer alone, overnight at 4°C. To cover a rather complete allergen spectrum, blots were incubated with sera from seven different latex-allergic individuals. Sera were not pooled to avoid dilution of individual specificities. After two washes in buffer A for 5 min and one

for 30 min, bound IgE was detected with ^{125}I -labelled antihuman IgE antibodies (Pharmacia RAST, Uppsala, Sweden) diluted 1:10 in buffer A overnight at room temperature. Strips were washed as above, dried and exposed to Kodak X-OMAT films using intensifying screens (Kodak, Heidelberg, Germany).

Determination of total and allergen-specific IgE levels

Total IgE and latex-specific antibodies were determined using the CAP RAST FEIA system (Pharmacia). RAST classes and kilo units of antigen per litre (kUA/L) are related as follows: <0.35 kUA/L = RAST class 0; 0.36–0.7 kUA/L = RAST class 1; 0.8–3.6 kUA/L = RAST class 2; 3.7–17 kUA/L = RAST class 3; 18–50 kUA/L = RAST class 4; 51–99 kUA/L = RAST class 5; >100 kUA/L = RAST class 6.

Latex-specific IgE antibodies were further measured by ELISA (Table 3). ELISA plates (Nunc-Maxisorb[®], Nunc, Roskilde, Denmark) were coated overnight at 4°C with 100 μL /well of equally PBS-diluted glove extracts (3 μL stock solution/100 μL PBS) obtained from inner glove surfaces of brands B, E, F, I, J, O, which represented different IgE binding patterns and protein contents. After two washings with 200 μL PBS, 0.05% v/v Tween-20, wells were blocked with 200 μL blocking solution (PBS, 0.05% v/v Tween-20, 1% w/v BSA) for 2 h. Sera were diluted 1:10 in PBS 0.05% v/v TWEEN-20, 0.5% w/v BSA and 100 μL aliquots added to each well and incubated overnight at 4°C. After five washings with 200 μL PBS, 0.05% v/v TWEEN-20, bound IgE antibodies were detected with mouse monoclonal antihuman IgE antibodies (Pharmingen, San Diego, CA, USA) diluted 1:1000 and a peroxidase-coupled sheep antimouse Ig antiserum (Amersham, Amersham, UK) diluted 1:2000 in PBS, 0.05% v/v TWEEN-20, 0.5% w/v BSA. The optical density was determined at 405 nm in an ELISA reader (Dynatech MR 7000, Denkendorf, Germany) after 30 min of incubation in the dark at room temperature. Results (Table 3) are expressed as mean OD values of duplicate determinations. They correspond with the amount of bound IgE antibodies.

IgE ELISA inhibition experiments

ELISA plates were coated with extracts obtained from the inner surfaces of glove brands B, E, F, I, J, O as described for the IgE ELISA experiments above. Serum from patient 20 (Table 1) which contained the highest latex-specific IgE levels was selected as a representative serum for the IgE competition experiments. Serum aliquots of 260 μL were diluted 1:5 in PBS, 0.05% v/v TWEEN-20, 0.5% w/v BSA and pre-incubated overnight at 4°C with 100 μL

Table 1. Demographic, serological and clinical characterization of latex-allergic patients and a control individual

Patient no.	Age (years)	Sex	Profession	Total IgE (kU/L)	Latex CAP (kUA/L)
1	34	F	Florist, housewife	658	11.3
2	27	F	Laboratory assistant	561	1.49
3	45	M	Scientist	>2000	3.49
4	45	F	Cleaner	98.4	5.04
5	24	F	Nurse	41.6	9.35
6	25	F	Nurse	48.9	1.68
7	27	M	Economy student	>2000	6.9
8	38	F	Laboratory assistant	8.43	1.51
9	52	F	Saleswoman (meat)	16.7	2.57
10	22	M	Medical doctor (anaesthesia)	>5000	78.3
11	34	F	Housewife	562	5.25
12	46	F	Hairdresser	117	1.31
13	26	M	Medical student	1.18	2.29
14	20	F	Spina bifida patient	Not done	RAST 4
15	23	F	Medical student	491	0.88
16	39	M	Bricklayer	705	RAST 4
17	43	F	Cleaner	304	11.6
18	27	M	Medical student	462	4.65
19	72	F	Teacher	578	5.41
20	55	M	Retired	2414	>100
21	39	F	Cleaner	87	2.51
22	2	M	Child (3 surgical procedures)	339	9.23
23	52	M	Nurse	>5000	2.99
24	33	F	Nurse	206	40.1
25	29	F	Nurse	45.9	2.47
26	29	F	Housewife	60.7	2.2
27	16	F	Student	771	13.6
28	45	F	Technician	105	<0.35
29	48	F	Housewife	Not done	12.2
30	34	F	PhD student (biology)	468	0.61
31	20	M	Post-office worker	164	4.62
32	44	M	Entrepreneur	>2000	4.66
33	32	F	PhD student (biology)	59.2	<0.35
Normal serum	35	M	Scientist	37.8	<0.35

stock solution of each of the glove extracts or, for control purposes, with PBS containing 0.5% w/v BSA alone. Hundred-microlitre aliquots of the pre-adsorbed sera were then exposed to each well of the coated plates and bound IgE was detected as described for the IgE ELISA. The percentage of inhibition of IgE after pre-incubation with latex extracts was calculated as follows:

$$\% \text{ inhibition} = \frac{100 - (\text{OD}_{\text{extract}} \times 100)}{\text{OD}_{\text{PBS}}}$$

