

Allergens and natural rubber proteins

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Background: Allergy to natural rubber latex (NRL) results from exposure to proteins derived from *Hevea brasiliensis*. Type I latex hypersensitivity is observed in certain occupational and other high-risk groups with frequent exposure to NRL products. This includes health care workers (HCWs), workers in the latex industry, children with spina bifida, and atopic individuals.

Objectives: Early reliable diagnosis and avoidance are required for better patient care. Standardized reagents are not presently available for in vitro and in vivo testing and treatment of patients with latex allergy. However, a number of allergens have been isolated and characterized from *Hevea* latex and NRL products. Currently, a total of 11 major and minor allergens are designated by the International Allergen Nomenclature Committee. This article reviews the structural and functional characteristics of these latex allergenic proteins. **Results:** NRL-allergenic proteins include those involved in the biosynthesis of polyisoprene and coagulation of latex rubber elongation factor, small rubber particle protein, prohevein, and patatin. Pathogenesis-related proteins include β -1,3-glucanases, chitinases, and heveamine; and the structural proteins include microhelix protein complex, proline-rich protein, profilins, enolases, and manganese superoxide dismutase. Recombinant allergens demonstrated skin test reactivity in patients with latex allergy. The minimal level of skin test reactivity was about 70 pg/mL for NRL and 1 ng/mL for recombinant allergens. The use of selected recombinant latex allergens (Hev b 5, Hev b 6, and Hev b 7) in skin prick tests identified 93% of allergic individuals, mainly health care workers. **Conclusions:** Recombinant latex allergens are clinically reactive and can be produced in a standardized manner, which could potentially provide safe and sensitive reagents for the diagnosis and treatment of type I latex allergy. (*J Allergy Clin Immunol* 2002;110:S33-9.)

Key words: Natural rubber latex, latex allergenic proteins, health care workers, children with spina bifida, skin prick test

Abbreviations used

HCWs: Health care workers
MnSOD: Manganese superoxide dismutase
NRL: Natural rubber latex
REF: Rubber elongation factor
SPT: Skin prick test

Proteins present on various natural rubber latex (NRL) products have been implicated in causing allergy in specific risk groups.¹⁻⁷ One risk group of particular interest consists of health care workers (HCWs) exposed to powdered NRL gloves.⁸ Other important risk groups are workers in industries in which latex products are manufactured, including those collecting and processing latex from rubber trees.⁶⁻⁹ Patients with congenital anomalies such as spina bifida, those undergoing multiple surgeries, and patients with atopy are more susceptible to latex protein-induced allergy and asthma.¹⁰ Considerable overlap of the clinical and immunologic symptoms also occurs because of sensitization by some of the latex allergens that cross-react with other known allergens. Some shared allergens cross-react with fruits and cause clinical food allergy.¹¹⁻¹³

Although purified NRL allergens have been isolated and characterized, more research is needed for the development of standardized reagents to be used for reliable diagnosis of latex allergy. Similarly, no concerted attempt has been made to understand the structure-function relationship of the allergens in the pathogenesis of latex allergy. The major difficulty is that the latex from the rubber plant *Hevea brasiliensis* contains a large number of proteins, which constitute about 1% of the total weight.¹⁴ There is considerable batch-to-batch variation in the composition and protein content of rubber tree sap.

It is essential to have pure and standardized allergens to have consistent immunologic assays. A number of allergenic proteins have been produced by molecular cloning and expression of their genes.¹⁵ Although a large number of NRL allergens have been identified, it may not be necessary to use all these allergens for diagnosis of latex allergy.^{9,16,17} However, by careful evaluation, the minimum number of essential allergens will be identified and selected for use in skin tests and in vitro assays.

