

DIAGNOSTIC VALUE OF THREE DIFFERENT LATEX EXTRACTS

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Received October 10, 2006 – Accepted February 27, 2007

The diagnosis of latex allergy is made on clinical history, but a confirmatory skin prick test (SPT) or a serological assay based on a commercial latex extract is always recommendable. Different raw materials can be used in the preparation of commercial latex extracts. Such extracts can consequently show both different qualitative profiles and a different diagnostic potential. Therefore, the selection of a proper latex extract is essential for *in vitro* and *in vivo* diagnosis of latex allergy. In the present study three different latex extracts, prepared from different raw materials (ammoniated -AL-, serum -SL-, or rubber particles -RPE- latex), are compared by *in vitro* techniques using sera from twenty patients with latex allergy. SDS-PAGE technique was used to compare the antigenic profile of the three latex extracts. Subsequently, their allergenic profiles were evaluated by immunoblotting technique using the individual sera from the twenty latex allergic patients. The diagnostic potential of the three latex extracts was also evaluated using direct Radio-Allergo-Sorbent Test (RAST) as well as skin prick-tests (SPTs). In order to establish the more appropriate latex extract in a perspective of *in vivo* diagnosis of latex sensitization, the same latex extracts were subsequently compared by an *in vivo* SPT involving ten of the above subjects. The SDS-PAGE profiles of the three latex extracts examined were quite different. SL extract showed numerous bands comprised between 10-100 kDa. RPE extract was characterized by two intense bands at 14 and 20 kDa while AL extract showed the poorer antigenic composition. Analogously, immunoblotting analysis evidenced a different profile in relation to both different patients and extracts. For only two out of the twenty sera, direct RAST results showed a same positive class in relation to the different latex extracts used. SPT with SL extract showed, in respect to the other extracts (AL, RPE), a significantly higher wheal. This study showed that SL extract is able to express the best *in vitro* and *in vivo* diagnostic potential. Thus, its use should be preferred for the diagnosis of patients affected by latex allergy.

Latex has been used since XVII century to manufacture thousands of products (1). The first report of immediate-type reaction to natural rubber latex (NRL) was documented in 1927 (2). Clinical manifestations of latex allergy usually

include urticaria, rhinitis, conjunctivitis, asthma and anaphylaxis (3-5). Nowadays latex allergy is recognized as a serious and increasing healthcare problem (6-7).

The diagnosis of latex allergy is based on the

Key words: ammoniated latex extract, serum latex extract, latex rubber particles extract, SPTs, diagnostic potential

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0394-6320 (2007)

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clinical history of exposure to latex-containing devices with associated IgE-mediated allergic symptoms. Confirmatory skin prick tests (SPTs) or serological assays are recommended (8). Therefore a reliable preparation of latex extract is essential to diagnose latex allergy (8).

Latex glove extracts have been proposed for diagnostic purposes. The complex procedure required to obtain them, the variability of the amount of latex proteins present, and the high risk of appearance of systemic reactions during skin tests have discouraged allergen manufacturers from using them in the preparation of a diagnostic commercial latex extract (9). Ammoniated and non-ammoniated latex (AL and NAL respectively) have been introduced in the preparation of SPT extracts and used in the diagnosis of latex allergy (10-12).

Latex extracts derived from 3 different sources of material (NAL, AL and extracts of powdered latex gloves) were studied by Hamilton and Adkinson (12). The Authors concluded that both SPTs and intradermal skin tests with latex were inherently safe when the protein concentration was monitored. All 3 latex source materials (NAL, AL, and glove extracts) produced comparable diagnostic SPT results when normalized for protein concentration. NAL is the most attractive candidate for further development because of its stability and simplicity on quality control (12).

Latex is, however, a complex material made up of various organelles (e.g. rubber particles and non-rubber particles, particularly luteoids) suspended in aqueous liquid (B-serum and C-serum), each of them characterized by a different assortment of proteins (13-15). Therefore, the diagnostic potential of a latex extract might depend on the kind of materials and on the procedures used to prepare it (12).

The aim of this study is to evaluate, by *in vitro* techniques [SDS-PAGE, immunoblotting (IB) and RAST] and an *in vivo* method (SPT), the allergenic potential of three different latex extracts [AL, serum (B and C) latex mixture (SL) and rubber particles extract (RPE)] in order to select the most effective latex extract in identifying subjects affected by latex allergy.