OD_{PBS} and OD_{extract} represent the optical density after pre-incubation with PBS and glove extract, respectively.

Histamine release experiments

Granulocytes were isolated from heparinized blood samples of a representative symptomatic latex-allergic individual (Table 1: patient 2) and a nonatopic individual by dextran sedimentation [20]. Cells were incubated with various dilutions (1:10², 1:10³, 1:10⁴, 1:10⁵, 1:10⁶) of the 'stock solutions' prepared from glove brands B, E, I and O (Table 2) representing high, intermediate and low-allergen gloves in the IgE immunoblotting (Fig. 1). For control purposes cells were incubated with a monoclonal antihuman IgE antibody E 124-2-8 (Immunotech, Marseille, France) for 30 min at 37°C, and then centrifuged at 4°C. Histamine released into the supernatant was measured by

Other known allergens	Clinical symptoms
Tree, weed, grass pollen, fruits, vegetables, mite	Contact urticaria
Birch, grass pollen, cat, dog, mite	Anaphylactic shock, angioedema, atopy
Tree, weed, grass pollen, cat, dog, mite	Contact urticaria, atopic dermatitis
Kiwi fruit, banana	Angioedema, urticaria, conjunctivitis, dyspnoea
Chestnut	Contact urticaria, rhinoconjunctivitis, dyspnoea
Tree, grass pollen, kiwi, avocado, banana, apple, paprika	Contact urticaria, conjunctivitis, dyspnoea
Tree, grass, weed pollen, cat, dog, mite	Contact urticaria, atopic dermatitis
Grass pollen	Contact urticaria, atopy
–	Contact urticaria
–	Contact dermatitis, atopic dermatitis
–	Contact urticaria, atopic dermatitis
Grass pollen	Sub-clinical sensitization
Mite	Contact urticaria, atopic dermatitis
Birch, grass pollen, mite	Contact urticaria, dyspnoea, collapse
–	Sub-clinical sensitization atopic skin diathesis
Birch, grass pollen	Sub-clinical sensitization, atopy
Cat, birch pollen	Contact dermatitis, urticaria, atopy
Birch, grass pollen	Contact urticaria
Grass pollen	Sub-clinical sensitization, atopy
–	Urticaria, atopic skin diathesis
Grass pollen, cat	Contact urticaria, rhinitis, asthma
Bee	Sub-clinical sensitization
–	Contact urticaria, contact dermatitis
Grass pollen	Contact urticaria rhinitis, asthma
–	Contact urticaria, conjunctivitis
Banana, bee, wasp	Sub-clinical sensitization, atopy
Grass pollen, rye	Contact urticaria, atopy
Birch, grass pollen, banana	Sub-clinical sensitization, atopy
Banana, maracuja	Contact urticaria, atopy
Grass pollen	Contact urticaria, atopy
–	Contact urticaria
Mugwort, birch pollen	Contact urticaria
Birch pollen	Atopy, eczema (dorsum of hands)
Healthy nonallergic individual	

radioimmunoassay (Immunotech). Total histamine was determined after cell lysis in distilled water and freeze thawing of the cells. Results are expressed as mean values of triplicate determinations and represent the percentage of total histamine (Fig. 2).

Skin prick testing

After written consent was obtained from the volunteers, skin prick tests were conducted in accordance with the recommendations of the European Academy of Allergy and Clinical Immunology [21] in a representative symptomatic latex-allergic patient (Table 1: patient 3) and, for

control purposes, in a birch pollen-, mite- and mouse dander-allergic patient without latex allergy. Aqueous extracts (stock solutions) from inner surfaces of glove brands B, E, I, O were diluted in serial 1 : 4 steps in 0.9% NaCl up to a dilution of 1 : 1024. Extracts B, E, I were skin pricked from 1 : 1024 (containing a total protein content of 13 µg/mL in B, 10 µg/mL in E and I) to 1 : 32 (containing a total protein content of 430 µg/mL in B, 328 µg/mL in E and 325 µg/mL in I). The dilution 1 : 32 in the glove extracts thus covered the range of protein contents (300–400 µg/mL) described to be of highest sensitivity [22]. Glove extract O was skin prick tested in dilutions from 1 : 1024 (1.5 µg/mL total protein) to 1 : 4 (400 µg/mL total protein).

