

# Allergens, IgE, mediators, inflammatory mechanisms

## Serum reactivities to latex proteins (*Hevea brasiliensis*)

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**Background:** In recent years there has been an increasing incidence of allergy to latex among health care workers and children with spina bifida. The allergic response in these individuals can be severe and occasionally fatal. Several allergens have been identified with the use of sera from different patient groups. In our effort to identify reagents for in vitro testing and clinical use, we investigated the reactivities of latex proteins to sera collected from a wide range of patients with latex allergy.

**Methods:** Twenty-six serum samples were obtained from adult patients with latex allergy, both hospital workers and non-hospital workers. Serum pools were made either from sera of children with spina bifida or sera of adult patients with latex allergy. Proteins from C-serum and latex particles of latex sap (nonammoniated) were separated by different gel electrophoresis techniques and evaluated for specific IgE binding by immunoblotting.

**Results:** More than 50% of the sera tested reacted to an 18 kd protein, a 25.6 kd acidic protein with an isoelectric point of 3.5, or to both proteins; whereas only 23% of the individual serum samples tested reacted to the rubber elongation factor, which has been reported to be a major latex allergen. The immunoreactive patterns of children's and adults' serum pools were similar but not identical.

**Conclusions:** With the use of gel electrophoresis and immunoblotting techniques, different immunoreactive proteins were identified in C-serum and particles of latex. Rubber elongation factor, which reacted to only 23% of sera tested, did not appear to cross-react immunologically with other latex allergens. (*J ALLERGY CLIN IMMUNOL* 1995;95:1196-1205.)

**Key words:** Latex allergens, IgE-reactive proteins, latex particles, latex C-serum

Since IgE-mediated latex hypersensitivity was first reported in 1979,<sup>1</sup> a number of IgE-reactive proteins in rubber raw materials and in processed rubber products have been identified.<sup>2-9</sup> Different patient groups, different latex preparations (e.g., ammoniated latex, nonammoniated latex, and latex gloves), and different electrophoresis techniques used for these studies produced various results. Four proteins (14 to 100 kd) in the C-serum of ammoniated latex were shown to be reactive with sera of six allergic adults.<sup>3</sup> Sera from children with spina bifida reacted to a 14 kd

### Abbreviations used

|           |                                                           |
|-----------|-----------------------------------------------------------|
| IEF:      | Isoelectric focusing                                      |
| MW:       | Molecular weight                                          |
| pI:       | Isoelectric point                                         |
| REF:      | Rubber elongation factor                                  |
| SDS:      | Sodium dodecyl sulfate                                    |
| SDS-PAGE: | Sodium dodecyl sulfate-polyacrylamide gel electrophoresis |

protein in nonammoniated latex, and many of these sera also reacted to a 20 kd protein.<sup>3,4</sup> The 14 kd protein was also identified in ammoniated latex by sera from patients with latex allergy in Japan.<sup>5</sup> A 10 kd protein from glove extract and glove powder was reported to be the major allergen.<sup>6</sup> Alenius et al.<sup>7</sup> described six major allergenic proteins, of 14 kd to 75 kd, from a nonammoniated latex. The 14 kd protein was recently identified as

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**TABLE I.** Clinical manifestations and latex IgE of 26 patients

| Serum no. | Age (yr) | Sex | Occupation | Symptom        | Latex IgE (ng/ml) |
|-----------|----------|-----|------------|----------------|-------------------|
| 1         | 34       | M   | HP         | NS             | ND                |
| 2         | 28       | F   | NHCW       | CU             | 17.8              |
| 3         | 21       | F   | NHCW       | CU, AN         | 5.44              |
| 4         | 27       | M   | NHCW       | CU             | 20.6              |
| 5         | 32       | M   | NHCW       | CU             | <0.84             |
| 8         | 42       | M   | NHCW       | CU             | 21.5              |
| 9         | 27       | M   | NHCW       | CU             | ND                |
| 10        | 31       | F   | HN         | CU, AR, BA     | 30.0              |
| 11        | 26       | M   | HHK        | CU, AN         | 72.5              |
| 12        | 31       | F   | HN         | CU, AR, BA     | 29.5              |
| 13        | 33       | F   | HN         | CD, CU, BA     | 19.1              |
| 14        | 31       | F   | LT         | CU, AR, BA     | 16.8              |
| 15        | 31       | F   | HN         | AR, BA         | 198               |
| 16        | 29       | F   | HHK        | CU, AR         | 72.8              |
| 17        | 29       | F   | HN         | CD, BA, AN     | 57.8              |
| 18        | 27       | F   | HN         | CD, AR         | 35.8              |
| 19        | 57       | F   | NHCW       | AR, AN         | 18.3              |
| 20        | 42       | M   | HP         | AN             | 36.7              |
| 21        | 26       | F   | HN         | CU, AR, BA, AN | 87.6              |
| 22        | 21       | F   | NHCW       | CU, AN         | 113               |
| 23        | NA       | F   | NA         | NA             | 28.3              |
| 24        | NA       | F   | NA         | NA             | 1.68              |
| 25        | 37       | F   | HN         | CD, AR         | <0.84             |
| 26        | 52       | F   | HN         | CU             | 1.92              |
| 27        | 41       | F   | NHCW       | CD, AR         | 2.78              |
| 28        | 25       | F   | NHCW       | CU, AN         | 44.6              |
| 29        | 25       | F   | LT         | CU             | ND                |

Latex-specific IgE was measured by Pharmacia CAP System according to the manufacturer's specifications.