MATERIALS AND METHODS

Definitions

Latex allergy = patients with latex-specific IgE and symptoms consistent with IgE-mediated reactions to

latex-containing devices (8).

Atopy = positive SPT to one or more common aero- and food allergens (11-16).

Current asthma = current wheezing (wheezing or whistling in the chest during the previous 12 months) and asthma at any time during patients' lifetime (17).

Current rhinoconjunctivitis = itchy or runny nose, sneezing, itchy or watery eyes apart from colds during the past 12 months (17).

Current food allergy = clinical manifestations of food allergy during the past 12 months (18).

Patients

The subjects with latex allergy were referred to our Pediatric Allergy and Pulmonology Center for clinical reactions to rubber (16).

All subjects and/or parents answered a questionnaire (under the supervision of an allergist) revealing any current or previous presence of IgE mediated diseases, clinical reactions to rubber, number and dates of previous operations, and spina bifida (16-19). All subjects and/or parents gave their informed consent before the patients started this study.

The latex SPT and SPTs for common aero- and food allergens (16) were performed with commercial extracts as previously reported (16) and interpreted according to the EAACI guidelines (20).

Sera of twenty patients with latex allergy (16) and a pool of ten sera from a control group [without IgE mediated clinical manifestations, without atopy, and with undetectable levels of latex specific serum IgE (<0.35 kUA/l according to CAP RAST FEIA) and with negative skin reactions (SPT with wheal size < 3 mm)] were used in the study.

Preparation of latex extracts

Ammoniated latex (AL)

Fresh latex sap, immediately treated with 0.7% ammonium, was provided by Rubber Inst. of Malaysia, Dr. Yeang, Kuala Lumpur, Malaysia. This material was diluted (1:1, w/v) with ammonium acetate buffer, pH 6.8 and vigorously stirred for 30 minutes before centrifugation at 44,000 x g at 4°C for further 30 minutes. The aqueous layer containing soluble proteins was filtered through a 0.2 µm membrane and subsequently dialyzed across 5 kDa cut-off membrane tube against distilled water.

Serum (B and C) latex mixture (SL)

Fresh latex sap was collected in chilled containers surrounded by crushed ice before being centrifuged as described above. The rubber cream was recovered and then RPE fractions were constituted. The aqueous fraction (latex serum) contained the C-serum while the

bottom was made up of lutoid organelles, rich in the B-serum. To obtain B-serum, bottom fraction was subjected to alternate freezing and thawing (three times). In this manner lutoid membranes were broken and removed by further centrifugation. Supernatant (B-serum) was then added to C-serum fraction to obtain SL latex extract. Such extract was then dialyzed against distilled water and freeze-dried.

Rubber Particles Extract (RPE)

RPE fraction was treated with an equal weight of detergent comprising 0.1% Triton X-100 and 1% sodium dodecyl sulphate (SDS). The rubber cream was dispersed in the detergent mixture stirred for 1 hour and then centrifuged at 44,000 g for further 30 minutes. The recovered aqueous fraction dialyzed against distilled water constituted the RPE latex extract. The protein content of three latex extracts was determined by Bio-Rad method (Prodotti Gianni, Milan, Italy).

We bought the three different samples (AL, RPE and SL) from the same provider, Dr. Yeang of the Rubber Inst. of Malaysia, and we have information that they derive from the same clone of *Hevea brasiliensis*.

SDS-PAGE and Immunoblotting

Electrophoresis of latex samples (30 µg protein/30 µL) was performed in a 4-12 % polyacrilamide precast Nupage Bis-Tris gel according to the manufacturer's instructions (Novex, Prodotti Gianni, Milan, Italy) at 180 mA for 1 hour. The resolved proteins were transferred into a nitrocellulose membrane (Protran BA85, Schleicher and Schuell, Milan, Italy) according to Towbin and co-workers (21). The membrane was stained with 0.1% Coomassie Blue Brilliant or saturated in Tris (0.025 M) buffered saline (TBS) containing 5% defatted dry milk before being incubated with 1 ml of patients' sera and control sera from non-atopic subjects. Bound specific IgE were detected by peroxidase-conjugated goat anti-human IgE serum (KpL, Prodotti Gianni, Milan, Italy) at dilution 1:1000 in TBS buffer, using an ECL western blotting kit (Amersham, Milan, Italy) as substrate. Experiments by SDS-PAGE were performed three times with similar results.

