Allergens, IgE, mediators, inflammatory mechanisms

The rubber elongation factor of rubber trees *(Hevea brasiliensis)* is the major allergen in latex

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Background: *Allergy to latex-containing articles is becoming more and more important because it can result in unexpected life-threatening anaphylactic reactions in sensitized individuals.*
Methods: *A protein of 58 kd with an isoelectric point of 8.45 was purified from raw latex and from latex gloves and identified as the major allergen, completely blocking specific IgE antibodies in the serum of latex-sensitized subjects. The allergen is a noncovalent homotetramer molecule, in which the 14.6 kd monomer was identified, by amino acid composition and sequence homologies of tryptic peptides, to be the rubber elongation factor found in natural latex of the Malaysian rubber tree.*
Results: *Competitive immunoinhibition tests showed that the starch powder covering the finished gloves is the airborne carrier of the allergen, resulting in bronchial asthma on inhalation. The purified allergen can induce allergic reactions in the nanogram range.*
Conclusion: *The identification of the allergen* (Hev b *I) may help to eliminate it during the production of latex-based articles in the future. (J* ALLERGY CLIN IMMUNOL *1993;92:690-7.)*

Key words: *Rubber elongation factor, latex allergen, latex gloves, airborne latex allergen*

Worldwide rubber production from the rubber tree *Hevea brasiliensis* (family, Euphorbiaceae) has reached 6 million tons per year. Besides various medical uses, such as surgical gloves, drainage tubes, and dental inserts, an increasing number of everyday products ranging from condoms to sport gears to car upholstery are made from natural rubber.

IgE-mediated allergic reactions to various latex-containing articles have been increasingly recognized in recent years.[1-5] The hypersensitivity reactions in sensitized individuals range from skin rashes and mild rhinitis to more severe bronchial asthma and life-threatening anaphylactic reactions. It is estimated that approximately 7% of medical personnel are sensitized to latex, and more and more case reports on surgical and dental patients describe hypersensitivity reactions because of contact with medical instruments made of natural rubber.[6-8] The episodes of fatal and life-threatening anaphylactic reactions have made the identification of the responsible allergen high priority.

Despite the awareness of latex allergy, the corresponding allergen could not be identified unequivocally until now. According to several studies, the reported molecular weight of the latex allergens ranges from about 2 to 100 kd, and the

Abbreviations used

EAST:	Enzyme allergosorbent test
FPLC:	Fast protein liquid chromatography
REF:	Rubber elongation factor
SDS-PAGE:	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

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reactivity patterns with antibodies differ individually with an approximate 14 kd protein, reacting in 10 of 10 cases in pediatric patients.[5, 9, 10] Recently, the air concentration of the starch powder covering the surgical gloves was directly correlated to asthma attacks in medical personnel in the operating room.[11]

In the present study for the first time, proof is provided that a major latex allergen in raw latex, surgical gloves, and on the powder covering the gloves is identical to the biologically important rubber elongation factor (REF) of the rubber tree, which is necessary for the biosynthesis of the polyisoprene chains in latex.

METHODS

Patients

Blood was taken from 13 latex-sensitized subjects attending our outpatient department in order to file for workers' compensation claims, stating occupationally induced latex allergy. Eight patients were tested in a nasal challenge test and 10 in a bronchial challenge test[5] according to recommendations of the Ethics Commission of the German Society for Occupational Medicine, which require patient's consent. None of the tests was done in a double-blind fashion because of the necessary postchallenge monitoring of the patients. All subjects had immediate-type skin reactions to latex and were diagnosed as having latex allergy by the criteria described previously.[5] In addition, blood of three subjects who were occupationally exposed to wheat flour and who showed elevated specific IgE concentrations, was taken without anticoagulants and centrifuged, and serum samples were aliquoted and kept at −18° C until use.

Fast protein liquid chromatography

Preparation of the samples. Ammoniated native latex was a generous gift of the "Kautschuk GmbH" Company in Frankfurt, Germany. Latex was diluted with a 0.05 mol/L Tris-HCl buffer, pH 7.5, at 1:100 (vol/vol) and filtered through a 0.2 μm Amicon filter (Amicon Inc., Beverly, Mass.). Two hundred microliters of latex was then used for fast protein liquid chromatography (FPLC) (see below).

