Characterization of latex antigens and allergens in surgical gloves and natural rubber by immunoelectrophoretic methods

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Allergy to natural rubber (latex) products is increasingly frequent among both medical personnel and lay subjects. Although individual antigens and allergens in latex products have not been fully characterized, they are believed to derive from the sap of the rubber tree. Rabbit IgG and human IgE antibodies against rubber proteins were used to characterize antigens and allergens in surgical latex gloves and natural rubber by crossed immunoelectrophoresis and its modifications. The results from crossed-line immunoelectrophoresis demonstrated that the surgical latex gloves had at least 10 antigens in common with natural rubber sap and ammoniated latex. In crossed radioimmunoelectrophoresis, at least six of the 10 protein antigens in the surgical glove extract and natural rubber were allergens since they bound IgE antibodies from the latex-allergic patients' sera. The surgical glove extract also demonstrated one allergen not found in natural rubber, suggesting that rubber proteins may be altered during glove manufacture. (J ALLERGY CLIN IMMUNOL 1992;90:230-5.)

Key words: *Latex allergens, immunoelectrophoresis, cross-reaction, latex gloves, rubber, immediate hypersensitivity*

SAP obtained from the rubber tree *(Hevea brasiliensis)* and used as a source material in rubber manufacture is a liquid suspension of several compounds. At least three separate fractions, including polyisoprene and small amounts of water-soluble proteins, can be achieved with high-speed centrifugation. The major protein component is hevein, which has been isolated and characterized.[1] Various rubber-compounding chemicals added to the rubber latex during manufacture can cause contact dermatitis in subjects that use rubber gloves. This type IV contact allergy is mostly caused by thiuram chemicals and is easily diagnosed by patch testing.[2]

Type I hypersensitivity reactions to rubber gloves were reported with increasing frequency in the 1980s.[3-6] This immediate allergy, often called latex allergy, appears to be due to the rubber proteins eluting from the gloves. In sensitized subjects, the proteins may be absorbed by cutaneous or mucosal routes, and symptoms such as contact urticaria, angioedema, rhinitis, conjunctivitis, asthma, and even anaphylaxis may appear.[4, 7-9] In addition to surgical gloves, catheters and other medical rubber products can be the

Abbreviations used

RIE:	Rocket immunoelectrophoresis
RRIE:	Rocket radioimmunoelectrophoresis
CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
CLIE:	Crossed-line immunoelectrophoresis
CLRIE:	Crossed-line radioimmunoelectrophoresis
CIEWIG:	Crossed immunoelectrophoresis with an intermediate gel
SAP:	Natural rubber sap
AL:	Ammoniated latex, that is, natural rubber sap in 0.6% ammonia
GE:	Glove extract
GP:	Glove powder taken from the gloves
CSGP:	Cornstarch glove powder
MW:	Molecular weight

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allergen source during operations, delivery, and medical examinations. Allergic reactions have also been described from nonmedical rubber products, such as toy balloons, household gloves, and condoms.[5, 10, 11]

Our knowledge of latex allergens in rubber products is far from complete. Recent immunoblot, high-performance liquid chromatography, and gel-filtration studies suggest that the allergens derive from SAP and latex.[12-14] In the present investigation, we used various immunoelectrophoretic methods to study whether surgical latex gloves contain water-soluble antigens and allergens similar to those in natural rubber from which the gloves are manufactured. Using CRIE, we also examined various latex IgE antibody-binding patterns in the sera of patients allergic to latex gloves.

MATERIAL AND METHODS

Latex proteins

GE from a brand of surgical latex gloves (Exona, Semperit Technological Products/Medizintechnik, Vienna, Austria) was prepared as described previously.[14] Briefly, pieces of latex gloves (0.5 by 0.5 cm) were incubated in 40 mmol/L of potassium-phosphate buffer, pH 8.4, at 37° C overnight. The eluates were centrifuged at 4000 *g* for 10 minutes. Supernatants were collected and concentrated by air-drying in dialysis tubing. The concentrates were desalted by Sephadex G-25 column chromatography according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden). Protein-containing fractions were collected, concentrated by air-drying, and finally freeze-dried. The protein content of the freeze-dried GE was 140 μg/mg, determined by the Lowry method, with bovine albumin as the standard. This GE was used in rabbit immunization, immunoelectrophoretic studies, and skin prick testing.