HP, Hospital physician; NHCW, non-health care worker; HN, hospital nurse; HHK, hospital housekeeper; LT, laboratory technician; NA, information not available; NS, no symptom; CU, contact urticaria; AN, anaphylaxis; AR, allergic rhinitis; BA, bronchial asthma; CD, contact dermatitis; ND, not determined.

the rubber elongation factor (REF), which exists in a tetrameric form in latex.<sup>9</sup> Interestingly, REF was shown to be able to block 86% to 100% of latex-specific IgE binding in all 13 patients included in this study.<sup>9</sup> N-terminal amino acid sequences were determined in a number of the latex allergens, and some of the allergens were found to have sequence homologies to hevein and heveamine.<sup>10</sup> In these reports the term *major allergens* referred to latex proteins that reacted to sera from patients with latex allergy in high frequencies.

The purpose of this study was to identify the IgE-reactive proteins in C-serum and latex particles. When proteins related to allergic symptoms are identified, it will be possible to set standards for the regulation of latex extracts manufactured for use in diagnosis. Immunoblots of isoelectric focusing (IEF) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were used to analyze the IgE-reactive proteins with sera

from different groups of patients with latex allergy in the United States.

## METHODS

### Sera from patients with latex allergy

A total of 26 individual serum samples from patients with latex allergy and two serum pools were collected for this study. The clinical characteristics of these patients were provided by the individuals supplying the sera and are described in Table I. A control serum sample (no. 1) was taken from a healthy adult who had no symptoms of allergy to latex. Six serum samples, numbered 2, 3, 4, 5, 8, and 9 were purchased from Plasma Lab International (Everett, Wash.); these were drawn from adult patients (non-health care workers) who experienced contact urticaria when exposed to latex products. Seven serum samples, numbered 10 to 16, were generously provided by Dr. J. W. Yunginger (Mayo Clinic, Rochester, Minn.) and were drawn from health care workers and laboratory technicians. Twelve serum samples, numbered 17 to 28,

were a gift from Dr. B. Williams (Allergy Respiratory Institute of Colorado, Denver, Colo.) and were obtained from adult patients allergic to latex products. In addition, two serum pools, from adults (no. 6) and from children with spina bifida (no. 7), were a gift from Dr. R. Hamilton (Johns Hopkins University, Baltimore, Md.). Serum no. 29 was taken from a volunteer in our laboratory who experienced contact urticaria after exposure to latex gloves. All sera were stored frozen at  $-20^{\circ}\text{C}$  until use.

### Latex

Nonammoniated latex was collected by Dr. Esah Yip of the Rubber Research Institute in Kuala Lumpur, Malaysia. One volume of fresh latex from *Hevea brasiliensis* (Clone RRIM 600) trees was mixed immediately with 2 volumes of 0.1 mol/L sodium bicarbonate containing 50% wt/vol glycerol and 5 mmol/L cysteine. Glycerol was added to prevent protein degradation by proteolytic enzymes. Buffer/glycerol-diluted latex material was stored at  $4^{\circ}\text{C}$ , with the exception of 2 days at room temperature during shipping.

For comparative study, a sample of ammoniated latex and a glove extract (Baxter Triflex Gloves; Baxter Healthcare Corp., Valencia, Calif.) were obtained from Dr. R. Hamilton (Johns Hopkins University, Baltimore, Md.).

### Latex fractionation

The latex sample was centrifuged at 50,000 g at  $4^{\circ}\text{C}$  for 2 hours. The floating coagulum containing most latex particles was saved and stored at  $4^{\circ}\text{C}$  ("latex particles" were used throughout the study without specification). The yellow aqueous layer, known as the C-serum, was filtered in sterile fashion through a 0.22  $\mu\text{m}$  filter unit, adjusted to 54.5% vol/vol glycerol, and refiltered. This fraction was aliquoted into 2 ml fractions and stored at  $4^{\circ}\text{C}$  as C-serum.

### Extraction of proteins bound to latex particles

The extraction was performed according to the method of Dennis and Light.<sup>11</sup> Five grams of latex particles (wet weight) was extracted with 10 ml of 50 mmol/L Tris buffer, pH 8.0, with and without 2% sodium dodecyl sulfate (SDS) and mixed by horizontal rotation overnight at 250 rpm at room temperature. Large coagulated latex particles, which floated to the top of the tube, were removed; and the remaining fraction was centrifuged at 50,000 g at  $4^{\circ}\text{C}$  for 1 hour. Floating latex particles were discarded, and the aqueous layer, which contained proteins from the latex particles, was collected and stored at  $4^{\circ}\text{C}$ .

### Determination of protein concentration

The protein concentrations of the latex C-serum and the latex particle extract were determined by Ninhydrin (Sigma Diagnostics, St. Louis, Mo.) protein assay.<sup>12</sup>

### IEF electrophoresis

IEF was performed with Ampholine PAGplates, pH 3.5 to 9.5 (Pharmacia, Piscataway, N.J.). Twenty microliters of sample containing 56  $\mu\text{g}$  of protein was applied to each lane, and gels were run at 1500 V, 50 mA, 30 W,  $15^{\circ}\text{C}$  for 1 hour. IEF pI markers (Pharmacia) were run on the same gel.