H BRASILIENSIS LATEX PROTEINS

Natural rubber, a cis-1,4-polyisoprene, is processed from the latex of *H brasiliensis* trees. Although approximately 2000 plants produce latex, more than 99% of the latex used in commercial application originates from *H brasiliensis*.¹⁴ The protein content of NRL varies from

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1% to 1.8% and depends on a number of factors including genetic, chemical, and metabolic makeup of the rubber trees.^{16,17} This variability is also determined by fertilizer, chemical, and soil composition. The latex also contains lipids, carbohydrates, and many inorganic constituents such as potassium, manganese, calcium, sodium, zinc, copper, and iron.¹⁴

Many proteins from NRL have been implicated as having a role in latex allergy.¹⁸ Some of these allergenic proteins have been cloned and sequenced, and the proteins have been expressed in appropriate vectors. The majority of proteins detected in NRL have also been identified in their native form or, infrequently, in altered configuration in the finished latex products. Treatment of NRL with chemicals such as ammonia increases the formation of protein fragments and components. In nonammoniated latex, more than 240 separate peptides have been identified by 2-dimensional electrophoresis.^{16,17} However, only 25% of these proteins showed binding of IgE from the sera of patients with latex allergy,⁹ whereas the majority of these proteins strongly bind IgG from the sera of both patients with spina bifida and HCWs with latex allergy.¹⁷

THE BIOLOGIC FUNCTION OF THE ALLERGENS OF *H BRASILIENSIS* LATEX

The tropical rubber plant, *H brasiliensis*, grows best at temperatures between 20°C and 28°C in humid climates where it is constantly exposed to a multitude of microorganisms, insects, and fungi. As a consequence, rubber plants had to adapt, evolve, and develop efficient wound repair mechanisms to deal with invading pathogens such as fungi or insects. The sealing of wounds and the inhibition of growth of microorganisms that invade the tissues of the plant are of primary concern to the survival of the plant. Beneath the surface, adjacent to the phloem of the tree, exists a tube-like network of specialized cells called *laticifers* whose cytoplasm contains proteins that are involved in the biosynthesis of polyisoprene, coagulation of latex, and the plant's defense systems. In addition, several housekeeping enzymes and structural proteins are present. These types of proteins can trigger type I immune responses in individuals with latex allergy. To date, 11 *H brasiliensis* allergens have been accepted by the International Nomenclature Committee of Allergens and assigned official numbers (ftp://biobase.dk/pub/who-iuis/allergen.list).¹⁵ Selected data, subcellular localization, biologic function, and relevance as allergens are shown in Tables I and II.

ALLERGENS INVOLVED IN THE BIOSYNTHESIS OF POLYISOPRENE AND THE COAGULATION OF LATEX

Hevea prenyltransferase is a hydrophobic membrane-bound enzyme that generates multimers from the isoprene subunit to form natural rubber, a cis-1,4-polyisoprene. A 14.6-kd rubber elongation factor (REF) (Hev b 1) facilitates the prenyltransferase to elongate the mole-

cules to several thousand isoprene subunits.^{19,20} REF is tightly bound to large rubber particles (>350 nm in diameter) and coats the interface between the aqueous serum and the extremely hydrophobic polyisoprene molecules. REF, with its highly charged N-terminus, makes up 10% to 60% of the total protein found in latex. REF was cloned and its nucleotide sequence was determined as the first major latex allergen.^{21,22} Hev b 3, also described as the small rubber particle protein, is another significant protein present on the surface of rubber particles. Hev b 3 has a significant role in rubber synthesis because of its ability to synthesize long-chain polyisoprene.²³

Prohevein, Hev b 6.01, is processed to yield 2 allergenic fragments, the N-terminal hevein, Hev b 6.02, and the C-terminal portion, Hev b 6.03. Hevein, a cysteine- and glycine-rich acidic protein is one of the most abundant proteins in the luteoid bodies of *Hevea* latex.²⁴ Hevein shows homology to each of the 4 domains of wheat-germ agglutinin and other cereal lectins.²⁵ Hevein is released from the luteoids when the cells are damaged and interacts with a 22-kd receptor glycoprotein in the envelope around the rubber particles, resulting in the coagulation of latex.²⁶

