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Allergen concentration in natural rubber latex

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Summary

Background *Hevea brasiliensis* latex serum is commonly used as the *in vivo* and *in vitro* reference antigen for latex allergy diagnosis as it contains the full complement of latex allergens.

Objective This study quantifies the concentrations of the significant allergens in latex serum and examines its suitability as an antigen source in latex allergy diagnosis and immunotherapy.

Methods The serum phase was extracted from centrifuged latex that was repeatedly freeze-thawed or glycerinated. Quantitation of latex allergens was performed by two-site immunoenzymetric assays. The abundance of RNA transcripts of the latex allergens was estimated from the number of their clones in an Expressed Sequence Tags library.

Results The latex allergens, Hev b 1, 2, 3, 4, 5, 6, 7 and 13, were detected in freeze-thawed and glycerinated latex serum at levels ranging from 75 (Hev b 6) to 0.06 nmol/mg total proteins (Hev b 4). Hev b 6 content in the latex was up to a thousand times higher than the other seven latex allergens, depending on source and/or preparation procedure. Allergen concentration was reflected in the abundance of mRNA transcripts. When used as the antigen, latex serum may bias the outcome of latex allergy diagnostic tests towards sensitization to Hev b 6. Tests that make use of latex serum may fail to detect latex-specific IgE reactivity in subjects who are sensitized only to allergens that are present at low concentrations.

Conclusion Latex allergy diagnostics and immunotherapy that use whole latex serum as the antigen source may not be optimal because of the marked imbalance of its constituent allergens.

Keywords diagnostic, EST, *Hevea brasiliensis*, IEMA, immunoassay, immunotherapy, latex, latex allergy, natural rubber serology, skin prick test

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Introduction

The clinical diagnosis of latex allergy can be confirmed using a skin prick test (SPT) or *in vitro* serology for IgE anti-latex using one of the FDA-cleared immunoassays. Whole natural rubber latex is the preferred diagnostic reference antigen as it contains a comprehensive repertoire of clinically relevant latex allergens. Clinicians and researchers have used latex serum-based extracts under the assumption that all relevant soluble allergenic proteins are present in molar excess to latex-specific IgE in the skin and blood.

Presently, 13 latex allergens have been recognized by the International Union of Immunological Societies (IUIS). A latex-allergic subject could become sensitized to any of these 13 proteins, and may be very likely sensitized to a combination of several [1, 2]. No single latex allergen is dominant, based on skin testing, serology and immunoblot analyses with purified and/or recombinant allergens. At least six latex allergens – Hev b 1, 2, 3, 5, 6 and 13, and possibly Hev b 4 – have reported sensitization rates of 50% or greater among latex allergic healthcare workers [3].

In vivo and *in vitro* diagnostics for latex allergy have been reviewed by Turjanmaa et al. [4]. Several latex

diagnostic antigens have been reportedly used in latex allergy diagnosis and research. Of these, the preparations from Stallergenes (Antony, France) and Greer (Lenoir, NC, USA) have been those described in the greatest detail. In the method adopted by Stallergenes, non-ammoniated latex is collected in Malaysia and frozen before being transported to France. The frozen latex is then thawed and centrifuged to obtain the serum. Whole latex serum prepared in this manner has been used as a SPT antigen at a concentration of 10 or 22 µg/mL of protein [5, 6]. In the approach adopted by Greer, glycerol is added to the collected non-ammoniated latex as a stabilizer. Following shipping, the latex is centrifuged to obtain the latex serum. The Greer latex reagent has been used as a SPT antigen at protein concentrations of 1, 100 and 1000 µg/mL [7, 8]. Optimal diagnostic accuracy was reported at 100 µg/mL for SPTs [7].

Both latex preparations have also been used in immunotherapy of latex-allergic patients during which graded doses of latex extract are administered to induce tolerance to the allergenic proteins. Although various processes of standardization are used, essentially whole natural rubber latex serum is used [9, 10]. The rationale is that the whole latex serum is expected to contain whichever latex allergen to which the patient is sensitized.

It is generally agreed that whole latex serum contains all the principal latex allergens to which humans have become sensitized. Nevertheless, we have questioned what effect a concentration disparity and lack of molar excess status among the individual allergens present in latex might have on diagnosis and therapeutic efficacy. In this study, we examine the magnitude of this concern by quantifying the major allergen components in relevant latex serum preparations. The data demonstrate a serious latex allergen bias that raises far-reaching concerns about the accuracy of diagnostic and therapeutic results obtained with *Hevea* serum-based reagents.