Glove brand	type*	Hypo-allergenic label?	Powdered?	Protein content (mg/mL)	
				(outer surface)	(inner surface)
A	S	Yes	No	2.0	1.2
B	S	Yes	Yes	6.0	13.8
C	S	Yes	Yes	4.9	5.4
D	E	No	Yes	4.0	10.5
E	E	No	Yes	5.6	10.5
F	E	Yes	Lightly	5.1	6.0
G	E	No	No	2.3	2.4
H	E	No	No	3.0	2.6
I	E	No	Yes	3.2	10.4
J	S	Yes	No	1.1	0.8
K	S	Yes	No	1.5	1.0
L	E	Yes	Lightly	2.0	5.0
M	E	Yes	No	1.0	0.8
N	E	No	No	0.7	0.7
O	E	No	No	1.0	1.6

* Glove type: S = surgical, E = examination (E).

Table 2. Characterization of examined gloves.

Table 3. IgE-ELISA results of latex-allergic patients in comparison with total protein contents

	Glove brand					
	B	E	F	I	J	O
Protein content (mg/mL)	13.8	10.5	6	10.4	0.8	1.6
<i>Patient</i>						
1	0.012	0.002	0.012	0.010	0.016	0.005
2	0.016	0.014	0.017	0.023	0.008	0.013
3	0.013	0.005	0.012	0.014	0.011	0.006
4	0.008	0.005	0.017	0.028	0.011	0.013
5	0.008	0.008	0.011	0.014	0.009	0.006
6	0.007	0.008	0.016	0.011	0.015	0.010
7	0.020	0.006	0.015	0.007	0.025	0.009
10	0.061	0.031	0.078	0.118	0.024	0.015
14	1.686	1.100	1.598	1.720	0.180	0.538
16	0.338	0.251	0.283	0.177	0.156	0.116
20	2.166	0.985	1.613	1.300	0.109	0.373
22	0.147	0.095	0.280	0.133	0.070	0.066
29	0.120	0.031	0.102	0.053	0.027	0.033
Mean OD*	0.354	0.195	0.312	0.278	0.051	0.093

* The OD values corresponding with the IgE reactivity of the patients to glove extracts (B, E, F, I, J and O) are displayed. The mean IgE reactivities (mean OD) of the 13 sera to each of the glove extracts are shown in the bottom line.

Results

Skin manifestations predominate in latex-allergic patients

Table 1 displays the demographic, clinical and serological characterization of 33 latex-allergic patients. Twenty-four patients reported immediate symptoms after contact with latex products. Among these, 14 showed solely contact urticaria (contact urticaria syndrome stage I [10]) when wearing gloves, two had generalized urticaria or angioedema (contact urticaria syndrome stage II), six additionally suffered from rhinoconjunctivitis or dyspnoea (contact urticaria syndrome stage III), two patients collapsed (contact urticaria syndrome stage IV) when exposed to latex.

Four patients (patients 10, 17, 23 and 33) developed eczematous reactions in exposed skin areas: half of these reactions developed after a preceding immediate reaction (patients 17 and 23), and half occurred without a preceding immediate reaction (patients 10 and 33). One patient (patient 33) reproducibly exhibited characteristic hand eczema after contact, but a negative RAST to latex. By immunoblotting, serum from this patient reacted with bands in two glove extracts (gloves B and I) thus confirming an IgE-mediated sensitization (data not shown). History and skin prick testing showed that 61% (20/33) of patients were sensitized to aeroallergens and 21% (7/33) to cross-reactive fruits. In seven patients (patients 12, 15, 16, 19, 22, 26 and 28) a sub-clinical sensitization to latex without manifestation of clinical symptoms was diagnosed during routine prick testing. The cutaneous manifestations of latex

allergy thus represented by far the most predominant symptoms of latex allergy in our patients.

Different glove brands and their inner and outer surfaces contain widely varying amounts of proteins

In the present study we investigated 15 glove brands (Table 2: five surgical, 10 examination gloves; seven powdered, eight non-powdered) for their protein and allergen contents using a standardized aqueous extraction procedure. We found that the investigated glove brands, both surgical and examination gloves, contained widely varying protein contents ranging between 0.7 mg/mL to 13.8 mg/mL (Table 2). The protein content was higher in the powdered gloves (gloves B, C, D, E, F, I and L) than in the non-powdered gloves (gloves A, G, H, J, K, M, N and O) (Table 2). We also observed that the protein contents were highest on the inner surfaces of powdered gloves (Table 2).

Varying amounts of allergenic and nonallergenic proteins of different molecular weights can be detected in different glove brands and their inner and outer surfaces

The determination of protein contents in latex gloves has been suggested as a method to identify low-allergen gloves [12]. When we analysed aqueous extracts separately obtained from the inner and outer surfaces of 15 different glove brands for the presence of allergens and nonallergenic proteins, we found the following.

