

Immunoglobulin E reactivity to latex antigens in the sera of patients from Finland and the United States

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Background: Patients with latex sensitivity and latex antigens from the United States and Finland, two countries where allergic reactions to latex have been widely reported, were evaluated to determine the spectrum of immune responses.

Methods: Sera from 27 patients from Finland and 18 from the United States with latex allergy and control sera from nonsensitive individuals were studied for latex-specific IgE antibodies. Four antigen preparations were used: two extracted from gloves and one each extracted from rubber tree sap from Malaysia and India. All 45 patients had skin prick test results that were positive to latex antigens, and all sera were evaluated by enzyme-linked immunosorbent assay (ELISA) with the various antigens.

Results: There were considerable differences in the reactivity of patient sera with the different antigens. Only 50% of the sera from patients with latex allergy from Finland demonstrated significant levels of IgE to latex as determined by enzyme-linked immunosorbent assay. These patients showed more reactivity with rubber tree sap antigens than with glove antigens. However, 72% of the patients from the United States demonstrated antibodies to latex, and no marked differences were noted between the antigen extracts.

Conclusions: The results indicate that reagents such as rubber tree sap, which contain multiple clinically significant antigenic components, should be included in evaluation of latex allergy and that differences in patient populations may result in serologic variances. (*J ALLERGY CLIN IMMUNOL* 1993;91:1128-34.)

Key words: Latex, IgE antibody, ELISA, anaphylaxis, skin prick test, latex allergy

Recently, IgE-mediated hypersensitivity reactions to latex proteins have been reported in several countries.¹⁻⁸ The proteins that are present in natural rubber are considered to be the responsible antigens, and the hypersensitivity responses

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Abbreviations used

GE-1:	Glove extract from Perry X-AM
GE-2:	Glove extract from Triflex, Baxter
LE-1:	Rubber tree extract from Malaysia
LE-2:	Rubber tree extract from India
OD:	Optical density
PBS:	Phosphate-buffered saline
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

induced include urticaria, angioedema, rhinitis, bronchospasm, anaphylaxis, and death.^{1, 3, 7, 9} Individuals at high risk of experiencing latex hypersensitivity include patients with spina bifida and health care workers, especially those who are frequently exposed to latex gloves and other latex products.^{3, 10} Studies of such patients from Finland, other European countries, and the United States have been published.^{1, 3, 10-12}

The latex products used in medical care in different countries are often obtained from different manufacturers. Because the procedures and standards in the manufacturing process vary, the protein and antigen contents of such products may also show considerable diversity.^{13, 14} Hence, the immunologic responses of the populations exposed to such proteins may also differ significantly, and there may be significant differences in the immune responses of the populations affected because of differences in exposure patterns or underlying disease states. Because there is no study that compares patients with latex allergy from different countries and their immunologic responses to diverse antigens, we have undertaken this investigation. We have evaluated sera of patients from Finland and the United States who demonstrated latex allergy as evidenced by clinical symptoms and skin test reactivity to locally available antigens. In vitro studies were carried out with antigen preparations used in the two laboratories. The results indicated that crude rubber plant sap extract contains more proteins than extracts from gloves or other latex products and that major differences exist in the ability of different antigens to react with antibodies in the sera of patients with latex allergy from the two countries. The results also suggest that variances in the immune responses between the patient groups may be related to their clinical sensitivity, manner of exposure, or underlying disease.

METHODS

Patients

Patients were evaluated in the Department of Dermatology of Tampere University Hospital, Finland and in the Allergy Clinics of the Medical College of Wisconsin, Milwaukee. All patients had clinical evidence of latex hypersensitivity. The 27 Finnish patients ranged in age from 20 to 56 years, and all were women. Twelve were health care workers and 15 had local symptoms such as contact urticaria induced by latex. Seven of the patients had generalized symptoms of urticaria, angioedema, asthma, or anaphylaxis after latex contact. All of these subjects had immediate wheal and flare skin reactions to an extract of latex gloves commonly used in Finland when tested by the prick technique.⁴ Total serum IgE levels of these patients ranged from <5 to 16,040 IU. Eighteen Finnish subjects who ranged in age from 19 to 29 years and who had no clinical history of latex allergy were considered normal control subjects. None had skin reactivity to the latex glove extract.