Hev b 7 is a latex allergen with sequence similarity to the potato tuber storage proteins. Hev b 7 is a cytosolic protein with esterase activity.^{27,28} Hev b 7 has been shown to be an inhibitor of rubber biosynthesis by interfering with the incorporation of isopentenyl diphosphate into rubber.²⁹ Hev b 7 may also have a defense function in the plant because its sequence is related to patatins that have been shown to inhibit the larval growth of certain pest insects.³⁰

ALLERGENS WITH A PATHOGENESIS-RELATED FUNCTION

β -1,3-Glucanases are able to catalyze the hydrolytic cleavage of polymers of β -1,3-glucans that are essential cell wall components of most fungi. Therefore, they can help defend plants against fungal infection by degrading the cell walls of fungal pathogens. β -1,3-Glucanase activity was observed in extracts of luteoids from *Hevea* latex in which 2 acidic isozymes and 3 basic isozymes were identified.³¹ A clone encoding a basic β -1,3-glucanase was isolated from a complementary DNA library prepared from the latex of *H brasiliensis*.³² Sunderasan et al³³ gave the allergen designation *Hev b 2* for a basic β -1,3-glucanase isolated from luteoids. β -1,3-Glucanases exist as multiple structural isoforms that differ in size and have different IgE-binding capacities.³⁴

Chitinases are abundant proteins found in a wide variety of seed-producing plants. Nearly all plant chitinases isolated to date are endochitinases, which hydrolytically degrade chitin from within the polymer. Chitin is a major structural component of the cell wall of many fungi and of the exoskeleton of insects. Class I chitinases are basic and contain an N-terminal chitin-binding domain with homology to hevein.³⁵ Sensitization to *Hevea* latex hevein (Hev b 6.02) was described as the cause of IgE

TABLE I. Selected data of latex allergens

Allergen	Molecular weight of mature protein	pI	Accession No. DNA	Accession No. protein	Significance as allergen	Reference
Hev b 1	14.6 kd	4.9	X56535	P15252	Minor/major	22
Hev b 2	35.1 kd	9.5	U22147	P52407	Major	33
Hev b 3	22.3 kd	4.8	AF051317, AJ223388	NA	Major/minor	23, 53
Hev b 4	50-57 kd	4.5	NA	NA	NA	33
Hev b 5	16 kd	3.5	U51361, U42640	NA	Major	42, 43
Hev b 6.01	20 kd	5.6	M36986	P02877	Major	24
Hev b 6.02	4.7 kd	4.9				
Hev b 6.03	14 kd	6.4; 7.0; 7.4				
Hev b 7	42.9 kd	4.8	AJ223038, U80598	NA	Minor	28, 27
Hev b 8	13.9 kd	4.9	Y15402, AJ243325	NA	NA	Asturias JA, EMBL database; Ganglberger E, EMBL database
Hev b 9	47.7 kd 47.5 kd	5.6 6.4	AJ132580, AJ13258	NA	Minor	Wagner S, EMBL database
Hev b 10	22.9 kd	6.3	L11707, AJ249148	P35017	Minor	60; Wagner S, EMBL database
Hev b 11	33 kd	5.1	AJ238579	NA	Minor	O'Ríordáin G, EMBL database

pI, Isoelectric point; NA, not available.

TABLE II. Subcellular localization and biologic functions of *Hevea brasiliensis* allergens

Allergen	Subcellular localization	Biologic function
Hev b 1	Large rubber particles	Involved in biosynthesis of polyisoprene
Hev b 2	Lutoids	β -1,3-Glucanase, involved in defense against fungal pathogens
Hev b 3	Small rubber particles	Involved in biosynthesis of polyisoprene
Hev b 4	Lutoids	Component of the microhelix protein complex
Hev b 5	Cytoplasm	Structural protein
Hev b 6.01	Lutoids	NA
Hev b 6.02	Lutoids	Coagulation of latex
Hev b 6.03	Lutoids	NA
Hev b 7	Cytoplasm	Esterase, inhibitor of polyisoprene biosynthesis
Hev b 8	Cytoplasm	Profilin, cytoskeletal actin-binding protein
Hev b 9	Cytoplasm	Enolase
Hev b 10	Mitochondria	MnSOD, protection from reactive oxygen species
Hev b 11	Lutoids	Class I chitinase, involved in plant-pathogen interaction