1 Extracts from different glove brands (Fig. 1) and the inner and outer surfaces of the same glove brand (Fig. 1) contained widely varying amounts of proteins of different molecular weights as demonstrated by silver staining, e.g. gloves B and I contained proteins of a wide molecular weight range (5–50 kDa), whereas in extracts from gloves C, D, E low molecular weight moieties (5–15 kDa) only, and in extracts from gloves G (Fig. 1) and H (data not shown) high molecular weight moieties (approximately 50 kDa) only were stained. No proteins were stained in extracts from gloves A, F, J, K and O (Fig. 1) and L, M and N (data not shown). Little, or no protein was stained in extracts from the outer surfaces of gloves C, D, E and I, whereas extracts from their inner surfaces contained proteins detectable by silver staining.

2 Many of the proteins visualized by silver staining (e.g. low molecular weight moieties in three gloves [C, D and E] and high-molecular weight moieties in two gloves [G and H]) failed to react with IgE antibodies (Fig. 1). This result indicates that many of the extracted proteins do not represent allergens and that the allergenic components often constitute a minor fraction of the total proteins extracted.

3 Sera from the latex-allergic patients (Fig. 1: lanes 1–7) reacted strongly with extracts from the inner and outer surface of glove B (Fig. 1b), weaker with extracts from the inner surfaces of five gloves (C, D, E, F and I) and failed to react with eight other gloves (G, H, J, K, L, M, N and O). Extracts from the inner glove surfaces of gloves C, D, E, F and I contained more allergens than extracts from their outer surfaces.

4 Extracts from different glove brands contained IgE-reactive moieties of comparable molecular weights. Sera from two patients (patients 1 and 7) reacted with 46–50 kDa moieties in four glove brands (B, C, D, F). Sera 2 and 5 bound to bands in the same molecular weight range in gloves B, D, E and I.

5 Neither the patterns of antigens detected nor the intensity of IgE reactivity was associated with particular clinical manifestations, e. g. a patient (patient 4) with low or no IgE reactivity and three patients (patients 2, 5 and 6) with strong IgE anti-latex immunoreactivity exhibited severe and systemic reactions to latex products (Table 1; Fig. 1).

In summary, the data show that allergens can be identified by IgE immunoblotting, but not by protein staining alone.

IgE ELISA measurements allow the identification of glove brands with low-allergen contents

The results obtained by IgE immunoblotting were compared with ELISA measurements. Glove brands containing high (Fig. 1B), lower (Fig. 1E,F,I) and no (Fig. 1J,O) blot-detectable allergens were analysed by IgE ELISA (Table 3). Sera from 13 latex-allergic patients were exposed to ELISA plates coated with extracts from the inner glove surfaces. The bound latex-specific IgE antibodies were detected. The ELISA results confirmed those of IgE immunoblotting. For example, glove brand B was found by immunoblotting and ELISA to contain most allergens (Fig. 1, Table 3). Although this glove brand contained most protein, the IgE binding capacity of other glove brands was not always proportional to their protein contents. Brand F contained less proteins than glove brands E and I but had higher allergen contents (Tables 2 and 3). Gloves J and O containing no detectable allergens according to IgE immunoblotting also showed weak or no IgE binding capacity in the IgE ELISA (Fig. 1, Table 3). The fact that sera with rather low latex-specific IgE levels (sera 1–7; Tables 1 and 3) reacted weakly or not in the ELISA but gave clear results in the IgE immunoblot (Fig. 1) is likely due to the higher sensitivity of the immunoblot. In summary, IgE immunoblotting and ELISA delivered comparable results: both methods identified the same glove brands as the ones containing low-allergen contents. The protein contents alone, were often, but not always, correlated with the allergen contents.

