

Latex allergy diagnosis: *in vivo* and *in vitro* standardization of a natural rubber latex extract

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For the diagnosis of IgE-mediated (immediate) hypersensitivity to natural rubber latex (NRL), skin prick testing with extracts of latex gloves has been widely used, but such extracts are difficult to standardize. The present study aimed to produce on an industrial scale an NRL extract from freshly collected NRL and to evaluate, calibrate, and standardize the extract by both *in vivo* and *in vitro* testing. The source material, latex of the rubber tree, *Hevea brasiliensis* (clone RRIM 600), was frozen immediately after collection in Malaysia and shipped in dry ice to Stallergènes SA, France. Protein and allergen profiles were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), immunoblotting, isoelectric focusing (IEF), crossed immunoelectrophoresis (CIE), and crossed radioimmunoelectrophoresis (CRIE). Allergen quantification was effected by RAST inhibition. The capacity of the preparation to elicit immediate hypersensitivity reactions *in vivo* was measured by skin prick testing in 46 latex-allergic patients and 76 nonallergic control subjects. SDS–PAGE and immunoblot profiles of the extract and an NRL standard (E8) provided by the US Food and Drug Administration were almost identical, disclosing several distinct IgE-binding proteins with apparent molecular weights of 14, 20, 27, 30, and 45 kDa, conforming to reported molecular weights of several significant NRL allergens. An arbitrary index of reactivity (IR) of 100 was assigned to the extract at 1:200 dilution (w/v), having a protein content of 22 µg/ml. Skin prick testing of latex-allergic patients and controls using the extract at 100 IR revealed 93% sensitivity, 100% specificity, 100% negative predictive value, and 96% positive predictive value. In conclusion, a skin prick test reagent for diagnosis of type I NRL allergy was successfully standardized. The reagent was demonstrated to contain most, if not all, of the currently known clinically significant NRL allergens, and it showed high sensitivity and specificity.

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Immediate hypersensitivity reactions to natural rubber latex (NRL) proteins have been increasingly recognized during the past 15 years (1–3). Manufactured products, such as surgical and household gloves, catheters, condoms, baby pacifiers, and toy balloons may contain allergenic proteins capable of eliciting type I allergic reactions (4–7), and, probably, also causing sensitization of exposed subjects.

Accumulating knowledge on the potentially serious consequences of exposure to NRL proteins in sensitized patients, especially when the contact takes place via mucous membranes (5, 8, 9), has

made an accurate diagnosis of immediate-type NRL allergy increasingly important. Currently, the consensus is that the diagnosis of NRL allergy should be based on both a positive clinical history and a positive *in vivo* test result, the most reliable test being the skin prick (3, 10). Various *in vitro* tests, demonstrating the presence of specific IgE antibodies to NRL proteins, may provide valuable additional support to the diagnosis, but are not sufficient alone.

A complicating factor in establishing means for accurate diagnosis has been the multitude of

potentially significant NRL allergens discovered (2, 11–19). The most important allergen in NRL appears to be prohevein, a 20-kDa protein, against which 70–80% of adult latex-allergic patients show IgE antibodies (12). Other clinically significant allergens whose full or partial primary molecular structure has been elucidated include rubber elongation factor (REF, Hev b 1) (18), a 27-kDa NRL protein (14, 20), the 30-kDa heveamine (12), a 36-kDa NRL protein probably representing a glucanase enzyme in NRL (12), and a 46-kDa NRL protein (21). Obviously, there may be additional, currently unrecognized allergens, and the issue is further complicated by the plethora of cross-reacting allergens observed in several fruits and plants, such as avocado, banana, kiwi, and chestnut (22–27).

Although little is currently known of the exact antigenic and allergenic structures and molecular forms of NRL allergens eluting from manufactured products, it is possible that the several NRL allergens so far identified occur in the end-product, such as a surgical glove. It is well known that during the very first phases of rubber manufacturing, various amounts of ammonia are added to the raw material. Several studies have shown that ammoniated latex and latex glove extracts may contain similar allergens (2, 16, 28), but it has also been shown that both the quantity and immunologic properties of NRL proteins may alter after ammonia is added. As a consequence, the allergenic potential of ammoniated latex may change, decrease, and even be lost during storage, making standardization of such preparations difficult.

Since the crux of the clinical problem obviously lies in latex gloves, one could assume that extracts of highly allergenic gloves constitute an optimal reagent for diagnosis. Indeed, specific, sensitive, and safe extracts for skin prick testing have been prepared and extensively used, especially in Europe (3). However, tests based on glove extracts have not been standardized, and it appears that there will soon be a shortage of strongly allergenic gloves. Therefore, other source materials have to be used for standardization. The aim of the present study was to prepare, evaluate, and standardize an extract made of fresh NRL that would be suitable for latex allergy diagnosis and could be made commercially available. The various stages of this process are reported here.