SAP and AL, sap stored in 0.6% ammonia, were obtained from *H. brasiliensis* trees growing on a rubber plantation in Malaysia. SAP was extracted and centrifuged to remove the polyisoprene fraction.[14] Soluble components from AL were extracted by acid precipitation.[8] These components were dialyzed against 20 mmol/L of ammonium bicarbonate with a membrane (Spectapor, Spectrum Medical Industries, Inc., Los Angeles, Calif.) with a cutoff of 3.5 kd and finally freeze-dried. The protein content of the SAP extract was 300 μg/ml, and that of AL, 112 μg/mg, as measured by the Bradford (Bio-Rad Laboratories, Richmond, Calif.) method with bovine immunoglobulin as the standard. These extracts were used in immunoelectrophoresis.

GP was collected from one brand of sterile examination latex gloves (Glads, Ansell Rubber Co., Bangkok, Thailand) by gentle rubbing. The control glove powder (CSGP), pure cornstarch, was obtained directly from a glove manufacturer (Biosorb, Johnson & Johnson Co., Arlington, Texas) and was used in RIE.

Immunoelectrophoretic methods

RIE, RRIE, CIE, CRIE, CLIE, CLRIE, and CIE with an intermediate gel (CIEWIG) were performed, basically, according to the method described by Lowenstein.[15] The electrophoretic runs were conducted on plastic film (GelBondy, Pharmacia Diagnostic). Various amounts of antigen (0.015 to 4500 μg) and rabbit antiserum (5 to 16 μl/cm²) were used.

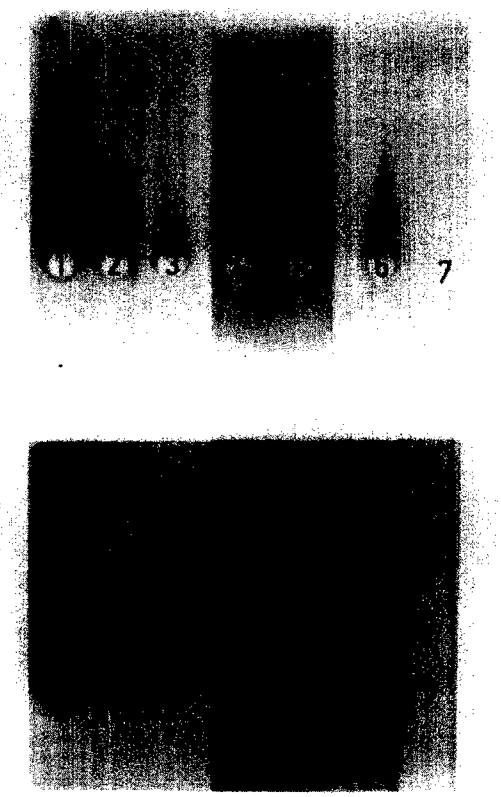

FIG. 1. A, RIE. **B,** RRIE. Rubber samples with pooled rabbit antilatex antiserum (16 μl/cm²) and a serum pool obtained from five latex-allergic subjects. Samples used were (*1* to *3*) AL containing 17, 1.7, and 0.17 μg of protein; latex GE *4* and *5*, 105 and 53 μg protein; GP, *6*, 1.5 mg of dry weight; cornstarch control *7*, 150 μg of dry weight.