### SDS-PAGE

SDS-PAGE was performed with Pre-Cast gels (Novex, San Diego, Calif.) according to the method of Laemmli.<sup>13</sup> The running buffer was purchased from Novex. Proteins were denatured in the presence (reduced) and in the absence (nonreduced) of 1% mercaptoethanol.

### Two-dimensional gel electrophoresis

The first dimension, Immobiliner DryStrip (pH 3 to 10, Pharmacia), was rehydrated in 25 ml of rehydration solution containing 12 gm of urea, 50  $\mu\text{l}$  of 1 mol/L acetic acid, and 0.25 mg of orange G for 8 hours. Fifteen microliters of C-serum was applied to the gel and run at 1400 V, 0.5 mA, 5 W for 16 hours at  $15^{\circ}\text{C}$ , for IEF.

The second dimension, Immobiliner DryStrip, was soaked in 10 ml of equilibration buffer containing 3.6 gm of urea, 1 ml of 0.5 mol/L Tris pH 6.8, 3 ml of glycerol, 0.2 gm of SDS, 500  $\mu\text{l}$  of  $\beta$ -mercaptoethanol, and a few grains of 0.1% pyronin Y for 20 minutes with gentle agitation. The equilibrated DryStrip was placed on an ExcelGel XL 12% to 14% (Pharmacia) and run at 1000 V, 40 mA, 40 W at  $15^{\circ}\text{C}$ , for SDS-PAGE.

### Transfer and immunoblot

The separated proteins on the gels were transferred to ProBlot membranes (Applied Biosystems, Foster City, Calif.) at 400 mA for 1 hour in 10 mmol/L 3-cyclohexylamino-1-propanesulfonic acid buffer (Sigma Diagnostics, St. Louis, Mo.) with 15% vol/vol methanol, pH 10.5. For IgE screening, the membranes were blocked with 1% nonfat dry milk in B114 solution (phosphate-buffered saline with 0.1% BRIJ 35 (Sigma Diagnostics) for 1 hour and cut into 3 mm strips. Strips were incubated overnight in patients' serum diluted 1:10 in B114. The strips were washed with B114 and incubated for 2 hours in phosphatase-labeled goat IgG anti-human IgE( $\epsilon$ ) antibody (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) diluted 1:1000 and then washed with B114 and developed with BCIP/NBT solution (Kirkegaard and Perry Laboratories). Protein detection was performed either with Coomassie brilliant blue (Sigma Diagnostics) on the membrane or with silver nitrate on the gel.

### Inhibition study of C-serum proteins and proteins associated with particles

Proteins associated with latex particles were further purified by a Pharmacia fast protein liquid chromatography with a HiTrap Q anion-exchange column (Phar-

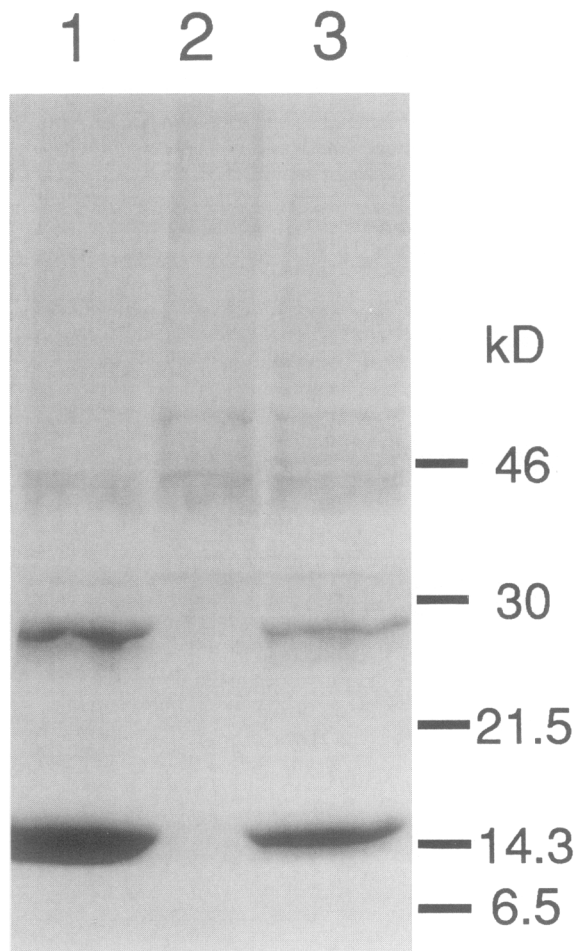


**FIG. 1.** Proteins in latex sap and in C-serum and proteins associated with latex particles. Proteins were separated by 4% to 20% SDS-PAGE under the reduced condition and stained with Coomassie Brilliant Blue. *Lane 1*, Proteins extracted from latex particles with 50 mmol/L Tris buffer, pH 8.0, and 2% SDS; *lane 2*, proteins from C-serum; *lane 3*, proteins extracted from latex sap with 50 mmol/L Tris buffer, pH 8.0, and 2% SDS.

macia). Buffer A contained 0.1 mol/L Tris buffer, pH 8.0; buffer B contained 1 mol/L NaCl in addition to buffer A. Both 14.6 kd and 24 kd proteins were eluted from the column at an NaCl concentration of 0.7 mol/L. C-serum proteins were used after centrifugation without further purification.

For inhibition immunoblotting, the children's serum pool, which reacted strongly with proteins from C-serum and from the latex particles, was first incubated either with the same volume of latex particle proteins or with C-serum proteins for 3 hours at room temperature before reaction with the membrane strips. The membrane strips were then processed and developed as mentioned above.

