Natural rubber latex allergens: new developments

Hoong-Yeet Yeang

Purpose of review

New allergenic latex proteins have been identified, whereas further information on known latex allergens has emerged in recent years. Although prevalence figures for sensitization to the various latex allergens have been published in several studies in the past, the data have not been collated to facilitate cross-comparison.

Recent findings

Salient characteristics of the three most recently identified latex allergens, Hev b 11, 12 and 13 are described, whereas new findings on some of the previously recognized allergens are examined. Hev b 2 is viewed from the standpoint of allergenicity and protein glycosylation, Hev b 4 in relation to its biochemical identity and molecular cloning, Hev b 5 with respect to its recombinant form, and Hev b 6 in connection with conformational IgE epitopes. Reports on sensitization or allergic reaction to purified latex allergens from recent and past work are summarized. The use of latex allergens in latex allergy diagnostics is reviewed and discussed.

Summary

Thirteen latex allergens have been recognized by the International Union of Immunological Societies. Based on the results of published studies, native Hev b 2, recombinant Hev b 5, native or recombinant Hev b 6, native Hev b 13, and possibly native Hev b 4 are the major allergens relevant to latex-sensitized adults. Although there is an increasing tendency to identify and characterize latex allergens largely on the basis of their recombinant forms, not all such recombinant proteins have been fully validated against their native counterparts with respect to clinical significance.

Keywords

latex allergens, latex allergy diagnostics, latex proteins, prevalence of latex sensitization, recombinant allergens

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Biotechnology and Strategic Research Unit, Rubber Research Institute of Malaysia, Malaysian Rubber Board, RRIM Experiment Station, Selangor, Malaysia

Correspondence to Hoong-Yeet Yeang, Biotechnology and Strategic Research Unit, Rubber Research Institute of Malaysia, Malaysian Rubber Board, RRIM Experiment Station, 47000 Sungei Buloh, Selangor DE, Malaysia
E-mail: hyyeang@lgm.gov.my

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Abbreviations

AMP	antimicrobial protein
IUIS	International Union of Immunological Societies
LTP	lipid transfer protein
SDS–PAGE	sodium dodecyl sulphate–polyacrylamide gel electrophoresis

1528-4050

Introduction

When the 'rubber elongation factor' was identified as the first latex allergen in 1993, there were those who thought the problem of latex allergy was well on its way to being resolved. It fact Hev b 1, as the protein was named in accordance with the nomenclature of the International Union of Immunological Societies (IUIS)–World Health Organization, was just the beginning. The count has reached 13 at the time of writing.

This review reports on the most recently named latex allergens, Hev b 11, 12 and 13, whereas research advances touching on some of the earlier allergens, Hev b 2, 4, 5 and 6, are also covered. With multiple latex allergens confronting researchers and clinicians, it is essential to establish the allergenicities of the individual allergenic proteins. Differences in outcomes between studies are to be expected. Therefore, results that are reviewed are not only those from the most recent publications; data from older studies are also drawn upon to complete the picture, thus enabling comparisons across a range of assay methodologies and test subjects. The management of occupational health problems related to latex allergy calls for reliable diagnosis as an important initial step. In this connection, difficulties surrounding the use of latex proteins as reference antigens are discussed.

The new latex allergens

Three new latex allergens were recently named by the IUIS. These are Hev b 11, a class I chitinase, Hev b 12, a lipid transfer protein that is a pan allergen, and Hev b 13, a lipolytic esterase that is a homologue of the early nodule specific protein of legumes.

Hev b 11: class I chitinase

O'Riordain *et al.* [1•] and Rihs *et al.* [2•] cloned complementary DNAs encoding a class I chitinase (Hev b 11), the former from latex RNA and the latter from leaf cDNA. The sequences of both cDNAs are very similar, giving the predicted molecular weight of the mature protein as 31 600 M_r and predicted isoelectric point of 5.6. Hev b 11 protein shows greater than 65% identity with several other plant endochitinases. Whereas early class I chitinases were generally basic vacuolar proteins [3], this classification has become less stringent with later findings. Hev b 11 is acidic and appears to be located in the cytosol (latex C-serum). As DNA sequences upstream of the translated mature proteins are unavailable, the absence of a protein signal peptide (that would suggest a non-cytosolic protein) cannot be confirmed.