For the autoradiographic studies, the unstained plates were incubated first with patient serum or serum pool (diluted 1:16) and then with ^{125}I-labeled anti-IgE (Pharmacia Diagnostic) with a total activity of 280,000 cpm. The plates were placed on x-ray film (Agfa-Gevaert-ABT Roentgen, Leverkusen, Germany) and exposed in a Kodak X-Omatic (Eastman Kodak Co., Rochester, N.Y.) cassette equipped with Trimax T4/T8 intensifying screens for 1 to 20 days at −40° C.

Sera

Rabbit sera. To produce IgG antibodies to rubber proteins, three white, local-strain rabbits were immunized subcutaneously with 0.6 mg of GE in physiologic saline biweekly eight times and then, intravenously, once. Freund's complete adjuvant was used in the first four injections, and Freund's incomplete adjuvant was used thereafter. Rabbit sera collected before and after immunization were stored at −20° C before use in electrophoretic studies. To confirm that the precipitating rabbit antibodies were specific to latex,

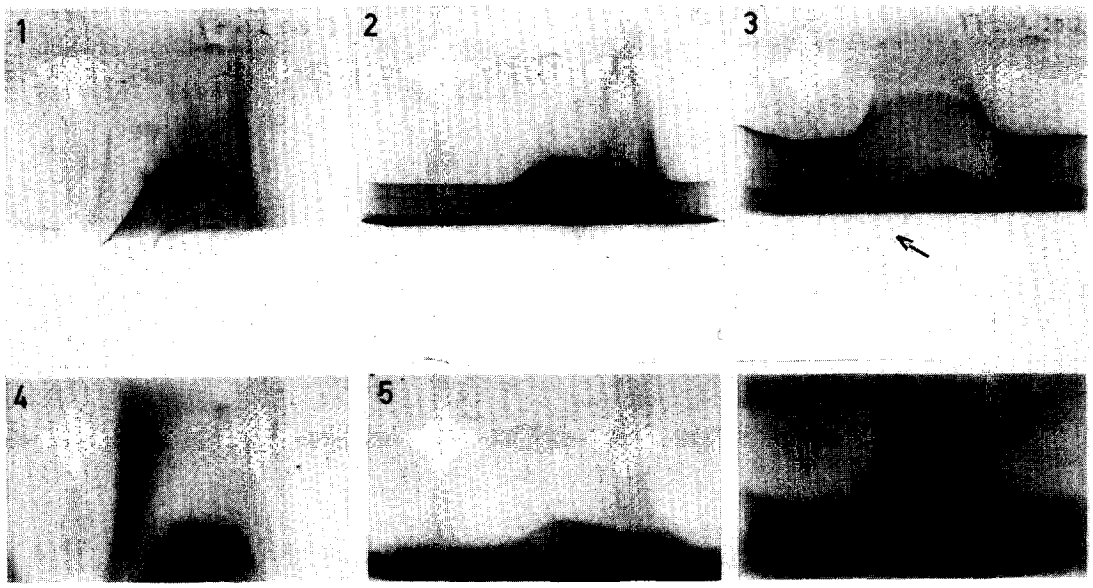

FIG. 2. CLIE and CLRIE of rubber samples; 140 μg of protein from GE applied in sample wells and rabbit antiserum (13 μl/cm²) in the upper gels were run without additional antigens *(1)* or with the addition of 30 μg of SAP proteins *(2)* or 112 μg of AL proteins *(3)* in the intermediate gel. In corresponding autoradiographic pictures, pool of sera obtained from five latex-allergic patients was used (*4* to *6*). Antigen detected only in glove extract is indicated with an arrow.

