

Characterization of a Major Latex Allergen Associated with Hypersensitivity in Spina Bifida Patients¹

Liang-jun Lu,* Viswanath P. Kurup,^{2*} Donald. R. Hoffman,[†] Kevin J. Kelly,* Pazhayannur S. Murali,* and Jordan N. Fink*

*Allergy-Immunology Division, Departments of Medicine and Pediatrics, Medical College of Wisconsin, Zablocki Veterans Administration Medical Center, Milwaukee, WI 53295; and [†]Department of Pathology and Laboratory Medicine, East Carolina University School of Medicine, Greenville, NC 27858

Hypersensitivity to latex proteins present in health care products, particularly in latex gloves, has emerged as an important public health problem in recent years. Most of these latex allergens have not been purified or characterized. Here we report the purification and characterization of a 23-kDa latex polypeptide from nonammoniated natural rubber latex. This purified 23-kDa polypeptide reacted with IgE from 13 of 17 (76%) latex-allergic spina bifida patients, but from only 1 of 5 health care workers with latex allergy. Furthermore, all the sera of patients with anaphylaxis to latex showed reactivity with the 23-kDa polypeptide. This allergen also induced significant lymphocyte proliferation by PBMC from spina bifida patients, but not from health care workers. The 90 amino acid residues sequenced from the 23-kDa polypeptide showed a 45.6% homology with a previously reported latex protein, rubber elongation factor. By using a mAb LAM-1, produced against the 23-kDa allergen, we could also detect this allergen or its epitope in ammoniated latex and natural rubber latex products used in health care management, but not in non-latex extracts studied. *The Journal of Immunology*, 1995, 155: 2721–2728.

IgE-mediated immediate hypersensitivity responses to latex products have emerged as an important public health problem in recent years (1–5). The increase in allergic reactions to latex has been attributed to the enormous increase in the use of latex gloves for protection against HIV and hepatitis, as mandated by OSHA (6, 7). Allergic reactions to latex include urticaria, asthma, anaphylaxis, and even death (1–3, 8, 9). Natural rubber latex proteins retained in gloves and other products such as condoms, balloons, barium enema catheters, and bladder catheters may be responsible for such reactions (4, 10–14). It has been reported that as many as 2.8 to 8.8% of all health care workers (HCW)³ (15–17) and 18 to 37% of patients

with spina bifida (SB) (18–21) have been sensitized to latex. The occupational use of latex gloves by HCW is believed to contribute to their sensitization to latex, while exposure through multiple surgeries and conditions requiring frequent use of latex products have likely contributed to the latex allergy in patients with SB (21, 22).

The diagnosis of latex allergy is based on clinical history and immunologic findings (5, 6, 23). The immunologic evaluation includes *in vivo* methods such as skin prick test with latex allergen and “use test” employing the suspected products, and *in vitro* tests for detection of specific IgE Abs in sera (23). Significant progress has not been made in the diagnosis of latex allergy due to the lack of well-characterized latex allergens as standardized reagents. Isolation of relevant allergens and their characterization are of paramount importance in developing a reagent for reliable and early diagnosis of latex allergy, to prevent untoward reactions in susceptible patients (5, 6, 14, 23).

We purified a 23-kDa latex polypeptide from natural rubber latex by electroelution from SDS-PAGE gels and demonstrated its significant role in both humoral and cell-mediated immunity in SB patients with latex allergy. The partial amino acid sequence of this protein was determined and a mAb was produced against this allergen.

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² Address correspondence and reprint requests to Dr. Viswanath P. Kurup, VA Medical Center, Research Service 151-I, 5000 West National Avenue, Milwaukee, WI 53295-1000.

³ Abbreviations used in this paper: HCW, health care worker; SB, spina bifida; AL, ammoniated latex; NAL, nonammoniated latex; SI, stimulation index; REF, rubber elongation factor.

Table I. *Diagnosis of latex-allergic patients and the reactivity of their IgE Abs to the 23-kDa latex polypeptide^a*

Subjects	Anaphylaxis	Skin Prick Test	Specific RAST	Total IgE (ng/ml)	ELISA of IgE to:		
					GE	NAL	23-kDa (OD)
Spina bifida patients							
AG	+	+	2.8	176	+	+	0.334 ^b
SJ	+	+	3.1	30	+	+	0.294 ^b
DL	+	+	2.6	55	+	+	2.931 ^b
MP	+	+	2.7	320	+	+	0.607 ^b
LL	+	+	18.5	1536	+	+	0.309 ^b
CS	+	+	1	290	+	+	0.293 ^b
AS	—	+	6	47	+	+	0.238 ^b
AT	—	+	7.39	47	+	+	0.277 ^b
NS	—	+	6	37	+	+	0.483 ^b
SJ	—	+	10.6	108	+	+	0.703 ^b
MB	—	+	2	31	+	+	0.346 ^b
AM	—	+	2.2	52	+	+	0.180
DS	—	+	30	2104	+	+	0.289 ^b
BS	—	+	1.5	<24	+	+	0.174
MS	—	+	2	<24	+	+	0.314 ^b
NN	—	+	5.5	81	+	+	0.126
AT	—	+	2.9	161	+	+	0.147
Health care workers							
LS	+	+	0.9	169	+	+	0.809 ^b
PS	—	+	nd	nd	+	+	0.108
MJ	—	+	nd	nd	+	+	0.196
AT	—	+	nd	nd	+	+	0.194
MK	—	+	3.6	367	+	+	0.104
Control ^c							
1	—	—	nd	nd	—	—	0.111
2	—	—	nd	nd	—	—	0.091
3	—	—	nd	nd	—	—	0.110
4	—	—	nd	nd	—	—	0.125
5	—	—	nd	nd	—	—	0.184
6	—	—	nd	nd	—	—	0.207
7	—	—	0.6	nd	—	—	0.168
8	—	—	0.5	26	—	—	0.143
9	—	—	0.3	nd	—	—	0.159
10	—	—	0.4	nd	—	—	0.140
11	—	—	0.3	<24	—	—	0.189
12	—	—	0.5	<24	—	—	0.155

Positive result to GE and NAL based on an ELISA log titer value equal to 1.8 or more calculated from five dilutions of the serum; negative result based on a log titer value less than 1.2. GE indicates Baxter glove extract; +, positive; —, negative; and nd, not done.