Aqueous extract of starch powder from the surface of the surgical gloves. Two hundred pairs of surgical gloves (see below) were used for the experiment. The gloves were put into a container, and compressed air was used to blow off the flock from the surface of the gloves. The powder was then collected on paper filters at the outlets of the chamber. This method for collecting the powder was chosen because scraping of the powder resulted in the presence of microscopic rubber pieces on the powder surfaces after analysis by scanning electron microscopy (results not shown). After that, 100 mg of the collected powder was suspended in 1 ml of 0.05 mol/L Tris-HCl buffer, pH 7.5. The suspension was then vigorously shaken for 120 minutes and allowed to stand for 18 hours at 4° C. The mixture was then centrifuged for 20 minutes at 10.000 *g*, the supernatant was filtered through a 0.2 μm Amicon filter, and 200 μl was applied to the column.

Glove extract. Fifty pairs of sterile latex surgical gloves ("Sempermed," Hypoallergic Gloves, Semperit, Austria) were cut into small pieces of approximately 1 × 1 cm with a scalpel. The cut rubber pieces were quickly rinsed once in distilled water and then submerged in an equal volume of distilled water of approximately 500 ml. The mixture was then vigorously shaken for 120 minutes and allowed to stand for 18 hours at 4° C. After that, the rubber pieces were removed, and the supernatant was lyophilized. The dry material was kept at −20° C until further analysis. For the FPLC, the dry substance was dissolved in 0.05 mol/L Tris-HCl buffer, pH 7.5, at a protein concentration of 10 mg/ml, and 200 μl was used for the FPLC.

Gel filtration chromatography. The FPLC unit of Pharmacia (Freiburg, Germany) was used, and it was fitted with a Superose 12 column (1.5 × 30 cm) with a molecular separation range of 10.000 to 300.000 kd. FPLC was performed isocratically with 0.05 mol/L Tris-HCl buffer, pH 7.5, and the flow rate was adjusted to 0.5 ml/min. The absorption was monitored at 280 nm. Before the run, the column was calibrated with the low molecular weight molecular markers of Pharmacia.

Anion-exchange chromatography

Anion-exchange FPLC elution profile of the aqueous glove extract. For analytical runs, 100 to 200 μg of glove protein was applied. Preparative chromatography was carried out by applying 5 mg of the glove protein to the column, and 500 μl fractions were collected. Each of these fractions was then tested in a competitive immunoinhibition test.

The FPLC unit was fitted with an ion-exchange column (Mono-Q, 0.5 × 5 cm, Pharmacia), and the unit was programmed to deliver a discontinuous gradient of 0% to 5% solution B in 6 minutes, 35% of B in 24 minutes, and 100% of B in 6 minutes. Solution B consisted of 0.05 mol/L Tris-HCl, pH 7.5, in 1 mol/L NaCl and the starting buffer (A) was 0.05 mol/L Tris-HCl, pH 7.5. The flow rate was adjusted to 1 ml/min, and the absorption was monitored at 280 nm.

Enzyme allergosorbent test and immunoinhibition assays

The routine latex enzyme allergosorbent test (EAST) with the purified allergen and/or crude latex extract was carried out as described,[5] except that murine monoclonal anti-human IgE alkaline phosphatase was used as second antibody with p-nitrophenyl-phosphate substrate.