Preparation of latex extract-coated solid phases

Polystyrene beads (6.4 mm diameter, Precision Plastic balls, Chicago, Illinois, USA) were activated by treatment with glutaraldehyde solution (2% w/v) in PBS buffer. After washing, activated beads were coated by incubation overnight at room temperature under shaking with a solution of each (AL, SL and RPE) latex extracts (at 25 µg/ml in PBS, 150 µl/bead). Coated beads were then washed before treatment with NaBH₄ 0.01M to stabilize the latex protein-bead binding. Finally, different latex-coated beads

were saturated with bovine serum albumin (BSA, 5% in PBS-Tween 20, 0.05%). After further washing, beads were stored at 4°C until use.

Direct RAST

For direct RAST, latex sensitized polystyrene beads were used. Direct RAST was performed by adding 50 µL of patients sera to tubes containing AL, SL and RPE extract-coated beads. After 6 hours of incubation at room temperature under shaking, tubes were washed and 50 µL of I125 labeled anti-human IgE goat serum (Sferikit, Lofarma S.p.A., Milan, Italy) was added and left overnight. At the end of incubation, the beads were washed and residual radioactivity on the beads was measured by gamma counter. Results were expressed as percentage of bound radioactivity on the total radioactivity added and compared to a reference curve, as indicated by the manufacturer's instructions, to establish the positive class.

SPT with three different latex extracts

AL, SL and RPE latex extracts were equalized at the concentration of 1 mg/mL and subsequently diluted 1:1 with solution containing 0.68% NaCl, 0.275% NaHCO₃, 0.4% phenol and 50% glycerol before using them in SPT. Then, ten out of the twenty patients allergic to latex performed SPT using these three different latex extracts.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), release 9. Means were compared using the Student t-test. Differences were considered to be significant when p values were <0.05.

RESULTS

Tables I, II and III summarize clinical data of the twenty patients with latex allergy included in the study.

SDS-PAGE profile of AL, SL and RPE latex extracts are shown in Fig. 1 (lane a of A, B and C, respectively). Such profiles are quite different among the three considered extracts. AL extract presents a poor antigenic composition with intense bands at about 5-6, 14, 23 and 46 kDa. SL extract shows a very complex antigenic pattern with numerous bands at significant intensity between 5-90 kDa. RPE extract has an intermediate pattern between AL and SL extracts. In RPE extract the components at 14 and 20-23 kDa are particularly well represented.

In Fig. 1 the IB profiles of AL, SL and RPE latex

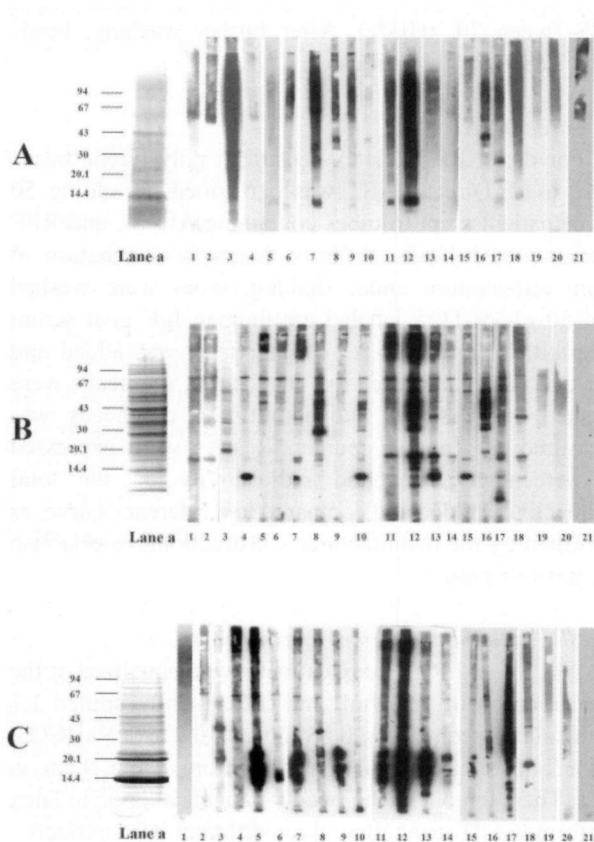


Fig. 1. SDS-PAGE profile (lane a) of the three different latex extracts considered (see text).

