Latex antigens

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Previous studies have demonstrated that some children with spina bifida have IgE to proteins in natural rubber. In this study we compare different sources of latex antigen and identify possible antigenic peptides by radioimmunoblotting technique. Sera were collected from 26 children with spina bifida, tested by RAST with ammoniated latex extract (AL), and frozen until use. Extracts were prepared from ammoniated and nonammoniated latex, and the proteins were separated by electrophoresis on a 15% sodium dodecyl suflate–polyacrylamide gel and transferred to polyvinylene difluoride (PVDF). Strips of PVDF were then incubated with individual sera and ^{125}I-labeled rabbit antihuman IgE before development by autoradiography; 18/26 sera were AL RAST positive; 0/8 AL RAST-negative patients had any binding to the latex proteins on PVDF. Sera from all patients were tested in a RAST with a nonammoniated latex extract (NAL), and the results were comparable to the AL RAST. Liquid-phase AL and NAL were comparable in their ability to inhibit the binding of patient's IgE to solid-phase AL and NAL. Sera from 14 RAST-positive patients were tested by immunoblotting with separated, reduced, and nonreduced AL and NAL. All 14 sera demonstrated IgE binding to a 14 kd peptide, which was more pronounced in reduced NAL. These results suggest that the 14 kd peptide in NAL is a major antigen in rubber allergy but that AL is an acceptable antigen source for in vitro diagnostic studies. (J ALLERGY CLIN IMMUNOL 1992;89:673-8.)

Key words: *Latex, rubber, immediate hypersensitivity, RAST*

Numerous case reports have appeared describing immediate hypersensitivity reactions to natural rubber products. Many of these reports have suggested that health care workers and children with spina bifida are at increased risk for rubber allergy, and some reports have provided credible evidence of an IgE-mediated mechanism.[1-6]

Natural rubber is *cis*-1,4-polyisoprene that has been extracted from the latex of *Hevea brasiliensis* and cross-linked for improved elasticity. Latex is a complex intracellular product that is 30% to 40% polyisoprene by weight. Although rubber production yields a product that is 93% to 95% polyisoprene, rubber crepe may be as much as 3% protein by weight.[7]

Abbreviations used

AL:	Extract made from ammoniated latex concentrate
NAL:	Extract made from nonammoniated latex
PAS:	Periodic acid-Schiff
PBS:	Phosphate-buffered saline, pH 7.4
PVC:	Polyvinylchloride
PVDF:	Polyvinylene difluoride
SDS–PAGE:	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
MW:	Molecular weight

Previous investigators have used several different source materials for rubber-allergy testing and have attempted to characterize the antigens of natural rubber with filtration, chromatography, and nitrocellulose immunoblots. Our laboratory has demonstrated that as many as 34% of children with spina bifida have evidence of rubber-specific IgE, with a RAST using AL.[6] In this article, we describe the immunochemical and electrophoretic characteristics of two different

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preparations of natural rubber. In addition, we describe the IgE radioimmunoblot characterization of the antigens of *H. brasiliensis* latex with the sera of 14 rubber-allergic patients.

MATERIAL AND METHODS

Latex sources

Nonammoniated latex was obtained from Dr. Katrina Cornish, United States Department of Agriculture, collected from *H. brasiliensis* in Costa Rica in Tris buffer (0.1 mol/L, pH 7.5) and sodium azide (0.01%), and shipped frozen on dry ice.[8] Ammoniated latex concentrate was obtained from Guthrie Latex, Baltimore, Md. Purified hevein was the generous gift of Dr. Manuel Soriano-Garcia, Mexico City.

Extract preparation

NAL. Frozen nonammoniated latex was thawed at room temperature. This slow thawing led to substantial coagulation of rubber that contracted and left a residual translucent serum (500 ml/L of latex). The serum was filtered, dialyzed in water across a 1000 dalton cutoff cellulose membrane, and lyophilized.

AL. Ammoniated latex was centrifuged at 100,000 *g* at 4° C for 1 hour. The rubbery supernatant was discarded, and the yellow aqueous layer was dialyzed extensively in PBS (pH 7.4), then in water across a 1000 dalton cutoff cellulose membrane, and lyophilized.