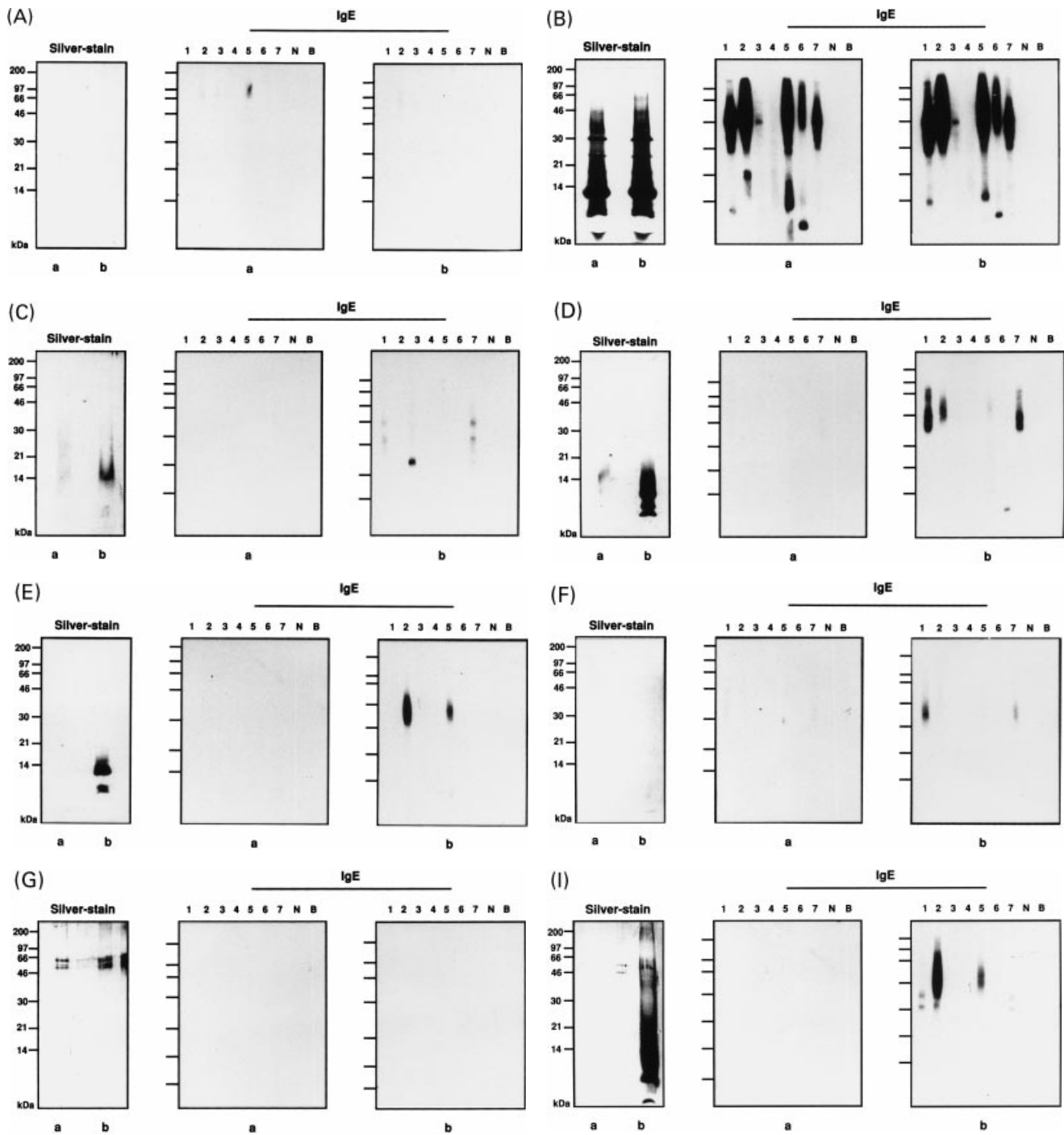


Fig. 1. Characterization of extracts from various glove brands (A–G, I–K, O) by SDS-PAGE, silver staining and IgE immunoblotting. Protein extracts obtained from the outer (a) or inner (b) glove surfaces of glove brands A–G, I–K, O were separated by SDS-PAGE, silver stained or exposed to serum IgE from latex-allergic patients (lanes 1–7), a nonallergic individual (lane N) or buffer alone (lane B).