Material and methods

Collection and handling of the source material for the allergen extract

Raw latex of rubber trees, collected in Teflon flasks from 15 trees of *Hevea brasiliensis*, clone

RRIM 600, was obtained from the Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia. The material was deep-frozen immediately to -70°C after collection and transported within 3 days in dry ice to Stallergènes, Fresnes, France, where it was stored at -20°C . In brief, the material was thawed, and the liquid was separated from the coagulate by pressing and ultracentrifuged at 4°C for 3 h at 100 000 g. The supernatant, designated "latex raw material", was then filtered through 0.45- μm membranes (Millipore, Saint-Quentin en Yvelines, France), dispensed as 5-ml aliquots, re-frozen, and stored at -20°C .

Preparation of in-house reference standard

The latex raw material was thawed and diluted (1:20, w/v) with a solution of 2% mannitol (Cooper, Melun, France) in distilled water. The solution was then filtered through 0.22- μm membranes (Millipore), dispensed into 1-ml aliquots, and lyophilized. The material was designated as in-house reference standard (IHRS). An NRL standard (FDA-E8) which was used for comparative analyses was obtained from the Food and Drug Administration (FDA) (USA).

Patients and sera

Reference patient sera used for in vitro characterization of allergen preparations. Reference sera were obtained from five latex-allergic patients showing IgE antibodies in immunoblotting to one or several of the most significant latex allergens, such as 14-, 20-, 27-, 30-, 45-, and 75-kDa NRL proteins. The main clinical symptoms and laboratory results of the patients are shown in Table 1. Four of the patients were adults and one was a child with spina bifida. Three sera (nos. 1–3) were from patients treated at Tampere University Hospital (Finland) and two (nos. 4 and 5) were supplied by Plasmalab International (Everett, WA, USA). Two separate

Table 1. Clinical symptoms and main laboratory findings of five latex-allergic reference patients

Patient no.	Age/sex	Main symptoms	Latex RAST (kU/l)	Total IgE (kU/l)	Associated condition
1	62/M	CU*	2.5	3335	–
2	32/M	CU	1.9	15 000	–
3	13/M	GU, FE, R, A	22	110	Spina bifida
4	29/F	CU	36	>52 000	–
5	29/M	R	17.5	>52 000	–

*CU=contact urticaria; GU=generalized urticaria; FE=facial edema; R=rhinitis; A=asthma.

bleedings were available from patient no. 4. A serum from a subject with no history of latex allergy served as a control in these experiments. A serum pool consisting of the two bleedings from patient no. 4 and of serum from patient no. 5 was used for immunoblotting after isoelectric focusing (IEF), crossed radioimmuno-electrophoresis (CRIE), and RAST inhibition.

Patients and controls subjected to skin prick testing in the biologic standardization procedure. The material comprised 46 latex-allergic patients (23 from Finland and 23 from France), all of whom had clinical histories and characteristic symptoms compatible with latex allergy. Pertinent clinical data and laboratory results of the patients are compiled in Table 2. All patients from Finland had earlier had positive reactions in skin prick testing with an eluate of latex gloves (Exona[®], Semperit, Vienna, Austria), the glove extract being prepared, and the prick test performed as previously described (10). All French patients had earlier been tested positive in skin prick testing using at least one non-standardized commercial latex extract, and all had also earlier tested positive in a commercial latex RAST. Forty-two of the patients were women and four men, ranging in age from 26 to 67 years. Twenty-two patients were health-care workers, and none of the patients had spina bifida or urogenital abnormalities. Of the 46 patients, 24 (52%) had histories of surgery and 25 of the 42 female patients (60%) had been pregnant.

The study was approved by the ethics committees of the hospitals involved, and informed consent was obtained from each patient or patient's guardian.

Latex RAST (Cap System, Pharmacia, Uppsala, Sweden) was used according to the manufacturer's instructions to measure the levels of latex-specific IgE in the patients' sera. As can be seen in Table 2, positive results (≥ 0.4 kU/l) were recorded in 41/46 (89%) of the patients.

Seventy-six healthy Finnish adults tested for suspicion of atopy but with no histories or symptoms suggesting latex allergy and with negative skin prick tests to a latex glove eluate (see above) served as controls for the skin prick test standardization procedure. Approximately 40% of the control subjects had positive skin prick tests to one or several common (nonlatex) allergens.

In vitro characterization of proteins and allergens in the extract

Measurement of total protein. Total protein content of the preparations at different phases of manufacturing was measured by the Bradford

method (Bio-Rad Protein Assay Kit, Richmond, CA, USA) according to the manufacturer's instructions, with bovine serum albumin as the reference.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. Allergen extracts before and after lyophilization were used as antigens in immunoblotting. Total protein concentrations of the antigen samples were adjusted to 1 mg/ml before SDS-PAGE, which was performed according to a method previously described in detail (14). Briefly, the samples were diluted 1:3 into sample buffer containing 2% SDS and 5% 2-mercaptoethanol. The samples were boiled for 3 min and then applied to a 13% polyacrylamide gel (170 μ l sample/20-mm-wide well in 0.75-mm-thick gel). Electrophoresis was carried out for 45 min at a constant 200 V (Mini Protean II Cell; Bio-Rad). Molecular weight protein markers (2.35–46 and 14.3–200 kDa, Amersham, UK) were run along with samples.