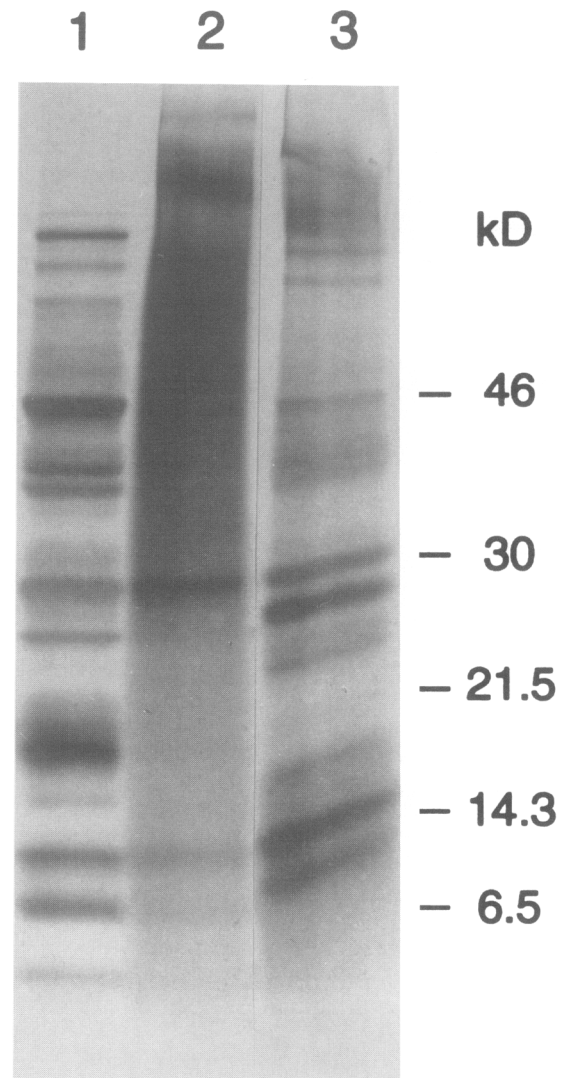


**FIG. 2.** Immunoblot of ammoniated and nonammoniated latex and latex glove extract. Proteins were separated by 16% SDS-PAGE under the nonreduced condition and transferred to a ProBlot membrane, which was incubated with a serum pool from adults with latex allergy. *Lane 1*, Sodium carbonate buffered and glycerinated latex (nonammoniated); *lane 2*, glove extract; *lane 3*, ammoniated latex.

#### N-terminal amino acid sequence and total amino acid analysis of REF

The 14.6 kd and 24 kd proteins from fast protein liquid chromatography were separated by SDS-PAGE and transferred to ProBlot membranes. The 14.6 kd protein band was excised from the membrane and sequenced directly by a 477A gas-phase microsequencer (Applied Biosystems) with an on-line model 120A analyzer. For total amino acid analysis, the protein was eluted from the membrane, hydrolyzed in 6N HCl at

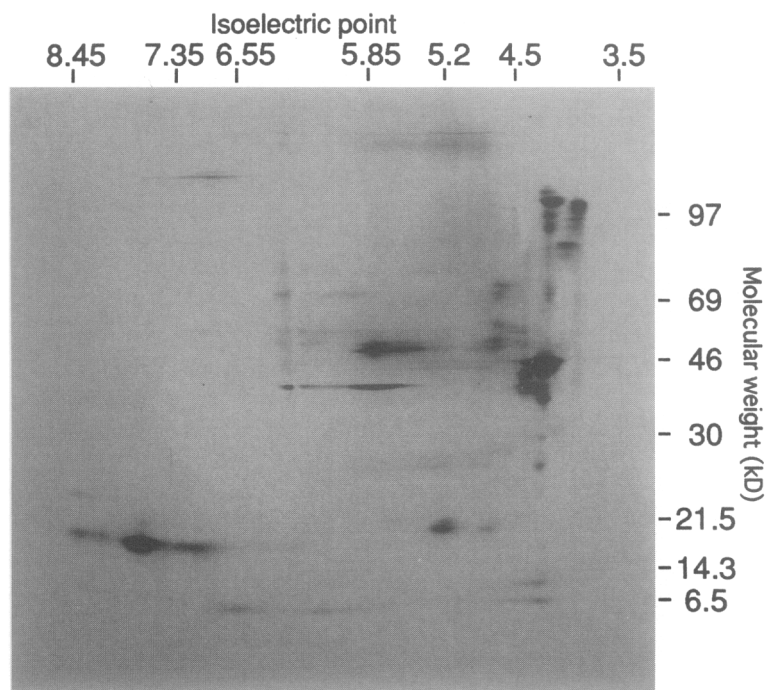


**FIG. 3.** Two-dimensional immunoblot. Proteins were first separated by pI (*horizontal axis*), followed by SDS-PAGE under the nonreduced condition (*vertical axis*). Separated proteins were transferred to a ProBlot membrane and incubated with a 1:10 diluted serum sample from a patient with latex allergy.

