

THE JOURNAL OF
ALLERGY
AND
CLINICAL IMMUNOLOGY

VOLUME 92

NUMBER 5

Editorial

Is rubber elongation factor the major allergen of latex?

In the past decade, latex has become recognized as an allergen capable of inducing life-threatening allergic reactions in an increasing number of sensitive individuals.¹⁻⁵ Identification of the major allergen or allergens in latex is important for several reasons. Identification of the major allergen(s) will make it possible to determine whether latex allergens are all found in native latex as it comes from the tree or whether some new allergic epitopes are created during processing.⁶ If new allergens are created during processing, it may be possible to alter processing to reduce the allergen content of finished products. If the major allergen(s) are all found in native latex, it may be necessary to learn the function of the allergenic proteins. Some proteins found in latex may contribute to the physical properties of finished rubber products, whereas removal of other proteins may not alter physical properties. Identification of major allergen(s) will also aid in the management of latex allergy. Knowledge of major allergen(s) should lead to the preparation of potent, standardized, and well-characterized latex extracts for diagnosis of latex allergy and to the development of more

Abbreviations used

EAST: Enzyme allergosorbent test
REF: Rubber elongation factor
CIE: Crossed immunoelectrophoresis

accurate tests of allergen content in finished products.

The International Union of Immunological Societies Subcommittee for Allergen Nomenclature has proposed criteria for defining and labeling allergens.⁷ The general criteria for identifying an allergen are identification of the source and physical form of the material yielding the allergen. Purified allergens are to be numbered with Roman numerals, usually in order of isolation, provided that a major allergen is number I. Ideally, any variation in the allergen derived from different strains of the same species should be identified. The purity of the allergen should be established with four or five criteria, including molecular weight; molecular charge; hydrophobicity; immunochemistry from crossed immunoelectrophoresis (CIE), crossed radioimmunoelectrophoresis, and immunoelectrophoresis; and identification of NH₂- and COOH- terminals plus amino acid composition. To be accepted as a major allergen, the importance of the allergen should be demonstrated in an epidemiologically defined subpopulation of 20 to 30 allergic individuals. The method used for testing the individuals and the criteria for determining positivity must be clearly stated.⁷

From the Division of Allergy and Clinical Immunology, Department of Internal Medicine, Henry Ford Hospital, Detroit.

Reprint requests: Dennis R. Ownby, MD, Division of Allergy and Clinical Immunology, Henry Ford Hospital, 2799 West Grand Blvd., Detroit, MI 48202.

J ALLERGY CLIN IMMUNOL 1993;92:633-5

Copyright © 1993 by Mosby-Year Book, Inc.

0091-6749/93 \$1.00 + .10 1/1/48670

In this issue of the JOURNAL, Czuppon et al.⁸ present information suggesting that rubber elongation factor (REF) is the major allergen of latex. REF was first identified and characterized by Light and Dennis⁹ who later published the complete amino acid sequence.^{9, 10} Molecular cloning and nucleotide sequencing of REF has also been reported.¹¹ REF is 137 amino acids long with a molecular mass of 14.6 kd.¹⁰ In native latex REF is bound to rubber particles.⁹ Washing rubber particles with sodium dodecyl sulfate results in the release of two proteins, the 14.6 kd REF and a 24 kd protein found on both the rubber particles and free in the serum.⁹ Light and Dennis⁹ found that REF must be present for prenyltransferase to be able to add multiple *cis*-isoprene units to rubber molecules; hence, they termed the protein rubber elongation factor.⁹

Czuppon et al.⁸ isolated an allergenic protein from raw latex, surgical gloves, and surgical glove powder. The isolated protein had an initial molecular weight of 58 kd as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; after pretreatment with sodium dodecyl sulfate and dithiothreitol, the protein had a molecular weight of 14.4 kd. On the basis of the apparent change in molecular weight, the authors suggest that the 58 kd molecule is a homotetramer composed of 14.4 to 14.6 kd molecules.⁸ The allergenicity of the isolated protein was demonstrated by skin tests, nasal and bronchial challenges, and inhibition of latex-specific IgE. Amino acid composition and sequence analysis of the isolated allergen are consistent with the composition and sequence of REF.^{8, 10}