The competitive inhibition assay was done by pipet-

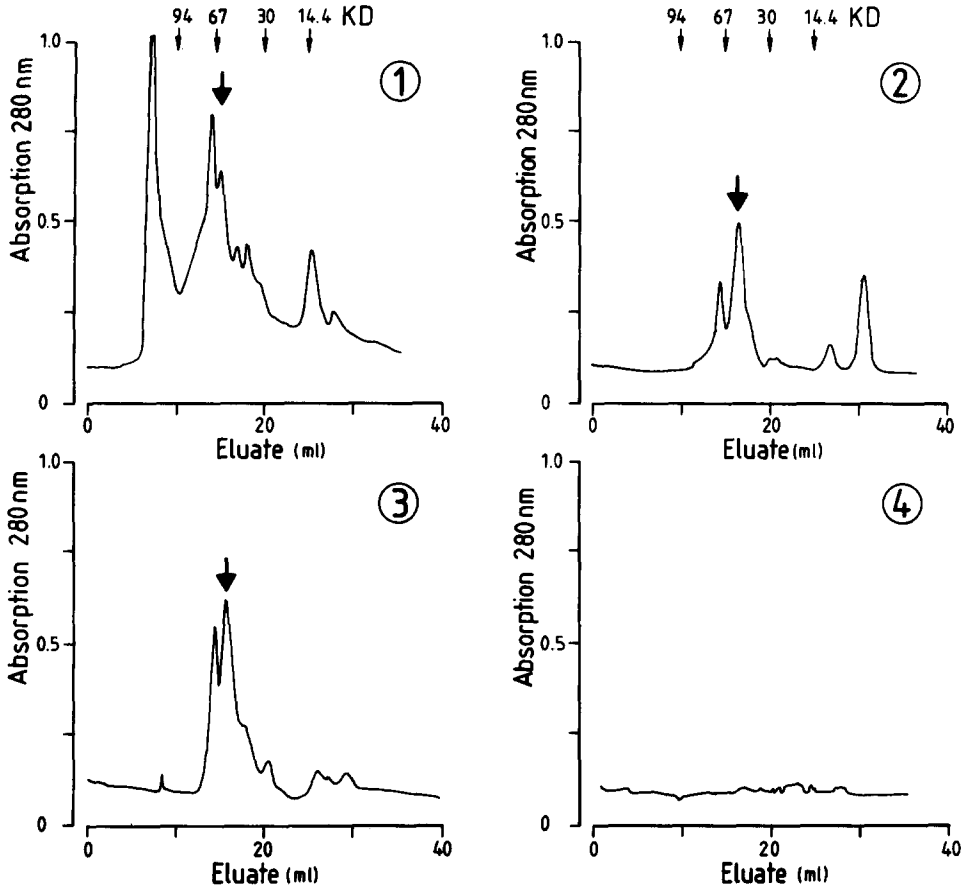

FIG. 1. FPLC of raw latex *(1),* aqueous extract of starch powder covering the surgical gloves *(2),* glove extract *(3),* and aqueous extract of native starch powder *(4).* Note that although the elution profiles of the three samples are quite different, one molecular species at around 58 kd *(vertical arrow)* is present in samples *1, 2,* and *3.* FPLC of starch powder that has never been in contact with surgical gloves but that has been otherwise treated, as sample no. 2, lacks any peaks.

ting 10 μl of each of the FPLC fractions or fraction no. 3 to 40 μl of sera from latex-sensitized subjects in the EAST. For the corresponding controls (0% inhibition) 10 μl of elution buffer was used instead of the fractions.

Two types of the water-insoluble starch powder suspensions were prepared at a concentration of 10% (wt/vol): (1) starch powder, which is used by the manufacturer to coat the gloves to prevent self-adhesion and was collected from the surface of the gloves; (2) the same starch powder, which had never been in contact with a glove surface. Serum samples from six latex-sensitized subjects were analyzed.

To check any nonimmunologic IgE binding on the particles, three subjects with high IgE titers to allergens other than latex were also tested with the glove powder and the corresponding target allergens.

The immunoabsorption test was carried out by incubating 40 μl of the 10% (wt/vol) powder suspensions with 160 μl of serum overnight at 4° C. After that, the mixture was centrifuged, and the supernatants were assayed in the EAST.[5]

Sodium dodecylsulfate–polyacrylamide gel electrophoresis

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of fraction no. 3 after FPLC of the glove protein was carried out in an 8% to 25% polyacrylamide gel gradient with the Phast system electrophoresis unit (Pharmacia) with precast polyacrylamide gels (43 × 50 × 0.45 mm) in a discontinuous buffer system according to the method of Laemli.