**TABLE II.** Amino acid composition of REF and the 14.6 kD protein extracted from latex particles

| Residue | REF*  | 14.6 kD protein |
|---------|-------|-----------------|
| Asp     | 10.2† | 10.0            |
| Thr     | 4.4   | 3.9             |
| Ser     | 8.0   | 7.9             |
| Glu     | 9.5   | 11.7            |
| Pro     | 6.6   | 6.3             |
| Gly     | 7.3   | 14.4            |
| Ala     | 11.7  | 11.6            |
| Cys     | 0     | 0               |
| Val     | 10.9  | 9.9             |
| Met     | 0     | 0               |
| Ile     | 6.6   | 4.7             |
| Leu     | 7.3   | 7.1             |
| Tyr     | 4.4   | 1.6             |
| Phe     | 4.4   | 4.0             |
| His     | 0     | 0               |
| Lys     | 5.8   | 3.9             |
| Arg     | 3.0   | 2.8             |
| Trp     | 0     | 0               |

\*Data from Dennis et al. J Biol Chem 1989;264:18618-26.

†Residue number.

150° C, and analyzed on a Beckman 6300 amino acid analyzer (Beckman, Columbia, Md.).

## RESULTS

### Proteins in latex

The latex preparation used for this study contained 25% (wet weight) latex particles. The total protein concentrations in whole latex sap and in C-serum were 4.5 and 2.8 mg/ml, respectively. Proteins could be extracted from particles by shaking with aqueous buffers with or without SDS (2%). The addition of SDS increased the efficiency of extraction. The concentrations of protein extracts were 2.6 mg/ml with SDS and 1.1 mg/ml without SDS.

Fig. 1 shows the Coomassie brilliant blue-stained protein patterns in the different latex preparations as determined by SDS-PAGE. The major proteins associated with particles were 14.6 kD and 24 kD (Fig. 1, *lane 1*). With the exception of glycine and tyrosine, the amino acid composition of the 14.6 kD protein was consistent with that of the REF reported by Dennis and Light<sup>14</sup> (Table II). The high value of glycine is mostly likely derived from

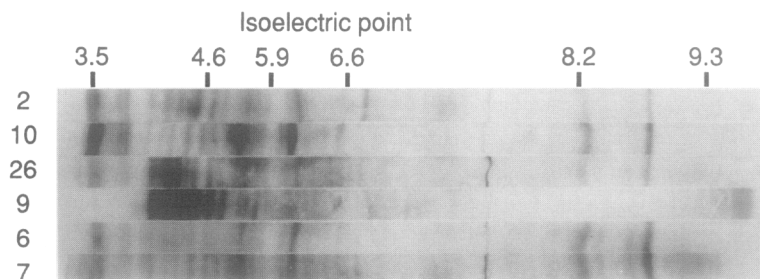


**FIG. 4.** IEF immunoblot of C-serum proteins with sera from patients with latex allergy. C-serum proteins were separated by IEF gel (pI, 3.5 to 9.5). Isoelectric point markers are on the top. Serum numbers are on the left.

**TABLE III.** IgE reactivity to latex proteins separated by IEF

| Serum* | Isoelectric point |     |     |     |     |     |     |     |     |          |
|--------|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|----------|
|        | 3.5, 3.7, 3.8     | 4.1 | 4.2 | 4.4 | 4.8 | 5.1 | 5.5 | 5.9 | 6.1 | 8.1, 8.6 |
| 1      |                   |     |     |     |     |     |     |     |     |          |
| 2      | +                 | +   | +   | +   |     |     | +   | +   | +   | +        |
| 3      |                   |     |     |     |     |     | +   | +   |     |          |
| 4      |                   |     | +   | +   |     | +   | +   | +   | +   |          |
| 5      |                   |     |     |     |     | +   |     |     |     |          |
| 6      | +                 | +   | +   | +   | +   | +   | +   | +   | +   | +        |
| 7      | +                 | +   | +   | +   | +   | +   | +   | +   | +   | +        |
| 8      |                   |     |     | +   | +   | +   |     |     |     |          |
| 9      |                   | +   | +   | +   | +   | +   |     |     | +   |          |
| 10     | +                 |     | +   | +   | +   | +   | +   | +   | +   | +        |
| 11     | +                 | +   | +   | +   | +   | +   | +   | +   | +   | +        |
| 12     |                   | +   | +   | +   |     |     | +   | +   |     | +        |
| 13     | +                 |     |     |     |     | +   | +   | +   |     | +        |
| 14     | +                 |     |     |     |     | +   | +   | +   |     | +        |
| 15     | +                 | +   | +   | +   | +   | +   | +   | +   | +   |          |
| 16     |                   | +   | +   | +   | +   |     | +   |     | +   |          |
| 17     | +                 |     |     |     |     | +   | +   | +   |     | +        |
| 18     |                   |     |     |     |     | +   | +   | +   |     | +        |
| 19     |                   | +   | +   | +   | +   | +   | +   | +   | +   | +        |
| 20     | +                 |     |     |     |     | +   | +   | +   | +   | +        |
| 21     | +                 | +   | +   | +   | +   | +   | +   | +   | +   | +        |
| 22     | +                 | +   | +   | +   | +   | +   | +   | +   | +   | +        |
| 23     | +                 |     |     |     |     | +   | +   | +   | +   | +        |
| 24     |                   |     |     |     |     |     |     |     |     |          |
| 25     |                   |     |     |     |     |     |     |     |     |          |
| 26     | +                 | +   | +   | +   | +   | +   | +   |     | +   | +        |
| 27     | +                 |     |     |     |     |     |     |     |     |          |
| 28     |                   |     |     |     |     | +   | +   | +   |     |          |
| 29     |                   |     |     |     |     |     |     |     | +   |          |

\*Serum 1 is a negative control, serum 6 is an adult serum pool, serum 7 is a children's serum pool, and others are individual serum samples from adults.

the contamination on the ProBlot membranes, and the low value of tyrosine is due to the high temperature used for hydrolysis. In addition, like REF the N-terminal amino acid of the 14.6 kd protein was blocked, suggesting that it is REF. The

REF was not detected in the C-serum fraction of latex (Fig. 1, lane 2). Latex sap contains proteins found in both particles and in the C-serum (Fig. 1 lane 3).