MnSOD, Manganese superoxide dismutase.

cross-reactivity to class I chitinases in latex-avocado cross-reactivity.³⁶ Allergy to avocado, banana, and chestnut has been associated with the latex-fruit syndrome.¹³ In each of these fruits, a class I endochitinase has been identified as the major cross-reactive allergen.¹¹⁻¹³ Recently, a cDNA coding for class I chitinase from *H. brasiliensis* latex has been cloned and its designation, Hev b 11, was approved by the Allergen Nomenclature Committee.¹⁵ Whether the cross-reactivity between latex allergens and fruit class I chitinases is restricted to the hevein domain remains to be determined. Class II chitinases are acidic, lack the N-terminal chitin-binding domain, and have a high sequence similarity to class I chitinases within the catalytic domain.³⁶ Such a class II

chitinase was identified as an NRL allergen by microsequencing the N-terminus of an IgE-binding protein in a 2-dimensional immunoblot.¹⁶

Hevamine is a 29.5-kd basic protein from the lutoids of NRL and is believed to play an important role in the defense of the tree against pathogenic fungi. The tertiary structure of hevamine has been elucidated.³⁷ Hevamine catalyzes the cleavage of β -1,4-glycosidic bonds of chitin, the main constituent of the fungal cell wall, and of the sugar moiety of peptidoglycan. Hevamine was classified as a defense-related bifunctional enzyme with chitinase and lysozyme activity, although its cleavage specificity differs from that of lysozyme.³⁸ Lysozymes of higher plants can also be regarded as defense-related pro-

teins because of their bacteriolytic activity, which results in resistance to bacterial diseases.³⁹ A 27-kd lysozyme from NRL was identified as an allergen with enzymatic similarity to fruit lysozymes.⁴⁰

ALLERGENS AMONG STRUCTURAL PROTEINS AND HOUSEKEEPING ENZYMES

Hev b 4, an acidic protein, was isolated from the luteoids and shown to be a component of the microhelix protein complex. The N-terminal amino acid sequence of Hev b 4 does not match any sequence information deposited in the databases.⁴¹ Hev b 5 is a proline-rich protein with a predominantly random secondary structure whose deduced protein sequence showed 46% homology to an acidic protein from kiwi.^{42,43} It has been suggested that Hev b 5 is a cytoplasmic structural protein.⁴³ The actin cytoskeleton is composed of a network of actin filaments whose precise organization is regulated by a number of actin-binding proteins, one of them being profilin. Profilins have been identified as allergens in many plant species. IgE-binding inhibition demonstrated that profilin is also an allergen of NRL.⁴⁴

Immunoprobings of a 2-dimensional electrophoresis of NRL protein and microsequencing of IgE-binding protein spots identified an enolase and a manganese superoxide dismutase (MnSOD) as allergens.³⁶ Enolase is a key enzyme of the glycolytic pathway. MnSOD protects against highly reactive oxygen species and therefore provides protection against the toxicity of oxygen-related radicals. The NRL enolase has been accepted as Hev b 9 (AJ132580, AJ132581), and the latex MnSOD, as Hev b 10 (L11707, AJ249184) by the Allergen Nomenclature Committee. Both proteins were recently produced as recombinant proteins and shown to be minor *Hevea* latex allergens (Wagner S, unpublished data).

CHARACTERISTICS OF INDIVIDUAL LATEX ALLERGENS

Hev b 1 (rubber elongation factor)

Hev b 1 is a 137-amino acid protein that tetramerizes with a molecular mass of about 58 kd. Czuppon et al²² evaluated 13 patients with latex allergy and found high levels of Hev b 1-specific IgE in the sera of 9 patients. In another study, Hev b 1 reactivity was found to be present in 81% of the patients with spina bifida and latex allergy.⁴⁵ In contrast, only 50% of the HCWs showed REF IgE reactivity. Yeang et al⁴⁶ also found Hev b 1 reactivity with IgE from the sera of patients with spina bifida and adult HCWs with latex allergy. In a multicenter study, we showed that 13% to 32% of HCWs and 54% to 100% of patients with spina bifida and latex allergy reacted to Hev b 1.⁴⁷ When Hev b 1 was used in PBMC stimulation, about 52% of the patients had a proliferation response.^{48,49} In a recent study, we found a PBMC proliferation response in 57% of HCWs with latex allergy, but no correlation with high Hev b 1-specific IgE in the serum was demonstrable.⁵⁰