The anodic buffer strip consisted of 0.3 mol/L Tris–0.3 mol/L acetate buffer, and the cathodic buffer strip contained 0.8 mol/L Tris and 0.08 mol/L glycine buffer, pH 7.1. Both buffer strips contained 0.4% sodium dodecyl sulfate. The running conditions were preprogrammed at 250 V and 10 mA (3.0 W) for 40 minutes at 14° C. The following molecular weight standards (Pharmacia), were included in each run: α-lactalbumin 14.4 kd, soy bean trypsin inhibitor 20.1 kd, ovalbumin 43 kd, bovine serum albumin 67 kd, and phosphorylase B 94 kd. All samples were applied with a calibrated sample applicator (4 μl at a protein concentration of

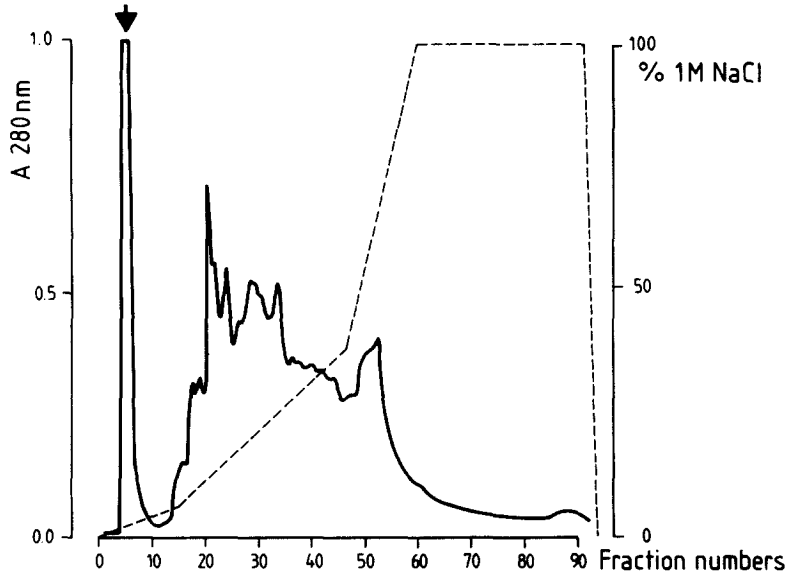

FIG. 2. The FPLC unit was fitted with an ion-exchange column (Mono-Q, 0.5 × 5 cm, Pharmacia), and the unit was programmed to deliver a discontinuous gradient of 0% to 5% solution B in 6 minutes, 35% of B in 24 minutes, and 100% of B in 6 minutes. Solution B consisted of 0.05 mol/L Tris-HCl, pH 7.5 in 1 mol/L NaCl and the starting buffer (A) was 0.05 mol/L Tris-HCl, pH 7.5. The flow rate was adjusted to 1 ml/min, and the absorption was monitored at 280 nm. For analytical runs, 100 to 200 μg of glove protein was applied. Preparative chromatography was carried out by applying 5 mg of the glove protein to the column, and 500 μl fractions were collected. Each of these fractions was then tested in a competitive immunoinhibition test. The first peak contained most of the allergenic activity, and fraction no. 3 showed the highest inhibition of latex-specific IgE antibodies in EAST *(arrow)*.

100 μg/ml). For reducing conditions, the samples were heated for 5 minutes in 5 mg sodium dodecyl sulfate and 50 mg dithiothreitol per milliliter in a water bath at 90° C. The gels were stained with silver nitrate.

For preparative runs, the gels were stained with Coomassie Brilliant Blue (Serva Feinbiochemica, Heidelburg, Germany), and the 14 kd protein band was excised, extracted from the gel, and subjected to amino acid analysis and partial sequencing after tryptic digestion.

Estimation of isoelectric point of the latex allergen

The isoelectric point of the purified latex allergen was estimated with the pH 3 to 9.5 ampholite and standards in the Phast system electrophoresis unit (Pharmacia).