Sera. For immunoblotting experiments, sera were obtained from 22 patients with spina bifida, of which 14 were RAST positive for AL; of these RAST-positive patients, five had histories consistent with rubber allergy.[6] Sera were collected in a study approved by the Children's Hospital Institutional Review Board.

For RAST-inhibition studies, sera from four additional patients, AL RAST-positive, rubber-allergic, and with spina bifida were used.

Forty-five unidentified control sera were obtained from the outpatient clinical laboratory at Children's National Medical Center.

RAST. AL RAST was performed as previously described.[5] Briefly, 1 μg of AL was bound to each well in a PVC microtiter plate and incubated sequentially with patient's serum and ^{125}I-labeled rabbit antihuman IgE (Pharmacia Diagnostics, Piscataway, N. J.). Results are expressed as counts per minute (bound)/counts per minute (total added). NAL RAST was identical to the AL RAST, except that 1 μg of NAL was used as the antigen.

RAST-inhibition studies were performed by adding AL, NAL, or hevein (up to 10 μg) to the wells along with the sera. In addition, NAL (1 μg) was bound to PVC plates, and a RAST was performed with and without added NAL or AL.

Electrophoresis. SDS–PAGE was performed with the mini-Protean II system (Bio-Rad Laboratories, Richmond, Calif.). Approximately 30 μg of latex, with or without 5% β-mercaptoethanol, was applied to each lane, and 15% gels were run at 200 V for 45 minutes.

Transfer and immunoblotting. The separated latex peptides were transferred to a PVDF membrane (Millipore Corp., Bedford, Mass.) at 240 A for 2 hours in Towbin's buffer.[9] The PVDF was stained with Coomassie blue (0.1% in 50% methanol) and cut into strips for immunoblotting. These strips were blocked in PBS containing 3% bovine serum albumin, 0.05% Tween, and 0.01 of sodium azide ("blocking buffer") for 2 hours and incubated overnight in patient's serum (1:10). The strips were then washed in PBS/Tween and incubated for 6 hours in ^{125}I-labeled antihuman IgE (Pharmacia; 3.3 ng/ml) in blocking buffer. Finally, the strips were washed and developed.

PAS. The staining was done as previously described.[10] PVDF strips with separated latex peptides were incubated sequentially with 5% PAS reagent (1:3, vol/vol) and sodium metabisulfite (0.5%).

Statistics. Pearson's correlation coefficient was determined with a statistical software package.

RESULTS

RAST was performed with the AL extract on all 26 patients with spina bifida (Table I). With the same technique, the geometric mean binding of serum from 45 control subjects was 0.41% (95% confidence interval, 0.17% to 0.95%). Therefore, we chose a cutoff of 1% binding. Sera from 18 patients with spina bifida were positive; 12 of these patients had experienced symptoms consistent with rubber allergy. Of the eight AL RAST-negative patients, none had symptoms of rubber allergy or idiopathic anaphylaxis. A RAST was also performed on these sera with NAL as the antigen. Binding was generally somewhat higher in the NAL RAST (Table I). When the AL and NAL RAST results were compared, Pearson's correlation coefficient was 0.95.

When AL or NAL extract was added to microtiter wells concurrently with four of the patients' sera, significant inhibition of IgE binding was noted (Fig. 1). Under all conditions, for each of the four patients, the 50%-inhibition amount was <2 μg of added extract. The ability of the two latex extracts to inhibit IgE binding to immobilized latex was essentially the same.

Hevein,[11-13] purified for x-ray crystallography, was added to wells in an AL RAST concurrently with five positive sera. No inhibition of specific IgE binding was observed (data not presented).

As illustrated in Fig. 2, when the peptides of nonreduced NAL were separated by SDS–PAGE and stained with Coomassie blue, major bands appeared at 45, 28, 21, and 14 kd, along with multiple minor bands; in reduced NAL, a doublet appeared at 27 and 28 kd, and a new major band appeared at around 10 to 12 kd. In contrast, nonreduced AL had only faint, minor bands at 60, 45, and 33 kd; with reduction, additional faint bands appeared at 28 and <14.3 kd. PAS staining was negative.