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Recombinant Hev b 11 is recognized by IgE from latex-allergic and fruit-allergic patients. Rihs *et al.* [2•] found that 29% of their 58 patients were sensitized to Hev b 11, whereas O'Riordain *et al.* [1•] recorded 19% IgE positive individuals among 57 patients. The chitin-binding domain of Hev b 11 displays 56–58% identity to hevein (Hev b 6.02), but this segment of the molecule does not appear to play a dominant role in the IgE reactivity of the protein [1•].

Hev b 12: lipid transfer protein

Lipid transfer proteins (LTPs) facilitate the transport of phospholipids and galactolipids across membranes. The pan-allergen, LTP, was cloned from *Hevea brasiliensis* RNA and produced as a recombinant protein by Beezhold *et al.* [4•]. The DNA sequence predicts a mature Hev b 12 protein of 9300 M_r with an isoelectric point of 10.8. The in-vivo location of Hev b 11 in natural rubber latex is uncertain because the authors did not detect the native protein in latex. A 24 amino acid signal peptide precedes the amino acid sequence of the mature protein, but no clear vacuole targeting sequence at the *C*-terminus (that might suggest a lutoid protein) has been identified.

Immunoblots of the recombinant protein demonstrated Hev b 12-specific IgE in the sera of nine out of 37 latex-allergic individuals (24%). It is by no means certain if sensitization to Hev b 12 might commonly be caused by crossreactions with food proteins, and *vice versa*. The reactivity of IgE from latex-allergic patients to Hev b 12 occurred under reducing conditions of the protein, but fruit LTP-allergic sera reacted only with the non-reduced form of Hev b 12. Configuration in the molecule might therefore play a role in IgE recognition.

Hev b 13: lipolytic esterase

Frequent reports of a highly allergenic 42 000–46 000 M_r protein in *H. brasiliensis* latex appeared to have been resolved with the discovery of a 43 000 M_r allergenic latex protein that was a homologue to patatin. However, the low to moderate prevalence of sensitization to the protein, Hev b 7, could not adequately explain the frequent observations of the 42 000–46 000 M_r allergen. This led to the thinking that another protein of a similar molecular size was responsible. The allergen, Hev b 13, is a 42 980 M_r glycoprotein isolated from *Hevea* latex by Arif *et al.* [5]. In some earlier publications, this protein was referred to as Hev b 7b [6,7••]. The protein predicted from its cDNA (GenBank accession number AY283800) has 391 amino acids, the first 26 of which constitute a putative signal peptide. The deduced molecular weight of the mature protein is 40 400 M_r. The discrepancy between the predicted and observed molecular weights might be caused by glycosylation. The protein shows protein sequence homology to the early nodule-specific proteins of legumes and has lipase and esterase properties. The allergenicity of Hev b 13 has been assessed by various approaches. IgE reactivity was 61% ($n = 36$) by IgE-dot blot [6] and 78% ($n = 67$) by IgE-enzyme-linked immunosorbent assay [7••], whereas allergic sensitization was demonstrated by skin-prick test in 63% ($n = 62$) of healthcare workers with latex allergy [8••].

Latex allergens revisited

Recent research has provided new insights on various molecular characteristics of the latex allergens Hev b 2, Hev b 4, Hev b 5 and and Hev b 6.

Involvement of glycans in allergenicity of Hev b 2

Although several isoforms of latex glucanase have been encountered [9], latex glucanase, Hev b 2, commonly appears as a doublet of approximately 35 000 M_r on sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). Latices from different clones (cultivars) of the rubber tree may show different proportions of the larger or smaller component of the doublet [10]. Churngchow *et al.* [11] demonstrated by purification on a concanavalin A affinity column that the larger Hev b 2 peptide was glycosylated whereas the smaller peptide was not. More recently, Yagami *et al.* [12•] showed that the smaller (faster-migrating) protein band on an SDS–PAGE gel comprised two co-migrating peptides, one of which was glycosylated and the other was not. They opined that whereas much of the IgE affinity to Hev b 2 was caused by the glycan moiety of the glucanases, the allergic reaction to Hev b 2 in patients was attributed more to the unglycosylated isoform. Nevertheless, this proposition requires further study and confirmation in view of the very small sample size used to determine allergenicity by skin-prick test ($n = 7$).