Amino acid analysis and sequencing

Fraction no. 3 of the ion-exchange FPLC was subjected to SDS-PAGE under reducing conditions, and the gel was stained with Coomassie Brilliant Blue. The single band at 14 kd was excised from the gel and extracted with distilled water by vigorous shaking for 120 minutes to remove excessive dye. After that, the gels were put in 0.05 mol/L acetate buffer, pH 8.5, and shaken for 18 hours at room temperature. The gels were then removed and the solution lyophilized. The lyophylisate (0.4 μg protein) was taken up in 100 μl of 6 N HCl and hydrolyzed for 1 hour at 150° C. The liberated amino acids were coupled to phenylisothiocyanate, and the resulting phenylthiocarbamoyl amino acid derivatives were identified with an amino acid analyzer (model 130, Applied Biosystems, Weiderstadt, Germany).

The 14 kd allergen was extracted directly from the gel after SDS-PAGE, and 500 pmol was digested with 1 μg trypsin (Promega Corp., Madison, Wis.). The peptide mixture was then separated on a C_{18} column (2 × 100 mm) with a 0.1% trifluoroacetic acid–0.08% trifluoroacetic acid 84% acetonitrile gradient at a flow rate of 0.15 ml/min.

Four peptides showing baseline separation were collected and subjected to Edman degradation with a Protein/Peptide Sequencer (model 470A, Applied Biosystems).

EAST with REF

Various concentrations were tried in order to achieve maximal IgE binding. The purified latex allergen (1 mg/ml) and the crude latex fraction at 1:100 dilution in the coupling buffer was then coupled to CNBr-activated paper discs,[5] and the EAST with serum samples of the 13 sensitized subjects was carried out as

TABLE I. EAST and immunoinhibition tests with sera of 13 latex-sensitized patients

Outpatient no.	EAST (U/ml) REF (crude extract)*	Competitive immunoinhibition tests		
		Percent inhibition of EAST with REF (25 μg/ml)	Percent inhibition of EAST with glove powder† (2% wt/vol, final concentration)	Percent inhibition of EAST with native powder‡ (2% wt/vol, final concentration)
1401	4.09 (1.49)	92	75	5
1411	12.88 (4.47)	98	70	0
1498	8.01 (13.5)	87	73	2
1594	4.74 (1.33)	92	85	3
1633	1.03 (10.08)	86	95	10
1645	4.02 (0.62)	97	90	7
1667	6.12 (2.21)	96	ND	ND
1778	0.58 (0.52)	98	ND	ND
1881	13.44 (2.01)	85	ND	ND
1873	8.08 (1.98)	90	ND	ND
1917	0.39 (0.66)	95	ND	ND
1918	2.38 (0.46)	93	ND	ND
1920	0.69 (0.51)	100	ND	ND

ND, Not determined.

*Primary screening was done with one serum sample (no. 1594; 3.3 U/ml)[5] in a competitive immunoinhibition test. Of all FPLC fractions (1 to 70), fraction no. 3 produced the highest inhibition. This fraction was then tested with an additional panel of serum from 13 latex-sensitized subjects. The results show that it blocked the latex-specific IgE antibodies in all cases at a protein concentration of 25 μg/ml. This fraction was subjected to further analysis.

†Note that up to 95% of the latex-specific IgE is blocked by the powder collected from the surface of the gloves.

‡Powder that was never in contact with gloves.

described above. The results are expressed as units per milliliter according to the standard curve by Pharmacia.

RESULTS

It has been shown that one molecular species at around 58 kd is common in all samples except for the native starch powder (Fig. 1). To purify this fraction, the aqueous glove extract was subjected to ion-exchange FPLC (Fig. 2) in which each of the fractions was collected and tested in a competitive immunoinhibition assay with the serum of a sensitized subject who had a high IgE antibody concentration to raw latex. Fraction no. 3 blocked latex-specific IgE antibodies in the solid-phase assay completely and was further analyzed. Serum samples of 12 additional subjects, all of whom had latex allergy and showed high titers of IgE antibodies, were consequently tested in the competitive immunoinhibition assay with fraction no. 3. This FPLC fraction blocked specific IgE binding to the latex target allergen up to 100% (Table I).

The results of the EAST with purified latex allergen and with the crude latex allergen are shown in Table I. In some cases (no. 1594, 1811, 1918, and others) considerably higher IgE binding occurred with the purified allergen.