A. (ammoniated latex extract); B. (serum latex extract); C. (rubber particles latex extract);

Lanes 1-20, single sera from different allergic people; Lanes 21, negative control (pool of ten sera from non-allergic people).

extracts (A, B and C, respectively) are also indicated using each sera from the twenty allergic patients (lanes 1-20) and a pool of ten sera from non-allergic people (lane 21). Allergenic recognition within the same extract consistently varied from one serum to another and the same serum showed different allergenic profile in relation to the different latex extract used. The IB profile of AL extract showed weak and not easily distinguishable bands for the major part of sera checked. Only six sera (7, 8, 11, 12, 16 and 17) reported a picture with some defined bands. A consistent number of sera did not recognize any bands. SL extract showed numerous and well defined bands at about 5, 14-25, 30-60 and more than 80 kDa for almost all the sera checked. Only

the serum number 20 showed a weak and ill-defined signal. For the RPE extract, the zone 14-25 kDa was particularly well represented, even if some sera also show a very intense and diffuse signal along the whole lane. Sera 1, 2, 4, 15, 19 and 20 seemed to recognize no band at all. As expected control human serum did not show any significant recognition against each of the three considered extracts.

In Table IV direct RAST data of the three different latex extracts considered are shown. All sera from allergic patients were positive against SL extract with different positive classes. On the contrary, using the AL and RPE extracts, 4 and 7 out of twenty were respectively negative. Direct RAST against SL extract showed, in respect to the other extracts (AL, RPE), a significantly higher class.

In Table V the SPT results performed on ten of the twenty subjects initially considered are represented. It was not possible to perform them on all the patients. For these subjects the SL extract proved to be the one endowed with the more allergenic potential, being able to induce a more significant cutaneous response in almost all the subjects. Only in two patients affected by spina bifida did RPE extract give a cutaneous response more intense than that observed using SL extract.

DISCUSSION

We performed a comparison of three different latex extracts (AL, SL and RPE) by *in vitro* and *in vivo* assays such as SDS-PAGE, IB, direct RAST and SPT. Data on sensitivity, specificity and predictive values are not presented because our study refers to a particular population already known as being allergic to latex. We found that the SL extract was the one with the more allergenic potential.

Other authors have studied the allergenic profile of different latex extracts (12, 22). Hamilton and Adkinson have studied the allergenic profile of NAL, AL and glove extracts and have shown that NAL extract had an higher diagnostic capability with respect to the other two extracts (12). La Grutta and co-workers compared SDS-PAGE and immunoblotting profiles of AL and NAL extracts using serum from children with well-ascertained latex allergy and found that the NAL extract, with respect to AL extract, had a higher *in vitro* diagnostic

Table I. General and clinical features of 20 patients with latex allergy.

Sex	
Female	10 (50%)
Male	10 (50%)
Mean $\pm$ SD age (years) (blood extraction)	10.5 $\pm$ 3.75 (range, 5-21)
Positive CAP RAST FEIA to latex (> 0.35 KUA/L)	20 (100%)
Positive SPT to latex (wheal > 3 mm)	19 (95%)
Previous surgical procedures	10 (50%)
Atopy	20 (100%)
Current asthma	9 (45%)
Current rhinoconjunctivitis	12 (60%)
Current food allergy	7 (35%)
Spina bifida	4 (20%)

value in the diagnosis of latex allergy (22). Moreover, Yeang and co-workers have recently evidenced that probably the exclusive use of the latex serum extract is not optimal for diagnosing latex allergy (23). Therefore, it is important to establish which latex material should be used to prepare a diagnostic tool for latex allergy. Recombinant latex allergenes might be useful instruments to obtain further progress in diagnosing latex allergy (24).