Although sensitization to native Hev b 2 is above 50% in most investigations, the allergenicity of recombinant Hev b 2 from two studies, one involving serological assays and the other involving skin-prick tests, was very low (Table 1). The absence of carbohydrate in the recombinant protein may explain the discrepancy between the native and recombinant species, but other factors such as structural conformation of the molecules may also play a part.

Identity and molecular cloning of Hev b 4

Hev b 4 is a protein complex rather than a single protein. Although it was shown to be associated with the latex microhelix [31], its functionality and biochemical identity remained unclear. Under reducing conditions of SDS–PAGE, Hev b 4 appears as a triplet with a narrow protein band of approximately 56 000 M_r and two broader bands of approximately 50 000 M_r that often merge to appear as a single broad band. In its undenatured state, Hev b 4 takes the form of a single protein on a native PAGE gel and defies various attempts at the chromatographic separation of its sub-units.

Table 1. Reactivity of adult latex-allergic patients or patient sera to purified latex allergens

Reference	Assay	Sample size	Hev b 1	Hev b 2	Hev b 3	Hev b 4	Hev b 5	Hev b 6.01	Hev b 6.02	Hev b 6.03	Hev b 7	Hev b 8	Hev b 9	Hev b 10	Hev b 11	Hev b 12	Hev b 13
[8••]	SPT	62	23	**63**	24	39	**(65)**	**63**			45						**63**
[13]	SPT	29		(7)	(7)		**(62)**	**(66)**			(41)	(3)					
[14][a]	CAP	53	(19)	(0)	(9)		**(68)**	**(70)**				(15)	(4)	(4)	(25)		
	EAST	57		**74**													**87**
[7••][b]	ELISA	65	17	**83**	17	20	**(65)**	**75**			32						**79**
[15]	ELISA	31	32	**65**	32	**65**		**(55)**			(42)						
	RAST		13	48	19	23		(45)			(23)						
	RAST		19	**61**	19	**61**		(45)			(45)						
[6][b]	DIB	36	3	28	0	**75**	(31)				8						**61**
[16]	CAP	71	(23)														
[17]	EAST	105	**52**														
[18]	WIB	32	3														
	ELISA		6														
[19]	WIB	11[c]		**55**													
	ELISA	13[c]		46													
[12•]	SPT	7		**57**													
[20]	RAST	13					**(92)**										
[21]	ELISA	25						**(84)**	**(88)**	(40)							
	SPT	15						**(80)**	**(80)**	(33)							
[22]	SPT	21							**81**								
	EAST	64							**75**								
[23]	ELISA	52[d]						**69**		21							
	ELISA	43[e]							**56**								
	WIB	20						**75**		15							
	SPT	4							**75**								
[24]	ELISA	35									49						
[25]	WIB	36									11						
											(11)						
[26]	WIB	40									23						
[27]	WIB	50										(24)					
[28]	CAP	42										(17)					
[29]	WIB	110											(15)				
[30]	WIB	15												(27)			
[1•]	WIB	57													(18)		
[2•]	CAP	53													(25)		
[4•]	WIB	37														(24)	

CAP, Pharmacia ImmunoCAP; DIB, dot-immunoblot; EAST, enzyme-linked allergosorbent test; ELISA, enzyme-linked immunosorbent assay; RAST, radioallergosorbent test; SPT, skin-prick test; WIB, Western-IgE immunoblot.
Prevalence of skin reaction (skin-prick tests) or IgE reactivity expressed as a percentage. Results based on recombinant proteins in parentheses. Prevalences above 50% (suggesting major allergens) in bold. The data show mainly results from adult test subjects. Data from children are excluded where there is information in the paper to enable this.
[a]Results of the CAP assays and partial results of the EAST assays were presented in the paper. The full results of the EAST assays were obtained in a private communication with Dr Monika Raulf-Heimsoth, BGFA, Germany.
[b]In these studies, Hev b 13 was referred to as Hev b 7b.
[c]Samples that were unreactive with non-ammoniated latex omitted; the original sample size was 15.
[d]Including 20 samples from children.
[e]Including up to 20 samples from children.

