

Latex allergens

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SUMMARY: Several natural rubber latex (NRL) allergens have recently been purified and characterized at the molecular level. This progress has been essential for the assessment of their roles and clinical significance. Current allergen nomenclature system lists 7 NRL allergens. It is now well established that prohevein (Hev b 6) and hevein (Hev b 6.1) as well as an acidic 16 kDa protein (Hev b 5) represent major NRL allergens. Hydrophobic rubber particle proteins, Hev b 1 (REF) and Hev b 3, seem to be important allergens to latex-allergic patients with spina bifida. Hev b 1, Hev b 3, Hev b 5 and Hev b 6.1 or IgE-binding peptides derived from them have been identified in NRL gloves. These proteins or peptides are thus resistant to the highly denaturing conditions of glove manufacturing and, therefore, could act as initial sensitizers and lead to the development of Type I latex allergy.

KEY-WORDS: Allergy – Latex proteins – Allergens – IgE.

Immediate hypersensitivity reactions to natural rubber latex (NRL) proteins have been increasingly recognized during the past 15 years. Currently, approximately 3 % of hospital employees and 5-10 % of physicians and nurses working in operation units are allergic to NRL (reviewed in [19]). Manufactured NRL products, such as surgical and household gloves, catheters, condoms, baby pacifiers and toy balloons may contain allergenic proteins capable of eliciting type I allergic reactions. NRL, the raw material of natural rubber products, is obtained during the process of tapping of the rubber tree *Hevea brasiliensis*. NRL is formed in the cytoplasm of specialized cells situated beneath the bark of the rubber tree, and its protein content is approximately 1-2 %. Proteins or peptides remaining in the NRL products are obviously responsible for sensitization processes in NRL allergy. In fresh NRL, over 200 different polypeptides have been demonstrated by 2-dimensional immunoblotting

but only some 25 % them bound IgE antibodies from latex-allergic patient sera and can be considered as allergens [6, 16]. Recently, substantial progress has been made in the purification and molecular characterization of several NRL allergens which has facilitated the assessment of their significance and given grounds for proper comparison of the results obtained by different researchers. In the present communication the current knowledge of NRL allergens will be reviewed.

NATURAL RUBBER LATEX ALLERGENS CHARACTERIZED AT THE MOLECULAR LEVEL

Rubber elongation factor (Hev b1)

Rubber elongation factor (REF) is tightly bound to rubber particles but can be solubilized

e.g. by detergents [12]. REF was the first NRL allergen characterized at the molecular level and was suggested to be the major allergen in NRL and the only allergen present in one latex surgical glove [11]. In more recent studies, however, IgE-antibodies to highly purified REF, detected by immunoblotting and ELISA, were practically confined to the subgroup of patients with spina bifida [5]. Yeang et al. [20] reported similar results using recombinant REF and it thus appears that IgE response to Hev b 1 is for some reason rare in adult latex-allergic patients but common in patients with spina bifida and/or other congenital anomalies and histories of multiple surgical operations.

Prohevein (Hev b 6) and mature hevein (Hev b 6.1)

Hevein is the most predominant protein in NRL, compartmentalized in luteoids, the vacuolar structures of the laticifer cells present in the rubber tree [9]. It is a chitin-binding protein suggested to be involved in the coagulation of latex and to play a role in the protection of rubber tree wounds by inhibiting the growth of several chitin-containing fungi [9, 13]. Hevein is synthesized as a 20 kDa preproprotein (prohevein) that is posttranslationally processed into aminoterminal hevein (43 amino acids) and the carboxyterminal 138-amino-acid C domain.

Purified prohevein bound IgE by immunoblotting and ELISA in sera from 69-83 % of NRL-allergic patients and elicited positive SPT reactions in the majority of NRL-allergic patients [2, 4]. Only a minority (15 %) of patients with antiprohevein IgE antibodies had IgE against the prohevein C-domain whereas IgE antibodies to purified hevein, the 43 amino acid N-terminal fragment of prohevein, were found by ELISA in 56 % of NRL-allergic patient sera [4]. Purified hevein also elicited positive SPT reactions in all patients showing IgE to the N-terminus of prohevein. The roles of prohevein and hevein as major NRL allergens were recently confirmed in articles published by Beezhold et al. [7] and Chen et al. [10].

Hydrophobic rubber particle protein (23-27 kDa) (Hev b 3)

Most pediatric patients with spina bifida seem to react, in addition to REF, to another hydrophobic rubber-particle-associated protein which showed partial homology to REF and had an apparent molecular weight of 27 kDa [3]. The amino-acid sequences of tryptic peptides from the 27 kDa protein and from a 23 kDa protein later described by Lu et al. [14] were in essence identical suggesting that these proteins are the same or

they represent different isomers or modifications of the same protein. This protein has later been designated as Hev b 3 by Yeang et al. [20], who also reported that the molecule appears to be relatively unstable with a tendency to fragment into several polypeptides of lower (from 24 kDa to about 5 kDa) molecular weight.