Hev b 2 (β -1,3-glucanase)

Hev b 2, a basic β -1,3-glucanase, showed binding to IgE from sera of patients with latex allergy.^{33,47} The protein expressed in different vectors failed to react with IgE from the sera of patients with latex allergy.³² It is not known whether the failure to bind to IgE is due to the lack of glycosylation of the expressed protein or the conformational changes occurring in the recombinant protein. Both patients with spina bifida and HCWs with latex allergy showed strong IgE-binding reactivity to the native Hev b 2.^{33,47} Depending on the methods used, the reactivity varied from 20% to 61% in patients with latex allergy. However, only 2 of 29 patients showed skin prick test (SPT) reactivity to Hev b 2.⁵¹ This allergen also showed proliferation of PBMCs in more than 56% of the HCWs.⁵⁰ PBMC proliferation showed more than 70% correlation with the IgE levels in the sera of the patients with latex allergy. Thus, Hev b 2 appears to be a significant latex allergen with IgE-binding capacity and T-cell stimulation properties.^{47,50}

Hev b 3

Hev b 3 forms an integral part of the small rubber particles of 70 nm or less.⁴⁶ This protein showed strong IgE-binding activity in sera from patients with latex allergy and spina bifida.^{9,46,52,53} The reactivity of Hev b 3 with serum IgE of HCWs was weaker and less frequent, and only 7% showed SPT reactivity to recombinant Hev b 3.^{47,51} PBMC stimulation of sera from patients with spina bifida and latex allergy was reported, although our studies of HCWs indicated just 25% proliferation of PBMCs in response to Hev b 3.⁵⁰ No correlation of IgE levels with PBMC proliferation was evidenced in these patients. The T-cell epitopes of Hev b 3 have been determined.⁵⁴

Hev b 4

Hev b 4 showed IgE-binding in 65% of the HCWs and 77% of the patients with spina bifida and latex allergy.⁴⁷ Only 14% of those patients showed PBMC proliferation in response to Hev b 4.⁵⁰ This allergen has not yet been cloned and expressed.

Hev b 5

Hev b 5 has been isolated from ammoniated latex and from heat-treated latex and glove extracts.⁴² Even after autoclaving, this acidic protein retains allergenicity. Hev b 5 stimulates histamine release from basophils of patients with latex allergy. Hev b 5 is a major latex allergen as indicated by its strong IgE binding with IgE from sera of both HCWs and patients with spina bifida; 92% of HCWs showed reactivity to Hev b 5, and 56% of patients with spina bifida showed specific IgE binding. Sixty-two percent of HCWs who were skin tested with Hev b 5 had a positive response.⁵¹ This allergen also demonstrated strong T-cell responses in patients with latex allergy.⁵⁵

Hev b 6 (prohevein)

Hev b 6 demonstrated reactivity with IgE from sera of HCWs and patients with spina bifida.⁵⁶ Both immunoblots

and ELISA of serum IgE of patients with spina bifida showed more reactivity with the N-terminal of Hev b 6 than with the C-terminal alone. PBMC proliferation was detected with Hev b 6 in HCWs with latex allergy.⁵⁰

Hev b 7

Hev b 7 showed IgE-binding reactivity in 23% of HCWs with latex allergy.⁴⁷ Both patients with spina bifida and HCWs with latex allergy reacted to this allergen; and SPT reactivity was demonstrated in 41% of HCWs. PBMC proliferation was detected in 15% of the patients with latex allergy, but no correlation was detected with specific IgE in the sera and the Hev b 7-induced PBMC proliferation.⁵⁰

Hev b 8 (latex profilins)

Cross-reactivity of latex allergens with allergens from banana, grass, and birch pollen have been reported.⁴⁴ The *H brasiliensis* profilin also reacted with IgE from 36 patients with ragweed allergy.⁵⁷ The purified latex profilin, when used in skin prick testing, showed positive reactions in all 24 patients with spina bifida and latex allergy but in only 6 of 17 HCWs with latex allergy.^{57,58} All other subjects, including patients with fruit allergy, had negative responses to this allergen.