Recent work by Sunderasan *et al.* [32•] revealed the heaviest component of the Hev b 4 triplet as having amino acid sequences matching the published sequences of several plant glucosidases, including those of cyanogenic glucosidases. Enzyme activity was demonstrated when linamarin was used as the specific glucosidase substrate, demonstrating that the protein was indeed a cyanogenic glucosidase (linamarase). Its full cDNA sequence has since been published (GenBank accession number AY297039). Partial amino acid sequences of the two other (lighter) sub-units of Hev b 4 suggest that they are largely identical. The full cDNA sequence (GenBank accession number AY437086) shows homology with the myrosinase-associated protein of *Arabidopsis*, and lipases of *Arabidopsis*, *Oryza* and *Brassica*.

Non-fusion recombinant Hev b 5

Although Hev b 5, as originally described by Akasawa *et al.* [33], was isolated from latex, most subsequent research on this allergen has used the maltose-binding protein expression vector and glutathione-*S*-transferase expression vectors to generate allergenic recombinant fusion proteins [6,20,34,35]. Attempts at cleaving Hev b 5 from the vector protein had not been fruitful because IgE binding ability was subsequently lost [6,34]. Unlike the maltose-binding protein expression vector or glutathione-*S*-transferase vectors, the histidine expression vector employed by Sutherland *et al.* [36•] added only marginally to the mass of Hev b 5 and was readily reactive with IgE. That notwithstanding, Western blots depicting this reaction were not always easy to interpret, as the binding of IgE and monoclonal antibodies on Western blots did not always correspond with protein bands visible on the blot.

Conformational IgE epitopes of Hev b 6

Karisola *et al.* [37•] introduced a novel approach to construct conformational IgE-binding epitope domains of hevein (Hev b 6.02) using an antimicrobial protein (AMP) from *Amaranthus caudatus* as a three-dimensional molecular template. Hevein and AMP share a structurally identical core region but have different *N* and *C* terminals. Whereas several sera from hevein-allergic patients were mainly unreactive with AMP, all showed IgE binding when both the hevein *N*-terminal and *C*-terminal regions were fused with the AMP core. Chimeric AMP bearing the hevein *N* terminus alone or *C* terminus alone was recognized by IgE from 88 and 38% of the patients ($n = 16$), respectively. The study indicated that the major IgE-binding epitopes of hevein are conformational because linear synthetic peptides corresponding to various hevein regions in the AMP chimeras showed no significant IgE binding capacity. The results are, however, at variance with those reported by Beezhold *et al.* [38] and Banerjee *et al.* [21], who independently demonstrated IgE binding to a number of linear hevein oligopeptides, including segments corresponding with the AMP core region that Karisola *et al.* [37•] found poorly reactive.

Reactivity of purified latex allergens

A collation of results from recent and earlier studies on sensitization to purified latex allergens is given in Table 1. The first six studies listed in the table involve comparisons of multiple allergens, whereas the other studies mainly show reactivity of individual latex proteins. The data presented pertain to adult latex-allergic subjects, mainly healthcare workers. (Spina bifida children are known to be more sensitized to certain latex allergens, notably Hev b 1 and Hev b 3 [39•].) The variation in the prevalence of allergen reactivity that is observed in different studies may be caused by differences in the assay employed, the sample population or the test reagent (e.g. whether native or recombinant, extent of protein denaturation). Prevalences of reactivity to Hev b 5, 8, 9, 10 and 11 have been estimated only from recombinant proteins. Although serological assays demonstrated IgE sensitization that may not always involve an allergic reaction, there is broad agreement between the results from skin-prick tests and in-vitro assays (Table 1).

Latex proteins are deemed to be major allergens when 50% or more of latex-allergic patients are sensitized to them [40]. As shown in Table 1, the prevalence of IgE sensitization or allergic reaction among adult latex-sensitized individuals frequently exceeds 50% for native Hev b 2, recombinant Hev b 5, native or recombinant Hev b 6 and native Hev b 13. Native Hev b 4 is borderline.

Latex allergens in allergy diagnostics

Accurate diagnosis is an important first step to address the problems arising from latex allergy and to provide healthcare support. While commercial diagnostics are available for serologic assays, skin-prick tests, interpreted with clinical history, provide the most reliable diagnosis of latex allergy. Commercial latex test reagents for the latter are available in several countries, but not yet in the United States.