^b Indicating positive reactions with O.D. values larger than Mean + 2 SD of control sera. O.D. values showed here are means of duplicate.

^c Controls 1 to 10 are laboratory workers; 11 and 12 are SB patients without latex allergy. Mean + 2 SD = 0.219.

Materials and Methods

Subjects

All of the latex-allergic patients were diagnosed by clinical history, immediate wheal and flare skin test reactivity to a latex glove extract and the presence of elevated specific IgE Abs to glove extract and nonammoniated latex (NAL) in their sera (Table I). Sera from 22 latex-allergic patients were selected at random from serum samples collected from patients evaluated in the Allergy-Immunology Division of the Medical College of Wisconsin and were studied by ELISA. Seventeen of the random samples were from patients who had SB, including six patients who had anaphylactic reactions to latex; five were from symptomatic HCW with latex allergy, one of whom had anaphylaxis on latex contact. Sera of control subjects (from 10 laboratory workers and 2 SB patients who had neither clinical symptoms nor IgE Abs against latex Ag) were also studied.

Peripheral blood was drawn from three SB patients and three HCW with latex allergy to study PBMC responses to latex proteins by Ag-induced lymphocyte proliferation, while peripheral blood donated by two non-latex atopic patients were used as controls. The human study protocols were approved by the Institutional Human Studies Committee, Medical College of Wisconsin, Milwaukee, WI 53226.

Antigens

Natural rubber latex. Natural rubber latex was collected from rubber trees (*Hevea brasiliensis*), in containers with or without ammonia (0.7% v/v), in Malaysia. Clear liquid containing latex proteins was collected after autocoagulation of rubber particles without ammonia and designated as NAL. The addition of ammonia converts natural rubber cytosol to liquid and solid phases. The liquid phase was collected and designated as ammoniated latex (AL). Both NAL and AL were obtained through Ansell International (Melbourne, Australia) from a Malaysian rubber plantation and were immediately frozen and shipped in dry ice to our laboratory. After thawing, the liquid phases were dialyzed against PBS across 1-kDa cutoff dialysis membranes. The retentates were then filtered through 0.45- μ m filters and stored at -70°C until used.

Latex glove antigens. Latex gloves were extracted in PBS for 24 h at 4°C , as previously described (24).

Antigens from non-latex materials. Ag extracts were prepared from avocado, banana, banana peel, kiwi, mango, papaya fruit, papaya seeds, potato, oat, and wheat, as previously described (25). Protein concentrations of all Ags were determined using a micro biconinonic acid (BCA) Protein Assay Reagent Kit (Pierce Chemical Co., Rockford, IL) according to the manufacturer's instructions.

Purification of latex polypeptide by electroelution

Approximately 10 mg of NAL proteins were electrophoresed semi-preparatively in 15% SDS-PAGE gels, after reduction with 5% β -mercaptoethanol (26, 27). The Ag was applied over the stacking gel, covering the whole length. The separated proteins were stained with 0.2% Coomassie brilliant blue to demonstrate latex proteins. The relevant polypeptide bands, as determined by their reactivity with patient serum in immunoblot, were carefully cut out and loaded into an Electro-eluter (Model 422, Bio-Rad, Richmond, CA). The polypeptide was eluted from the gel pieces with a buffer (25 mM Tris, 192 mM glycine, and 0.1% SDS) at a constant current of 10 mA/tube for 4 h. After electroelution, the protein retained in the membrane caps was collected and dialyzed against PBS for 24 h at room temperature. The retentate was filtered through a 0.22- μ m filter and stored at -70°C until used.

Amino acid sequencing

The polypeptide eluted from SDS-PAGE gels was evaluated for purity in two-dimensional electrophoresis. It was then acetone precipitated, vacuum dried, and dissolved in 25 μ l of solution (8 M urea, 0.4 M ammonium bicarbonate, pH 8.0). Then it was reduced with 7.5 mM of DTT and alkylated with 15.3 mM of iodoacetic acid. The concentration of urea was reduced by adding 60 μ l of 0.1 M ammonium bicarbonate, pH 8.0. The denatured protein was digested with 3 μ g of HPLC-purified staphylococcal V8 protease or trypsin (Sigma Chemical Co., St. Louis, MO) by stirring overnight at 37°C . Peptides were isolated by reversed-phase chromatography on a Vydac 2.1×150 mm C18 column (The Separations Group, Hesperia, CA) using a gradient of 0.1% trifluoroacetic acid (TFA) in water and 0.07% TFA in acetonitrile at a flow rate of 0.2 ml/min. Fractions of 0.2 ml were collected (28). Protein sequencing was performed on a Milligen-Bioscience Model 6625 ProSequencer (Millipore Corp., Burlington, MA). This is a solid-phase, fully automated sequencer/phenylthiohydantoin analyzer. Proteins were coupled to 1,4 phenylene diisothiocyanate disks, and the covalently bound proteins were reduced and alkylated in the presence of 6 M of guanidine HCl, as described above. The disks were then washed five times each in water and methanol and air dried. Separated peptides from digests were coupled to arylamine disks for sequencing.

Production of mAbs

A mAb was produced against the purified 23-kDa polypeptide using methods similar to those described before (29). Briefly, BALB/c mice were immunized with the purified 23-kDa polypeptide obtained by electroelution by s.c. injection (20 μ g of protein/injection). The spleen cells from the immunized mice were fused with NS-1 myeloma cells, using polyethylene glycol (Sigma Chemical Co.). Ab-secreting cells were selected and cloned. A purified mAb, LAM-1, with a strong reactivity with the polypeptide (which was isotyped as IgG1), was used in this study.

