

Purified Natural Rubber Latex Antigens Show Variable Reactivity with IgE in the Sera of Latex Allergic Patients

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ABSTRACT

Immediate-hypersensitivity reactions related to latex products have increased rapidly in recent years. However, a reliable diagnosis is hampered by lack of purified and well characterized latex allergens. We have purified allergens in natural rubber latex (NRL) after SDS-PAGE by electroelution, and tested specific IgE-binding activities of these isolated allergens by ELISA. Ten sera from spina bifida patients and 5 sera from health care workers with latex allergy were evaluated for specific IgE antibody in the sera using ELISA. Five normal controls with no evidence of latex allergy were also studied. None of the isolated allergens reacted with normal control sera used. Among the latex allergic patients' sera studied, 11, 9, 9, 2, and 6 sera reacted with 27, 23, 21, 17, and 10 kDa isolated allergens, respectively. Each latex allergic serum reacted with at least one of the purified allergens, indicating a diversity in response to latex. The isolated and purified latex allergens may, however, be useful to identify latex allergy in patients. (Allergy and Asthma Proc 17:209–213, 1996)

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Although IgE-mediated immediate hypersensitivity responses related to latex products have been known for many years,¹ the diagnosis of latex allergy still depends initially upon clinical histories of patients.² The immunological tests such as *in vivo* skin test and *in vitro* RAST, ELISA, and immunoblots are supportive, but there is considerable variability in the specificity and sensitivity of the reagents currently used in such assays, due to the lack of standardized latex allergens.^{2,3} In addition, crude antigens may vary in their allergenicity, which may lead to adverse reactions, including anaphylaxis during skin testing.⁴ Although purified and well characterized latex allergens may be helpful in the diagnosis of latex allergy, their variability in reactivity must be considered. This study examines that variability.

Because latex polypeptides with molecular weights between 10–30 kDa have frequently been reported to react with IgE antibodies in the serum of patients with latex hypersensitivity,⁵ we concentrated on this fraction. In the present study, we have isolated five latex allergens by electroelution from NRL and demonstrated their allergenicity by binding IgE antibodies from latex allergic patients. These allergens have molecular sizes of 27, 23, 21, 17, and 10 kDa respectively. None of them reacted with all the sera used in this study and that variability must be considered when isolated allergens are used as standardized reagents in the immunodiagnosis of latex allergy.

METHODS

Subjects

All patients were diagnosed with latex allergy by their clinical history of latex induced asthma, anaphylaxis, or urticaria with latex contact, and confirmed by positive

immediate wheal and flare skin prick tests to a latex glove extract and positive ELISA results demonstrating IgE antibodies to a glove extract and natural rubber latex (NRL).⁶ Fifteen latex allergic patient sera were randomly selected from serum samples collected from patients evaluated in the Allergy-Immunology Division of the Medical College of Wisconsin. Ten were from spina bifida (SB) patients and five from health care workers (HCW) (Table I). Five patients (four from SB and one from HCW) previously had anaphylactic reactions to latex. The total IgE level measured in 10 of these 15 sera ranged from 30 to 2104 ng/mL. The control sera were from five laboratory workers who had neither clinical symptoms nor detectable IgE antibodies against latex antigens, but were frequently exposed to natural rubber products during their work.

NATURAL RUBBER LATEX (NRL)

NRL was provided by Ansell Corporation (Melbourne, Australia) and was prepared as described previously.⁷ It was frozen immediately without addition of any chemicals upon tapping from rubber trees (*Hevea brasiliensis*) in Malaysia, and was shipped to our laboratory on dry-ice. The protein concentrations of NRL and other latex samples were determined by using a Micro Bicinchoninic acid Protein Assay Reagent Kit (Pierce, Rockford, Ill.) according to the manufacturer's instructions.

Isolation of Latex Polypeptides and SDS-PAGE

To obtain purified latex polypeptides, NRL was first separated by SDS-PAGE. Approximately 10 mg NRL was reduced with sample buffer containing 5% β -mercaptoethanol and loaded onto 15% polyacrylamide gels containing 0.1% SDS. Electrophoresis was performed by using

Bio-Rad Protean II xi (Bio-Rad, Richmond, Cal.) at a constant voltage of 300 V for 8 hours. After this, the gels were stained by 0.2% Coomassie blue to demonstrate latex proteins. The molecular weights of the latex polypeptides were determined by comparing them with molecular weight standards (GIBCO BRL, Gaithersburg, Md.) run on the same gel. The gel pieces containing a single protein band were carefully cut out and loaded into an Electro Elutor (Model 422, Bio-Rad). Electroelution was carried out according to the manufacturer's instructions. The bands were eluted with protein elution buffer (25mM tris, 192mM glycine, and 0.1% SDS) at a constant current of 10 mA/tube for 4 hours. After electroelution, the proteins retained in the membrane caps were collected and dialyzed against PBS for 24 hours at room temperature. The retentate was filtered through a 0.22 μ m filter, aliquoted, and stored at -70°C .