After electrophoresis, proteins were transferred from the gel to a 0.2- μ m pore size nitrocellulose filter (Trans-Blot, Bio-Rad) by electroblotting at 100 V for 1 h (Mini Trans-blot Cell, Bio-Rad). After blotting, the membrane was stained with Ponceau-S red to demonstrate successful transfer of proteins. The membrane was then cut into 3-mm strips, washed with Tris-buffered saline (TBS) containing 0.1% Tween-20, and incubated for 1 h in a blocking buffer containing 5% nonfat milk powder in TBS. The strips were incubated overnight at 4°C with patient sera and with control serum (diluted 1:5). The strips were then washed and incubated with biotinylated goat antihuman IgE antibody (Vector, Burlingame, CA, USA) diluted 1:1000. An extra blocking was carried out for 2 min after washing, and the membranes were then washed again. Streptavidin-conjugated alkaline phosphatase (Zymed), diluted 1:24 000 in TBS, was added and the strips were incubated for 30 min at room temperature. The strips were placed in a color-development solution (Bio-Rad) after washing, and the reaction was stopped after 8 min by rinsing in tap water.

Isoelectric focusing (IEF) in polyacrylamide gel. IEF was performed with preformed Ampholine PAG-Plates (pH 3.5–9.5), as described by the manufacturer (Pharmacia). pI markers (Text Mixture 9) were supplied by Serva (Heidelberg, Germany). Gels were stained with Coomassie brilliant blue G-250. The amount of protein applied was 40 μ g/lane for each sample.

Immunoblotting after IEF. After IEF, the proteins were transferred from the gel to 0.45- μ m pore size

Table 2. Clinical data, laboratory results, and skin prick test results for 46 latex-allergic patients used for biologic standardization of NRL extract

Patient no.	Age	Sex	Center	Main symptoms of latex allergy	Latex RAST (kU/l)	Wheal mean diameter (mm)				
						PC	NC	Latex		
								A	B	C
1	32	F	FIN	CU, E	1.70	4.0	1.0	11.0	7.2	5.0
2	47	M	FIN	CU, FE, R, loA	0.64	5.6	1.7	10.0	6.2	4.4
3	63	M	FIN	E	1.40	6.1	1.4	7.9	6.0	4.2
4	32	F	FIN	CU, E	2.70	5.5	1.7	13.7	8.9	5.4
5	52	F	FIN	CU, E	<0.4	8.9	0.7	5.3	5.3	3.6
6	52	F	FIN	CU, EA, eye	0.50	10.6	1.4	11.4	8.9	4.7
7	49	F	FIN	CU, R	4.30	5.5	2.5	6.2	4.8	4.7
8	36	F	FIN	CU, FE, R, eye	2.50	9.9	1.9	13.9	11.8	10.9
9	26	F	FIN	E	8.90	9.6	2.1	11.9	8.8	5.9
10	37	F	FIN	CU, E, FE, A, loA	15.50	7.4	1.4	19.6	12.5	11.4
11	48	F	FIN	CU, E	2.50	4.3	1.6	3.3	2.2	1.6
12	46	F	FIN	CU, E, FE	<0.4	5.6	2.0	8.5	7.3	5.2
13	26	F	FIN	CU	14.90	3.9	1.4	4.0	2.0	3.3
14	35	F	FIN	CU, E	5.30	7.2	2.4	16.0	17.4	11.0
15	51	F	FIN	CU, R, eye	<0.4	11.2	2.2	3.6	3.2	4.4
16	44	F	FIN	E	<0.4	10.5	2.2	3.5	3.5	2.9
17	36	F	FIN	CU, FE, eye	0.50	3.6	2.6	9.3	7.1	5.2
18	37	F	FIN	CU, FE, eye	6.40	6.2	2.7	16.3	12.2	9.0
19	35	F	FIN	CU, E, R, eye	2.40	6.9	2.6	12.6	10.5	6.7
20	32	F	FIN	CU, A, loA	1.20	9.5	2.1	15.8	14.5	10.5
21	40	F	FIN	CU, E	2.80	6.3	1.7	11.2	10.0	9.0
22	49	F	FIN	CU, E	<0.35	11.9	3.0	14.0	9.2	6.7
23	38	F	FIN	CU	2.00	9.2	2.4	10.6	9.1	7.5
24	30	F	FR	CU, FE	2.82	11.1	0.0	12.0	8.1	1.0
25	16	F	FR	CU, FE	1.41	7.7	0.0	9.4	7.7	0.1
26	37	M	FR	CU, FE, E, A, R	23.37	7.5	0.0	10.5	10.2	1.8
27	39	F	FR	CU, FE	36.00	7.7	0.2	11.8	7.9	6.3
28	38	F	FR	CU, R, loA	4.70	9.9	0.0	10.9	8.1	7.2
29	56	F	FR	CU, FE	17.50	9.9	0.0	15.1	13.8	11.5
30	38	F	FR	CU	3.50	10.7	0.2	14.1	10.2	9.7
31	44	F	FR	CU, FE, loA	6.70	7.9	0.0	11.7	11.4	9.0
32	36	F	FR	CU, FE, loA	2.00	8.1	0.2	9.6	8.4	6.8
33	32	M	FR	CU, FE	5.10	10.5	0.0	8.9	6.8	6.6
34	33	F	FR	CU	0.70	6.5	0.0	9.0	8.3	6.1
35	32	F	FR	CU	7.70	6.5	0.0	11.8	8.7	6.6
36	28	F	FR	CU	12.00	7.5	0.0	9.4	6.2	5.5
37	29	F	FR	CU	6.10	11.9	0.2	8.9	7.7	5.2
38	40	F	FR	CU, FE	3.50	9.2	0.0	9.3	8.0	8.5
39	64	F	FR	CU, FE	3.50	8.3	0.0	8.6	7.5	4.7
40	20	F	FR	CU	2.25	7.5	0.0	4.8	4.0	0.6
41	34	F	FR	CU, FE, E	0.90	8.4	0.0	12.2	11.9	6.3
42	27	F	FR	E	2.61	9.5	0.2	9.4	7.4	5.8
43	60	F	FR	CU	0.40	11.0	0.0	13.3	10.3	9.1
44	48	F	FR	CU, FE, loA	15.80	7.9	0.0	10.1	5.3	0.2
45	37	F	FR	CU, FE	3.40	8.1	0.0	13.2	10.8	8.9
46	26	F	FR	CU, FE, A	4.20	5.7	0.2	6.3	5.7	0.2
Geometric mean								9.7	7.6	4.3