Materials and methods

Latex serum preparation

Latex was collected under chilled conditions from clone RRIM 600 *Hevea brasiliensis* trees growing in two fields at the Rubber Research Institute of Malaysia Experiment Station, Sungei Buloh, Selangor. The latex from each field was divided and subjected to two treatments to prepare non-ammoniated latex serum. The first treatment involved centrifuging the latex to remove the rubber cream. The non-rubber fractions (comprising mainly the C-serum and the lutoids) were then subjected to repeated freeze thawing to rupture the lutoids and release their contents (the B-serum) into the serum mixture. The resulting 'freeze-thawed latex serum' was a mixture of B-serum and C-serum that was analogous to the Stallergenes latex

reagent. The second treatment involved addition of glycerol to latex in a 2:1 mixture. This was followed by gentle stirring and storage at 4 °C overnight. The following day, the latex was centrifuged to obtain the latex serum, which was similarly a mixture of B-serum and C-serum. This 'glycerinated serum' was analogous to the Greer latex reagent. In addition to the above two latex serum preparations, the insoluble rubber particle membrane proteins Hev b 1 and Hev b 3 were extracted for quantification. This was accomplished by adding sodium dodecyl sulphate (1%) to whole latex and then boiling the mixture for 20 min. The aqueous extract was recovered by centrifugation.

Immunological analyses

The threshold for skin reactivity of purified individual latex allergens was assessed by performing SPTs on 62 latex-allergic American adults as previously described by Bernstein et al. [2]. Multiple 10-fold dilutions of each of the purified native latex allergens, Hev b 1, 2, 3, 4, 6 and 13, and recombinant Hev b 5 were administered to the volar aspect of the forearm to determine the lowest allergen concentration (the endpoint percutaneous threshold concentration) that could elicit a positive skin reaction.

In a separate assessment, 101 European patients from the Allergy Clinic Reumannplatz and the General Hospital, both in Vienna, Austria, and the Allergy Unit at the National Health Service in Rome, Italy, were picked based on compelling histories of latex allergy (Table 1). Their allergy to latex was confirmed by serologic assay (Pharmacia CAP, Phadia, Uppsala, Sweden). When subsequently skin-tested with latex, however, only 74 patients had tested positive while the remaining 27 tested negative. *In vitro* IgE-ELISA experiments were performed on sera from all 101 patients to test for sensitization to rHev b 6.02 (Biomay, Vienna, Austria). As the test antigen in the immunoassay was a recombinant fusion protein of Hev b 6.02 attached to the maltose binding protein (MBP), all the sera were also tested for IgE antibody reactivity to MBP (New England Biolabs Inc., Beverly, MA, USA). The proteins were coated at a concentration of 5 µg/mL overnight, blocked and incubated with individual patient sera at 1:5 dilution overnight. Bound IgE was detected with alkaline phosphatase-conjugated anti-human-IgE (BD Pharmingen, San Diego, CA, USA). The threshold for positive values was determined as the mean optical density + 3 standard deviation with serum from five non-latex-allergic patients.

Two-site immunoenzymetric assays (IEMAs) for the concentrations of the latex allergens Hev b 1, 2, 3, 5, 6 and 13 in latex were performed as described previously [11]. Owing to the nature of latex as a complex biologic mixture, proportionality of allergen concentration to the assay photometric absorbance was observed only over a

Table 1. Symptoms of European test subjects positive or negative to latex skin prick test

Latex skin prick test	<i>n</i>	Occupational contact with latex	Allergy symptoms						
			Urticaria	Angiooedema	Asthma	Eczema	Rhinitis	Rhinoconjunctivitis	Anaphylaxis
Positive	74	45	62	24	20	12	11	22	1
Negative	27	41	63	7	4	30	11	0	4
Total	101	44	63	20	16	17	11	16	2

The number of subjects in each skin prick test category is denoted by *n*. Other values are percentages of *n*.

narrow band of allergen dilutions. An assay result was accepted when there was agreement between readings for two or three different dilutions of the allergen. To quantify the level of Hev b 4 and Hev b 7 in the latex preparations, two polyclonal antibodies for each protein were utilized. In each assay, the detection antibody was biotinylated and detected with an avidin-alkaline phosphatase conjugate. The IEMA for Hev b 6 could not distinguish between Hev b 6.01 (prohevein) and 6.02 (hevein). In the present study, hevein was used to construct the calibration curve, and the Hev b 6 concentration is thus expressed as hevein molecular equivalents.