The purity of the latex polypeptides obtained after electroelution was determined by SDS-PAGE using a Bio-Rad Mini Protean II Cell as previously described.⁸ Purified latex polypeptides (500 ng) or NRL (14 μ g) were reduced and loaded onto the gels, and the electrophoresis was carried out for 45 minutes at a constant voltage of 200 V. The gels were then stained with Coomassie blue and inspected for presence of a single protein band.

ELISA

The ability of the isolated latex antigens to bind human IgE was evaluated by ELISA as previously described.^{4,8,9} Microtiter plates (Immulon II, Dynatech, Chantilly, Va.) were coated with separate isolated latex polypeptide (1 μ g/mL) overnight at 4°C . The wells were then washed with PBS-T. Five serial dilutions (1:10, 1:20, 1:40, 1:80, and 1:160) of each latex allergic patient or control sera

TABLE I
Details about the Patients Included in the Study

Subjects	No.	Skin Test	Anaphylaxis to Latex Exposure	Total Serum IgE ng/mL	Latex-Specific IgE ku/L (RAST)*
Spina bifida (SB)	1	+	—	30	3.1
	2	+	—	2104	30
	3	+	+	320	2.7
	4	+	+	176	2.8
	5	+	—	NT+	NT
	6	+	+	108	10.6
	7	+	—	81	5.5
	8	+	—	37	6
	9	+	+	55	2.6
	10	+	—	104	30
Health care workers (HCW)	11	+	—	NT	NT
	12	+	+	169	0.9
	13	+	—	NT	NT
	14	+	—	NT	NT
	15	+	—	NT	NT

* Malaysian non-ammoniated latex protein; +NT: Not Tested

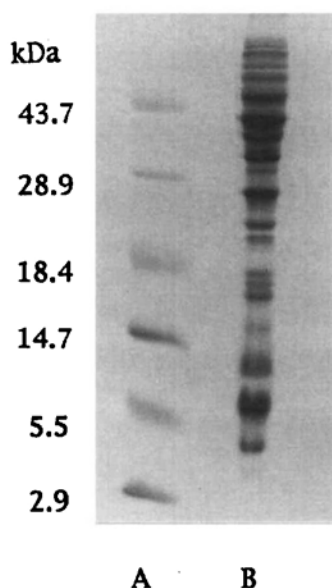


Figure 1. Protein profile of NRL is shown by a 15% SDS-polyacrylamide gel stained with Coomassie blue. A: Molecular weights standard. B: NRL showing over 23 protein bands.

were added to the wells and incubated for 3 hours. This was followed by the addition of biotinylated anti-human IgE (1:1000, Sigma, St. Louis, Mo.) and streptavidin-HRP (1:5000, Sigma), and the plates were incubated with each reagent for 1 hour at room temperature with washing three times with PBS-T between additions of the reagents. After a final wash cycle, the substrate (0.06% O-phenylenediamine containing 0.015% H_2O_2) was added to the wells and the color developed. The reaction was stopped after 20 minutes with 4N H_2SO_4 , and the O.D. was read in an

automatic ELISA Reader (Dynatech) using a 490 nm filter. Crude NRL antigen (14 $\mu g/ml$) coated plates were processed similarly and served as a control.

The O.D. values of the ELISA were converted to log titers for better comparison of results.^{3,9} The titers were calculated for each serum from the most linear part of O.D. value of the 5 serum dilutions and converted into log titer with a base line cut off of 0.5 O.D., using a computer software package (Microsoft, Seattle, Wa.). The mean + 2SD (standard deviation) value of log titers of NRL reacting with IgE from 50 control sera tested previously under similar condition. Log titers of greater than 1.0 (mean + 2S.D) were considered as positive.

RESULTS

The profile of NRL on a 15% SDS-polyacrylamide gel stained with Coomassie blue is shown in Fig. 1. Nine bands ranged between 10 and 30 kDa were defined in a scanning densitometer using a software package (Ambis, San Diego, CA) (Fig. 2). Five of them, 27 kDa, 23 kDa, 21 kDa, 17 kDa and 10 kDa, were then isolated by electroelution and their purity was then determined on SDS-PAGE. Each polypeptide showed a single band in SDS-PAGE (Fig. 3).