ELISA and competitive inhibition ELISA

ELISA was performed as previously described (24, 26, 29). Briefly, microtiter plates (Immulon II, Dynatech, Chantilly, VA) were coated with 1 μ g/ml of purified, or 5 μ g/ml of crude, latex Ag overnight at 4°C . Sera from patients and control subjects were used at a dilution of 1:10 for latex-specific IgE determinations. Affinity-purified mAb was used at a concentration of 1 μ g/ml. Human sera or mAb were added to the wells and incubated for 2 h at room temperature. This was followed by the additions of biotinylated anti-human IgE (1:1000, Vector, Burlingame, CA) or biotinylated anti-mouse IgG1 (1:2000, Sigma Chemical Co.), streptavidin (1:5,000, Sigma Chemical Co.) and substrate *o*-phenylenediamine (Sigma Chemical Co.). The wells were washed between each addition with PBS Tween-20 (0.05%). The reaction was stopped after 20 min with 4 N H_2SO_4 and the OD was read in an ELISA reader (Dynatech) using a 490-nm filter. Appropriate controls were included in each plate.

To determine the relative concentration of the 23-kDa polypeptide in the different latex extracts, an inhibition ELISA assay was employed. Microtiter ELISA plates (Dynatech) were coated with 50 μ l/well of a PBS solution of the 23-kDa polypeptide at a concentration of 0.5 μ g/ml. The coating was conducted overnight at 4°C . To a constant concentration of the mAb LAM-1 (0.2 μ g/ml), serial dilutions of latex Ag extracts were

added and incubated for 3 h at room temperature. Fifty microliters of these mixtures was then added to the coated wells, which had been washed and blocked with PBS Tween-20 (26). After incubation for 45 min, the wells were washed and a secondary Ab, biotinylated anti-mouse IgG1 (1:2000, Sigma Chemical Co.), was added. The subsequent steps were the same as described above for ELISA. The concentration of each extract in micrograms needed for a 50% inhibition of the reactivity of mAb LAM-1 with purified 23-kDa polypeptide was calculated by extrapolation of the OD values from the competitive ELISA.

One- and two-dimensional electrophoresis

Latex Ags were studied using SDS-PAGE, as previously described (26). About 14 to 20 μ g of protein of NAL or other crude extracts, or 0.5 μ g of purified polypeptide, was reduced and loaded onto a 15% gel, and was electrophoresed at a constant voltage of 200 V for 45 min. A Bio-Rad mini gel electrophoresis system was used.

Two-dimensional electrophoresis was performed according to a modified method of O'Farrell, as previously described (27). Isoelectric focusing was used in the first dimension, and SDS-PAGE was used in the second dimension. Twenty millimolar of NaOH was used as the upper chamber buffer and 10 mM H_3PO_4 as the lower chamber buffer. Protein was electrophoresed at a constant voltage of 500 V for 10 min, and then at 700 V for 5 h. SDS-PAGE was performed in the second dimension, as previously described (27).

Immunoblot

The reactivity of the Ags was studied against human latex-specific IgE or mAb using immunoblots, as previously described (27). After SDS-PAGE, the proteins were electrophoretically transferred from the gels to nitrocellulose membranes (Bio-Rad). The membranes were then incubated with a blocking buffer (5% nonfat milk in TBS) for 1 h, followed by incubation with human sera (1:5) or mAb (1 μ g/ml) overnight at 4°C . After washing the membranes, they were incubated with biotinylated anti-human IgE (1:1000, Vector) or biotinylated anti-mouse IgG1 (1:1000, Sigma Chemical Co.), followed by streptavidin-conjugated alkaline phosphatase (1:20,000, Zymed, San Francisco, CA). The substrate NBT/BCIP (Bio-Rad) was added to aid in visualizing the reacting bands. The reaction was stopped after 10 min by rinsing the membranes with deionized water. Between each step, the membranes were washed three times each with TBS containing 0.1% Tween-20.

Ag-specific lymphocyte proliferation

Proliferative assays were performed in 96-well, flat bottom microtiter plates (Falcon, Becton Dickinson, Lincoln, NJ) in medium alone, and with differing final concentrations of latex Ags, to determine the optimal dose. The preliminary studies indicated a concentration of 1 μ g/ml of latex protein was ideal for the stimulation of PBMC. PBMC were obtained from heparinized blood as reported (30). Cells (2×10^5 /well) were cultured in RPMI 1640 supplemented with glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μ g/ml), and fetal bovine serum (10%; HyClone, Logan, UT) for 7 days, at 37°C in a humidified atmosphere containing 5% CO_2 . The cultures were pulsed with 0.5 μCi /well of [^3H]thymidine (sp. act., 6.7 Ci/mmol; New England Nuclear, Boston, MA) for the final 18 h of culture, harvested on glass fiber filter, and the radioactivity counted on a beta scintillation counter (Rack Beta; LKB Pharmacia, Uppsala, Sweden). Results were expressed as stimulation index (SI) as follows:

$$\text{SI} = \text{cpm (antigen-stimulated cultures)} / \text{cpm (medium alone cultures)}.$$

Results

Amino acid sequence of the 23-kDa polypeptide

The 23-kDa latex polypeptide was purified by electroelution from NAL separated on SDS-polyacrylamide gels. The m.w. of this polypeptide was determined by computerized analysis using a densitometer scanner (Ambis, San Diego, CA), after comparison with several commercial

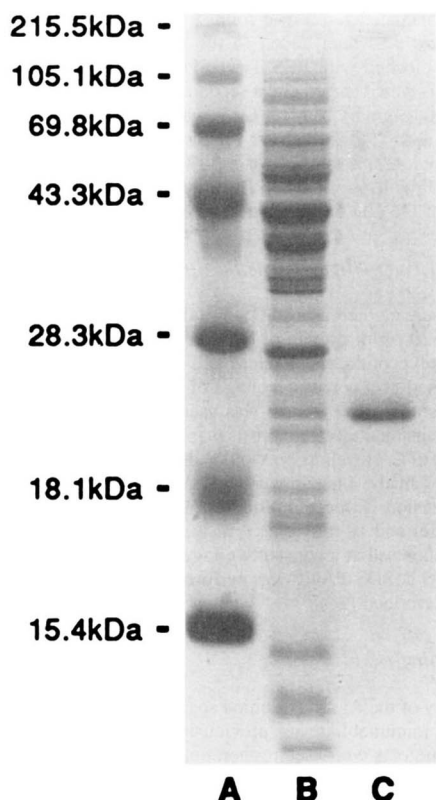


FIGURE 1. Purified 23-kDa polypeptide showing a single band on SDS-PAGE (15% gel) stained with Coomassie brilliant blue. Fourteen micrograms of NAL protein (*lane B*) and 0.5 μ g of purified 23-kDa polypeptide (*lane C*) were loaded. *Lane A*, Molecular weight standard.

m.w. standards. The calculated molecular mass in different runs varied from 22.6 to 23.4 kDa. The purified 23-kDa polypeptide showed a single band on SDS-PAGE (Fig. 1) and one spot with an isoelectric point of 4.8 on a two-dimensional electrophoresis gel (Fig. 2).