Hev b 9 (enolase)

Hev b 9 is a 47.7-kd enzyme with very high homology to *Ricinus communis* enolase, tomato enolase, and *Cladosporium* enolase.^{16,59} This allergen is a component of the NRL C-serum. A high degree of cross-reactivity can be expected because of the general homology of enolases. However, our studies of 26 HCWs with latex allergy failed to show IgE binding with any of the latex or fungal enolases (unpublished results). No PBMC response to this minor allergen is known.

Hev b 10 (MnSOD)

MnSOD has been detected in a number of fungi, bacteria, and humans.^{60,61} *Aspergillus fumigatus* MnSODs have been recognized as major allergens in various *Aspergillus*-induced allergic diseases.⁶² Patients with allergy to these molds demonstrate strong PBMC proliferation when stimulated with the respective MnSODs.⁶¹ IgE binding was demonstrated in only 1 of 26 patients with latex allergy by using latex MnSOD (unpublished results). Hev b 10 showed strong similarity to *A fumigatus*, *Escherichia coli*, and human MnSODs.⁶¹ No PBMC stimulation results are available for Hev b 10.

Hev b 11

Hev b 11 is a type I chitinase of NRL. It has a molecular size of 33 kd and an isoelectric point of 5.1.⁶³ A number of chitinases from NRL have been described.^{18,63} Chitinases and lysozymes constitute about 25% of the proteins in the lutoid fraction of rubber latex (B-serum). The class I chitinases share homology with N-terminal hevein domain and probably also share epitopes with similar allergens from avocado and banana.⁶⁴ Although Hev

b 11 is considered a minor allergen in latex allergy, detailed cross-reactivity with other allergens and its immune responses and role in allergy are not known.

ADDITIONAL ALLERGENS

Several other components in NRL, such as hevine and lysozyme, show binding to IgE from patients with latex allergy.^{65,66}

RECOMBINANT PROTEIN SKIN TEST DATA

Six recombinant proteins were expressed in *E coli* and tested as intact fusion proteins (Hev b 2, Hev b 5, Hev b 6, and Hev b 8) or as purified proteins (Hev b 3 and Hev b 7). Skin prick testing was done by using 10-fold serial dilutions on 31 subjects with known latex allergy to determine the minimal level of reactivity to each recombinant allergen. The minimal level of protein for a positive SPT response was 70 pg/mL for NRL and 1 ng/mL for one purified recombinant allergen⁵¹ (Hev b 7). In these patients, the use of a combination of recombinant latex allergens Hev b 5, Hev b 6, and Hev b 7 identified latex allergy with 93% sensitivity and 100% specificity. These data indicate that SPT with a panel of recombinant allergens may be nearly as effective as SPT with NRL in the diagnosis of latex allergy in our HCW population. In addition, it has been demonstrated that anti-carbohydrate IgE antibodies, found in many patients with plant allergies, will bind to the glycosylated plant proteins and that they are not allergen-specific. Recombinant proteins are not glycosylated, which eliminates a source of cross-reactivity. Because of these and other problems, the significance of a weak positive reaction in some in vitro assays will make interpretation of the results impossible without clinical symptoms identified by history. The use of a select panel of recombinant proteins may also significantly improve the diagnostic sensitivity of in vitro assays. Such assays would be limited by the reactivity of the patients and the appropriate selection of recombinant allergens.

Other advantages of using recombinant proteins are that large quantities of purified protein can be produced by a standardized method. There would be no contamination by other latex protein allergens. Recombinant allergens increase our understanding of latex hypersensitivity and provide a potential pool of improved diagnostic and therapeutic modalities.

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