The results of the ELISA using individual polypeptides reacting with serum IgE from latex allergic patients and controls are shown in Table II. None of the purified polypeptides reacted with any control sera. The purified allergens showed diverse and variable IgE-binding abilities with latex allergic patient sera; none reacted with all the latex allergic patients' sera. The 27, 23, 21, 17, and 10 kDa allergens reacted with 11, 9, 9, 2, and 6 of 15 patients' sera respectively. Among the five

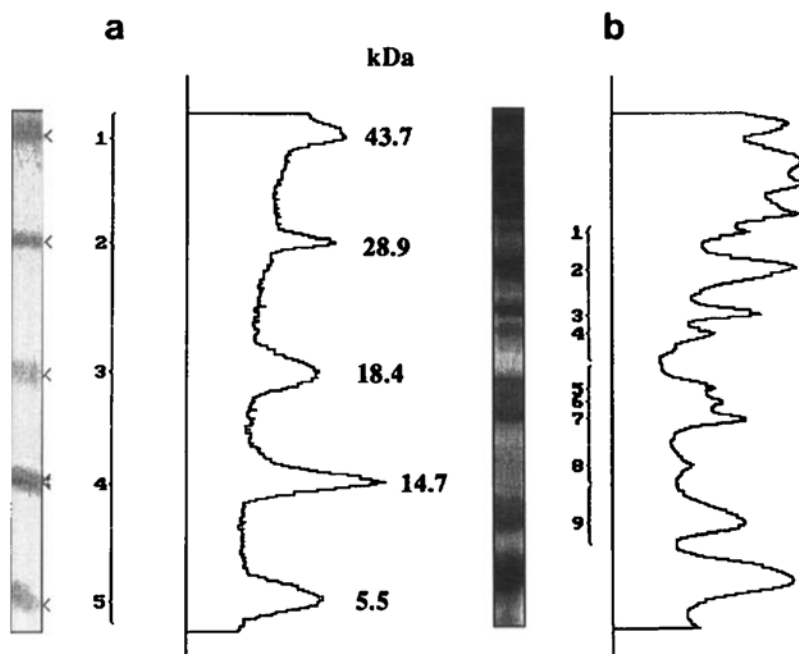
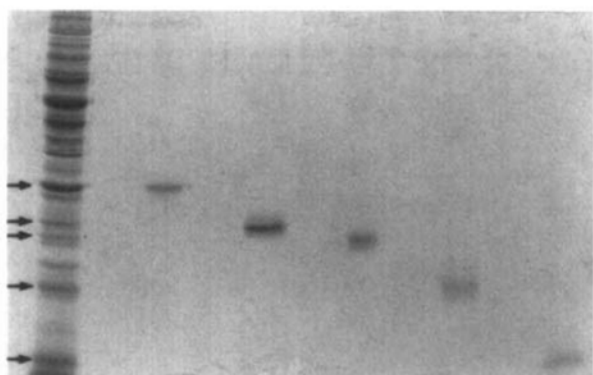


Figure 2. Scanned SDS-PAGE of NRL (shown in Fig. 1) using a densitometer shows 9 latex polypeptides having molecular sizes between 10 to 30 kDa. a: Molecular weight standard; b: NRL. Protein bands 1 to 9: 30, 27, 23, 21, 18, 17.6, 17, 15, and 10 kDa.



27kDa 23kDa 21kDa 17kDa 10kDa

Figure 3. SDS-PAGE of purified latex polypeptides stained with Coomassie blue. Arrows indicate corresponding protein bands of the purified latex proteins in NRL.

purified allergens, the 23 and 10 kDa reacted more frequently with SB sera (8/10 and 6/10) than with HCW sera (1/5 and 0/5), whereas the 27 and 21 kDa allergens reacted with all five (27 kDa) or three of five (21 kDa) of the HCW sera. These two allergens also reacted with a majority (6/10) of SB sera tested. The 17 kDa polypeptide reacted with 2 of 10 SB sera and none of the HCW sera.

Specific IgE antibodies could be detected in 14 of 15 latex allergic patient sera when the 23 or 27 kDa allergens were used simultaneously. One serum (No. 7) negative to 23 and 27 kDa

allergens reacted only with the 10 kDa allergen. Thus, all the latex allergic patients' sera used in the present study demonstrated latex specific IgE antibodies against at least one of the 10, 23, or 27 kDa isolated allergens, but in a variable fashion.