TABLE I. Clinical history and laboratory results of five latex-allergic patients whose sera were examined in CRIE

Patient No.	Sex/age	Occupation	Glove type causing symptoms	Types of symptom	Latex tests		Serum total IgE (kU/L)
					Prick*	RAST† (kU/l)	
1	F/37	Surgeon	S,H	A,CU,GU	4+	26.0	460
2	F/31	General practitioner	S	CU	4+	25.0	4000
3	F/31	Dentist	S,H	CU, GU	4+	18.0	1730
4	F/47	Nurse	H	CU, AN	3+	4.3	3380
5	F/29	Kitchen assistant	H	CU, GU, AN	3+	3.3	4388

S, Surgical glove; *H*, household glove; *A*, asthma; *AN*, anaphylaxis; *CU*, contact urticaria; *GU*, generalized urticaria.
*Results compared with histamine, 10 mg/ml; 4+, wheal size twice that of histamine; 3+, wheal size same as histamine; 2+, wheal size half of histamine.
†Positive result, ≥0.35 kU/L.

absorption experiments were performed with preimmune rabbit sera added to the intermediate gel in CIEWIG.[16]

Patient and control sera. A pool of three to eight sera from latex-allergic patients was used for the autoradiographic studies with RRIE, CLRIE, and CRIE. In addition, five sera from five different patients were examined in CRIE for latex IgE antibody patterns. The five sera were from adult patients who had experienced allergic symptoms when they were using latex gloves or after being exposed to surgical or household latex gloves (Table I). All patients were prick tested with GE as described previously[17] and had positive results. The patients' sera examined with Phadebas latex RAST according to the manufacturer's instructions (Pharmacia Diagnostics) also demonstrated specific IgE binding (Table I). Values >0.35 kU/L were considered positive. Three latex RAST-negative but inhalant- or food-specific IgE-positive sera with high total IgE values (up to 6600 kU/L) were used as negative controls in RRIE and CRIE.

RESULTS

RIE and RRIE

All samples from GE, GP, SAP, and AL revealed several distinct rocket precipitates with the pooled rabbit antiserum (Fig. 1; SAP not illustrated). One of the three rabbit antisera appeared to precipitate fewer antigens (data not presented) and was not used in ad-