The suggestion by Czuppon et al.⁸ that REF is the only major allergen in the latex gloves used in this study is based on immunoblots with pooled sera and glove extracts demonstrating a single 58 kd band, the fact that REF blocked from 85% to 100% of the binding of latex-specific IgE from 13 individual serum samples in the enzyme allergosorbent test (EAST), and the ability of the purified glove allergen to induce symptoms.⁸ It is difficult to reconcile the findings of Czuppon et al.,⁸ suggesting a single major latex allergen in surgical gloves, with multiple other studies suggesting that latex allergens have molecular weights ranging from 2 kd to more than 100 kd. A review of 14 studies of latex allergens revealed that 6 of the 14 studies detected an allergen of approximately 14 to 15 kd and 11 studies detected allergens of 10 to 20 kd.¹² The sources of the latex products in these studies varied, but nine of the studies used gloves as at least one source of latex

allergen. Given the potential variations in determining molecular weights, REF with a molecular weight of 14.6 kd could possibly be detected with a molecular weight other than 14 to 15 kd. Only three of the 14 studies detected allergens with weights between 40 and 60 kd. This low rate of recognition of allergens with molecular weights similar to the 58 kd reported by Czuppon et al.⁸ for the tetrameric form of REF may result from variable use of detergents and reducing agents with gel electrophoresis. The latex or brand of glove used may also be a source of variation. Nine studies reported an allergen of 30 kd, including a report for which Czuppon was a coauthor.¹³ Three other laboratories reported an allergen of approximately 25 kd. This allergen with a molecular weight of 24 to 30 kd may be the same protein as the 24 kd protein mentioned by Light and Dennis⁹ as being found both in the serum and attached to rubber particles in latex. The structure and function of the 24 kd molecule have not yet been reported.

The strongest evidence indicating that REF is the only major allergen in latex gloves presented by Czuppon et al.⁸ is the consistently high degree of inhibition produced by REF in the EAST.⁸ The near total inhibition of anti-latex IgE from 13 individuals by REF is consistent with a single major latex allergen with two caveats. The first caveat is the question of whether the 13 patients whose sera were used in the inhibition study are representative of latex-sensitive individuals in general. If these patients were selected in a manner that biased the selection toward individuals sensitized only to REF, the high degree of inhibition by REF would be expected. Selection biases might include the source and route of the sensitizing latex exposure, the latex extract used to detect latex sensitivity, or the use of a particular assay for detection of anti-latex IgE. The second caveat is the question of whether all other potentially allergenic latex proteins are appropriately bound to the EAST solid phase. If REF were the dominant protein on the solid phase, REF would again be expected to produce high levels of inhibition because IgE antibodies specific for other proteins would not bind to the solid phase even in the absence of REF. Immunoblots with individual serum samples and extracts of native latex and glove extracts combined with inhibition of the immunoblots by REF would help to clarify the question of the number of latex allergens.

The article by Kurup et al.¹⁴ in this issue reports on the production of a monoclonal antibody specific for latex allergen(s). The reported monoclo-

nal antibody reacted with proteins found both in native latex and in 2 of 10 glove extracts. The molecular weights of the three protein bands recognized by the monoclonal antibody ranged from 55 to 65 kd. In crossed enzyme immunoelectrophoresis, the monoclonal antibody reacted with three precipitin arcs, which appeared to be identical to the three precipitin arcs recognized by IgE from latex-sensitive donors. The 55 to 65 kd weights of the protein(s) recognized by the monoclonal antibody are similar to the 58 kd weight of the REF tetramer reported by Czuppon et al.⁸ However, the 55 to 65 kd value was apparently obtained in the presence of sodium dodecyl sulfate, when only the 14.6 kd REF monomer should have been detectable. Another difference between the results of Kurup et al.¹⁴ and those of Czuppon et al.⁸ is that Kurup et al.¹⁴ found significant levels of anti-latex IgE antibody in 30% of 42 patients with crude latex extract and in 26% of the same patients with monoclonal antibody-purified allergen, but only 16% of patients had IgE antibodies to both crude and purified allergens. This level of discrepancy between IgE reacting with the crude and purified allergens suggests that significant allergens are present in the crude extract other than the purified allergen. It is also interesting that the monoclonal antibody only reacted with extracts of 2 of 10 different brands of gloves.¹⁴