The starch powder that coats the gloves was blown off by compressed air, and an immunoinhibition test showed that the starch powder reacted like a solid-phase latex allergen (Table I). No reaction could be observed with wheat flour–specific IgE. Furthermore, aqueous extracts of this powder also resulted in positive skin tests.

The biologic activity of the purified glove allergen with regard to clinical relevance was also checked in connection with occupationally induced latex allergy. The following IgE-mediated reactions could be observed: (1) positive skin reaction in 12 of 13 sensitized subjects with 4 ng protein, (2) nasal obstruction in 7 of 8 subjects at a protein concentration of 400 ng/ml, and (3) asthmatic reactions in 8 of 10 subjects at protein concentrations of 400 to 4000 ng/ml.

When fraction no. 3 was subjected to SDS-PAGE, the allergen showed an apparent molecular weight of 58 kd under nonreducing conditions, and sodium dodecyl sulfate/dithiothreitol treatment resulted in a single polypeptide of slightly more than 14.4 kd, indicating a homotetramer molecule (Fig. 3). Immunoblotting of the crude or purified glove extract with the same pooled sera resulted in a single band at 58 kd. The isoelectric

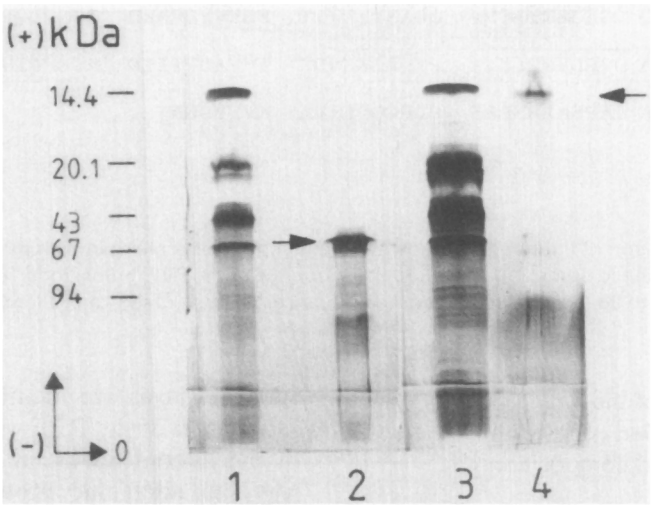

FIG. 3. SDS-PAGE of the latex allergen. *Lane 1,* Molecular weight markers; *lane 2,* fraction no. 3 after FPLC; *lane 3,* molecular weight markers reduced; *lane 4,* fraction no. 3 reduced. Fraction no. 3 of ion-exchange FPLC shows an apparent molecular weight of around 58 kd beside some species of higher molecular weights. Under reducing conditions, this fraction dissociates into a single protein band with an apparent molecular weight of about 14 kd. It is interesting to note, however, that a protein band of about 110.000 kd is indicated to be contained in the allergenic fraction in nonreduced, and to a lesser degree, in reduced samples (*lanes 2* and *4*). This would indicate a polymeric association of the 14 kd polypeptide that does not dissociate completely under the experimental conditions. This assumption is further supported by the fact that amino acid analysis and amino acid sequencing of tryptic fragments of the 14 kd allergen under reducing conditions *(lane 2)* indicate a homogeneous monomeric subunit (Table I and Fig. 4).

point was estimated to be 8.45 for the tetramer. The glove allergen was also extracted from the gel after SDS-PAGE and subjected to amino acid analysis and protein sequencing. The amino acid composition is shown in Table II. A search in the peptide library indicated that the REF *(Hevea brasiliensis)* had a similar molecular weight of 14.6 kd and an amino acid composition that was not significantly different from the latex glove allergen analyzed here. A simultaneously attempted sequencing of the allergen failed because no N-terminal amino acid residue could be detected, presumably because of a blocked amino N-terminus. Tryptic digestion and high-performance liquid chromatography separation of the purified glove allergen resulted in peptide fractions, four of which had clear-cut baseline separations. These peptides were then subjected to amino acid sequence analysis, and all four of them showed a 100% homology with the corresponding partial sequences published for REF[12] (Fig. 4).

DISCUSSION

The 58 kd molecule is not only present in natural rubber but also in the finished surgical gloves and in the starch powder covering the gloves but not in starch powder without glove contact.