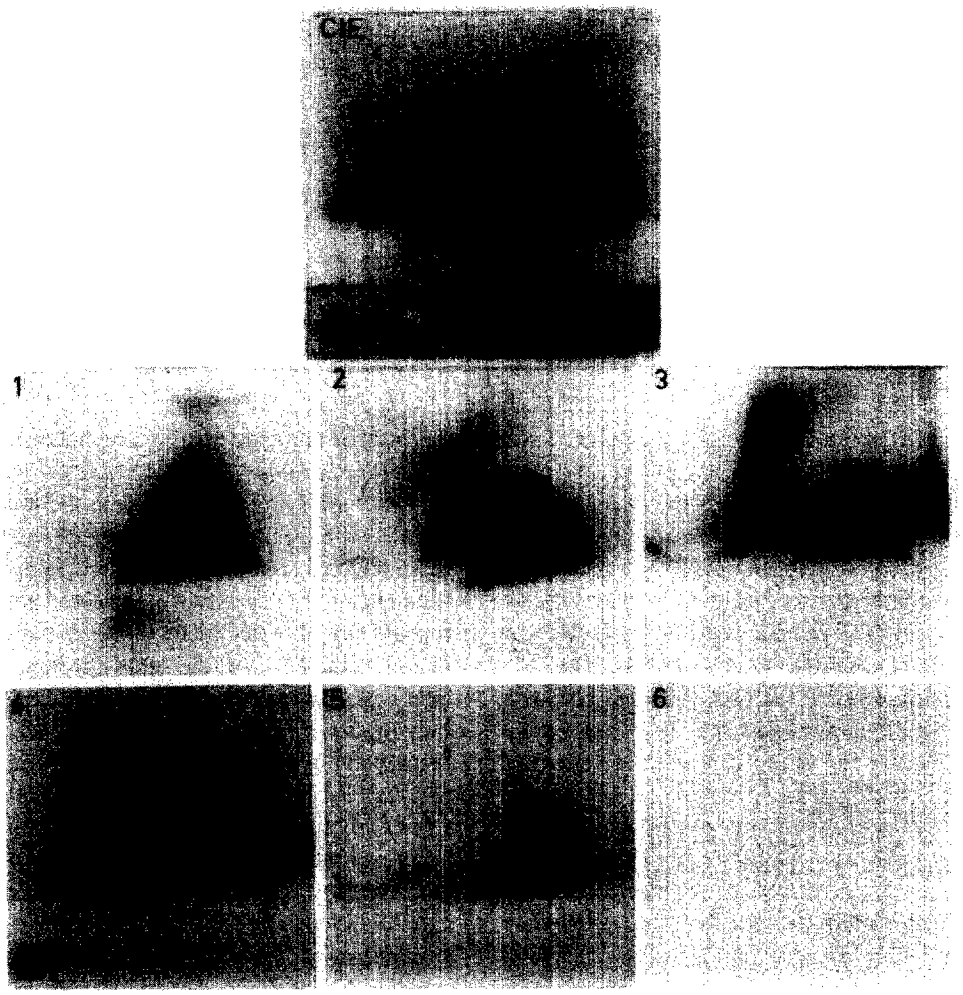

FIG. 3. CIE and CRIE of AL; 17 μg of protein used as sample and rabbit antiserum (13 μl/cm²) in *upper gel* was stained wth Coomassie brilliant blue (CIE) or incubated with an individual serum obtained from five latex-allergic patients (*1* to *5*), or with a control serum *(6).* Antigens that bound IgE antibodies are indicated with a letter (*A* to *F*).

ditional experiments. In RRIE, at least three of the precpitated antigens bound human IgE. No precipitates or IgE binding could be demonstrated with CSGP.

CLIE and CRLIE

In CLIE and CLRIE, all protein antigens in the GE, with the exception of one antigen, were fused with the corresponding antigens in SAP and AL (Fig. 2).

CIE and CRIE

CIE revealed at least 10 precipitates in GE, SAP, and AL. In CRIE, at least six of the latex antigens bound IgE antibodies from the pooled sera and individual patient's sera. The CIE and CRIE patterns of GE are illustrated in Fig. 2 (*plates 1* and *4*). The CIE and CRIE results with AL and five individual sera are presented in Fig. 3 and in Table II. In contrast to patient's sera, no binding was detected with the control serum (Fig. 3, *plate 6*).

CIEWIG

In absorption experiments, the antigen-precipitation pattern was not affected by the preimmune rabbit sera.

DISCUSSION

In the present study, rubber protein extract from latex gloves was used to produce IgG antibodies in rabbits. The antisera were used in immunoelectrophoretic studies to determine whether similar antigenic proteins occur in the surgical latex gloves and natural rubber. The rubber proteins from GE, SAP, and AL were precipitated and tested for their capacity to bind IgE antibodies from the sera of the latex-allergic patients.

CRIE demonstrated that in GE, SAP, and AL, at

TABLE II. Allergens in AL detected with CRIE in sera of five latex-allergic patients

Patient No.	Allergen					
	A	B	C	D	E	F
1	1+	—	—	—	5+	—
2	4+	4+	4+	—	4+	4+
3	4+	—	5+	—	3+	—
4	4+	3+	—	—	4+	—
5	1+	4+	2+	3+	3+	—

1+ to 5+ denotes increasing intensity of radiostaining.

least six antigens bound human IgE antibodies. Cornstarch, a powdering agent, did not reveal any allergens, whereas GP obtained from the manufactured gloves was contaminated with rubber proteins. This result is in agreement with the results of our immunoblot studies and supports the notion that airborne exposure by rubber protein-contaminated GP can cause asthmatic symptoms in sensitized subjects.[6]

The antigenic patterns of SAP, AL, and GE were all very similar, demonstrating that only minor alterations appear to occur in the composition of natural rubber proteins during glove manufacture. In the glove extract, however, there was one protein that was not detected in the SAP and AL by CLIE. This particular protein antigen may, therefore, be formed during glove manufacture and may also act as a specific sensitizing allergen because it bound IgE antibodies from the pooled sera of patients. This finding agrees with the immunoblot result of our group that revealed two protein in latex gloves not detectable in the SAP and AL.[14]