In their discussion Kurup et al.¹⁴ make the provocative statement that "all patients and normal subjects showed recognizable levels of IgE against latex as determined by ELISA" even though seven of the normal serum donors had negative skin test results. This suggests that either the ELISA was more sensitive than skin testing or that ELISA results should be interpreted with caution because of false positivity.

These two articles demonstrate that there is still much to be learned about the allergen composition of both native latex and latex products. Both articles are significant because they present methods for purifying allergens that can be evaluated by other laboratories. There are many variables that may complicate the recognition and isolation of pure allergens from latex. Clones of rubber trees have been selected for optimal latex yield, and these are often grafted onto root stock from different clones. There is little information concerning the variation in the protein composition between different clones. There is also limited information concerning the variation in latex composition from different countries. Beyond the variations in the native latex, there are many

variations in the handling of latex and in the production of finished rubber products. On the basis of the information presented by Czuppon et al.,⁸ it appears likely that REF is a latex allergen. If other investigators can confirm that REF is a major allergen, then REF will become the first well-characterized latex allergen. Hopefully, all latex allergens can be clearly defined in the near future so that ultimately the risk of latex-induced allergic reactions can be eliminated.

Dennis R. Ownby, MD

Division of Allergy and Clinical Immunology

Department of Internal Medicine

Henry Ford Hospital

2799 West Grand Blvd.

Detroit, MI 48202

REFERENCES

1. Leynadier F, Pecquet C, Dry J. Anaphylaxis to latex during surgery. *Anaesthesia* 1989;44:547-50.
2. Slater J. Rubber anaphylaxis. *N Engl J Med* 1989;17:1126-30.
3. Gerber A, Jorg W, Zbinden S, Seger R, Dangel P. Severe intraoperative anaphylaxis to surgical gloves: latex allergy, an unfamiliar condition. *Anesthesiology* 1989;71:800-2.
4. Sussman G, Tarlo S, Dolovich J. The spectrum of IgE-mediated responses to latex. *JAMA* 1991;265:2844-7.
5. Ownby DR, Tomlanovich M, Sammons N, McCullough J. Anaphylaxis associated with latex allergy during barium enema examinations. *Am J Roentgenol* 1991;156:903-8.
6. Makinen-Kiljunen S, Turjanmaa K, Palosuo T, Reunala T. Characterization of latex antigens and allergens in surgical gloves and natural rubber by immunoelectrophoretic methods. *J ALLERGY CLIN IMMUNOL* 1992;90:230-5.
7. Marsh DG, Goodfriend L, King TP, Lowenstein H, Platts-Mills TAE. Allergen nomenclature. *J ALLERGY CLIN IMMUNOL* 1987;80:639-45.
8. Czuppon AB, Rennert S, Engelke T, Meier H, Heber M, Baur X. The rubber elongation factor of rubber trees (*Hevea brasiliensis*) is the major allergen in latex. *J ALLERGY CLIN IMMUNOL* 1993;92:690-7.
9. Light DR, Dennis MS. Rubber elongation factor from *Hevea brasiliensis*. *J Biol Chem* 1989;264:18608-17.
10. 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;264:18618-26.
11. Attanyaka DPSTG, Kekwick RGO, Franklin FCH. Molecular cloning and nucleotide sequencing of the rubber elongation factor gene from *Hevea brasiliensis*. *Plant Mol Biol* 1991;16:1079-81.
12. Hamann CP. Natural rubber latex protein sensitivity in review. *Am J Contact Dermatitis* 1993;4:4-21.
13. 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.
14. Kurup VP, Kumar A, Kelly KJ, Fink JN. Characterization of a monoclonal antibody against latex protein associated with latex allergy. *J ALLERGY CLIN IMMUNOL* 1993;92:638-43.