The 18 patients from the United States had spina bifida and were evaluated because of clinical histories that suggested latex sensitivity, including perioperative

anaphylaxis. The ages of the patients ranged from 3 to 17 years, and six were female. Nine of the 18 had histories of anaphylaxis as a result of latex exposure, and the remaining 9 had allergic symptoms after latex contact but no anaphylaxis. All of the patients from the United States had immediate wheal and flare skin reactivity to latex glove extract when tested by the prick technique with extract prepared in the U.S. laboratory. Total serum IgE levels of these patients ranged from less than 10 to 1860 IU. Nine subjects from the United States who ranged in age from 1 to 16 years and who had no clinical history of latex allergy were considered normal control subjects. None of these subjects had skin reactivity to latex antigens.

Antigens

Rubber tree latex extracts. In the present study, four different latex antigen preparations were used to demonstrate specific IgE antibodies in the sera of patients and normal control subjects. Two preparations from the sap of the rubber tree (*Hevea brasiliensis*), one from Malaysia (LE-1) and the other from India (LE-2), were used. The former was preserved as a frozen sample, and the latter was preserved in 0.5% formalin. The extract obtained from India was refined by centrifugation of latex plasma from 0.5% formalin-preserved rubber tree sap.⁹ The sap separated into three layers after centrifugation at 13,000 g for 30 minutes. The middle layer of the clear fluid, which contained the majority of proteins, was carefully removed and extracted with an equal volume of phosphate-buffered saline (PBS). After further centrifugation at 13,000 g for 30 minutes, the supernatant was collected and dialyzed with a 1000 d molecular weight cutoff membrane against deionized water for 48 to 72 hours and stored frozen at -70° C or freeze-dried.

Glove extracts. Two antigen preparations were obtained from gloves, one used in the United States (GE-1; Perry X-AM, Smith and Nephew, Ohio) and the other in Finland (GE-2; Triflex, Travenol Laboratories, Lessines, Belgium). The antigens were prepared in the U.S. laboratory by extracting 1 cm² pieces cut from a pair of gloves, which had been stirred overnight in PBS at 4° C, as previously described.⁹ The supernatants were collected and the glove pieces were extracted once more with PBS. The extracts were dialyzed as described above, the dialyze was centrifuged at 13,000 g for 15 minutes, and the supernatants were filter-sterilized and freeze-dried. The antigen prepared from gloves used in the United States was the only one used to test U.S. patients. The protein content as determined by the bicinchoninic acid method with reagents from Pierce Chemical Co. (Rockford, Ill.) was 410 µg/gm of gloves for the Finnish GE-2 extract and 360 µg/gm for the U.S. GE-1 glove extract.

The glove antigen for skin testing of Finnish patients was prepared in Finland as previously

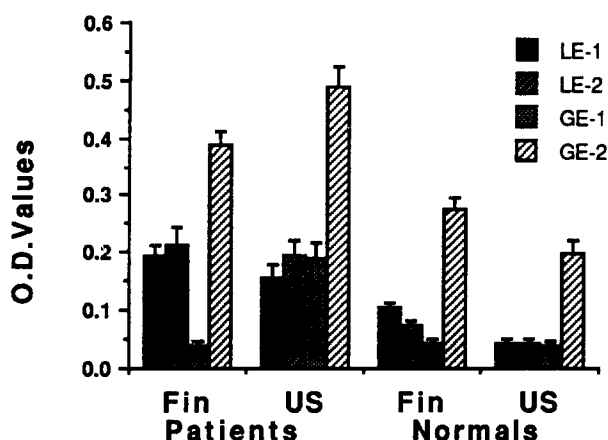


FIG. 1. Anti-latex IgE antibodies measured by ELISA in the sera of patients and normal control subjects from the United States and Finland. The plates were coated with the four antigens, and 1:100 dilutions of sera were used. The mean OD values obtained with different antigens were plotted; bars indicate standard error.

described.⁴ In brief, 400 mg of 1 cm² glove pieces were incubated with 5 ml of sterile physiologic saline solution. The gloves were obtained from a sealed bag to avoid contamination from external sources. After the glove pieces were removed, the solution was stored at 4° C for 1 month without addition of any preservatives.