Acidic 16 kDa NRL protein (Hev b 5)

Using cDNA library from *Hevea brasiliensis* latex and IgE from serum of a latex-allergic health care worker Slater et al. [17] identified, cloned and sequenced a novel NRL allergen, designated Hev b 5, against which 92 % of health care workers and 50 % of spina bifida patients with NRL allergy had IgE antibodies. At the same time Akasawa et al. [1] reported molecular identification and mass spectrometric data of an identical protein. The latter authors also isolated and cloned the cDNA coding for this protein from a latex cDNA library and showed that the deduced amino acid sequence form the cDNA was nearly identical to that obtained by amino acid sequencing of enzymatically digested internal peptides. Importantly, Hev b 5 was isolated also from 6 of 8 commercial NRL glove brands analyzed [1]. These data suggest that Hev b 5 may play a significant role in latex allergy.

Hevamine

A 30-kD NRL protein, purified and identified as hevamine showed IgE binding only in 3 % of NRL-allergic patients [2] suggesting that hevamine plays a minor role as an NRL allergen.

Endo-1,3-beta glucosidase (endoglucanase) (Hev b 2)

Alenius et al. [2] purified a 36-kD NRL protein which showed high homology to several plant endo-1,3- β -glucosidases. This 36 kD protein bound IgE in sera from 21 % of NRL-allergic patients and was therefore considered as a significant NRL allergen. Molecular data on an endoglucanase from the rubber tree luteoid-body fraction, designated as Hev b 2 by Sunderasan et al. [18], show that the IgE-binding protein purified by Alenius et al. [2] represent the same protein.

Patatin-like NRL protein (Hev b 7)

Beezhold et al. [8] showed that 23 % of latex-allergic patients recognize a latex protein that has sequence homology to a broad class of plant proteins known as patatins. In the current allergen nomenclature system this protein has been assigned as Hev b 7.

NATURAL RUBBER LATEX ALLERGENS IDENTIFIED IN MANUFACTURED RUBBER PRODUCTS

Until now, relatively little has been known about the exact molecular specificities and forms of allergens eluting from NRL gloves or other manufactured products. A recent study showed that hevein (Hev b 6.1) constituted up to 70 % of the total allergen activity of a high-allergen glove [4]. Preliminary results suggest that, in quantitative terms, hevein indeed can be the most important NRL allergen eluting from at least high-allergen medical gloves [15]. In addition, the presence of Hev b 1 [11, 15], Hev b 3 [14] and Hev b 5 [1] in NRL gloves has been documented but additional studies are needed to elucidate to what extent these and other proteins or peptides are responsible for the total allergenic potential of various manufactured products.

COMMENTS AND PROSPECTS FOR FUTURE RESEARCH

The primary molecular structure of several significant NRL allergens have recently been solved (Table I) and also the three-dimensional structure of a major NRL allergen, hevein, is known. Obviously, there may be additional, currently unrecognized allergens and the issue is further complicated by the multitude of cross-reacting allergens observed in several fruits and plants,

Table I. – Natural rubber latex allergens: Nomenclature 1997

Conventional/original name	Systematic name	MW (kDa)	Sequence data	References
Rubber elongation factor (REF)	Hev b 1	14.6	C*	11, 10
β -1,3-glucanase	Hev b 2	34-36	C	2, 18
23-27 kD rubber particle protein	Hev b 3	23-27	P	3, 14, 20
Microhelix protein complex	Hev b 4	100-115	P	18
Acidic C-serum protein	Hev b 5	16	C	1, 17
Prohevein	Hev b 6	20	C	8, 2
Hevein	Hev b 6.1	4.7	C	8, 4, 6, 9
Prohevein C-domain	Hev b 6.2	14	C	4
Patatin-like protein	Hev b 7	46	C	7

* C = complete sequence known, P = partial sequence known.

such as banana, avocado, kiwi, and chestnut (for review, see [19]). Future studies will undoubtedly be focused on the analysis of immunodominant epitopes in the allergen molecules and in possibilities to modify or destroy them to decrease their allergenic potential. Knowledge of the whole spectrum of NRL allergens will help researchers to develop more specific *in vivo* and *in vitro* tests for diagnostic purposes and the production of pure allergens could provide tools for immunotherapy. This information will also be helpful for rubber manufacturers in developing ways to produce less allergenic gloves and other NRL products.