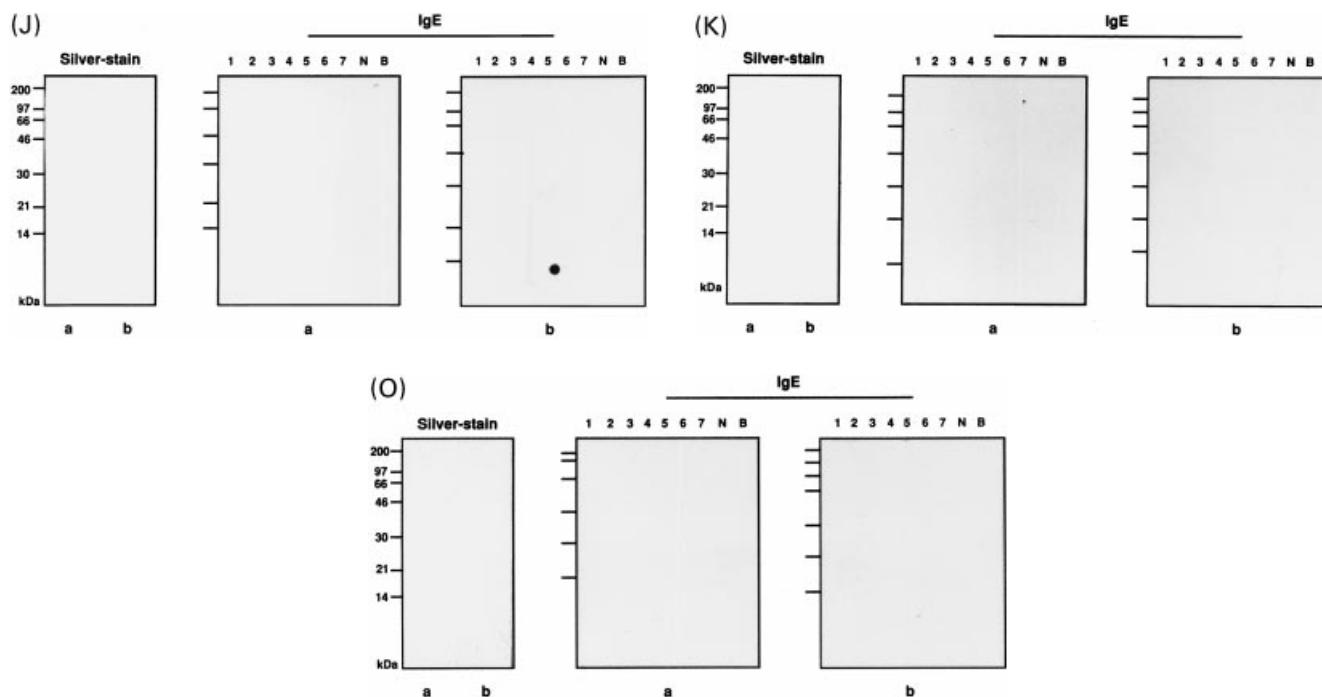


Fig. 1. continued

Different glove brands share IgE epitopes

The fact that extracts from different glove brands contained IgE-reactive moieties of comparable molecular weight indicated the presence of common IgE epitopes in these extracts (Fig. 1). We therefore investigated by IgE ELISA competition assays the presence of common IgE epitopes in the various extracts. When serum from a high-titre latex-allergic individual (Table 1: serum 20) was pre-absorbed with extracts from six different gloves (B, E, F, I, J and O) we found that:

- 1 pre-incubation with three glove extracts (B, F and I) inhibited binding to each other in the range from 83–88%;
- 2 pre-incubation with glove extract E inhibited binding to a lower degree 62–76%; and
- 3 the weakest inhibition (in the range of 40%) was observed after pre-incubation of the serum with extracts J and O. These results show that extracts from different glove brands contained cross-reactive IgE epitopes.

Extracts from latex gloves with low-allergen contents have a reduced capacity to induce basophil histamine release and immediate skin reactions

In order to investigate whether gloves with high- and low-allergen contents exhibit a different capacity to elicit

biologically relevant reactions, basophil histamine release assays and skin prick tests were performed. We found that glove extracts with medium to high-allergen contents (B, E and I) elicited a dose-dependent histamine release at a 5000-fold lower concentration compared with the extract prepared from a low-allergen glove O (Fig. 2a). No significant histamine release was induced from basophils of a nonatopic individual with the same extracts and dilutions (Fig. 2b). Anti-human IgE antibodies induced a dose-dependent histamine release from basophils of both donors (Fig. 2a,b: anti-IgE).

The biological significance of the *in vitro* experiments was confirmed by skin prick testing. The medium- to high-allergen glove extracts B, E and I elicited weal and flare reactions in all tested dilutions (1 : 1024 to 1 : 32) whereas low-allergen extract O failed to yield any skin reaction up to a dilution of 1 : 4 (Fig. 3a). The four latex extracts tested did not induce any skin reactions in an atopic individual without latex allergy in all dilutions (1 : 4–1 : 1024) tested (Fig. 3b). Both individuals showed clear weal reactions to birch pollen extract and histamine but failed to react to sodium chloride (Fig. 3a,b). The basophil histamine release as well as skin test experiments were in good agreement with the serological data (immunoblot, ELISA) as all of these determinations identified brand O as low-allergen latex product.