In addition to surgical latex gloves, other medical and nonmedical rubber products, such as catheters, household gloves, toy balloons, and condoms also contain latex allergens and can evoke type I hypersensitivity reactions. To our knowledge, rubber protein patterns in these products have not yet been studied by immunoelectrophoretic or immunoblot methods. Clinical observations and prick test results suggest that these rubber products also contain at least some allergens deriving from natural rubber.[5, 8]

The sera of five patients examined with CRIE revealed different latex IgE response patterns (Fig. 3). Usually, patients responded to more than one antigen. Some allergens were detected in several sera, whereas some allergens were detected in only one serum (Table II). However, a larger series of latex-allergic patients should be studied to establish a more precise definition of the allergens in natural rubber.

The advantage of the present immunoelectrophoretic methods in characterizing latex antigens is that the protein antigens can be examined in their native form. The samples are not treated with any reducing or other modifying agents, such as the sodium dodecyl sulfate, dithiotreitol, or mercaptoethanol used in sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting. However, the present immunoelectrophoretic methods do not allow determination of the MWs of the rubber protein antigens and allergens. Because we used only latex GE for rabbit immunization, it is possible that the antisera did not contain antibodies against all potential antigens in the natural rubber. Immunoblot studies have disclosed several allergens with MWs ranging from 10 to 100 kd in natural rubber.[8, 18, 19] Allergens of 14 and 29 kd have recently been detected in both natural rubber and extracts of surgical gloves.[14] Since the MW determinations were performed under reducing conditions, it cannot be ruled out that some of these proteins are subunits of higher MW proteins.

In conclusion, the use of various immunoelectrophoretic techniques with polyclonal rabbit antibodies to rubber proteins and human IgE antibodies to latex allows the detection and characterization of latex antigens and allergens in natural rubber and manufactured rubber products, such as surgical latex gloves. The present immunoelectrophoretic methods were also helpful in examinations of the individual IgE antibody response pattern to rubber proteins.

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Effect of environmental factors on the development of allergic disorders in infancy

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A total of 1167 infants were followed for 1 year in a population-based prospective study to assess the effect of environmental factors on the development of allergic disorders. Some of these environmental factors are interdependent. Mothers who formula fed their infants smoked more often ($p < 0.001$) and tended to belong to lower social classes ($p < 0.01$). Logistic regression analysis was performed to adjust for these confounding variables. Maternal smoking adversely affected the prevalence of asthma ($p = 0.003$) defined as three or more separate episodes of wheezing and total allergy ($p = 0.02$). Infants in lower socioeconomic groups developed asthma significantly more often ($p = 0.03$) than infants born in higher socioeconomic groups. There was a nonsignificant trend for infants born in summer to develop asthma more than infants born in winter ($p = 0.08$). No effect of these factors was observed on eczema, food intolerance, or on the subgroup of infants with definite allergy (clinical disorder with positive skin prick test [SPT]). Exposure to animal dander did not influence the prevalence of clinical disorder, but positive SPT reaction to cat dander was more prevalent in infants who were exposed to cats and/or dogs ($p = 0.04$). Positive SPT to house dust mite occurred significantly more often in infants who were formula fed ($p = 0.05$). The environmental factors had a profound effect on the prevalence of asthma but not on other allergic disorders. (J ALLERGY CLIN IMMUNOL 1992;90:235-41.)

Key words: *Environmental factors, maternal smoking, season of birth, asthma, allergic disorders*

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The interplay of genetic and environmental factors in the development of allergic disorders remains a subject for extensive investigation. The possibility of manipulating environmental factors to prevent the development of allergy attracts attention. Factors encountered in infancy may be of special importance.[1] Since the pioneer study of Grulee and Sanford,[2] there have been numerous studies of the protective effect