The total protein content in each of the latex preparations was determined by the ASTM D5712–99 modified Lowry assay.

Generation and analysis of expressed sequence tags

A library of 10 000 expressed sequence tags (ESTs) that represented transcribed latex genes was constructed from a *Hevea brasiliensis* latex c-DNA library. As a large number of these sequences were redundant, this redundancy could be used to estimate the relative abundance of mRNA transcripts. To check the frequency of each latex allergen, published cDNA sequences encoding these allergens were used to screen the database following the BLASTN algorithm. BLASTN sequence hits for each allergen cDNA were selected at a significance cut-off level of $E = 10^{-4}$. The National Center for Biotechnology Information (NCBI) GeneBank Accessions used in this analysis were X56535 (Hev b 1), U22147 (Hev b 2), AF051317 (Hev b 3), AY297039, AY437086 (Hev b 4), U51631 (Hev b 5), M36986 (Hev b 6), AF113546 (Hev b 7), AF119365 (Hev b 8), AJ132580 (Hev b 9), AJ249148 (Hev b 10), AJ238579 (Hev b 11), AY057860 (Hev b 12) and AY283800 (Hev b 13).

Results

Allergen content variation due to differences in the latex collection and preparation methods

All eight allergens (Hev b 1, 2, 3, 4, 5, 6, 7 and 13) that were measured in this study were detected in both the glycerinated and freeze-thawed latex serum preparations (Fig. 1). While the overall allergen profiles were similar between

the two latex collections and the two methods of latex serum preparation, several differences were noted between the allergen concentrations in latex collected from the trees of same clone at the same time of the year, but planted in two different fields (Fig. 1). Hev b 2 was most prominent in this respect, showing up to sixfold differences between fields. The method of latex preparation also affected allergen concentration in the latex serum, with Hev b 3 showing higher levels in glycerinated latex serum.

Latex allergen composition

There were marked differences in the relative concentrations of individual Hev b allergens (Fig. 1). Hev b 6 was by far the most abundant soluble allergen in latex in both freeze-thawed and glycerinated preparations, with Hev b 4 and Hev b 13 being the least abundant. Allergen concentrations are also plotted logarithm-transformed in Fig. 1 (right panels) to accommodate the huge disparity in Hev b 6 levels vs. those of the other latex allergens. Depending on the method of latex serum preparation and the field from which the latex was collected, Hev b 6 concentration (expressed in molar concentration) ranged from 380 to 7500 times that of Hev b 4. Averaged across latex collections and serum preparation methods, the concentrations of the allergens (in nmol/mg total protein) in descending order were: Hev b 6 (75.05), Hev b 3 (3.73), Hev b 2 (3.06), Hev b 7 (2.00), Hev b 1 (1.04), Hev b 5 (0.59), Hev b 13 (0.28) and Hev b 4 (0.06). Hence, the mol ratios of the allergens relative to Hev b 6 were, respectively, 1 : 0.05 : 0.04 : 0.03 : 0.01 : 0.008 : 0.004 : 0.001.

Allergen concentration reflected in mRNA transcripts

As Hev b 1 and Hev b 3 are insoluble rubber particle proteins, they are poorly represented in latex serum prepared either by freeze-thawing or by glycerol addition. When extracted with sodium dodecyl sulphate to facilitate solubilization of Hev b 1 and 3, however, the amount of these proteins in latex was substantial (Fig. 2). A correlation was observed between the Hev b allergen concentration and the relative abundance of mRNA of the corresponding allergens, the latter being reflected in the number of cDNA clones that were identified in the EST library. With both variables logarithm-transformed, the