The exact mode of transfer of the latex allergen from the gloves to the powder remains to be elucidated; however, a capillary transfer from the hot, vulcanized (moist) rubber gloves to the dry powder seems to be conceivable, thus resulting in a transfer of the allergen from the gloves into an airborne inhalant dust.

All of these results, seem to confirm the hypothesis that the allergen is transferred from the surgical gloves to the starch powder covering the gloves.[4]

When the results of the aqueous extract of surgical gloves purified here are compared with published results of the REF, the following properties are common to both proteins and support the hypothesis of an identity: (1) molecular mass; (2) blocked N-terminal amino acid, amino acid composition; (3) purification of the REF by the original method[12] that results in a molecular species of similar molecular mass to our allergen purified from latex gloves and blocking of latex-specific IgE antibodies by REF in sensitized subjects; (4) amino acid compositions that are not significantly different; (5) four identical overlap-

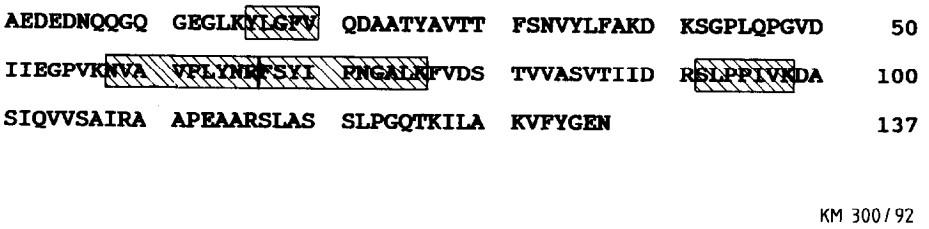

FIG. 4. Comparison of the tryptic peptide fragments of the latex allergen with the known primary amino acid sequence of the REF.[12] The partial sequence of the REF in positions 16 to 20, 58 to 66, 67 to 76, and 91 to 98 *(shaded sequences)* are identical to those of the latex allergen purified from gloves.

TABLE II. Comparison of the amino acid composition of the purified glove with the published amino acid composition of REF

Residue	REF*	Latex allergen*
Ala	12.0	9.4
Cys	0.0	0.0
Asp	10.5	8.8
Glu	9.8	12.5
Phe	4.5	4.4
Gly	7.5	7.8
His	0.0	1.3
Ile	6.0	4.4
Lys	6.0	5.0
Leu	7.5	9.7
Met	0.0	0.9
Pro	6.0	5.6
Arg	3.0	5.0
Ser	7.5	6.9
Thr	4.5	4.1
Val	10.5	9.1
Trp	0.0	0.0
Tyr	4.5	5.0
Total	100.0	100.0

The data show nearly identical amino acid composition of the REF to the allergen isolated from latex gloves. In another analysis the number of amino acid residues was compared by the chi-square test. No statistical significance of the differences could be found ($\chi^2 = 12.26$, d.f. $= 15$, $p < 0.05$, data not shown).

The values given are an average of three analyses, and they are not corrected for destruction/slow liberation of the amino acids under the experimental conditions.

An attempted amino acid sequence analysis (0.8 μg) of the same sample resulted in no sequence data, presumably because of a blocked N-terminal residue. The latter was reported by the authors for REF.[12]

*Residues are expressed as mol/mol (%).

ping REF peptides after tryptic digestion of the glove allergen.

All of the above-mentioned comparisons imply that the REF and the allergen isolated here from surgical gloves are identical. The reported close association of the REF with the lipophilic surface of the luteoid bodies in raw latex might explain why the allergenic protein purified here from surgical gloves resists the rather harsh treatment of rubber vulcanization in the process of glove manufacturing.

Furthermore, the results here indicate that REF exists in a native homotetrameric configuration with a calculated molecular weight of 58.4 kd and dissociates into its subunits only on treatment with detergent. Because the molecule does not contain any disulfide bridges, the coherent interaction of the four subunits must be noncovalent.