In our study SDS-PAGE profiles of the three latex extracts were quite different, demonstrating that the amount of protein molecules and their qualitative antigenic composition can vary considerably among different sources of raw materials. The SL extract is certainly the most complex, being made up of numerous bands in a molecular range between 5-6 and about 90 kDa. RPE extract is characterized by the presence of two very intense bands corresponding to the proteins known as Hev b1, rubber elongation factor, and Hev b3, rubber particle protein (25). AL extract is made up of a few bands, the most evident corresponding to the components known as prohevein C-domain or Hev b1, or both (25).

In order to establish the allergenic profile of three different latex extracts, IB experiments using the single sera of twenty patients with latex allergy and a pool of human negative control sera were performed. The allergenic profiles of AL, SL and RPE appear to be quite different. Single sera against the same

extract often gives rise to different IgE-binding profile as well as the same serum against different extracts.

Comparing the three different latex extracts we observe that the AL extract contains only a few defined components, showing an intense band at 14 kDa corresponding, probably to prohevein C-domain and Hev b 1 allergens. Several sera appear negative. Some of them are also negative when tested by RAST but, in general, contradictory results between IB and RAST are evident. In addition, allergic people were selected by performing SPT with an AL extract (16), to avoid missing suitable subjects to be included in the study due to the low sensitivity of serologic methods. These results confirm that, in the diagnosis of latex allergy, SPT is a more sensitive method than serological assay which in turn is more sensitive than IB. Extract to be used for the RAST method requires that all allergenic proteins remain fixed to the solid phase during the coating. On the other hand, for IB experiments a previous treatment of extract with denaturant reagents is required. Both methods can lead to a lack of some allergenic specificity in respect to SPT, because the SPT extracts do not require any previous treatment.

The RPE extract shows a very intense band at about 14 kDa (very probably, Hev b 1 allergen) and another at about 20 kDa (very probably, Hev b 6 or prohevein proteins). Looking at the IB profile of the RPE extract, a zone between 14-25 kDa showed an intense IgE reactivity (Fig. 1, A; in particular sera 5, 7, 9, 11, 12, 13, 14 and 17). Some other sera seem negative in terms of IgE-binding or show ill defined profiles. Six such sera (1, 2, 3, 4, 10, 15) are also negative when analyzed by RAST technique (Table IV).

A different situation is observed using SL latex extract. In this case all sera checked, except for serum 20, are able, even if with different patterns, to react with SL latex proteins, with the bands at 14-20, 30-70 and more than 80 kDa being the most recognized ones (Fig. 1, B). Moreover, comparing the IB profiles of SL and RPE extracts we can observe that, in some cases, the results were different (e.g.: sera 5, 6, 7, 8, 9, 10, 13, 15) suggesting that different allergens or, alternatively, different concentrations of the same allergen can be contained in the extracts under consideration. This difference appears evident

Table II. *Etiology of the 121 episodes of latex allergy in 20 patients with latex allergy.*

Surgical glove	51 (42%)
Surgical or medical procedures by a dentist	24 (20%)
Rubber balloon	18 (15%)
Pacifier	10 (8%)
Domestic glove	4 (3%)
Swimmer cap	2 (2%)
Rubber game	2 (2%)
Tire	2 (2%)
Operation in total anaesthesia	1 (1%)
Raincoat	1 (1%)
Sponge	1 (1%)
Cream filled-doughnut contaminated with latex	1 (1%)
Rubber mask	1 (1%)
A slip's elastic	1 (1%)
Rubber hose	1 (1%)
Shower cap	1 (1%)
Knee protection	1 (1%)

considering the IB profile of sera 4, 10 and 15. A band at about 11 kDa was clearly observed in the IB profile of the SL extract (Fig. 1, B) while it was completely lacking in the RPE (Fig. 1, C) extract. Vice versa, sera 5, 6, 9 and 11 appear highly positive against the RPE extract, at range 11-25 kDa, unlike that which happens for the SL extract.

These findings confirm that the SL extract has the highest allergenic potential with respect to the other two extracts. Even clearer evidence of the superiority of the SL extract was shown by direct RAST data (Table IV). All sera tested contain latex specific IgE antibodies with positive class from two to four only when testing is performed using SL-coated latex solid phases. Indeed, using AL or RPE-coated solid phases some sera give negative results. Four sera and seven sera, using respectively AL and RPE-coated latex extracts, give negative results as RAST. Control sera are negative against all three extracts.