No sequence data were obtained from the purified 23-kDa polypeptide coupled to 1,4 phenylene diisothiocyanate disks, suggesting that the N-terminus of the polypeptide was blocked. Proteolytic fragments were prepared from the reduced and alkylated 23-kDa polypeptide by digestion with staphylococcal V8 protease and trypsin. Nine of the tryptic peptides and three V8 peptides were successfully sequenced. The peptide sequences were compared by computer with the PIR (Protein Identification Resources, National Biomedical Research Foundation) database release 41 and the Entrez databases release 13 (National Center for Biotechnology Information). Several of the peptides showed significant similarity to the reported sequence for rubber elongation factor (31). The peptides were then aligned with this sequence as shown in Figure 3. Of the 90 residues aligned, 41 (45.6%) were identical, and a further 29 (32.2%) were conservative, single-base changes. This indicates that the polypeptide is a member of the same family. There are no cysteine residues

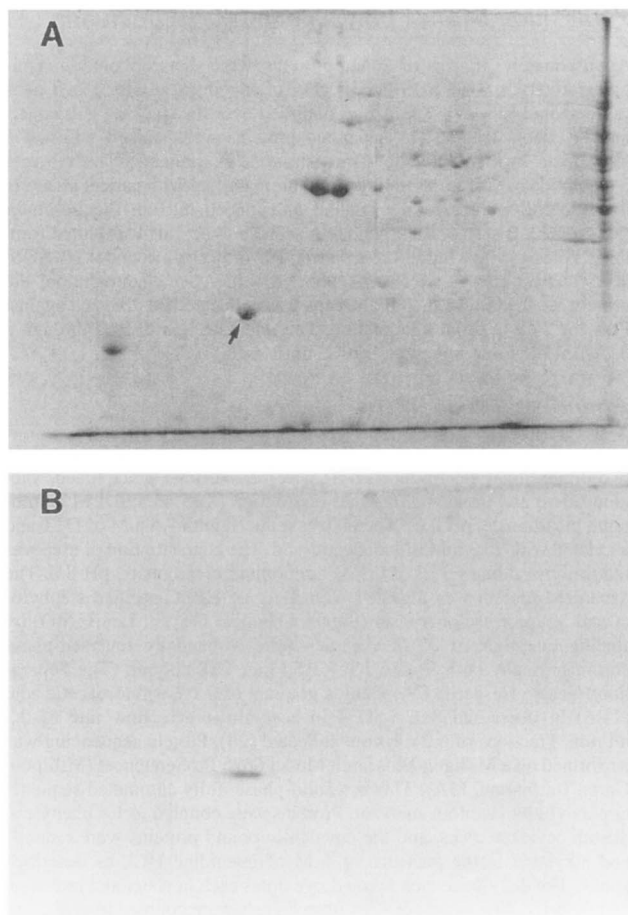


FIGURE 2. The 23-kDa polypeptide appears as an isolated spot in *A* (the arrow denotes the 23-kDa polypeptide) and as a single spot in *B*. The isoelectric point of this polypeptide was found to be about 4.8 on a two-dimensional electrophoresis gel. Five micrograms of purified polypeptide, or 140 μ g of NAL protein, was loaded in the first dimension while SDS-PAGE (12% gel) was used in the second dimension. Over 100 polypeptides were visible with NAL (*A*), while only one spot was visible with the 23-kDa polypeptide (*B*). Both gels were stained with Coomassie brilliant blue stain.

in rubber elongation factor, and none were found in the sequenced regions of the 23-kDa polypeptide. Several of the peptides could not be aligned; and a variant of the peptide starting at position 28 was found, SVDSFSTVY. The calculated m.w. of 14,590 for rubber elongation factor is significantly less than the estimated 23,000 for this latex allergen; therefore the allergen would be expected to have a longer sequence than the rubber elongation factor. No N-linked glycosylation sites were detected in the regions of the polypeptide sequenced, and none are present in rubber elongation factor.

Reactivity of the purified 23-kDa polypeptide with IgE Abs of latex allergic patients

The IgE-binding activity of the purified 23-kDa polypeptide was tested by ELISA using sera from 22 latex-allergic

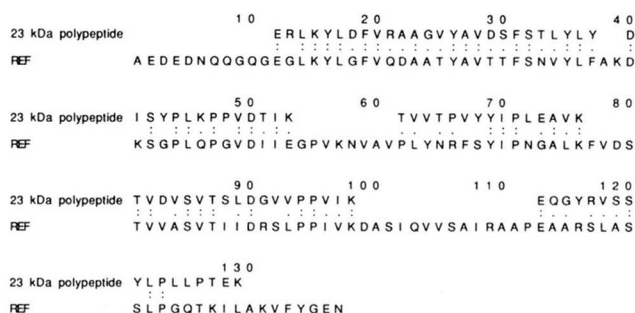


FIGURE 3. The amino acid sequence of the peptide fragments from the 23-kDa polypeptide compared with that of rubber elongation factor (REF). The N-terminus of the 23-kDa polypeptide was blocked. The amino acid sequence of five fragments formed from the 23-kDa polypeptide after digestion with V8 protease and trypsin showed significant similarity to REF. Symbol : indicates identical amino acid residues, and . indicates conservative substitution.

patients and 12 control subjects. The reaction of the 12 control sera with the 23-kDa polypeptide showed a mean OD of 0.149 ± 0.035 (mean $\pm$ SD). Therefore, we used the mean + 2 SD (0.219) as a cutoff value. Thirteen out of the seventeen (76%) sera from SB patients with latex allergy showed positive reactions, while only one of the five sera from HCW with latex allergy reacted with the purified 23-kDa polypeptide (Table I). Furthermore, all the sera of patients with anaphylactic reactions to latex, including the one HCW, showed reactivity with the 23-kDa polypeptide (Table I).