CU=contact urticaria; FE=facial edema; R=rhinitis; A=asthma; E=eczema; loA=intraoperative anaphylaxis; eye=eye symptoms; PC=positive control; NC=negative control; FIN=Finland; FR=France; A=latex w/v 1/20; B=latex w/v 1/200; C=latex w/v 1/2000.

nitrocellulose paper (Schleicher & Schuell, Basel, Switzerland), at 0.8 mA/cm² for 1 h, with a semidry transfer kit Novablot (Pharmacia). After blotting, the membrane was stained with Ponceau-S red to demonstrate successful transfer of proteins. The membrane was then washed three times for 10 min

in TBS, incubated for 1.5 h in a blocking buffer containing 5% of nonfat milk powder in TBS, and then washed three times for 10 min in TBS with shaking.

Reference patient serum (pool of two separate bleedings from patient no. 4 and serum from

patient no. 5; Table 1), diluted 1/10 in TBS containing 0.1% Tween 20 and 1% nonfat milk powder (TTBS), was added, and the strips were incubated overnight at room temperature with continuous shaking. The strips were then washed three times for 10 min in TTBS with shaking. Then, rabbit IgG antihuman IgE antibody (Dako A/S, Copenhagen, Denmark), diluted 1/500 in TTBS, was added, and the strips were incubated for 1.5 h at room temperature with agitation. The strips were again washed and then treated by the Immun-Lite II Assay Kit (Bio-Rad) according to the method described by the supplier (chemiluminescence). The allergens were revealed after 1-h contact with X-OMAT film (Kodak). Measurement of isoelectric points of the bands was done with an image reader (Epholab, Aries program, Montrouge, France).

Crossed immunoelectrophoresis (CIE). CIE was performed according to the method described by Weeke (29) with 1% agarose gel. Briefly, 15 µg of protein was run in the first-dimension electrophoresis at 150 V for 1 h. The second-dimension electrophoresis was performed at 100 V for 16 h using 11 µl of rabbit antiserum/cm³. The rabbit antiserum raised to NRL proteins was obtained from the Department of Immunobiology, National Public Health Institute, Helsinki, Finland. Control experiments used preimmune rabbit serum.

Crossed radioimmuno-electrophoresis (CRIE). CRIE was performed as described by Løwenstein (30). Briefly, the unstained CIE preparation was incubated overnight with patient sera (nos. 4 and 5; Table 1) diluted 1:3. Specific IgE was revealed overnight at room temperature by ¹²⁵I-radiolabeled anti-IgE (Stallergènes) adjusted at 20 000 cpm/50 µl. Allergen precipitation arcs were revealed by autoradiography after 7-day contact with X-OMAT film (Kodak) at -70°C with an x-ray-intensifying screen.

Radioallergosorbent (RAST) inhibition. In brief, dilutions of latex extracts (IHRS and FDA-E8) were incubated with a pool of sera from two patients (nos. 4 and 5; Table 1) for 1 h at room temperature. An allergen (latex raw material)-coated cyanogen bromide-activated paper disk (Stallergènes) was added to each dilution and incubated for 3 h. Disks were then washed and incubated with radiolabeled mouse monoclonal anti-human IgE antibody (BioMérieux, Lyon, France) for 1 h. Radioactivity bound to the disks was measured in a gamma-counter (Gammamatic II, Kontron). The relative potency of the extract to be assayed compared to that of the reference was the ratio of the volumes producing the same inhibition level (50%).