An interesting observation can be made

Table III. *Symptoms and/or clinical manifestations of the 121 episodes of latex allergy in 20 patients with latex allergy.*

Urticaria	96 (79%)
Asthma	22 (18%)
Rhinitis	15 (12%)
Conjunctivitis	14 (12%)
Orbit edema	9 (7%)
Angioedema	7 (6%)
Lip edema	6 (5%)
Anaphylaxis	2 (2%)

considering sera 9, 12, 17. Such sera, which belong to subjects affected by spina bifida, show a higher positive class when tested using RPE extract. It is known that Hev b1 and Hev b3 allergens are frequently involved in latex allergy of the above subjects (25) and RPE extract seems particularly rich in both of these allergens (25). This result can, therefore, explain the reason why sera with spina bifida show a higher positive class when tested against the RPE in respect to the SL latex extract (Table IV). However, even if RPE extract could be considered optimal for diagnosis of latex allergy in patients affected by spina bifida, it is useful to remember that none of these patients would be lost using SL-coated latex extract. All these sera are in fact RAST positive using SL extract, even if at a lower class (Table IV). We can speculate that the SL extract seems to contain enough quantities of both the Hev b1 and Hev b3 components.

Finally, to confirm the possibility that SL latex extract can be considered as having the best diagnostic potential, SPTs with the three different extracts were newly performed on 10 out of the 20 patients considered in the study. In all cases the SL extract gave rise to a more intense cutaneous response. In particular, in the case of a patient (sera

Table IV. Results of direct RAST of latex allergic patients.

Sera	Direct RAST (positive class)		
	AL	RPE	SL
1.	0	0	2
2.	0	0	2
3.	1	0	3
4.	0	0	2
5.	2	2	3
6.	1	1	2
7.	4	3	3
8.	1	0	3
9.	3	4	3
10.	2	0	3
11.	3	3	3
12.	3	4	3
13.	3	3	3
14.	0	2	2
15.	1	0	2
16.	2	1	3
17.	3	4	3
18.	2	1	2
19.	2	1	2
20.	2	1	3
21.	0	0	0

Direct RAST classes (AL): mean $\pm$ SD = 1.75 ± 1.20 (A),
 Direct RAST classes (RPE): mean $\pm$ SD = 1.50 ± 1.50 (B),

Direct RAST classes (SL): mean $\pm$ SD = 2.60 ± 0.50 (C).
 A vs B: $p = 0.26$; A vs C: $p = 0.001$; B vs C: $p = 0.002$.

4) with a borderline SPT response using AL and RPE extracts, the median diameter of both wheal and erythema was significantly higher using the SL extract (10/12 for SL; 4/6 and 3/5 for AL and RPE extracts) ($p < 0.05$). This suggests that, in cases of patients with a low level of latex sensitization, the

Table V. SPT results using AL, SL, and RPE.

Sera	SKIN PRICK TEST		
	(median diameter in mm of wheal/median diameter in mm of erythema)		
	AL	RPE	SL
*9	6/10	11/28	13/25
*12	4/19	6/18	3/15
*14	3/4	4/7	3/4
1	9/19	15/22	22/40
2	4/17	5/15	6/23
3	5/10	5/8	9/15
4	4/6	3/5	10/12
5	2/10	5/20	3/20
8	5/18	4/18	9/22
11	4/15	5/15	10/15

*Sera from patients affected by spina bifida;
 Mean $\pm$ SD (AL wheal) = 4.60 ± 1.89 (A),
 Mean $\pm$ SD (RPE wheal) = 6.30 ± 3.75 (B),
 Mean $\pm$ SD (SL wheal) = 8.80 ± 5.80 (C),
 A vs B: $p = 0.049$; A vs C: $p = 0.010$; B vs C: $p = 0.059$.

use of AL or RPE latex extracts can induce the risk of false positive results which, *vice versa*, are excluded using the SL latex extract.

In conclusion, our study demonstrates that the SL extract is able to express the best in vitro and in vivo diagnostic potential. Thus, its use should be preferred for the diagnosis of patients affected by latex allergy.

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