Characterization of various extracts

All four extracts were characterized for their protein content and their profile by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein content of the different extracts was determined by the bicinchoninic acid method. The protein profiles of the different extracts were studied in a 15% continuous polyacrylamide gel that contained 0.5% sodium dodecylsulfate as previously described.⁹ The resolved proteins were stained with Coomassie brilliant blue, and the molecular size of the peptides was compared with standards. Approximately 15 to 20 µg of protein was boiled with sample buffer and used in the SDS-PAGE. The extracts were also compared for their reactivity with rabbit anti-latex antibodies by means of crossed immunoelectrophoresis to determine the number of precipitin bands formed against each antigenic preparation.

Skin tests

All of the patients that were seen in the United States were skin tested by the prick technique with serial 10fold dilutions of GE-1 antigen in PBS, which ranged from 1:1,000 to 1:1,000,000 wt/vol.¹⁵ All sites were read at 20 minutes for wheal and flare reactions. Diluent and histamine were used as negative and positive controls, respectively. In Finland patients were prick tested with freshly prepared GE-2 glove antigen

extract and control solutions, and the reactions were read as previously described.¹¹ Latex sap extract was not used for skin testing because of the induction of anaphylaxis during the skin test procedures in several patients when even high dilutions of this antigen were used.³

ELISA

A biotin-avidin-linked immunosorbent assay was used to detect latex-specific IgE antibodies and was carried out in duplicate.¹⁶ This method was found to be sensitive and reproducible, and all four antigens were used to test all sera. Briefly, the procedure is as follows. One hundred microliters of a 1 µg/ml of protein in PBS, pH 7.4, was coated to the microwells of polystyrene microtiter plates and incubated at 37° C for 3 hours and then at 4° C overnight. The plates were washed and blocked with 0.1% Tween-20 in PBS for 1 hour.¹⁷ This was followed by washing three times with PBS that contained 0.05% Tween-20 for 5 minutes each. The dilutions of the sera made in PBS-Tween-20 (1:100) were then added to appropriate wells (100 µl/well) and incubated at room temperature for 3 hours. The wells were then washed as before. One hundred microliters of 1:1000 dilution affinity-purified goat anti-human heavy-chain-specific IgE (Sigma Chemicals, St. Louis, Mo.) was added to the wells. The plates were incubated for 1 hour at room temperature. Again, the plates were washed, and 100 µl of 1:5000 dilution of biotinylated rabbit anti-goat IgG was added to each well. After incubation for 30 minutes and washing as before, 100 µl of 1:20,000 dilution of streptavidin peroxidase was added to the wells. After incubation, the plates were washed, and ortho-phenylene-diamine was added to the wells. The color developed was read at 450 nm in an automated ELISA reader. Appropriate controls and known positive and negative sera were run in each plate.

The data were discarded, and the assay was repeated when the optical density (OD) value of these standard sera varied in any plate by more than 10%. OD values exceeding the mean plus 2 standard deviations of the control values were considered to be elevated.

Statistical analysis

The different groups of patients, normal subjects, and the antigens were compared by unpaired *t* test with the use of Statworks (Computer Associates International, Islandia, N.Y.).

RESULTS

Latex-specific IgE in serum

The four different latex antigens reacted diversely with the different groups of sera studied (Fig. 1, Table I). The GE-1 antigen, which was prepared from gloves used in the United States, demonstrated elevated levels of specific IgE against latex in the sera of only 1 of 27 patients

TABLE I. Skin test reactivity and latex-specific IgE in the sera of patients and control subjects from Finland and the United States

Features evaluated	Patients		Control subjects	
	Finland (27)	U.S. (18)	Finland (18)	U.S. (9)
Skin test reactivity	27	18	0	0
Anaphylaxis or systemic symptoms	7	9	0	0
Specific IgE response to				
LE-1	14*	10	1	0
LE-2	14	11	0	0
GE-1	1	13	0	0
GE-2	7	13	0	1
LE-1 and LE-2	18	11	1	0
GE-1 and GE-2	7	15	0	1
At least 1 of 4 antigens	19	16	1	1

Number of sera in each group are indicated in parentheses.