DISCUSSION

In the present study, we have isolated five latex allergens from NRL and individually demonstrated their allergenicity. Nonammoniated NRL contains almost all relevant unaltered native latex allergens that can be demonstrated by SDS-PAGE when compared to extracts of natural rubber latex products and ammoniated latex.¹⁰ We therefore used NRL as a source for purification of latex allergens in the present study. Although there is no direct evidence that these allergens are present in the intact forms in latex health care products, we have previously demonstrated that the antigenic epitopes of NRL are present in both ammoniated latex and latex glove extracts.⁷ Therefore, these results can be extrapolated to the latex end products.

Numerous latex allergens have been identified from different sources such as nonammoniated natural rubber latex, ammoniated rubber latex and extracts from manufactured latex products by demonstrating reactivity with IgE antibodies in latex allergic patient sera.⁵ A few latex proteins including prenyltransferase, rubber elongation factor (REF), hevein, and hevine have been isolated in pure forms and their amino acid sequences determined.¹¹⁻¹⁴ One protein,

TABLE II

Latex-Specific IgE Antibodies to the Isolated Polypeptides in the Sera of Patients Expressed as ELISA Log Titer

Subjects	No.	Isolated Latex Polypeptides					Crude NRL
		10 kDa	17 kDa	21 kDa	23 kDa	27 kDa	
Spina bifida (SB)	1	1.44*	0.4	0.72	1.52	0	1.56
	2	0.21	0	1.29	0.95	1.41	1.4
	3	0.9	0.4	1.52	1.6	1.71	1.73
	4	0	0	1.56	1.31	1.58	1.71
	5	0.6	0	1.27	1.62	1.38	1.34
	6	1.46	0	1.02	1.37	0	1.47
	7	1.21	0	0	0	0.34	1.3
	8	1.26	0.33	0	1.52	0.25	1.38
	9	1.77	1.81	1.81	2.19	2.14	2.18
	10	1.5	1.23	0.94	1.23	1.61	1.49
Health care workers (HCW)	11	0.56	0	1.33	0.67	1.32	1.18
	12	0.97	0.79	1.56	1.56	1.53	1.76
	13	0	0	0.85	0.7	1.29	1.06
	14	0.64	0.89	0.85	0.97	1.39	1.39
	15	0.41	0.53	1.29	0.98	1.41	1.39
Control	1	0	0	0.27	0.34	0	0.66
	2	0.230	0	0	0	0	0.39
	3	0	0	0	0	0.67	0.78
	4	0	0	0	0	0.24	0.45
	5	0	0	0	0	0	0.21

* Results with log titers larger than 1.0 were considered positive; equal or less than 1.0 were considered negative

REF, has been considered to be an important allergen associated with latex allergy.¹⁵ Latex allergens have also been purified using affinity chromatography,^{16,17} gel filtration,¹⁸ and high-performance liquid chromatography.¹⁹ However, some of these allergens do not represent individual pure proteins and may infrequently react with IgE from latex allergic sera. In the present study, the five isolated latex allergens each showed a single band on SDS-PAGE, and had substantial albeit variable reactivity with latex specific IgE antibodies.

SB and HCW are the highest risk groups for development of latex allergy.^{5,10} Most HCW are sensitized by contact with latex gloves during work, whereas SB patients are generally sensitized during repeated surgical or other procedures.^{2,3} It is possible that the major allergens involved in these two groups are different.^{2,3} In the present study using isolated antigens, the 10 and 23 kDa allergens reacted more frequently with sera of allergic SB patients when compared to allergic HCW. Interestingly, the 10 and 23 kDa allergens have been demonstrated as major unextractable latex antigens present on surgical latex gloves⁶ and the 23 kDa antigen has been shown to be relevant in SB latex allergy.¹⁹ Two other purified allergens of 21 and 27 kDa molecular weights reacted with most sera from either group. These results suggest that there may be specific reactivities in different sensitized populations that could be based on genetics, immune response variability, and presentation of a variety of antigens.

Thus, in the evaluation of patients with suspected latex allergy, the variability of reactivities with latex antigens must be considered. Such variability may account for the lack of reactivity up to 25% of sera from clinically sensitive individuals when tested by ELISA or RAST (Yunginger J., Williams B., personal communications). However, purified antigens may be useful in diagnosis in specific groups of latex allergic patients.

In conclusion, we have demonstrated that purified latex allergens can be used to identify latex-allergic patients and the antigenic reactivity may be specific for special groups, but the variability of reactions must be considered.

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