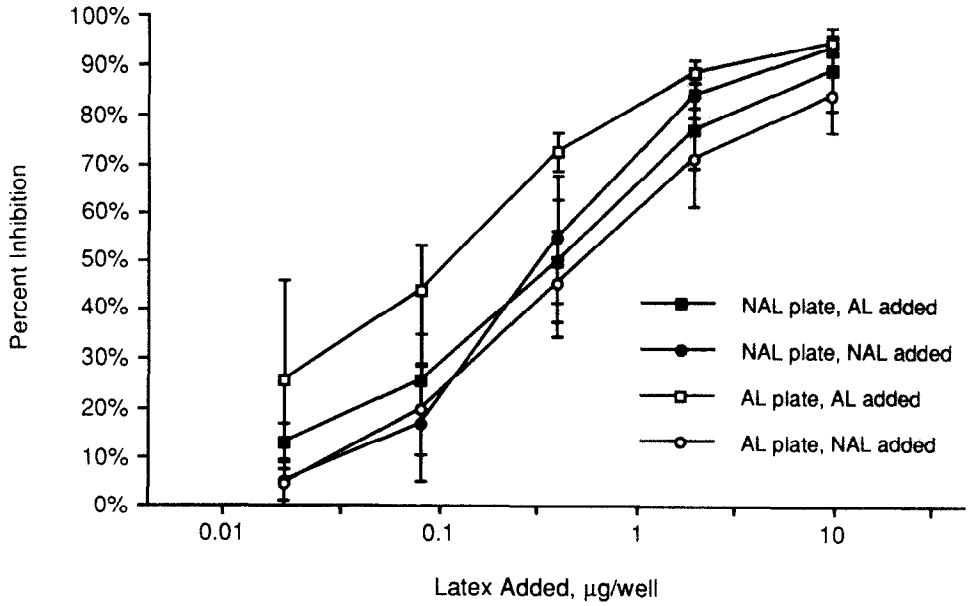

FIG. 1. RAST-inhibition studies. NAL or AL, 1 μg, was bound to microtiter wells. After wells were washed, serum containing rubber-specific IgE was added together with between 0 and 10 μg of NAL or AL. After overnight incubation, wells were washed, and ^{125}I-labeled antihuman IgE was added. *Each point* represents pooled data from four individual sera ± SD.

TABLE I. Latex (AL or NAL) RAST on sera from patients with spina bifida (r = 0.95)

Patient No.	Initials	AL % binding	±	NAL % binding
1	M. A.	8.2	+	1.1
2	O. B.	5.0	+	5.5
3	W. H.	4.4	+	5.4
4	A. P.	16.4	+	20.6
5	A. N.	1.0	+	1.9
6	M. M.	27.1	+	37.9
7	F. S.	7.5	+	10.7
8	B. T.	12.9	+	7.2
9	D. D.	11.1	+	12.6
10	B. L.	11.3	+	13.0
11	A. M.	1.6	+	1.0
12	L. E.	1.4	+	2.7
13	G. A.	17.2	+	22.0
14	D. E.	2.3	+	3.1
15	S. M.	0.7	−	0.4
16	F. A.	0.9	−	0.4
17	F. O.	0.9	−	1.2
18	S. H.	0.6	−	0.9
19	J. E.	0.5	−	0.5
20	B. A.	0.2	−	0.5
21	P. A.	0.5	−	0.5
22	F. A.	0.5	−	0.9
23	B. A. R.	22.9	+	27.0
24	F. R.	6.8	+	12.8
25	M. E.	8.4	+	17.0
26	H. A.	18.9	+	19.3