Reference latex for immunoassays

Diagnostic tests for latex allergy [41•] employ reference latex reagents to elicit an allergic reaction from the subject in a skin-prick test or to act as an allergosorbent (capture antigen) in a serological assay. Latex allergy is perhaps more complex than many other allergies in that it stems not from a single protein, but from no fewer than 13 known latex allergens (Hev b 1 to Hev b 13) with no single allergen deemed to be dominant. The proteins vary widely in their relative abundance in natural rubber latex. In this situation, well-characterized and reproducible reference test reagents are difficult to prepare from unpurified whole latex.

To regulate precisely and reproducibly the dosage of each latex allergen in a reference reagent, mixtures of purified antigens can be formulated. Most latex-allergic patients are sensitized to more than one latex allergen [6,7••,42]. It may thus not be necessary to have a blend containing all the known latex allergens and yet keep false-negative results manageable. Kurup *et al.* [15] reported that a combination of native Hev b 2 and recombinant Hev b 7 was sufficient to identify approximately 80% of latex-allergic healthcare workers ($n = 31$) and spina bifida patients ($n = 13$). Two major latex allergens, Hev b 5 and Hev b 13, were not on the panel of Kurup *et al.* [15]. With their inclusion in a more recent investigation involving 62 latex-allergic subjects, Bernstein *et al.* [8••] found that the combination of native Hev b 2, native Hev b 3, native Hev b 4, recombinant Hev b 5 and native Hev b 13 identified 92% of individuals who were latex skin-prick positive. The same tests carried out on 49 atopic non-latex-allergic control subjects gave a diagnostic specificity of 98% [8••].

Hev b 5 as a capture antigen in immunoassays

Despite Hev b 5 being a major latex allergen, Beezhold *et al.* [34] reported that its level in natural rubber latex was very low. Chen *et al.* [43] posted a confirmation, but they also found that sera that tested negative to latex could be reactive to recombinant Hev b 5. This led to the suspicion that the content of native Hev b 5 in latex was too low to elicit a positive response in a patient, but could yet concentrate in latex products such as gloves to pose a hazard to sensitized patients [36•].

If native Hev b 5 were lacking in latex, spiking the latex with recombinant Hev b 5, it is reasoned, should increase sensitivity of the diagnostic assay. Such an allergen preparation is now available as a commercial product [44]. Enhanced test sensitivity using the modified latex was borne out in a study by Hamilton *et al.* [45], in which serological positives in 68 samples increased from 51.5 to 61.8% when the latex allergosorbent was enriched with non-fusion recombinant Hev b 5. Seeing how the supplementation of latex reagent with recombinant Hev b 5 is fast becoming accepted practice, there should perhaps be greater urgency to demonstrate unequivocally the equivalence of native Hev b 5 with recombinant Hev b 5. That recombinant Hev b 5 is highly allergenic is not in doubt (Table 1). What is lacking are results that show purified native Hev b 5 matching the recombinant protein in this respect.

Conclusion

Continuing research on latex allergens sustains a stream of new information that serves to widen our understanding and appreciation of the intricacies of latex allergy. Of the 13 recognized allergens originating from natural rubber latex, Hev b 2, Hev b 6, Hev b 13 (and possibly Hev b 4) are the major allergens to which latex-sensitized adults react. Recombinant Hev b 5 is the major latex allergen that is paradoxically not found in natural rubber latex or latex products. The allergenicity of its native counterpart is widely accepted, but has yet to be unequivocally demonstrated.

Hev b 5 is by no means exceptional in being better characterized as a recombinant protein than in its native form. Latex allergens identified and named in the early years of research were principally isolated from natural rubber latex. Their recombinant forms were subsequently synthesized when their encoding cDNA became available. Among the four most recently named proteins, Hev b 10–13, only Hev b 13 has been isolated and characterized in its native form. The allergenicity of purified Hev b 10, Hev b 11 and Hev b 12 has been mainly demonstrated on recombinants. Mindful of the view that IgE-binding epitopes are mainly conformational [46,47], the trend towards using recombinant models in latex allergy research deserves careful attention. The allergenic equivalence between natural latex proteins and their recombinant counterparts should therefore rank among the priority research areas in latex allergy.

With the 13 latex allergens on the IUIS list, it is the author's view that all the major allergens have now been accounted for. Nevertheless, others have made the same pronouncement in the past and have been proved wrong. Time will tell.

References and Recommended Reading

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- • of special interest
- •• of outstanding interest

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