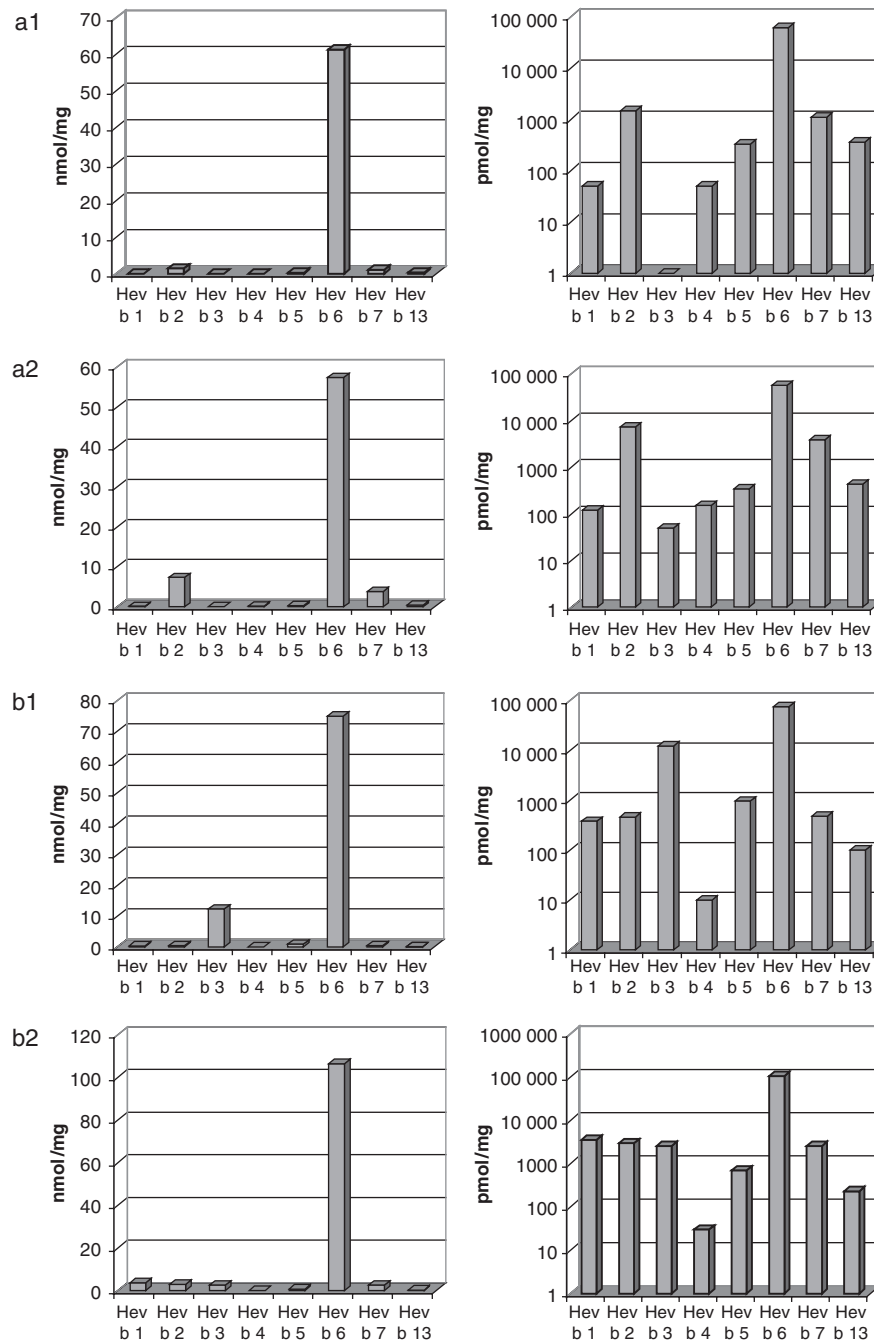


Fig. 1. Allergen concentration in freeze-thawed latex serum (a) and glycerinated latex serum (b). Results are from latex collected from two fields (1 and 2). Allergen concentrations are standardized against total protein content. Results in the right-hand panels are presented on a logarithm scale.

correlation coefficient was 0.729 ($r^2 = 0.531$, $P < 0.05$). Thus, the variation in mRNA abundance accounted for more than 50% of the variation in latex allergen concentration detected in the latex preparations.