Similar immunoreactive patterns were observed

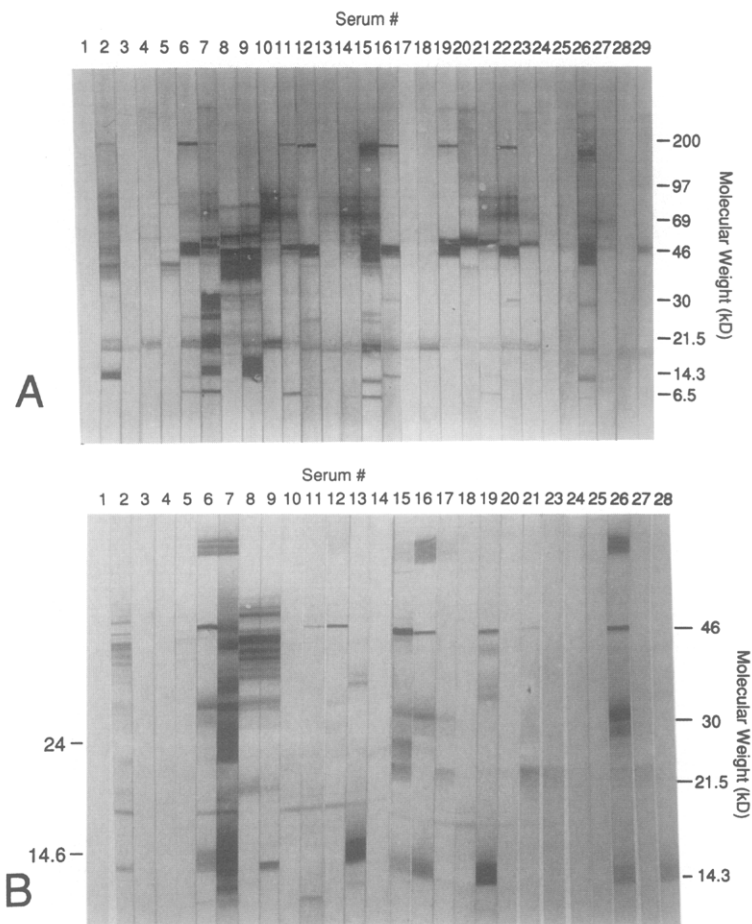


**FIG. 5.** SDS-PAGE immunoblots of latex C-serum proteins (**A**) and particle proteins (**B**) with sera from patients with latex allergy. C-serum proteins were separated by 4% to 20% SDS-PAGE under the reduced condition, and particle proteins were separated by 16% SDS-PAGE under the reduced condition. Protein MW markers are on the right.

in the ammoniated and the nonammoniated latex preparations (Fig. 2). Glove extract, however, produced a diffuse pattern, indicating that most of the proteins were degraded during the manufacturing process.

In a two-dimensional gel stained by silver nitrate, more than 150 dots were detected in the C-serum fraction of latex (data not shown). These proteins were widely distributed from pH 3 to pH 10 and from molecular weights (MWs) of 6 kD to 160 kD. Only two major proteins, 14.6 kD (isoelectric point [pI] 6) and 24 kD (pI 6.2), were detected in the latex particles when extracted with Tris buffer without SDS.

#### **Binding of latex proteins to serum IgE of patients with latex allergy**

Proteins in the latex C-serum and particle extracts were used for IgE-binding studies, which

included two-dimensional gel, IEF, and SDS-PAGE under reduced and nonreduced conditions.

In a two-dimensional immunoblotted membrane approximately 35 to 40 immunoreactive dots were detected in the C-serum fraction of latex (Fig. 3). There were 13 major IgE-reactive bands in the C-serum on the IEF-immunoblotted membrane (Fig. 4). Serum samples no. 24 and no. 25 did not react with any proteins on the IEF-immunoblotted membrane. Reactivities of each serum sample and serum pool to various IgE protein bands are summarized in Table III.

In the reduced SDS-PAGE, there were 18 IgE-reactive bands in the C-serum (Fig. 5, *A*). Similar patterns were observed for proteins under the nonreduced condition (Fig. 6). The 35 kD protein could only be seen under the nonreduced condition. The reactivity to the 38 kD protein band under the nonreduced condition was not shown because a