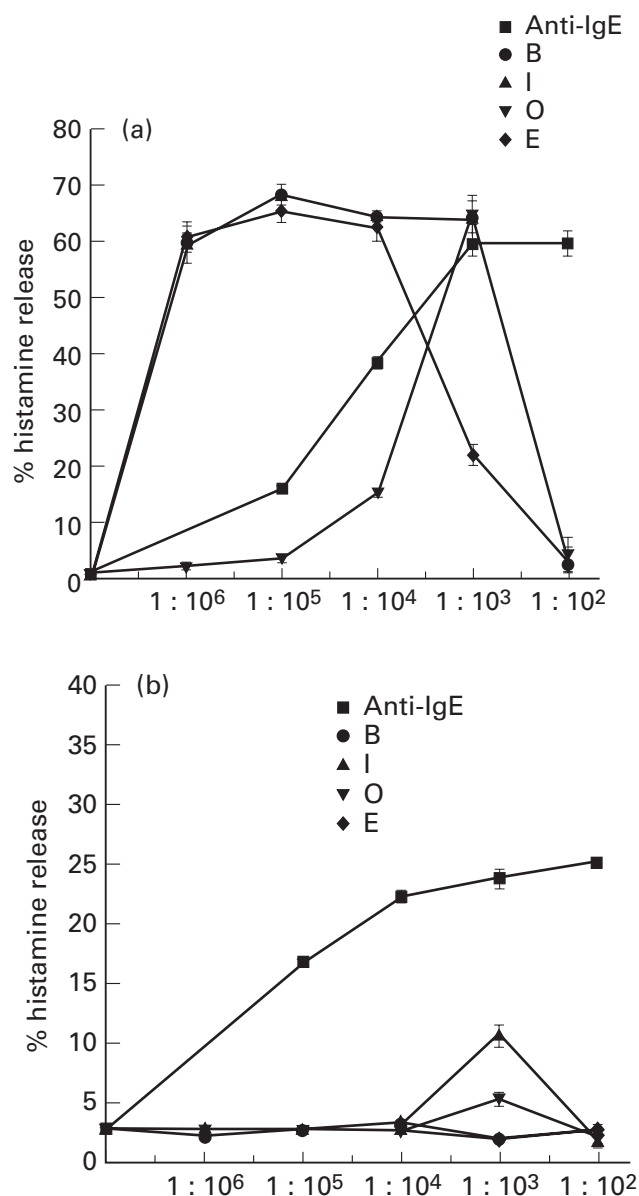


Fig. 2. Extracts from different glove brands have a different capacity to induce basophil histamine release. Granulocytes from a latex-allergic patient (a) and a nonatopic individual (b) were exposed to various dilutions (x-axis) of four latex extracts (B, I, O and E) or a monoclonal antihuman IgE antibody.

Discussion

Latex products represent important sources for localized and generalized IgE-mediated skin reactions and systemic anaphylactic reactions. Several ways of sensitization have been hypothesized. Since certain latex-allergic individuals have latex-specific IgE antibodies which cross-react with other plant proteins present in pollen or plant-derived food,

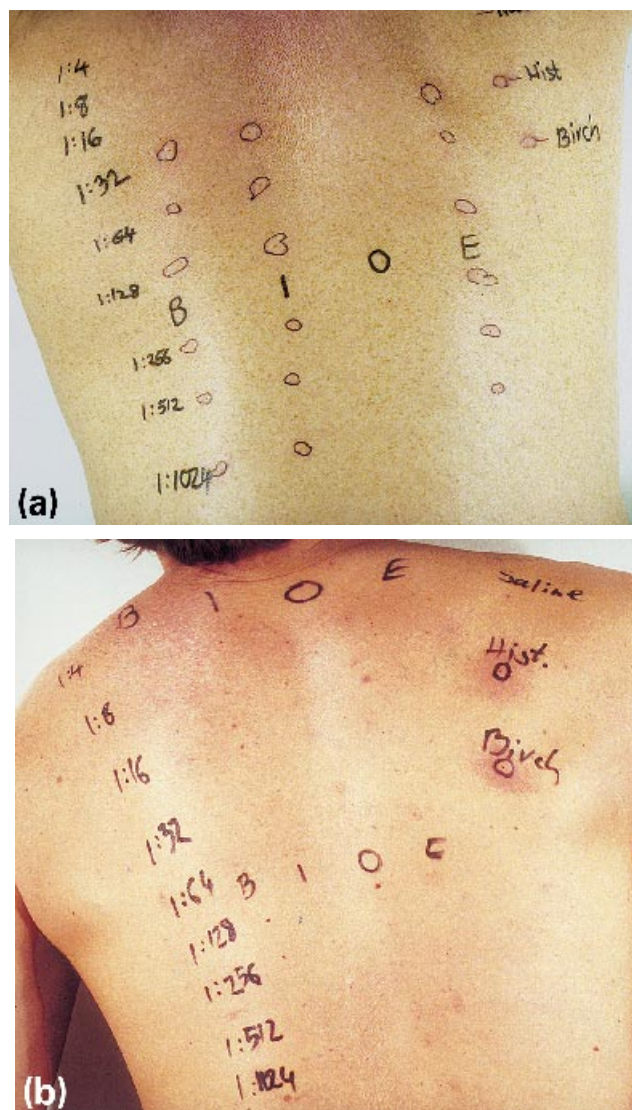


Fig. 3. Extracts from different glove brands have a different capacity to induce immediate-type skin reactions. A latex-allergic individual (a) and an allergic patient without latex allergy (b) were skin prick tested on their back. Patient A was tested with diluted glove extracts B, E and I (1:32–1:1024), O (1:4–1:1024), birch pollen extract (birch), histamine (hist.) and sodium chloride (saline; right upper corner). Patient B was tested with diluted glove extracts B, E, I and O (1:4–1:1024), birch pollen extract, histamine and sodium chloride. The weal reactions are encircled.