Biologic standardization of the extract

Determination of index of reactivity (IR) by skin prick testing. Skin tests were made to determine the *in vivo* reactivity of the allergen extract. An arbitrary index of reactivity (IR) of 100 was assigned to a dilution of an allergen extract which, when evaluated by prick test with a Stallerpoint® device on 30 people sensitized to the allergen, gave a geometric mean wheal size of 7 mm in diameter. Three 10-fold dilutions of IHRS in glycerol-saline diluent (1:20, 1:200, and 1:2000; w/v) were used. The skin reactivity of these patients was tested at the same time with 9% codeine phosphate as a positive control. Patients in whom the negative control was less than 3 mm and the positive control greater than 4 mm were included. Skin tests were made for latex-allergic patients in quadruplicate with a Stallerpoint® device on the anterior face of the forearm in a predetermined order. The negative (glycerol-saline solvent) and positive (9% codeine phosphate) controls were done in duplicate. The wheals and flares were outlined after 20 min and the figures transferred with adhesive tape to test record sheets. The mean diameter of each wheal was measured. The diameters of the wheals induced by different dilutions of the allergen extract were given as geometric means.

The dilution of IHRS that caused a geometric mean wheal size of 7 mm was calculated from the equation of the dose-response curve:

$$\log \text{diameter wheal} = a + b \log \text{concentration}$$

(where diameter wheal = geometric mean of the diameter of the wheals obtained, *a* = intercept, *b* = slope, concentration = concentration of the extract (w/v) tested).

Results

Protein and allergen content and composition of the latex raw material and the in-house reference standard (IHRS)

Total protein content. The total protein content of the latex raw material found by the Bradford method was 7.5 mg/ml. In IHRS, the total protein was 219 µg/ml. When standardized at 100 IR, the protein concentration of the extract was 22 µg/ml. In the FDA-E8 extract, the total protein was 1200 µg/ml.

Total allergen activity measured by RAST inhibition. The total allergen activity of the IHRS preparation at 100 IR (protein concentration 22 µg/ml) and of the FDA-E8 standard (protein concentration 1200 µg/ml) were compared by RAST inhibition.

The total allergen activity of the FDA E8 standard was 55 times higher than that of the IHRS. When measured at the same protein concentration (219 µg/ml), latex allergen activities in both extracts were similar (approximately 1000 IR).

Characterization of proteins by electrophoresis. Total protein staining after SDS-PAGE and blotting (Total Protein Staining Kit, Bio-Rad, used according to the manufacturer's instructions) revealed a large number of protein bands (20–30) in the source material and in the IHRS (Fig. 1). For comparison, results for the NRL standard preparation obtained from the FDA (FDA-E8), as well as for another freshly collected deep-frozen in-house standard NRL preparation that has been successfully used in several studies at the National Public Health Institute, Helsinki, Finland (NPHI-Fin; see references 13, 14, 20), are shown. The staining patterns were remarkably similar for all preparations.

IgE immunoblot analysis. IgE from four reference sera showed practically identical binding patterns with both the source material and the final product (Fig. 2). More than 10 allergens could be detected in the preparations, including proteins of approximately 14, 20, 27, 30, and 45 kDa, previously reported to be significant latex allergens. Results for the two other NRL extracts (FDA-E8 and NPHI-Fin), shown for comparison, revealed very similar binding profiles, except that, with regard to the FDA-E8 preparation, serum A (containing antiprohevein IgE antibodies) showed only a faint reaction at the 20-kDa zone, and serum D (containing anti-REF IgE antibodies) did not produce visible bands at the 14-kDa zone.

Isoelectric focusing (IEF). IEF showed approximately 40 bands between pI 4 and 6.5. Immunoprint after IEF revealed bands binding IgE between pI 4 and 6.0, as well as two bands at pI 7.3 and at pI above 9.4. The IEF profiles of the source material and the IHRS as well as the FDA-E8 standard were practically identical (Fig. 3).

Crossed immunoelectrophoresis (CIE) and crossed radioimmuno-electrophoresis (CRIE). Two-dimensional electrophoresis of the IHRS against hyperimmune rabbit sera showed 22 arcs of precipitation. CRIE done with a human serum pool showed four allergen arcs (Fig. 4).

Biologic standardization of the extract

Skin prick tests with three concentrations of the IHRS (dilutions 1:20, 1:200, and 1:2000; w/v)

Total protein staining

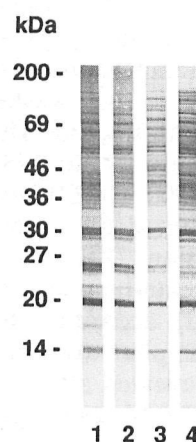


Fig. 1. Total protein staining after SDS-PAGE of four NRL extracts. Lane 1) latex raw material; lane 2) IHRS; lane 3) FDA-E8 extract; lane 4) NPHI-Fin NRL extract.