*Sera were considered positive for IgE antibody when the OD was ≥ 2 SD above the mean of normal control sera.

with skin test reactivity from Finland but reacted with the sera of 13 of 18 patients with skin test reactivity from the United States. No significant differences could be detected in the antibody levels against these antigens in the nine U.S. patients who experienced perioperative anaphylaxis when compared with the other nine U.S. subjects with latex skin reactivity and allergy. The antigen prepared from gloves used in Finland reacted with all the sera of the Finnish patients as determined by skin prick test, although only 7 of 27 showed significant specific IgE levels to that latex antigen when compared with Finnish control subjects. The Finnish GE-2 antigen, however, showed elevated levels of latex-specific IgE in 13 of 18 of the sera from the U.S. patients, with no differences between patients with and those without clinical histories of anaphylaxis. Both the Finnish and U.S. patients demonstrated similar levels of specific IgE antibodies to the two latex sap preparations LE-1 and LE-2. Control sera from both countries had low levels of specific IgE to all antigens studied, whereas only one control subject from each country showed a false-positive reaction to the antigens (Fig. 1, Table I). There was no correlation between the specific IgE titers and total serum IgE levels.

Characterization of antigens

The rubber tree sap antigens (LE-1 and LE-2) showed 10 to 15 protein bands that were stainable with Coomassie brilliant blue in SDS-PAGE (Fig. 2), whereas only two to four bands could be demonstrated with the two glove extracts (GE-1 and GE-2, Fig. 2, *A* and *B*). The protein bands of

the sap antigens showed a wide range of molecular weights, 14 to 90 kd (Fig. 2, *C* and *D*). GE-1 showed a peptide at 65 to 70 kd, which was also visible in LE-1 and LE-2. LE-1 and LE-2 also showed a band at 14 kd similar to that of GE-1, whereas GE-2 showed bands at 30 to 40 kd as with LE-1 and LE-2. In crossed immunoelectrophoresis (Fig. 3), the number of antigens reacting with hyperimmune rabbit sera (produced against LE-2) were higher in LE-1 and LE-2 preparations (14 to 16 precipitin arcs) when compared with the glove extract GE-1 (2 to 4 arcs). The GE-2 extract revealed several more precipitin arcs and consistently showed more reactivity in the sera than the GE-1 extract did.

DISCUSSION

This study indicates that there are considerable differences in the IgE antibody responses of patients to different latex antigen preparations as demonstrated by ELISA. The glove antigens from the United States and Finland were both sensitive and specific for the antibody in the sera of patients from the United States but not for the Finnish patients. On the other hand, the GE-2 antigen prepared from the Triflex gloves used in Finland reacted with sera of more patients, as well as sera of normal subjects from both countries. However, to some extent, nonspecific absorption of IgE to this antigen cannot be excluded, since the OD values were much higher than with the other antigens (Fig. 1).

The noted differences in the serologic reactions of the two groups of patients from the two countries may be the result of differences in clinical