It is not clear why immunoblottings by other investigators show a broad molecular weight range of reactive allergens, although in some reports it is not stated whether the samples were all subjected to sodium dodecylsulfate/dithiothreitol or mercaptoethanol treatment before electrophoresis.[5, 9]

The two most likely explanations should be considered: (1) various stages of breakdown/aggregation of REF during different isolation procedures or vulcanization of the gloves (130° C, 120 minutes) result in common epitopes with different molecular masses, and (2) some of the allergens of different molecular weights represent various lectins binding IgE in a nonimmunologic manner. However, as the results of the SDS-PAGE and competitive immunoinhibition show here, the first possibility seems to be more likely at this stage, because no allergenic molecular species other than the 14 kd and 58 kd could be detected here in the latex gloves. On the other hand, these results do not imply that REF is the only allergen in the original latex, because some patients showed a considerably higher binding of specific IgE with crude latex under optimal conditions. However, considering the results, it can be postulated that in this brand of surgical gloves REF is the only latex allergen of major clinical relevance

that would cause all the clinical symptoms, because no other allergen could be detected either by immunoblotting or by highly sensitive protein staining in fraction no. 3.

This would conclude the rather long journey of the REF allergen from the Malaysian rubber tree over latex gloves and starch powder, covering these gloves, into the respiratory tract, to be presented to the immune system of some sensitized subjects.

REF has a unique role in the biosynthesis of polyisoprene chains in rubber trees and serves as a carrier to add multiple cis-isoprene units to rubber molecules.[13]

It remains to be seen whether other prenyl-transporting molecules with similar biologic functions, such as transcortin or retinol-binding protein, might prove to have such allergenic properties. In view of the fact that 35 triplet motifs and three quadruplets between REF and human transcortin and one triplet between the partial sequence of the 16 amino acids of the retinol-binding protein and REF have been identified, the above-mentioned possibility of common allergenic motifs to the three molecules does not seem to be unlikely.

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REFERENCES

1. Turjanmaa K, Reunala T. Incidence of immediate allergy to latex gloves in hospital personnel. Contact Dermatitis 1987;21:197-8.
2. Spaner D, Dolovich J, Tarlo S, Sussmann G. Hypersensitivity to natural latex. J ALLERGY CLIN IMMUNOL 1989;83:1135-7.
3. Turjanmaa K, Reunala T, Tuimala R, Karkkainen T. Allergy to latex gloves unusual complication during delivery. Br Med J 1988;297:1029.
4. Czuppon AB, Jaeger D, Baur X. Purification and characterization of a 67 kDa latex *(Hevea brasiliensis)* major allergen from surgical gloves. J Allergy Clin Immunol News 1991;(suppl 1):201.
5. Jaeger D, Kleinhans D, Czuppon AB, Baur X. Latex-specific proteins causing immediate-type cutaneous, nasal, bronchial and systemic reactions. J ALLERGY CLIN IMMUNOL 1992;89:759-68.
6. Axelsson JG, Johansson SGO, Wrangsjö K. IgE-mediated anaphylactoid reactions to rubber. Allergy 1987;42:46-50.
7. Slater JE. Rubber anaphylaxis. N Engl J Med 1989;320:1126-30.
8. Leynadier F, Pecquet C, Dry J. Anaphylaxis to latex during surgery. Anaesthesia 1989;44:547-50.
9. Chambeyron C, Dry J, Leynadier F, Pecquet C, Tran Xuan Thao. Study of the allergenic fractions of latex. Allergy 1992;47:92-7.
10. Slater JE, Trybul DE. Affinity purification of latex (L) antigens [Abstract]. J ALLERGY CLIN IMMUNOL 1992;89:226.
11. Swanson MC, Bubak ME, Hunt LW, Reed CE. Occupational respiratory allergic disease from latex [Abstract]. J ALLERGY CLIN IMMUNOL 1992;89:227.
12. Dennis MS, Henzel WJ, Bell J, Kohr W, Light DR. Amino acid sequence of rubber elongation factor protein associated with rubber particles in *Hevea* latex. J Biol Chem 1989;31:18618-26.
13. Light DR, Dennis MS. Purification of a prenyltransferase that elongates cis-polyisoprene rubber from the latex of *Hevea brasiliensis.* J Biol Chem 1989;264:18589-97.