IgE immunoblot reactivity of four NRL allergic patients (A-D) against four NRL extracts (1-4)

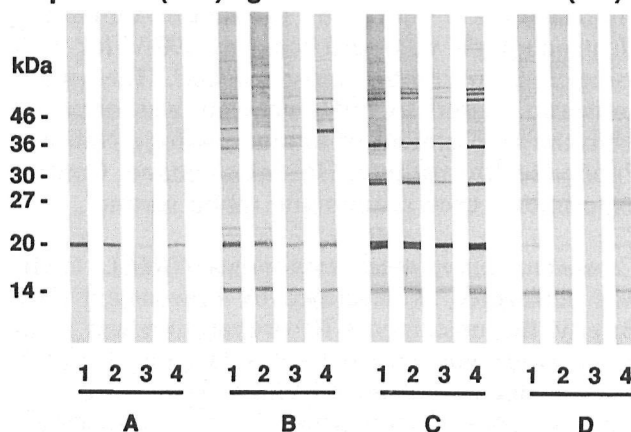


Fig. 2. IgE immunoblot analysis of sera from four NRL-allergic patients and four NRL extracts. Sera: A) serum from patient no. 1 (Table 1); B) serum from patient no. 2 (Table 1); C) serum from patient no. 4 (Table 1); D) serum from patient no. 3 (Table 1). 1) NRL extracts: IHRS, 2) final skin test reagent, 3) FDA-E8 standard, and 4) NPHI-Fin.

were made in quadruplicate on 46 latex-allergic patients. The ranges of the measured individual wheal diameters and their geometric means, including means of skin reactions from duplicate measurements to both positive (9% codeine phosphate) and negative (glycerol-saline) control solutions, are compiled in Table 2. Overall geometric means for the three IHRS concentrations used are summarized at the bottom line of the table. The concentration of IHRS that caused a geometric mean wheal size of 7 mm (to correspond to an IR of 100; see Material and methods for details) was calculated as 1:172 (w/v). The regression line of the equation obtained is depicted in Fig. 5. An IR

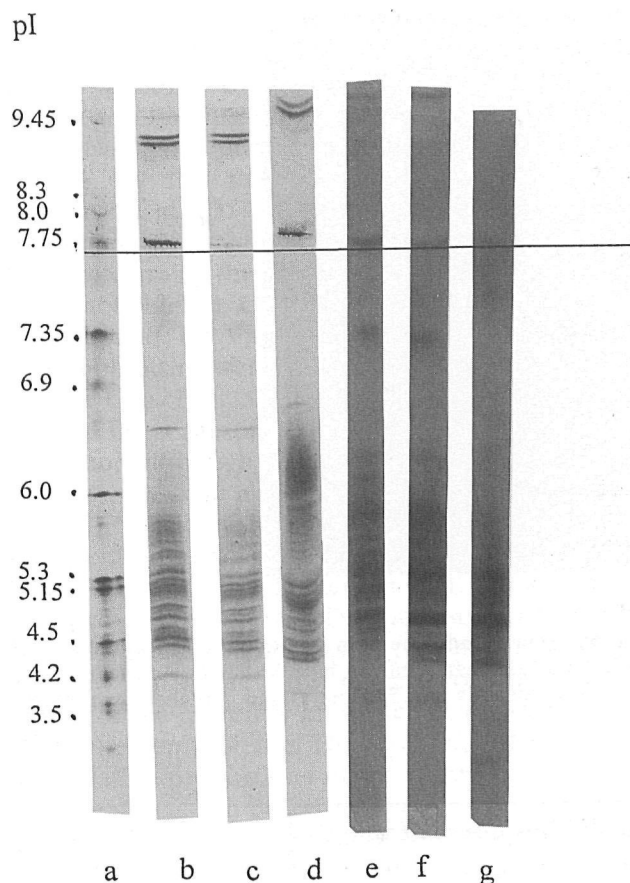


Fig. 3. Analytic IEF on Ampholine PAG-plates (pH 3.5–9.5) of latex raw material (b), IHRS (c), and FDA-E8 standard (d). For each sample, 40 µg protein was applied. Gel was stained with Coomassie brilliant blue. Immunodetection of IgE binding after IEF was effected by serum pool (patients 4 and 5; Table 1). Samples (b), (c), and (d) are shown in (e), (f), and (g) lanes, respectively.

of 100 was then assigned to a rounded concentration of w/v: 1/200 of the IHRS.

Forty-three out of the 46 latex-allergic patients had a positive response to the prick test. Thus, the sensitivity of the prick test was 93%. None of the 76 control subjects, tested with 100 IR of the IHRS, presented a positive response. Accordingly, the specificity of the test was found to be 100%, the negative predictive value 100%, and the positive predictive value 96%.

The standardized IHRS gave a positive reaction on prick test also in five latex-allergic Finnish patients in whom latex RAST was negative (patients 5, 12, 15, 16, and 22; Table 2).

Adverse effects

Mild rhinitis was observed in two subjects after skin prick testing. Otherwise, no adverse reactions were observed.

Stability of the finished product

Three batches stored for 1 year at 4°C, 24°C, and 37°C, respectively, were subjected to stability analysis; no degradation in allergen activity was observed.