Allergen concentration in relation to reactivity threshold

The relative degree of allergenicity for the different latex allergens was reflected in their reactivity threshold con-

centrations as determined by the lowest concentration of allergen required to produce a positive percutaneous skin reaction. These analyses were obtained by SPTs performed on a sensitized American test population. On a molar basis, the median reactivity threshold was the lowest for Hev b 13 (0.001 μM) and Hev b 4 (0.009 μM) (Tables 2 and 3). Hence, molecule for molecule, Hev b 13 and Hev b 4 were potentially the most potent latex allergens when introduced into the skin of sensitized individuals. Their higher

allergenic potency therefore appears to partially compensate for their relatively low concentrations in the latex extracts. In contrast, Hev b 1 and Hev b 6 showed the highest median threshold concentrations (0.188 and 0.026 μM) required to produce a positive skin reaction. They were

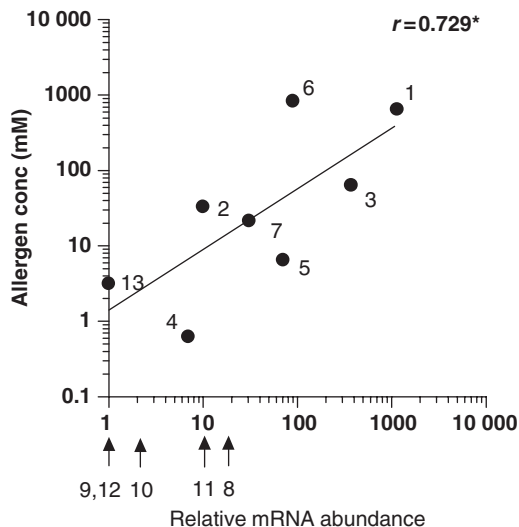


Fig. 2. Correlation between latex allergen concentration and the abundance of their corresponding mRNA estimated from the number of clones in the expressed sequence tags (EST) library. Numbering of the points on the graph refers to the allergens Hev b 1, 2, 3, 4, 5, 6, 7 and 13. The number of cDNA clones found for Hev b 8, 9, 10, 11 and 12 (for which protein concentration data are unavailable) are indicated on the x-axis. No cDNA clones were found for Hev b 9, 12 and 13, but a score of 1 has been assigned to facilitate logarithm transformation. Protein concentrations for Hev b 2, 4, 5, 6, 7 and 13 are the means of the freeze-thawed serum and glycerinated serum readings from latex collected in two fields. For this experiment, the insoluble membrane proteins, Hev b 1 and Hev b 3, were solubilized with sodium dodecyl sulphate to facilitate their extraction from the surface of rubber particles.

also the two allergens in highest absolute concentration in both latex preparations (Fig. 2).

Latex sensitization detected and undetected by SPTs

The efficiency by which the different latex preparations could detect IgE antibody in the skin was evaluated in a sensitized test population. The freeze-thawed latex serum (10 and 22 $\mu\text{g}/\text{mL}$ total proteins) produced fairly similar results. Hev b 6 concentration was well above the 75th percentile thresholds of skin prick reactivity, meaning that more than three quarters of tested subjects would respond positively (Table 2). For all the other allergens, however, their concentrations in latex serum preparations fell below the 75th percentile, and thus they were insufficient to elicit a positive reaction from at least one quarter of subjects who were sensitized to those individual allergens alone. More than half of the subjects who were mono-sensitized to Hev b 1, 3, 4 or 5 would falsely skin test negative with freeze-thawed latex serum antigens, as their median threshold for reactivity was not reached.

As the glycerinated latex was used at 1, 100 and 1000 $\mu\text{g}/\text{mL}$ of protein, there was a 1000-fold difference in concentration that provided a larger margin for detecting IgE specific for the Hev b allergens poorly represented in the extract. Even at the 1 mg/mL dose, Hev b 4 and Hev b 13 would not always elicit a positive skin prick response in at least one quarter of subjects mono-sensitized to these allergens (Table 3). At a dilution of 100 $\mu\text{g}/\text{mL}$ protein, some subjects who were sensitized only to Hev b 5 (and with some latex collections, to Hev b 2 and 7 as well) would obtain a false-negative skin test result as well. With the 1 $\mu\text{g}/\text{mL}$ dose of glycerinated latex, all the latex allergens in this study, with the exception of Hev b 6,

Table 2. Allergen content in freeze-thawed latex serum preparations

	Allergen content (μM) in freeze-thawed latex serum				Skin prick reactivity threshold (μM)		
	22 $\mu\text{g}/\text{mL}$ total protein		10 $\mu\text{g}/\text{mL}$ total protein		<i>n</i>	Median	75th percentile
	Field 1	Field 2	Field 1	Field 2			
Hev b 1	0.001	0.003	0.0006	0.001	14	0.188	0.343
Hev b 2	0.032	0.161	0.015	0.073	39	0.014	0.143
Hev b 3	0	0.001	0	0.0005	15	0.023	0.125
Hev b 4	0.001	0.003	0.0005	0.002	24	0.009	0.093
Hev b 5	0.007	0.007	0.003	0.003	40	0.017	0.313
Hev b 6	1.351	1.262	0.614	0.574	39	0.026	0.263
Hev b 7	0.024	0.085	0.011	0.039	28	0.011	0.114
Hev b 13	0.008	0.009	0.004	0.004	39	0.001	0.116