A pool of four randomly selected sera from SB patients showed strong reactivity with the 23-kDa polypeptide on immunoblot (Fig. 4), while another pool of sera selected from four HCW without anaphylactic reactions failed to show any reactivity with the 23-kDa-purified allergen or with the same component in the NAL (data not shown).

23-kDa latex polypeptide induced PBMC proliferation in latex-allergic SB and HCW patients

A significantly elevated proliferative response to 1 μ g/ml of the 23-kDa Ag was detected in PBMC from all three of the SB patients studied when compared with the HCW patients with latex allergy (Table II). All three of the HCW patients showed low SI, only slightly more than the SI of the two non-latex atopic controls tested.

Reactivity of the mAb LAM-1 with the 23-kDa allergen

The mAb LAM-1 showed strong reactivity with the purified 23-kDa latex allergen and with the 23-kDa component of NAL (Fig. 5). A weak band at the 46-kDa region, which was not present in NAL, appeared on the immunoblot of 23-kDa Ag (Fig. 5, lane 3). This band also showed reactivity with IgE Ab in the sera of patients with latex allergy

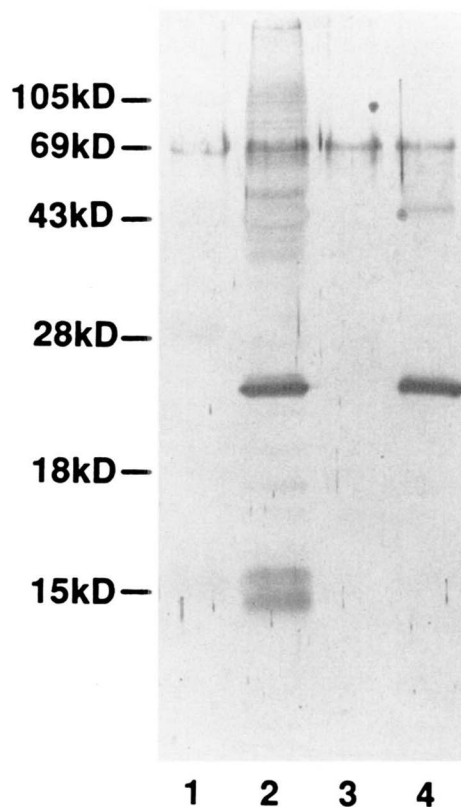


FIGURE 4. Immunoblot result using a serum pool from four SB patients with latex allergy. Strong IgE reactivity with the 23-kDa polypeptide is present in the crude NAL (lane 2), as well as with the purified allergen (lane 4). Lane 1 shows prestained m.w. weight standards. Lane 3 is blank. The 69-kDa band may be a nonspecific band, as it appears in all lanes including the blank lane. A band at about the 46-kDa position appearing in the purified 23-kDa sample in lane 4, which is not present in NAL in lane 2, may be a doublet of the 23-kDa polypeptide. The SDS-PAGE was carried out as described in Figure 1.

(Fig. 4, lane 4). Because of its molecular size, i.e., double the size of the 23-kDa polypeptide, and its reactivity with LAM-1 mAb, it is possible that the 46-kDa band may be a dimer of 23-kDa and not a contaminating protein co-eluted with the 23-kDa allergen.

Presence of the 23-kDa latex allergen in latex products and non-latex extracts

In addition to NAL, AL and extracts of latex surgical gloves, examination gloves, and tubing also reacted with LAM-1, indicating the presence of the 23-kDa and/or its subunit epitopes in these latex extracts (Table III). The inhibition ELISA using these latex extracts indicated that the crude raw latex samples contained more specific allergen than did glove extracts (Table IV). There was considerable difference in the content of the 23-kDa allergen among glove extracts from different commercial sources,

Table II. The proliferative response of PBMC to 23-kDa Ag by spina bifida patients (SB) and health care workers (HCW)

Subjects	Stimulation Index ^a
SB	
1	26.67
2	4.80
3	9.84
HCW	
1	1.68
2	1.20
3	0.70
Control	
1	0.45
2	0.55

^a Stimulation Index = cpm (antigen-stimulated cultures)/cpm (medium alone cultures). 23-kDa antigen (final concentration 1 µg/ml) was added to PBMC cultures for 7 days and the [³H]thymidine uptake was measured.

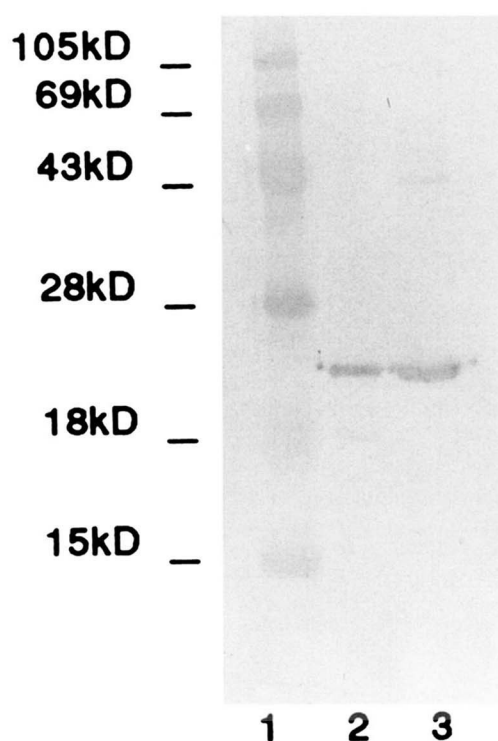


FIGURE 5. Immunoblot result of mAb LAM-1 reacting with the 23-kDa polypeptide in crude NAL (lane 2) and with a purified 23-kDa polypeptide (lane 3). Lane 1 shows prestained m.w. standards. SDS-PAGE was carried out as in Figure 1. This mAb also cross-reacts with a 19-kDa band present in NAL (lane 2). The 46-kDa band, also detectable in this blot (lane 3), is similar to that seen in lane 4 of Figure 4.

which may be due to the diverse procedures involved in the manufacturing of latex gloves.