Discussion

Standardized allergen extracts are needed for reliable and reproducible diagnosis of allergic diseases. As yet, standardized extracts have not been available for the diagnosis of immediate hypersensitivity to NRL proteins, a relatively recently recognized "new" disease. In the present study, an extract made of freshly collected latex of the rubber tree, *H. brasiliensis*, was prepared, characterized, and evaluated by *in vivo* and *in vitro* tests and standardized for use in skin prick testing.

Total protein staining of the IHRS and the final allergen preparation compared well with both the FDA-E8 standard and a well-characterized in-house standard NRL preparation used in earlier studies (Fig. 1). Several *in vitro* techniques provided evidence that the IHRS and the final skin test reagent contained significant latex allergens. In immunoblotting after IEF, a large number of IgE-binding bands could be identified (Fig. 3), and CRIE analysis revealed several IgE-binding protein arcs (Fig. 4). By means of immunoblotting (Fig. 2), the allergen preparation was shown to contain IgE-binding proteins with apparent molecular weights of 14, 20, 27, 30, 36, and 45 kDa, a group of proteins previously shown to represent significant NRL allergens (15).

Conclusions on the presence or absence of specific allergen molecules in a mixture of proteins such as NRL extract should be made with caution. The bands seen in immunoblotting often contain multiple peptides (13), and small molecular weight peptides may escape detection in standard SDS-PAGE. Heteroimmune antibodies recognizing specifically various NRL allergens or, more desirably, immunodominant epitopes (as yet uncharacterized) of the molecules would be helpful but, for the time being, such reagents are not commercially available. Likewise, there are no commercial sources of purified NRL proteins with which to carry out specific immunizations or immunologic inhibition tests. Therefore, the identification of allergens in the present extracts could be evaluated only by means of human antisera in which seemingly monospecific IgE antibodies had been earlier verified by direct binding and inhibition experiments with purified NRL proteins, as characterized in our earlier studies (12, 20, 31).

Given the above precautions, we could identify (using serum from patient no. 1; Table 1) the most

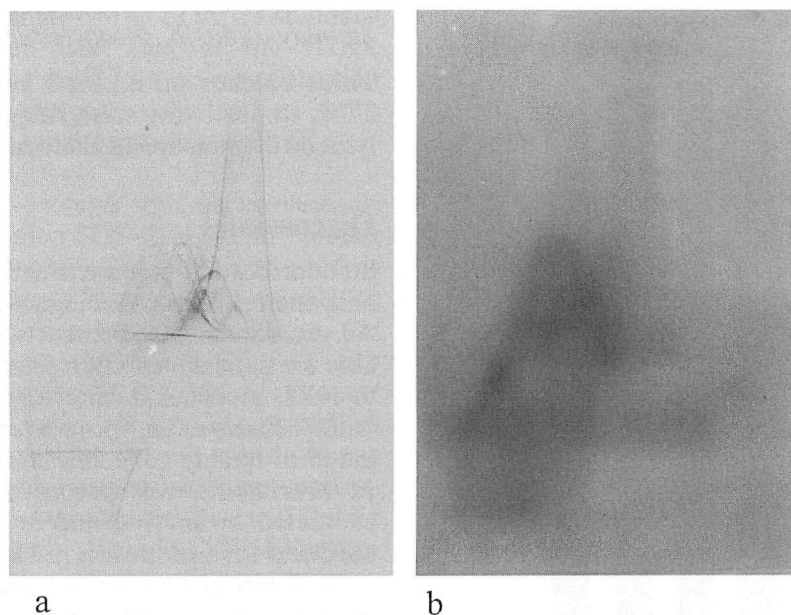


Fig. 4. a) CIE pattern of IHRS. In first dimension, 15 µg protein was run. For second dimension against antibody-containing gel, 11 µl/cm³ of rabbit antilatelx antiserum was used. Proteins were stained with Coomassie brilliant blue. b) CRIE. Autoradiography of CIE plate after sequential incubations with antilatelx IgE serum pool (patients 4 and 5; Table 1) and ¹²⁵I-labeled antihuman IgE. Four separate IgE-binding arcs can be seen.