REFERENCES

1. Akasawa A., Hsieh S.H., Martin B.M., Liu T., Lin Y. – A novel acidic allergen, Hev b 5, in latex. *J. Biol. Chem.*, 1996, 271, 25389-25393.
2. Alenius H., Kalkkinen N., Lukka M., Reunala T., Turjanmaa K., Mäkinen-Kiljunen S., Yip E., Palosuo T. – Prohevein from the rubber tree (*Hevea brasiliensis*) is a major latex allergen. *Clin. Exp. Allergy*, 1995, 24, 659-665.
3. Alenius H., Kalkkinen N., Lukka M., Turjanmaa K., Reunala T., Mäkinen-Kiljunen S., Palosuo T. – Purification and partial amino acid sequencing of a 27 kD natural rubber allergen recognized by latex allergic children with spina bifida. *Int. Arch. Allergy Immunol.*, 1994, 106, 258-262.
4. Alenius H., Kalkkinen N., Reunala T., Turjanmaa K., Palosuo T. – The main IgE-binding epitope of a major latex allergen, prohevein, is present in its N-terminal 43 amino acid fragment, hevein. *J. Immunol.*, 1996, 156, 1618-1625.
5. Alenius H., Kalkkinen N., Turjanmaa K., Mäkinen-Kiljunen S., Reunala T., Palosuo T. – Significance of the rubber elongation factor as a latex allergen. *Int. Arch. Allergy Immunol.*, 1996, 109, 362-368.
6. Alenius H., Kurup V., Kelly K., Palosuo T., Turjanmaa K., Fink J. – Latex allergy: Frequent occurrence of IgE antibodies to a cluster of 11 latex proteins in patients with spina bifida and histories of anaphylaxis. *J. Lab. Clin. Med.*, 1994, 123, 712-720.
7. Beezhold D.H., Kostyal D.A., Sussman G.L. – IgE epitope analysis of the hevein preprotein; a major latex allergen. *Clin. Exp. Immunol.*, 1997, 108, 114-121.
8. Beezhold D.H., Sussman G.L., Liss G.M., Chang N.S. – Latex allergy can induce clinical reactions to specific foods. *Clin. Exp. Allergy*, 1996, 26, 416-422.
9. Broekaert I., Lee H., Kush A., Chua N., Raikhel N. – Wound-induced accumulation of mRNA containing a hevein sequence in laticifers of rubber tree (*Hevea brasiliensis*). *Proc. Natl. Acad. Sci. USA*, 1990, 87, 7633-7637.
10. Chen Z.P., Posch A., Lohaus C., Raulfheimsoth M., Meyer H.E., Baur X. – Isolation and identification of hevein as a major IgE-binding polypeptide in hevea latex. *J. Allergy Clin. Immunol.*, 1997, 99, 402-409.
11. Czuppon A.B., Chen Z., Rennert S., Engelke T., Meyer H.E., 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, 690-697.
12. Dennis M., Henzel W., Bell J., Kohr W., Light D. – Amino acid sequence of rubber elongation factor protein associated rubber particles in Hevea latex. *J. Biol. Chem.*, 1989, 264, 18618-18626.
13. Gidrol X., Chrestin, H.L. Tan, Kush A. – Hevein, a lectinlike protein from *Hevea brasiliensis* (rubber tree) is involved in the coagulation of latex. *J. Biol. Chem.*, 1994, 269, 9278-9283.
14. Lu L., Kurup V., Hoffman D., Kelly K., Murli P., Fink J. – Characterization of a major latex allergen associated with hypersensitivity in spina bifida patients. *J. Immunol.*, 1995, 155, 2721-2728.

15. Palosuo T., Alenius H., Mäkinen-Kiljunen S., Reunala T., Turjanmaa K. – Concentrations of hevein and rubber elongation factor (REF) in relation to total allergen activity in extracts of medical latex gloves (Abstract). *J. Allergy Clin. Immunol.*, 1997, 99, 342.
16. Posch A., Chen Z.P., Wheeler C., Dunn M.J., Raulfheimsoth M., Baur X. – Characterization and identification of latex allergens by two-dimensional electrophoresis and protein microsequencing. *J. Allergy Clin. Immunol.*, 1997, 99, 385-395.
17. Slater J.E., Vedvick T., Arthur-Smith A., Trybul D.E., Keckwick R.G.O. – Identification, cloning, and sequence of a major allergen (Hev b 5) from natural rubber latex (*Hevea brasiliensis*). *J. Biol. Chem.*, 1996, 271, 25394-25399.
18. Sunderasan E., Hamzah S., Hamid S., Ward M.A., Yeang H.Y., Cardoso M.J. – Latex B-serum β -1,3-glucanase (Hev b II) and a component of the microhelix (Hev b IV) are major latex allergens. *J. Nat. Rub. Res.*, 1995, 10, 82-99.
19. Turjanmaa K., Alenius H., Mäkinen-Kiljunen S., Reunala T., Palosuo T. – Natural rubber latex allergy (review). *Allergy*, 1996, 51, 593-602.
20. Yeang H.Y., Cheoung K.F., Sunderasan E., Hamzah S., Chew N.P., Hamid S., Hamilton R.G., Cardoso M.J. – The 14.6 kD (REF, Hev b 1) and 24 kD (Hev b 3) rubber particle proteins are recognized by IgE from spina bifida patients with latex allergy. *J. Allergy Clin. Immunol.*, 1996, 98, 628-639.