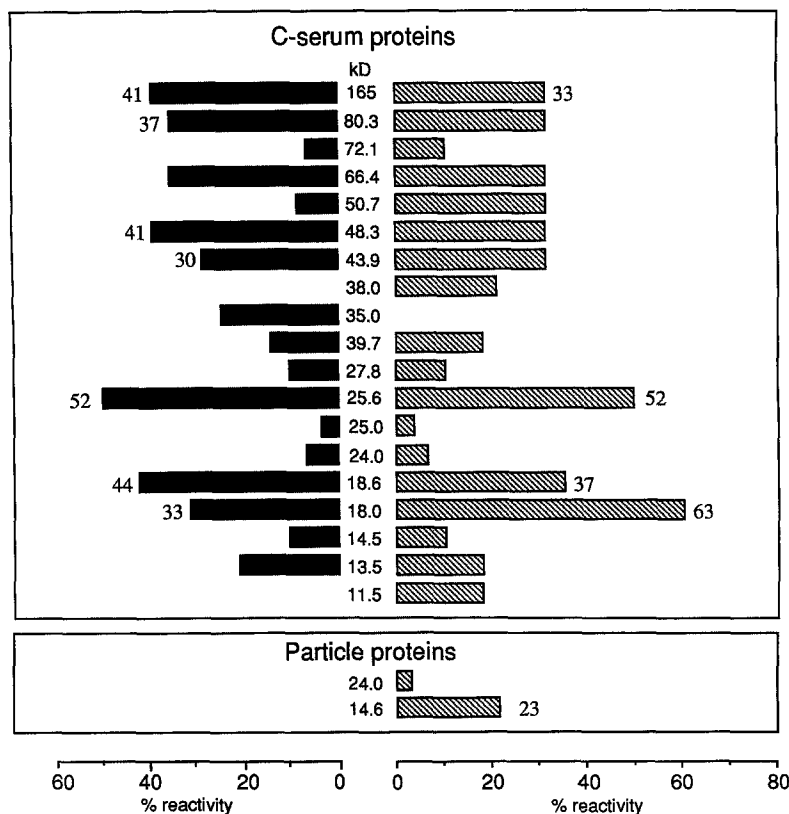


**FIG. 6.** Percent reactivities of sera from patients with latex allergy toward different proteins in C-serum and in latex particles. *Shaded bars* represent reactivities of sera when gels were run under the reduced condition. *Solid bars* represent reactivities under the nonreduced condition.

protein of the same MW reacted nonspecifically to all sera, including the control serum.

Reactivity rates (percent of sera reactive to a specific protein) of each protein under different conditions are summarized in Fig. 6. The reactivity rates were calculated based on the results obtained with the 26 individual serum samples. The positive reactivity rates of two proteins from latex particle extract were 23% for the 14.6 kd protein and 4% for the 24 kd protein (Fig. 5, *B*; Fig. 6). Most of the sera that reacted to the particle proteins also reacted to proteins from C-serum. Patient 13, however, reacted almost exclusively to the 14.6 kd protein from the latex particle extract.

#### Inhibition study of C-serum proteins and proteins associated with particles

Immunoblots were prepared with a mixture of C-serum and particle extract proteins. Prior adsorption of the children's serum pool with particle extract proteins removed reactivity to the 24 and 14.6 kd bands (Fig. 7, *lane 2*). However, adsorption of the serum pool with C-serum did not remove the

24 and 14.6 kd bands (Fig. 7, *lane 3*), suggesting that C-serum has no significant amount of 24 and 14.6 kd proteins.

#### DISCUSSION

The driving force behind this study was to identify a latex reference reagent that can be used both in vitro for quantification of allergens in rubber products and in vivo for skin testing in human beings. Native latex, nonammoniated, was used as starting material for this study, even though in the manufacture of rubber goods, latex is treated with ammonia immediately after collection from rubber trees. Proteins in ammoniated latex are often subjected to hydrolysis and denaturation during prolonged storage.<sup>3</sup> In addition, ammonia must be removed from the latex preparation before skin testing. Glove extracts might be an ideal source for the preparation of the reference reagent; however, proteins were badly damaged during the manufacturing process (Fig. 2, *lane 2*). To prevent degradation of the nonammoniated latex by proteolytic enzymes, a high concentration of

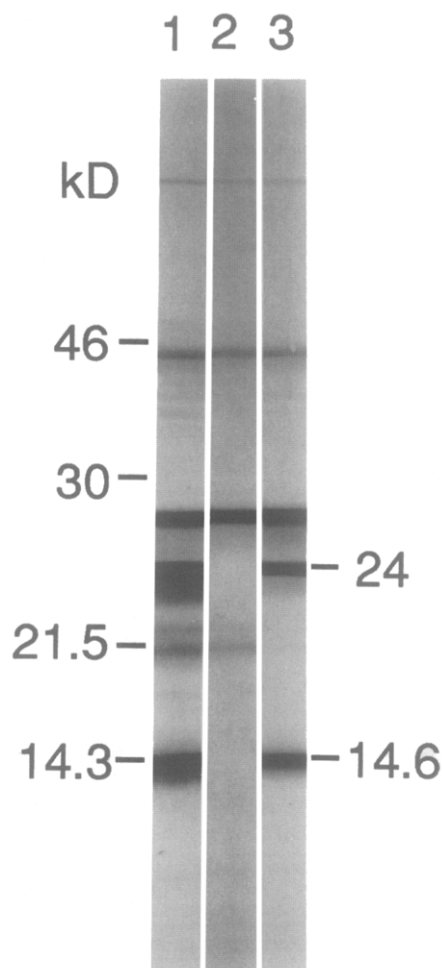


**FIG. 7.** Inhibition immunoblotting of latex proteins with a children's serum pool, which had been previously incubated with particle proteins or C-serum proteins. Latex proteins, containing both C-serum proteins and particle proteins, were separated by 16% SDS-PAGE under the nonreduced condition and transferred to a ProBlot membrane. *Lane 1*, Membrane strip was incubated with children's serum pool diluted 1:10 without prior incubation. *Lane 2*, Membrane strip was incubated with children's serum pool diluted 1:10 inhibited by latex particle proteins. *Lane 3*, Membrane strip was incubated with children's serum pool diluted 1:10 inhibited by C-serum.

glycerol was added to the initial latex preparation. There was no observable degradation to the source material as indicated by the clear protein banding pattern of the glycerinated latex (Fig. 2, *lane 1*). Later, the concentration of glycerol was adjusted to 54.5% in the C-serum, and the final product was stored at 4° C. Under these storage conditions, there was no detectable degradation of proteins for at least 1 year (unpublished observation). These results indicate that the nonammoniated

latex is the best choice as a source material for a reference reagent.