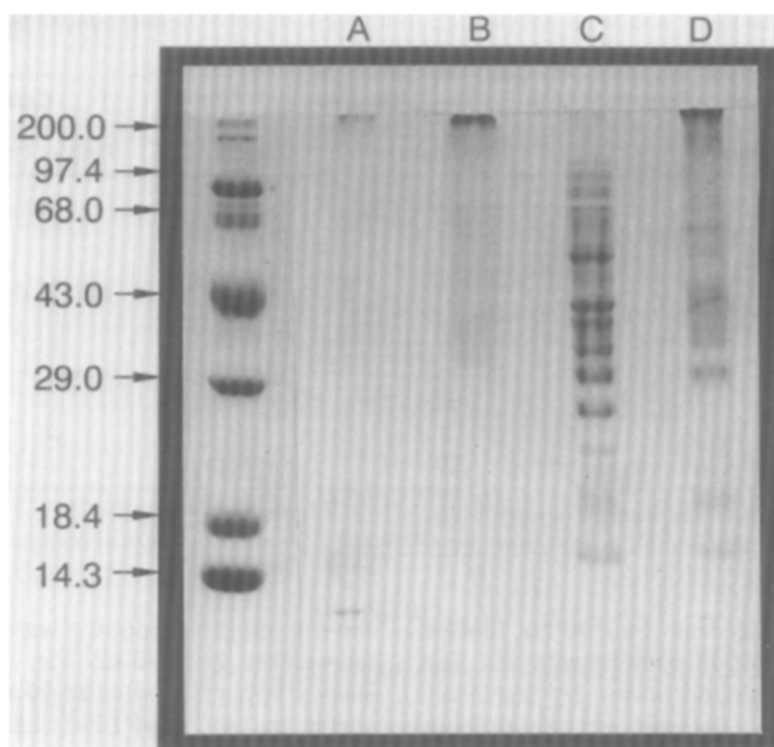


FIG. 2. SDS-PAGE showing the 4 latex antigens used in the study: **A**, GE-1; **B**, GE-2; **C**, LE-1; **D**, LE-2; and high molecular weight standard.

reactions of those groups or differences in antigen profiles of the tested preparations. The U.S. patients had spina bifida and were more susceptible to anaphylaxis,³ those from Finland were health care workers and others who mainly had only local dermal reactions.^{4, 11} There were also notable differences in the age distributions of both the patient and control groups, which may have influenced the observed serologic responses. The clinical immune response to rubber proteins may also be related to differences in latex antigens to which the individual is exposed or to variances in the immune reactivity of the several patient groups.

The protein profiles of the two tree sap antigens, LE-1 and LE-2, were comparable as determined by SDS-PAGE (Fig. 2). These extracts also showed similarity in the number of precipitin arcs produced against hyperimmune rabbit serum in CIE. Both antigens clearly demonstrated high specificity and sensitivity in detecting IgE antibodies in the sera of patients (Fig. 1).

The results of this study demonstrate that no single antigen can be used to detect antibodies to latex in all patients from all geographic locations.

Some of these patients may have been exposed to different cross-reactive antigens through exposure to diverse proteins in the variety of latex products available or through exposure to novel antigenic epitopes developed during the manufacturing process. Such cross-reactivity has been detected because ELISA antibody titers can be selectively inhibited with both glove and latex preparations.⁹ Western blot studies have shown that latex antibodies react with several antigenic components with different molecular weights, which are present in latex antigens from rubber tree sap extracts.¹⁸ However, only some of these components may be present in different gloves or other latex products because of diverse manufacturing procedures. Brand-to-brand differences¹³ or batch-to-batch variations in the antigenic content¹⁴ are likely to occur and make obtaining dependable antigens difficult. Since different latex products and batches of products differ in their protein contents, a pooled mixture of well-characterized proteins that have predictable reactivity and sensitivity is needed for use in the diagnosis of latex allergy.

A representative source of antigens for the detection of relevant antibodies is likely the rub-