The presence of protein cross-reactive with the 23-kDa latex Ag in avocado, banana, banana peel, kiwi, mango, papaya fruit, papaya seed, potato, oat, and wheat extracts was studied by immunoblot using LAM-1. Although some of these extracts revealed proteins at the 23-kDa position SDS-PAGE (Fig. 6A), no reaction was detected against

Table III. ELISA results of mAb LAM-1 reacting with latex extracts

Latex Extracts	OD ^a (Mean ± SD)
NAL (Malaysia)	3.120 ± 0.14
AL (Malaysia)	2.334 ± 0.20
Ansell glove	0.168 ± 0.03
Baxter glove	0.802 ± 0.04
Glad glove	1.091 ± 0.11
Perry glove	0.124 ± 0.02
Sima glove	0.746 ± 0.08
Vacuum tubing	0.264 ± 0.04
Control (vinyl glove)	0.066 ± 0.01

^a Mean of quadruplicate determinations. A reaction with mean OD value larger than mean + 2 SD of control was considered positive.

Table IV. Concentrations of latex extracts yielding 50% inhibition of the reactivity of mAb LAN-1 with the 23-kDa antigen

Latex Extracts	Concentration (µg/ml)
NAL (Malaysia)	2.7
AL (Malaysia)	4.1
Ansell glove	372.4
Baxter glove	38.4
Glad glove	27.8

^a The concentrations of different extracts resulted in 50% inhibition of the ELISA OD values of the purified 23-kDa polypeptide reactivity with its mAb. Lower inhibitory concentration indicates greater amount of specific Ag in the product.

LAM-1 in immunoblots (Fig. 6B), indicating that LAM-1 recognized the 23-kDa allergen unique to latex. The few bands showing reactivity with the mAb at a high m.w. region (Fig. 6B, lanes 8, 10 and 11) may represent non-specific reactions, as those were also visible in the lane of m.w. standards (Fig. 6B, lanes 1 and 13). Only papaya extract (Fig. 6B, lane 8) showed a weakly cross-reacting band, at about the 50-kDa region, with the mAb LAM-1.

Discussion

We previously purified five latex polypeptides from nonammoniated natural rubber latex (32). Our preliminary results indicate that one of them, the 23-kDa polypeptide, reacted frequently with IgE Abs from SB patients with latex allergy. In the present study, this polypeptide is further evaluated for its role in both humoral and cell-mediated immunity in SB patients with latex allergy. ELISA results using the purified 23-kDa polypeptide demonstrated that it is a significant allergen associated with latex-allergic SB patients. It also significantly stimulated PBMC proliferation in SB patients, but not in HCW with latex allergy. Hence, the 23-kDa allergen has diagnostic significance for SB patients with latex allergy. Furthermore, this allergen may also be associated with anaphylactic reactions to latex. All seven patients with anaphylaxis to latex reacted strongly with the 23-kDa allergen. Sera from other patient groups (non-SB and non-HCW) with anaphylactic reactions to latex also reacted with the purified 23-kDa

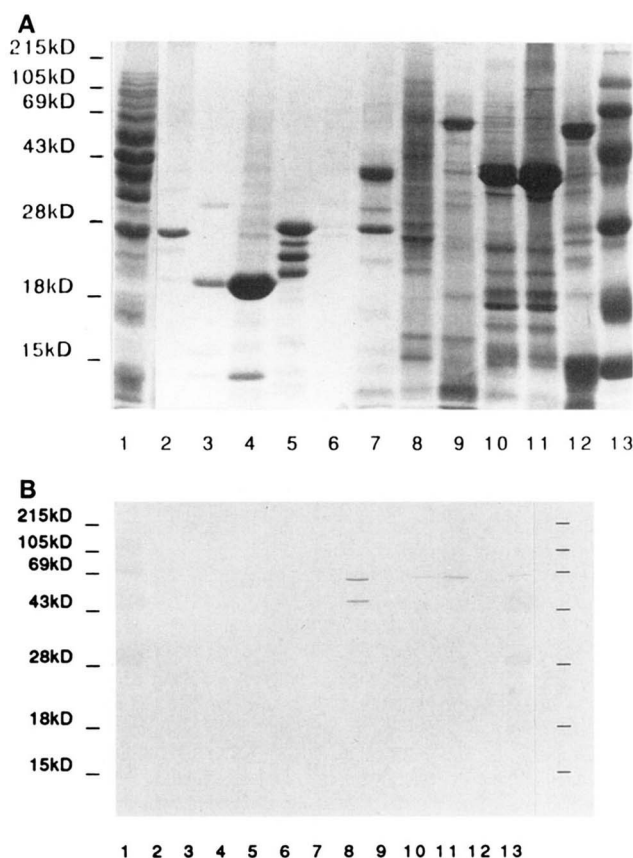


FIGURE 6. A, SDS-PAGE reveals multiple protein bands in extracts of NAL, avocado, banana, banana peel, kiwi, mango, oat, papaya plant, papaya seed, two different potato extracts, and wheat from lanes 1 to 12, respectively. Lane 13 shows m.w. standards. Approximately 20 μ g of protein of each extract was loaded in 15% gel. B, Immunoblotting with mAb LAM-1 failed to react with the 23-kDa polypeptide in extracts other than latex, indicating that LAM-1 recognizes the 23-kDa allergen unique to latex. Lanes 1 and 13 represent prestained m.w. standards. Lanes 2 to 12 in this figure represent the extracts in the same order as in Figure 6A from lanes 2 to 12. SDS-PAGE was carried out as described above. The 69-kDa band is detected in lanes 8, 10, and 11 and in m.w. standards (lanes 1 and 13), indicating that the band is nonspecific. Papaya plant (lane 8) shows a band near the 50-kDa region that has weak cross-reactivity with LAM-1.

allergen (data not shown). Although additional patients need to be studied before any definite conclusions can be made, our data demonstrated a correlation between anaphylactic reaction to latex and the presence of reactivity to the 23-kDa latex allergen.