they may be sensitized via plant products other than latex [23]. Other latex-allergic individuals, however, seem to be sensitized primarily against latex proteins. This hypothesis is supported by several findings: first, certain of the important latex allergens, e.g. Hev b 1, the rubber elongation factor, seem to represent genuine latex proteins with little or no cross-reactive homologues in sources other than latex

[11]. Second, the prevalence of latex allergy is higher in populations with more frequent mucocutaneous exposure to latex products (patients with chronic diseases: e.g. spina bifida, healthcare workers). These data are also supported by cohort studies demonstrating an increase of clinically relevant latex allergy in dental students from 2% in the 7th semester to 10.4% in the 10th semester [24].

Although, there are first experimental trials of DNA vaccination for allergen immunotherapy using the latex allergen Hev b 5 in an animal model [25,26], and oral latex desensitization has successfully been performed in three affected healthcare workers [27], there is no effective and specific treatment of latex allergy available, so far. Since latex gloves provide the best protection against infectious agents, an unnecessary restriction from the appropriate use of latex gloves cannot be recommended [13].

In the present study we analysed by a variety of methods (protein and IgE determinations, basophil histamine release, skin prick testing) the inner and outer surfaces of 15 different glove brands for their allergen contents. The IgE immunoblot and ELISA analysis of the 15 glove brands in our study revealed that they contained widely varying protein and allergen contents. Glove brands (e.g. glove B) with extremely high-allergen contents and other gloves (e.g. glove O) without detectable IgE-reactive components could be identified. Although protein and allergen contents were frequently in parallel, many of the extracted proteins did not exhibit IgE binding capacity. Our findings, therefore demonstrate that total protein and allergen contents did not always correlate and that the determination of protein contents is not sufficient for selection of low-allergen gloves.

In most of the extracts investigated we detected by protein staining a 14-kDa protein as predominant component whereas most sera displayed IgE reactivity to a 46-kDa moiety, presumably representing an oligomeric form of Hev b 1, the rubber elongation factor [28]. Other IgE-reactive moieties were detected at 5, 10–20, 21–25, 30, 35 and above 66 kDa with great heterogeneity. The great variation of allergen profiles revealed by IgE immunoblotting seems to be due to modifications of the allergens by the manufacturing processes because IgE competition experiments indicated that most of the different glove brands investigated contained cross-reactive IgE epitopes.

Our finding that most of the latex allergens could be extracted from the inner surface of powdered gloves is of note for two reasons. First, high-allergen content on the inner glove surfaces may explain the high rate of cutaneous manifestations observed in latex-allergic individuals. Although there are no experimental data, sensitization via the epidermis may be considered as a pathogenetic factor in people who are constantly exposed to high loads of latex allergens via skin contact (e.g. healthcare workers). Secondly, powdering of gloves represents a risk factor as

it is associated with a high-allergen load which may contribute to airborne latex exposure and thus to immediate reactions in already sensitized patients, and *de novo* sensitization via the respiratory tract [29]. Support for the hypothesis that powdered gloves may have a higher sensitization capacity comes from studies showing that compared with three dental services using powdered latex gloves with moderately high extractable protein levels, in the fourth dental service using non-powdered low protein latex gloves no sensitization to latex occurred [30]. Similarly, in 1075 skin prick-negative healthcare workers followed over a 1-year period, a 1% incidence of clinically relevant symptomatic latex allergy was observed in a subgroup wearing powdered latex-gloves, whereas there was only a non-symptomatic conversion in the skin prick test in two individuals wearing non-powdered gloves of low protein content [2].

During the last few years, several rubber industries have performed profound research to reduce allergen contents in their products. By certain manufacturing changes such as dry-gel leaching in addition to wet-gel leaching, chlorination, application of proteolytic enzymes and natural rubber derived from alternative species such as *Ficus elastica* and *Parthenium argentatum* instead of *Hevea brasiliensis* [1,31–33] protein and allergen contents can be markedly reduced. In contrast with previous reports [34,35], where a repertoire of allergens as complete as in ammoniated and nonammoniated latex fluids could be demonstrated in latex glove extracts, our findings document that brands of surgical as well as examination gloves with low-allergen contents can be identified by a combination of *in vitro* methods (IgE immunoblotting, IgE ELISA, basophil histamine release). The biological relevance of our *in vitro* data was supported by *in vivo* skin test experiments, which demonstrate the greatly reduced anaphylactic activity of the low-allergen glove brands identified by the *in vitro* methods. We thus propose the identification and use of low-allergen glove brands as a preventive measure to reduce exposure to latex allergens. The lowering of latex allergen loads may lead to a reduction of symptoms in already sensitized individuals [36] and, perhaps, help to prevent *de novo* sensitization.

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