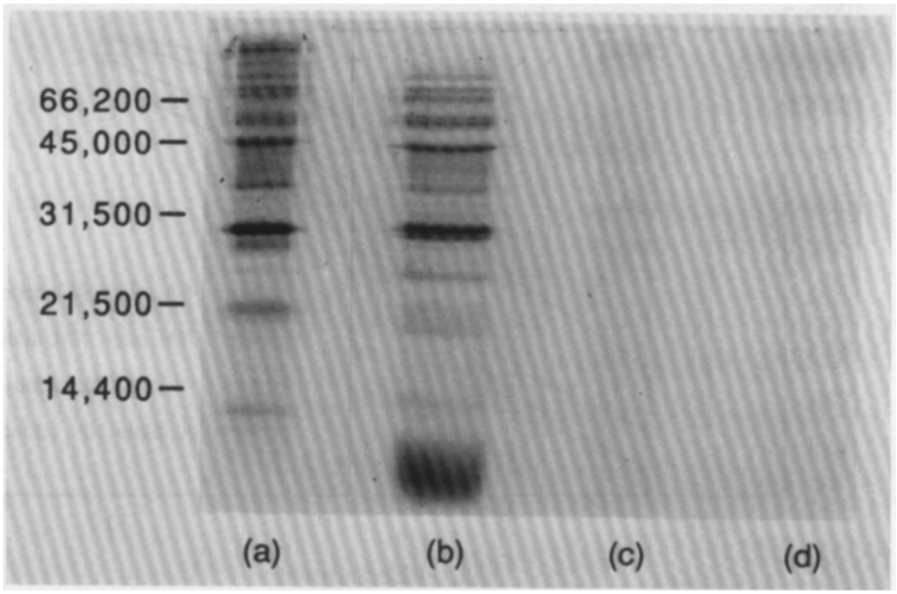

FIG. 2. Fifteen percent SDS–PAGE of *(a)* nonreduced NAL, *(b)* reduced NAL, *(c)* nonreduced AL, *(d)* reduced AL; MW markers are indicated *along the left.*

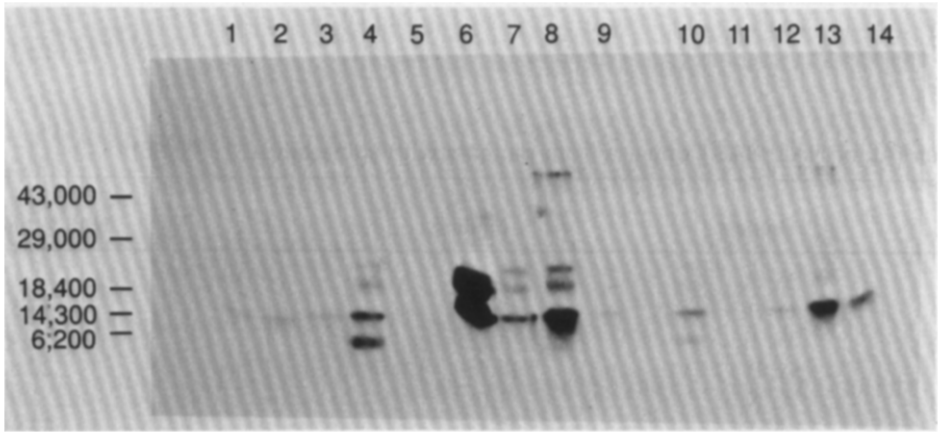

FIG. 3. Radioimmunoblot of reduced NAL, transferred to PVDF, and incubated with sera of 14 AL RAST-positive patients. Strips were then washed and incubated with ^{125}I-labeled antihuman IgE. MW markers are indicated *along* the *left.*

In Fig. 3, immunoblotting of electrophoresed, reduced NAL with sera from 14 AL RAST-positive patients revealed IgE binding to several bands that varied from patient to patient. Each serum demonstrated IgE binding to the 14 kd peptide in NAL. Binding to electrophoresed AL (reduced and nonreduced) was not distinct and highly variable (data not presented). The RAST-negative sera had no evidence of binding, even after film was exposed to the strips for 7 days.

Sera from five RAST-positive patients were used for immunoblots of nonreduced NAL as well. Although essentially the same pattern was observed, there appeared to be less immunoreactive protein in the 14 kd region in the nonreduced specimens (Fig. 4).

DISCUSSION

Allergy to natural rubber may occur by type I or type IV mechanisms. Reports of type I reactions have increased, and health care workers and patients with spina bifida appear to be at increased risk of this phenomenon. Recent episodes of fatal and life-threatening anaphylaxis have made the identification of the antigen(s) responsible for these reactions even more urgent.[14]

The most readily available product for allergy testing is the finished rubber product itself, but patients'