Significant homology of the 23-kDa polypeptide was observed with REF (45.6%, Fig. 3). REF is a 14-kDa protein bound to rubber particles in native latex and assists prenyltransferase to add multiple *cis*-isoprene units to rubber molecules (31, 33). Czuppon et al. (34) presented evidence suggesting that REF is a major allergen of latex. It is present as a 58-kDa homotetrameric protein with 14.5-

kDa subunits, in ammoniated and nonammoniated latex, and in latex glove extracts. REF has an isoelectric point of 8.5, while the 23-kDa polypeptide described in the present study has an isoelectric point of about 4.8, indicating that these two proteins are different. The relationship between the 23-kDa polypeptide and REF needs to be studied, as both share homology, even though significant differences are also present. Recently, Beezhold et al. (35) identified a 46-kDa allergen in nonammoniated and ammoniated latex. The N-terminal sequence from this protein was unique, suggesting that it is a previously unrecognized protein. Since the N-terminal of the 23-kDa allergen is blocked, we could not make any comparison with the 46-kDa allergen. Unlike the 23-kDa allergen, the 46-kDa allergen demonstrated strong reactivity with IgE from HCW (35).

Several other investigative groups have noted preferential reactivity of sera from SB patients to latex allergens with apparent m.w.s of 14,000 (36, 37), 24,000 (38), 25,000 (39), and 27,000 (37). According to the m.w.s and the reactivities, these latex allergens identified by different groups may be in fact the same polypeptides or may belong to the same family or even be a degraded product of a common large m.w. protein. This antigenic variability may be associated with differences in the strain of rubber trees producing latex with varied quality depending on commercial needs, or even preparation methods. The determination of the relationship of these putative relevant allergens to each other will require further purification and amino acid sequence analyses.

By using the mAb, we demonstrated that the 23-kDa latex allergen and/or its epitope was also present in ammoniated latex and extracts of natural rubber latex products. We have also demonstrated the presence of the 23-kDa allergen in latex glove powders and on natural rubber latex gloves as an unextractable latex allergen (40). On immunoblots, mAb LAM-1 failed to show strong reactivity with AL or glove extracts at the 23-kDa position, as it did with NAL. This may be due to the destruction of the 23-kDa polypeptide by ammonia treatment and/or to manufacturing procedures leading to a breakdown of proteins. However, the epitopes of the 23-kDa allergen are still preserved in active form in these materials, as evidenced by the ELISA results. Interestingly, the 23-kDa polypeptide could not be detected in extracts from natural products such as fruits and grains; these allergens have been reported to cross-react with latex-specific IgE Ab in the sera of latex-allergic patients (25, 41–45). These results indicate that the mAb LAM-1 can be used specifically to screen latex end products for the presence of this allergen.

In conclusion, the purified 23-kDa latex polypeptide can modulate both humoral and cell-mediated immunologic reactivity in SB patients with latex allergy. This polypeptide is a unique latex allergen present in natural rubber latex products that are used in health care management, and can induce sensitization in exposed patients. Hence,

this 23-kDa allergen may be of significant value in the diagnosis of latex allergy in SB patients.