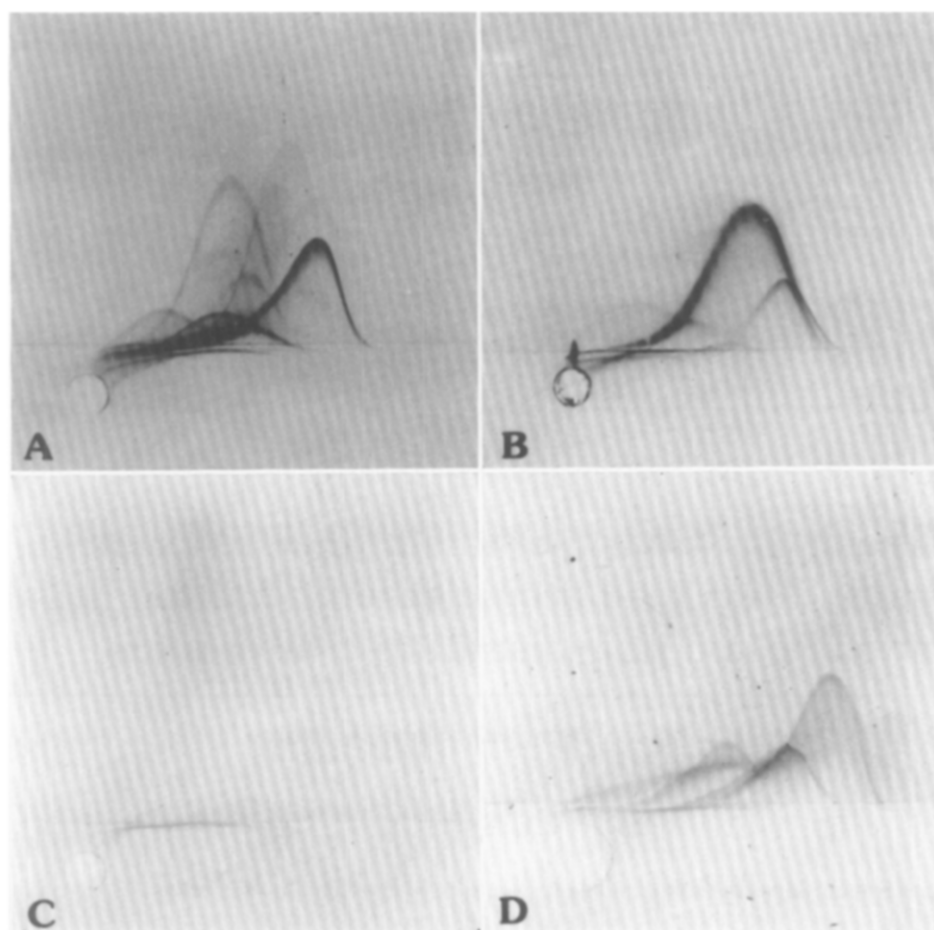


FIG. 3. Crossed immunoelectrophoresis of rabbit anti-latex serum against **A**, LE-1; **B**, LE-2; **C**, GE-1; and **D**, GE-2.

ber tree sap extract rather than extracts of latex products, because the latter may contain only a few proteins, although they may be more specific. Finally, differences in the patients and populations exposed (i.e., patients with spina bifida vs health care workers) or differences in the clinical response to latex (e.g., anaphylaxis vs contact urticaria) may play a role in defining the immune response of that group.

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Breast milk from mothers of children with newly developed atopic eczema has low levels of long chain polyunsaturated fatty acids

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Background: Infants at risk of atopic dermatitis have lower than normal levels of long chain polyunsaturated fatty acids. These fatty acids are normally present in substantial quantities in human breast milk.

Methods: Because of the equivocal evidence concerning the ability of breastfeeding to delay the onset or reduce the severity of atopic dermatitis, we have analyzed the fatty acid composition of breast milk from the mothers of children with newly developed disease with the use of gas chromatography.

Results: Breast milk lipids from mothers of children with newly developed atopic dermatitis had increased proportions of linoleic acid and significantly decreased proportions of its long chain polyunsaturated derivatives compared with a control group. The ratio of linoleic acid to the sum of its metabolites, γ -linolenic acid, dihomo- γ -linolenic acid, and arachidonic acid was 11.78 in the atopic group and 9.02 in the control group ($p < 0.01$).

Conclusions: These results are consistent with previous findings of an abnormal fatty acid status in atopic subjects and may account for some of the inconsistent results from studies of the effect of breastfeeding on the subsequent development of atopic dermatitis. We conclude that further studies to examine the effects of supplementation of the diet of breastfeeding mothers with long chain polyunsaturates should be done. (*J ALLERGY CLIN IMMUNOL* 1993;91:1134-9)

Key words: Breast milk, atopic eczema, essential fatty acids, γ -linolenic acid, dihomo- γ -linolenic acid, arachidonic acid, prostaglandins

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