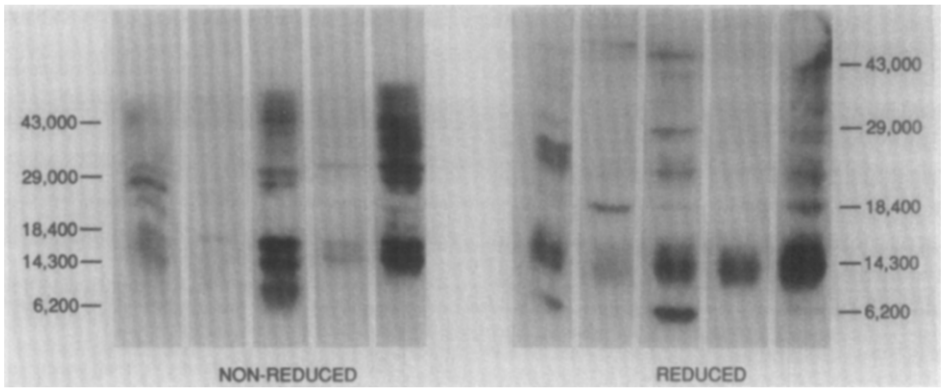

FIG. 4. Radioimmunoblot of nonreduced and reduced NAL, transferred to PVDF, and incubated with sera of five AL RAST-positive patients. Strips were then washed and incubated with ^{125}I-labeled antihuman IgE. MW markers are indicated *along the right* and *left*.

TABLE II. Latex antigen: Previous studies

Latex source	Methods	MW (s)	Ref No.
Gloves and latex	Filtration	>30	17
Gloves	HPLC	2, 5, 30	15
Condoms	HPLC	3-10	18
Latex	Immunoblot, nonreduced, pooled sera	10, 24, 35, 100	19
Glove powder	Immunoblot, nonreduced	10	20

Ref, Reference; *HPLC*, high-performance liquid chromatography.

experiences and a previous study[15] suggest that rubber gloves have a highly variable antigen content. Therefore, gloves are probably an unreliable source of testing material. The next most plentiful product is ammoniated latex concentrate, which may be obtained directly from rubber producers. Ammoniated latex is the source material for most of the medical latex products implicated in these reactions. If the allergens responsible for type I reactions are native plant peptides, it is reasonable to expect that the same allergens in the finished product will be present in AL. AL has been used successfully as a source of skin testing material[4] and RAST antigen,[5] but ammonia and prolonged storage may cause extensive hydrolysis and conformational changes in relevant peptides. Therefore, some investigators have used *H. brasiliensis* leaves[1, 4] as source material.

In this study, the electrophoretic and immunoblotting profiles confirm that AL contains fewer distinct peptides and, perhaps, a higher percentage of heterogeneous peptide fragments than NAL. Nonetheless, for most patients tested, AL and NAL were comparable as antigen sources for the RAST; the generally higher binding of patient IgE to NAL-coated wells may reflect somewhat greater antigen preservation in this preparation. RAST-inhibition data also indicate that the two preparations are immunologically comparable. Thus, both NAL and AL appear to be reasonable antigen sources for diagnostic studies. Larger, prospective studies will be necessary to evaluate the sensitivity and specificity of the assays performed with these antigens. However, for antigen characterization and immunoblotting, NAL is the preferable preparation.

Patients with rubber allergy produce IgE specific for several peptides in *H. brasiliensis* latex. In this study, all patients with latex-specific IgE had antibody to a 14 kd peptide present in NAL, and many sera recognized a 20 kd peptide as well. The increased amount of immunoreactive 14 kd peptide in NAL that had been reduced with β-mercaptoethanol suggests that the peptide is a subunit of a disulfide-linked polymer. Previous investigators have suggested that latex antigens range in MW from 2 to 100 kd (Table II). Recently, a patient with latex and banana allergy has been reported.[16] RAST-inhibition studies suggested cross-reactivity between latex and banana antigens. The nature of these cross-reactive determinants has yet to be studied.

H. brasiliensis latex contains several identified and

characterized proteins. Hevein has a MW of 5 kd and has been purified and sequenced; our work suggests that it is not the antigen in these patients. Other proteins include a prenyltransferase (38 kd) and rubber elongation factor (14 kd). Numerous other proteins doubtless exist in latex, and these data presented here are insufficient to identify definitively the major antigen or antigens. Nonetheless, these approaches will be useful in the further characterization of the immune response to natural rubber.

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