Shaded values are below the 75th percentile reactivity threshold. The concentration of the specific allergen is below that required to elicit a positive reaction in more than one quarter of latex-allergic subjects sensitized, resulting in their being potentially diagnosed as negative if the subjects were mono-sensitized to that particular allergen. Deep shaded values are below the median reactivity threshold. The concentration of the specific allergen is below that required to elicit a positive reaction in more than half of the latex-allergic subjects sensitized, resulting in their being potentially diagnosed as negative if the subjects were mono-sensitized to that particular allergen. The number of subjects that tested positive for each allergen is denoted by *n*.

Table 3. Allergen content in glycerinated latex serum preparations

	Allergen content (μM) in glycerinated latex serum						Skin prick reactivity threshold (μM)	
	1 mg/mL total protein		100 μg /mL total protein		1 μg /mL total protein		Median	75th percentile
	Field 1	Field 2	Field 1	Field 2	Field 1	Field 2		
Hev b 1	0.378	3.593	0.038	0.359	0.0004	0.0036	0.188	0.343
Hev b 2	0.461	2.986	0.046	0.299	0.0005	0.0030	0.014	0.143
Hev b 3	12.316	2.550	1.232	0.255	0.0123	0.0026	0.023	0.125
Hev b 4	0.005	0.035	0.001	0.003	0.00001	0.00003	0.009	0.093
Hev b 5	0.963	0.699	0.096	0.070	0.0010	0.0007	0.017	0.313
Hev b 6	74.951	105.502	7.495	10.550	0.0750	0.1055	0.026	0.263
Hev b 7	0.483	2.560	0.048	0.256	0.0005	0.0026	0.011	0.114
Hev b 13	0.099	0.231	0.001	0.023	0.00001	0.0002	0.001	0.116

Shaded values are below the 75th percentile reactivity threshold. The concentration of the specific allergen is below that required to elicit a positive reaction in more than one quarter of latex-allergic subjects sensitized, resulting in their being potentially diagnosed as negative if the subjects were mono-sensitized to that particular allergen. Deep shaded values are below the median reactivity threshold. The concentration of the specific allergen is below that required to elicit a positive reaction in more than half of the latex-allergic subjects sensitized, resulting in their being potentially diagnosed as negative if the subjects were mono-sensitized to that particular allergen.

would elicit positive skin prick reactions in fewer than half of the subjects sensitized only to these individual proteins.

Sensitization to Hev b 6 among latex-allergic subjects testing positive or negative to latex

European adult patients ($n = 101$) who were identified as latex-allergic based on clinical history and serologic tests were divided into two groups: 74 subjects who SPTed positive and 27 subjects who tested negative. Of the skin test-positive group, 45 subjects (61%) were found to have detectable IgE anti-Hev b 6.02 by *in vitro* analysis. In contrast, only two subjects (7%) from the skin test-negative group were sensitized to Hev b 6.02 using the same analysis. The difference in these percentages was statistically significant ($P < 0.0001$, 2×2 contingency matrix, followed by Fisher's exact probability test). These results support the hypothesis that if an allergic subject were not sensitized to Hev b 6, specific IgE may not be detected for the latex allergens that are recognized by the subject, but are present only in low concentrations in the antigen preparation.

Discussion

For many years, there has been concern about the low sensitivity of diagnostic latex skin test reagents and IgE anti-latex serology assays [2, 12, 13]. In the present study, we have investigated the hypothesis that the composition of latex in the reagents contributes to the low diagnostic sensitivity of these tests. Information on the actual concentration of the clinically important allergens, Hev b 1, 2, 3, 4, 5, 6, 7 and 13, in *Hevea brasiliensis* latex is important to study this issue. The latex serum preparations studied in

this project were prepared to reflect the latex preparations used clinically as skin test reagents. Besides their use in diagnostics, latex antigens are also used in immunotherapy. As with diagnostics, latex allergy immunotherapy is performed without detailed information on how much of the individual constituent latex allergens are being administered.