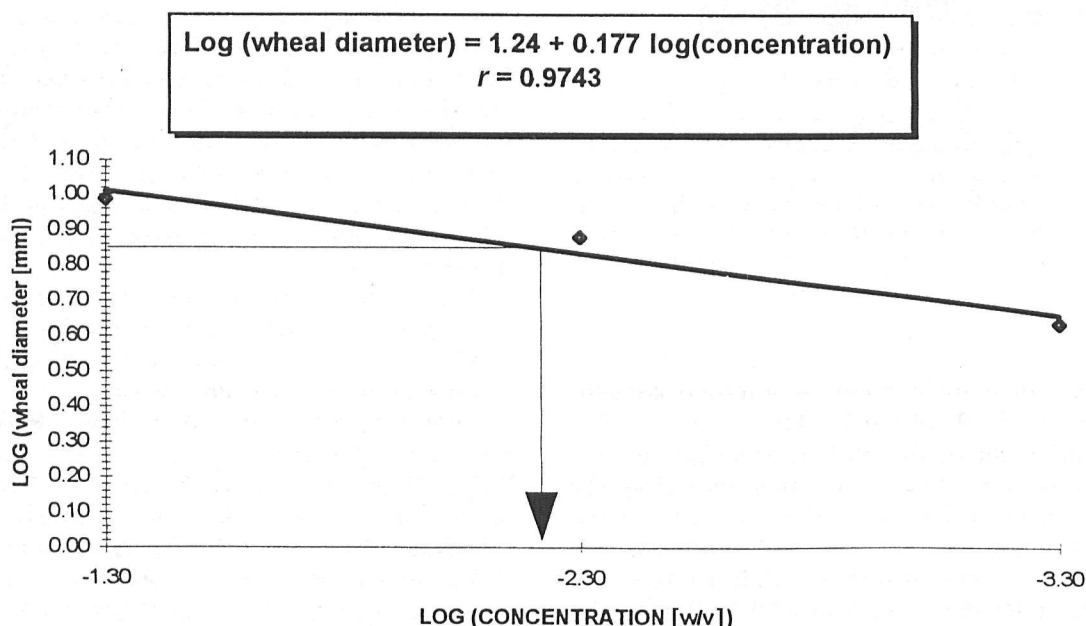


Fig. 5. Biologic standardization of skin prick test extract (IHRS). Dilution of IHRS that caused geometric mean wheal size of 7 mm was calculated as 1:172 (w/v) from equation (see Material and methods) of dose-response curve. Index of reactivity (IR) of 100 was assigned to rounded concentration of w/v: 1/200 of IHRS.

significant NRL allergen for adult latex-allergic patients, the 20-kDa prohevein (12), by immunoblotting in both the latex raw material and in the end product (but only faintly in the FDA-E8 extract). Likewise, a strong band of approximately 14 kDa, containing several proteins (13), including at least REF and the C-domain of prohevein, was

demonstrated by means of another reference serum from a patient with spina bifida (patient no. 3; Table 1). A 27-kDa, IgE-binding protein, typical of multioperated latex-allergic children (14), identified by the same patient with spina bifida, was also detected, although only as a faint band, in the preparations. Since it may be argued that it is

difficult to extract appreciable amounts of REF from fresh NRL without the use of detergents, we tested whether the standardized extract could inhibit the binding of human IgE anti-REF antibody to highly purified (electroeluted) REF in ELISA by the method recently described by Alenius et al. (31). A clear-cut, dose-dependent inhibition by serial dilutions of the preparation was seen, confirming the presence of REF in the extract (data not shown). Other distinct IgE-binding proteins bound by some of the reference sera in immunoblotting were seen at approximately 30, 36, and 45 kDa. Part of the IgE-binding reactivity at 29–30 kDa is presumably due to IgE antibodies to heveamine, although this protein has been considered to play only a minor role as a latex allergen (12, 21). A protein of approximately 36 kDa may represent an endo-1,3,β-glucosidase of the rubber tree, a significant NRL allergen (12), and a protein of approximately 45–46 kDa may also be an important allergen (21). The present results thus support the view that the skin test reagent contains most if not all of the currently known clinically significant NRL allergen moieties. However, no estimates of quantitative proportions of the different allergens in the extracts can be presented.

The NRL extract was successfully standardized for skin prick testing among latex-allergic patients from France and from Finland and among non-allergic control subjects. A concentration of the preparation giving an arbitrary index of reactivity of 100 at a protein level of 22 µg/ml was chosen for use. With this concentration of the reagent, a specificity of 100% and a sensitivity of 93% were achieved in the present series of patients and controls. The subjects chosen for the standardization procedure were patients with well-characterized symptoms and clinical histories compatible with latex allergy. All 23 Finnish patients had earlier been tested positive in skin prick testing with a reliable skin prick test reagent, an eluate of highly allergenic latex gloves (10), and all 23 French patients and 18 (78%) Finnish patients showed positive latex RAST results. Examining the skin prick test results in the patients (Table 2) revealed minor differences in measuring skin reactivity in France and in Finland; the negative (saline) control was recorded as a small positive (though insignificant) reaction in most Finnish patients, but as a negative reaction in the French patients. It should be emphasized, however, that this difference did not influence the results and conclusions drawn.

It may be argued that the present patients were highly selected (since they had earlier tested positive against various latex extracts), a fact which may have influenced the obtained sensitivity figure (93%). We agree that the patients were indeed

selected but, overall, they did not represent especially highly sensitized NRL-allergic patients, as shown by the range of their clinical symptoms and latex-RAST results (Table 2).

The preparation turned out to be safe, since undesirable effects (mild rhinitis) were seen in only two patients, although each patient received at one time 12 doses of the latex extract, of which four were of a high concentration (dilution 1/20, w/v, corresponding to protein concentration of 220 µg/ml).

In conclusion, a skin test reagent for the *in vivo* diagnosis of immediate-type latex allergy was developed, validated, and standardized. The preparation was shown to contain most if not all of the currently known significant NRL allergens, and it showed high sensitivity (93%) and specificity (100%). Adverse effects were rarely observed, suggesting that the reagent is sufficiently safe to be used in routine diagnostic work.

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