References

- Nutter, A. F. 1979. Contact urticaria to rubber. *Br. J. Dermatol.* 101:597.
- Carrillo, T., M. Cuevas, M. Munoz Hinojosa, and I. Moneo. 1986. Contact urticaria and rhinitis from latex surgical gloves. *Contact Dermatitis* 15:69.
- Slater, J. E. 1989. Rubber anaphylaxis. *N. Engl. J. Med.* 320:1126.
- Ownby, D., M. Tomlanovich, N. Sammons, and J. McCullough. 1991. Anaphylaxis associated with latex allergy during barium enema examinations. *Am. J. Roentgenol.* 156:903.
- Slater, J. E. 1994. Latex allergy. *J. Allergy Clin. Immunol.* 94:139.
- Fink, J. N., and K. J. Kelly. 1994. Latex hypersensitivity—an emerging problem. *Allergy Clin. Immunol. Newsl.* 6:4.
- Bubak, M. E., C. E. Reed, A. F. Fransway, J. W. Yunginger, R. T. Jones, C. A. Carlson, and L. W. Hunt. 1992. Allergic reactions to latex among health-care workers. *Mayo Clin. Proc.* 67:1075.
- Taylor, J. S., J. Cassettari, W. Wagner, and T. Helm. 1989. Contact urticaria and anaphylaxis to latex. *J. Am. Acad. Dermatol.* 21:874.
- Kelly, K. J., M. Setlock, and J. P. Davis. 1991. Anaphylactic reactions during general anesthesia among pediatric patients. *MMWR* 40:437.
- Boyle, K. T., M. C. Anderson, and J. G. Kenimer. 1992. Specific IgE to latex extracts. *J. Allergy Clin. Immunol.* 89:224.
- Turjanmaa, K., and T. Reunala. 1989. Condoms as a source of latex allergen and cause of contact urticaria. *Contact Dermatitis* 20:360.
- Turjanmaa, K., T. Reunala, H. Alenius, H. Brummer-Kowenkotio, and T. Palusuo. 1990. Allergens in latex surgical gloves and glove powder. *Lancet* 336:1588.
- Schneck, F. X., and M. F. Bellinger. 1993. The “innocent” cough or sneeze: a harbinger of serious latex allergy in children during bladder stimulation and urodynamic testing. *J. Urol.* 150:687.
- Hamann, C. P. 1993. Natural rubber latex protein sensitivity in review. *Am. J. Contact Dermatitis* 4:4.
- Turjanmaa, K. 1987. Incidence of immediate allergy to latex gloves in hospital personnel. *Contact Dermatitis* 17:270.
- Arellano, R., J. Bradley, and G. Sussman. 1992. Prevalence of latex sensitization among hospital physicians occupationally exposed to latex gloves. *Anesthesiology* 77:905.
- Berky, Z. T., W. J. Luciano, and W. D. James. 1992. Latex glove allergy: a survey of the US Army Dental Corps. *JAMA* 268:2695.
- Ellsworth, P. I., P. A. Merguerian, R. B. Klein, and A. A. Rozycki. 1993. Evaluation and risk factors of latex allergy in spina bifida patients: is it preventable? *J. Urol.* 150:691.
- Meeropol, E., R. Kelleher, S. Bell, and R. Leger. 1990. Allergic reactions to rubber in patients with myelodysplasia. *N. Engl. J. Med.* 323:1072.
- Meeropol, E., J. Frost, L. Pugh, J. Roberts, and J. A. Ogden. 1993. Latex allergy in children with myelodysplasia: a survey of Shriners hospitals. *J. Pediatr. Orthop.* 13:1.
- Tosi, L. L., J. E. Slater, C. Shaer, and L. A. Mostello. 1993. Latex allergy in spina bifida patients: prevalence and surgical implications. *J. Pediatr. Orthop.* 13:709.
- Shaer, C., and J. E. Slater. 1993. Latex allergy in children. *Curr. Opin. Pediatr.* 5:700.
- Kelly, K. J., V. P. Kurup, K. Reijula, and J. N. Fink. 1994. The diagnosis of natural rubber latex allergy. *J. Allergy Clin. Immunol.* 93:813.
- Kelly, K. J., V. P. Kurup, M. Zacharisen, A. Resnick, and J. N. Fink. 1993. Skin and serologic testing in the diagnosis of latex allergy. *J. Allergy Clin. Immunol.* 91:1140.
- Kurup, V. P., T. Kelly, N. Elms, K. J. Kelly, and J. N. Fink. 1994. Cross-reactivity of food allergens in latex allergy. *Allergy Proc.* 15:211.
- Kurup, V. P., K. J. Kelly, K. Turjanmaa, H. Alenius, T. Reunala, T. Palosuo, and J. N. Fink. 1993. Immunoglobulin E reactivity to latex antigens in the sera of patients from Finland and the United States. *J. Allergy Clin. Immunol.* 91:1128.
- Alenius, H., V. Kurup, K. Kelly, T. Palosuo, K. Turjanmaa, and J. Fink. 1994. Frequent occurrence of IgE antibodies to a cluster of latex proteins in patients with spina bifida and histories of anaphylaxis. *J. Lab. Clin. Med.* 123:712.
- Hoffman, D. R. 1993. Allergens in Hymenoptera venom XXV: the amino acid sequences of antigen 5 molecules. The structural basis of antigenic cross-reactivity. *J. Allergy Clin. Immunol.* 92:707.
- Kurup, V. P., A. Kumar, K. J. Kelly, and J. N. Fink. 1993. Characterization of a monoclonal antibody against latex protein associated with latex allergy. *J. Allergy Clin. Immunol.* 92:638.
- Murali, P. S., K. J. Kelly, J. N. Fink, and V. P. Kurup. 1994. Investigations into the cellular immune responses in latex allergy. *J. Lab. Clin. Med.* 124:638.
- Dennis, M. S., W. J. Henzel, W. Kohr, and D. R. Light. 1989. Amino acid sequence of rubber elongation factor protein associated with rubber particles of *Hevea brasiliensis*. *J. Biol. Chem.* 264:18618.
- Kelly K. J., L.-J. Lu, V. P. Kurup, and J. N. Fink. 1995. Purification of latex allergens and their IgE-binding activity with latex allergic patient sera. *J. Allergy Clin. Immunol. (Abstract)* 95:213.
- Dennis, M. S., and D. R. Light. 1989. Rubber elongation factor from *Hevea brasiliensis*: identification, characterization and role of rubber biosynthesis. *J. Biol. Chem.* 264:18608.
- Czuppon, A. B., Z. Chen, S. Rennert, T. Engelke, H. E. Meyer, M. Heber, and X. Baur. 1993. The rubber elongation factor of rubber trees (*Hevea brasiliensis*) is the major allergen in latex. *J. Allergy Clin. Immunol.* 92:690.
- Beezhold, D. H., G. L. Sussman, D. A. Kostyal, and N.-S. Chang. 1994. Identification of a 46 kD latex protein allergen in health care workers. *Clin. Exp. Immunol.* 98:408.
- Slater, J. E., and S. K. Chhabra. 1992. Latex antigens. *J. Allergy Clin. Immunol.* 89:673.
- Alenius, H., T. Palosuo, K. Kelly, V. Kurup, T. Reunala, S. Mäkinen-Kiljunen, K. Turjanmaa, and J. Fink. 1993. IgE reactivity to 14-kD and 27-kD natural rubber proteins in latex-allergic children with spina bifida and other congenital anomalies. *Int. Arch. Allergy Immunol.* 102:61.
- Avula, R. B., J. McCullough, and D. R. Ownby. 1995. Characterization of latex allergens for different latex sensitive groups. *J. Allergy Clin. Immunol.* 95:214 (Abstr.).
- Muguerza, J., C. Capo, F. Porri, J. L. Jacob, D. Vervloet, and J. L. Mege. 1995. Latex allergy: allergen identification in *Hevea brasiliensis* fractions by immunoblotting. *J. Allergy Clin. Immunol.* 95:212 (Abstr.).
- Lu, L.-J., V. P. Kurup, J. N. Fink and K. L. Kelly. 1995. Comparison of latex antigens from surgical gloves, ammoniated and non-ammoniated latex: effect of ammonia treatment on natural rubber latex proteins. *J. Lab. Clin. Med.* In press.
- Lavaud, F., C. Cossart, V. Reiter, J. Bernard, G. Deltour, and I. Holmquist. 1992. Latex allergy in patient with allergy to fruit. *Lancet* 339:492.
- Ceuppens, J. L., P. Van Durme, and A. Dooms-Goossens. 1992. Latex allergy in patient with allergy to fruit. *Lancet* 339:493.
- Anibarro, B., M. C. Garcia, and C. Pascual. 1993. Associated sensitization to latex and chestnut. *Allergy* 48:130.
- Mäkinen-Kiljunen, S. 1994. Banana allergy in patients with immediate-type hypersensitivity to natural rubber latex: characterization of cross-reacting antibodies and allergens. *J. Allergy Clin. Immunol.* 93:990.
- Blanco, C., T. Carrillo, R. Castillo, J. Quirarte, and M. Cuevas. 1994. Avocado hypersensitivity. *Allergy* 49:454.