The IUIS recognizes 13 latex allergens. Eight of these, Hev b 1, 2, 3, 4, 5, 6, 7 and 13, are the most significant latex allergens among healthcare workers because they are the allergenic proteins that elicit the highest prevalence of IgE antibody specificities that are detected in the skin and blood [3]. These proteins have average sensitization prevalences exceeding 30% (Fig. 3) and all eight allergens have been shown to be reactive in SPTs performed on latex-allergic subjects [2]. As multiple allergens are sensitizing most latex-allergic persons, no single latex allergen has been identified as the most representative marker allergen. The most abundant allergenic proteins in latex are Hev b 1, Hev b 6 and Hev b 3. However, Hev b 1 and Hev b 3 are insoluble rubber particle proteins that are significantly solubilized only in the presence of detergents (Fig. 2). Their content in freeze-thawed and glycerinated latex serum represents only a small fraction of the protein present in the whole latex. The low concentration of Hev b 4 in latex is surprising as Hev b 4 and Hev b 2 contents are comparable when these proteins are purified directly from latex B-serum [14]. There is the possibility that some Hev b 4 may be lost from the latex sera that were prepared in this study.

The allergens that we chose not to study (Hev b 8, 9, 10, 11 and 12) each has a sensitization rate below 30% (Fig. 3). As such, they are considered less significant allergens. The quantity of mRNA in latex does not always predict the concentration of its corresponding protein accurately as

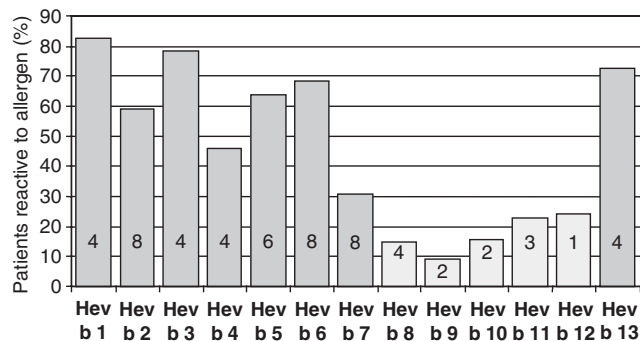


Fig. 3. Prevalence of reactivity to purified latex allergens by latex-allergic patients. Average of results from skin prick tests and serologic assays in published studies. The number of studies are indicated on the bars. Dark shaded bars are allergens analysed by immunoassay in this study. Hev b 1 and Hev b 3 results are from spina bifida children (who tend to be especially allergic to these two proteins); all other results are from adults. Results of Hev b 2 are from the native protein only. Results of Hev b 5 are from the recombinant protein only. Prevalence data are from Lu *et al.* [22], Wagner *et al.* [23] and the studies listed in Table 1 of Yeang [3].

factors such as protein stability and metabolism affect protein longevity and accumulation in the latex. Nevertheless, latex tapped from the rubber tree is replaced continually by *de novo* synthesis, and the specific mRNA in the latex may therefore be a reasonable indicator of protein concentration. In the present study, the mRNA levels (as reflected in EST numbers) explain more than half of the variation in protein concentration that is observed (Fig. 2). From the EST scores for Hev b 8, 9, 10, 11 and 12, it would appear that Hev b 8 and 11 might have a moderate presence in latex, whereas Hev b 9, 10 and 12 are likely to be relatively scarce. Overall, it would appear that omission of the direct measurement of Hev b 8, 9, 10, 11 and 12 protein levels should not compromise the main conclusions drawn from data collected in this study.

This study was not specifically designed to test for allergen variation in latex from different fields due to environmental and agronomic factors, but Fig. 1 suggests that such variations do exist. Seasonal effects on allergen concentration or composition have been reported previously [7, 15]. The manner in which latex is treated after collection also appears to affect allergen content. In this study, latex serum was prepared based generally on the methods adopted for the Stallergenes and Greer latex skin testing antigens. Many factors (arising from the field, season, agronomic practice, storage and laboratory handling differences) affect the concentration of the constituent proteins in a preparation of natural rubber latex. Therefore, no claim is made here that the two preparations are identical to those prepared by Stallergenes and Greer. Nevertheless, these preparations provide an indication of the levels of latex allergens that might be expected in these materials using broadly similar production approaches.