Dennis and Light<sup>11</sup> reported that one of the major proteins associated with the particle fraction of latex is the REF, which has been identified as the major allergen in latex.<sup>9</sup> In the commercial preparation of latex raw material, when latex particles are separated from the C-serum without high-speed centrifugation, both particle proteins and C-serum proteins are likely to be present. To determine the distribution of allergens in the latex, C-serum and particle-associated proteins were prepared, and each fraction was used to react with sera from patients with latex allergy. Martin<sup>15</sup> reported a relatively simple protein composition in latex with 70% to 80% of the soluble proteins in the pellet after centrifugation. In the presence of 36% glycerol, no significant pellet was found after 2 hours of centrifugation at 50,000 g. When proteins were separated in a two-dimensional gel and stained with silver nitrate, a large number of proteins were observed in the C-serum. Two proteins in high concentrations were found to be extractable from the particles (Fig. 1).

When individual serum samples and two serum pools were used for the immunoblot, rather complex and distinct reaction patterns were observed against C-serum and particle proteins. In the adult population, there were no significant differences between health care workers and non-health care workers in serum reactivities to latex proteins. Each individual, however, had an IgE response to different proteins. There is no obvious association between the immunoreactive pattern and the symptoms observed in different patients. The concentration of latex-specific IgE does not predict the severity of the allergic reaction (Table I). Most patients who reacted to REF also reacted to some proteins in the C-serum, with the exception of patient 13 who reacted almost exclusively to REF. The reactivity of the children's serum pool (Figs. 4 and 5, serum no. 6) occurred mainly with lower MW proteins (<30 kD). A similar observation was reported by Alenius et al.<sup>16</sup> Thus according to our study, there were no "major allergens" easily identifiable in the latex. Only two of the proteins in the C-serum reacted to more than 50% of sera screened in this study, and these proteins are not present in large quantities in latex. REF reacted with only 23% of sera, but it is present in large quantities in latex particles (Fig. 1) and might cause serious problems for those patients who are allergic to this protein. The high degree of inhibition of total IgE reactivity to other latex proteins

induced by REF<sup>9</sup> was not observed in our study. According to the results of inhibition immunoblot (Fig. 7), the addition of REF did not seem to inhibit the binding of serum IgE to other proteins in the C-serum.

As previously reported, major allergens were detected by using SDS-PAGE under the reduced condition. A slightly different pattern of reactivities could be observed when proteins from C-serum were analyzed under reduced and nonreduced conditions (data not shown). There were at least 21 IgE-reactive bands, ranging from 11.5 kd to 165 kd, under both reduced and nonreduced conditions (Fig. 5). The most reactive protein band was the 18.0 kd protein, which reacted with 63% of individual serum samples tested. The protein bands with over 30% reactivity rates were in rank order of reactivity: 25.6 kd, 18.6 kd, 48.3 kd, 165 kd, 66.4 kd, 80.3 kd, 18.0 kd, and 43.9 kd under the nonreduced condition; 18.0 kd, 25.6 kd, 18.6 kd, 43.9 kd, 48.3 kd, 50.7 kd, 66.4 kd, 80.3 kd, and 165 kd under the reduced condition. A 27 kd protein, reported by Alenius et al.<sup>7</sup> as a major allergen, was detected in less than 20% of our patients (Fig. 6). In a two-dimensional gel analysis, some of the protein bands had different pIs but shared the same MW. A more careful characterization of these proteins by two-dimensional gel is needed to reveal different allergens in latex. In the C-serum there is another protein with MW of 14 kd, which is not the REF, because patient 13 reacted strongly to the REF associated with the particles but did not react to the 14 kd protein in the C-serum (Fig. 5).

The allergenicity of proteins with low pIs, 3.5 to 4.0 (Fig. 4), MW 25.6 kd, has not been previously reported. These acidic proteins were transferred rapidly onto and through the membrane during electroblotting under normal conditions (400 mA, 1 hour) and did not stain well with Coomassie brilliant blue. When the transferring process was carried out under low-current conditions, over 50% of sera reacted to these proteins. Further characterization of these proteins is in progress.

In our study a number of low pI proteins and an 18 kd protein were most frequently detected by sera from patients with latex allergy. A number of higher MW proteins were also shown to be highly reactive to adult patients. Because different patients react to different proteins in both the C-serum and particle proteins, a reference reagent should contain the proteins from both fractions.

We thank Dr. Esah Yip of the Rubber Research Institute of Malaysia for providing us with the latex sap for our study. We also thank Drs. John Yunginger, Brock Williams, and Robert Hamilton for their generous contribution of serum and latex samples for this study; Teresa Liu and Cathy Miller for technical assistance; and Drs. Paul Turkeltaub and Arthur Karpas for their valuable suggestions and critical review of this manuscript.

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