The most striking characteristic of allergen composition in latex is the high concentration of Hev b 6, a primary allergen in natural rubber latex [16–18]. Thus, when diluted whole latex serum is used as the diagnostic antigen, the outcome could be expected to be biased towards sensitization to Hev b 6. Quantitative *in vitro* IgE antibody assays that depend on antibody–antigen interaction can similarly be influenced by a disproportionately large amount of any particular antigen, such as Hev b 6 in the present case, in the reaction mixture. In addition to diagnostic assays, the IgE-inhibition assay [19] that is used to quantify allergens in extracts of latex products (such as latex gloves) may also be affected. A critical variable of the IgE-inhibition assay is the reference latex antigen that is commonly whole latex serum in current practice. The use of this antigen could result in a bias of the assay towards the detection of Hev b 6, the binding of which to IgE might account disproportionately for the total IgE reaction.

Antigens used in SPTs and in the preparation of solid-phase allergens (allergosorbents) used in diagnostic serological assay have the potential to generate false-negative outcomes that would result in a loss of test sensitivity because of the proportional discrepancy in individual allergen concentrations. This could result in not only biasing quantitative concentration estimates of latex specific IgE antibody, but it can also bias positive/negative dichotomies. The *Pharmacia* company is sufficiently concerned about the low level of Hev b 5 in their latex allergosorbent to introduce an option to supplement with recombinant Hev b 5 in their ImmunoCAP assay [20, 21]. It is evident from the results of this study that several other significant allergens, besides Hev b 5, may also be critically low in reference test antigens.

In many cases, individuals who have become sensitized to specific allergens that are below reactivity threshold will still produce a qualitatively positive test result. This occurs because the individual is often concomitantly reactive to other allergens (e.g. Hev b 6) that are adequately present in the diagnostic antigen reagent. Allergic individuals who are especially liable to escape IgE antibody detection are those who are mono-sensitized to those latex allergens that are at a low concentration (but not to Hev b 6). For instance, among latex-allergic European patients tested in this study, only 7% of those who tested negative with latex had IgE antibody to Hev b 6. This contrasts with 61% of skin test-positive subjects who were sensitized to Hev b 6. These results suggest the existence of a sub-population of latex-allergic subjects who test negative against latex because they are not sensitized to Hev b 6, whereas the allergens they are actually sensitized to may not be present in sufficient amounts in the skin test antigen to trigger a response. A subject who is not sensitized to an allergen (such as Hev b 6) that is abundant in natural rubber latex may therefore

have an increased likelihood of being wrongly tested negative using diagnostics based on crude latex serum.

Latex allergy research in general is also affected when whole latex is used as the antigen in diagnostic tests to classify test subjects as either latex-allergic or non-allergic. Because the SPT is widely accepted as the gold standard, it is very commonly performed on study subjects to 'confirm' their latex allergy status. For example, in 25 studies that were performed to determine the prevalence of sensitization to individual latex allergens (summarized in Fig. 3), 21 studies or 84% adopted sensitization to latex as an inclusion criterion before accepting test subjects into the study. In view of this, the prevalence of Hev b 6 as depicted in Fig. 3 could possibly be overestimated if prospective study subjects had been excluded when they tested negative. Accordingly, the prevalence figures for the other allergens in Fig. 3 could be underestimated.

Do latex allergens that are low in concentration (to the extent that they cannot elicit a response in SPTs) have a real impact on the occupational exposure of a latex-allergic subject? Healthcare workers change latex gloves frequently through the course work and their exposure to latex allergen is therefore not from a single glove. Because exposure is cumulative from repetitive glove usage, one might expect that latex allergens even in relatively low concentrations can sensitize healthcare workers and may lead to symptom induction in individuals already sensitized.

Antigen preparations for latex allergy diagnostics in use today that are based on whole latex serum are not optimized because the allergen composition is unbalanced. Tables 2 and 3 provide estimates of the proportion of latex-allergic subjects who would potentially have been wrongly diagnosed as negative using a crude latex antigen, had they been mono-sensitized to the various latex allergens. The actual number of additional patients that can benefit from the higher sensitivity of a balanced antigen preparation may not be large, but the exact figure is hard to ascertain until diagnostics are available that capture even patients who are mono-sensitized to allergens low in concentration in whole latex. This can be achieved through the use of purified latex allergens, individually or in combination, as diagnostic antigens. With purified proteins, moreover, the patient's sensitization profile to individual latex allergens can be established. Should the patient elect immunotherapy subsequently, precise dosages of the relevant allergens can then be administered.

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