NUMBER 3, 2003

Natural rubber latex allergens: Characterization and evaluation of their allergenic capacity

Summary

In the last few years many efforts have been made to reduce the incidence of latex allergy. For this purpose the identification of the major latex allergens and their recombinant production and the determination of specific sensitization profiles for different patient groups (dependent on the various routes of exposure) are useful steps towards the understanding of latex allergy and its management. The use of well defined and standardized NRL extracts and/or purified proteins are the next objectives. In the diagnosis of NRL allergy, non-purified latex has practical utility as a test reagent and has generated useful research information. However, there are limitations in adopting it for use as a reference material, as variations in allergen content in latex due to different factors have been described. In this context, a test reagent formulated with appropriate proportions of purified allergens (native or evaluated recombinant latex allergens) has the advantages of better test sensitivity and reproducibility. Determination of an allergen protein profile of IgE antibodies may be a valuable strategy to enhance the diagnostic repertoire in certain cases. Complementary addition of natural latex extract by a single recombinant allergen, to compensate for shortage of that component, has been shown to be a useful approach to improve diagnostic performance.

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History of latex allergy

In 1927, Stern described for the first time allergic reactions to natural rubber latex (NRL) in a patient displaying urticaria and laryngeal edema after dental exposure to NRL [1]. At the same time Grimm reported asthma symptoms induced by exposure to fumes caused by the heating of a rubber-coated electric cable [2]. Thirty years later, Hansen introduced the term rubber allergy in an allergy textbook [3]. A clear case of an immediate-type skin reaction to NRL in an allergic patient, but not in control subjects, was demonstrated by Nutter [4] in 1979, using skin prick test (SPT) with latex-glove extract. Since then, IgE-mediated allergy to NRL has been recognized as an important occupational health problem with extensive economic implications. This has triggered intense research efforts directed towards latex allergen detection and quantitation, efficient diagnosis and strategies to prevent sensitization and disease.

Latex-associated reactions

Latex allergy refers to any latex-caused immune-mediated reaction associated with clinical symptoms and includes IgE-mediated reactions to the latex protein itself as well as the lymphocyte-mediated delayed reactions. The latter are also known as allergic contact dermatitis and is a result of T-cell-mediated sensitization to rubber products induced by additives like thiurams, carbamates or mercapto compounds. Between 48 to 72 hours after a repeated exposure, erythema is often observed. Finally, a third possible type of reaction is the so-called irritant contact dermatitis, which is often associated with latex but not caused by latex itself. The alkaline pH found in many powdered gloves has been implicated in this kind of reaction [5]. IgE-mediated allergy is caused by direct skin contact with latex products or by inhalation of airborne latex particles. Latex proteins are the inducer of the specific IgE response; thus latex-sensitisation is the prerequisite for developing symptoms like rhinitis, conjunctivitis and/or allergic asthma. Anaphylactic reactions are possible but rare.

Latex sources and application areas

Manufactured products, such as surgical and household gloves, catheters, condoms, chux (washable rubber pads), tourniquets, baby pacifiers, and toy balloons are only some examples of items which may contain allergenic proteins capable of eliciting IgE-mediated allergy and probably also causing sensitization of exposed subjects [6]. An expanded list of latex-containing products used in operating rooms, post anaesthesia care units as well as those which are actually available in the community, together with latex-free alternatives was published recently by Hepner and Castells [7].

Risk groups for latex allergy

NRL exposure can take place either through direct skin/mucus membrane contact or via inhalation. Children with spina bifida (SB), health care workers (HCW), persons with a history of multiple surgeries, non-health care workers with exposure to latex such as hairdressers, charwomen and food-service workers, as well as rubber industry workers or subjects with food allergy and atopy, belong to a group with an elevated risk of developing NRL allergy. Table 1 summarizes some studies reporting prevalences of latex sensitization in different risk groups [8-13].

Table 1: Prevalence of natural rubber latex (NRL) sensitization

Spina bifida patients [8]	up to 72 %
Occupational exposure in health care jobs [9,16]	up to 30 %
Occupational exposure in non-health care jobs [10,11]	up to 11 %
Atopic individuals with NRL exposure [12]	up to 36 %
General population with atopic background [13]	up to 8.6 %
General population without atopic background [13]	up to 2.3 %

Depending on the definition of the groups studied and the methods for assessment of latex sensitization and/or allergy, the observed prevalence among children with SB ranges between 25-72% [8,14,15], whereas the prevalence among health care workers ranges between 0 and 30% [9,16]. In adult blood donors, two separate studies reported a sensitization frequency of about 6% to latex [17,18]. In contrast, the prevalence in the general population is between 0 and 2.3% [13,20]. Individuals who are allergic to latex products may experience allergic reactions due to cross-reactivity with a variety of fresh fruits, vegetables and nuts.

Diagnosis of NRL allergy

The diagnosis of NRL allergy should be based on both a positive clinical history and a positive *in vivo* test result. The determination and quantification of NRL-specific IgE antibodies determined using *in vitro* tests are not always associated to clinical NRL protein sensitivity. Based on the specific symptoms (urticaria or acute erythema, respiratory symptoms from allergic rhinitis to asthma) of the patient, challenge tests may also be performed.

As prerequisites for correct *in vivo* and *in vitro* testing, standardized skin prick test extracts and well-characterized allergen mixtures for IgE determination are necessary tools. Sources of NRL are of varying quality and difficult to standardize for diagnostic purposes. Therefore, knowledge of the protein content and the corresponding allergenic potential are important items. Although research activities have resulted in purified natural allergens from NRL, so far no agreement exists concerning the selection of allergens appropriate for the development of standardized reagents for reliable diagnosis of latex allergy.

Allergenic components of natural rubber latex

Natural rubber latex (NRL) is the name of a milky sap collected after trapping the *Hevea brasiliensis* tree (Fig. 1). A main constituent of this sap is the polymeric hydrocarbon cis-1, 4-polyisoprene. Only 1-2% of the fresh weight of *Hevea* latex is made up of proteins, which are heterogeneously distributed in the latex sap [21]. After ultra-centrifugation of the fresh latex sap, Moir [22] distinguished as many as nine fractions of the *Hevea* latex, but basically three main fractions

Figure 1: Trapping latex from a rubber tree

(the rubber phase, the C-serum and the bottom fraction (B-serum)) are easily discerned. Fig. 2 shows the distribution of protein content and the localization of characterized latex allergens in non-ammoniated latex after centrifugation according to references [21,22]. The rubber phase comprises the rubber particles and two main, insoluble proteins, which are extractable from the surface of the rubber particles. Most of the C-serum and B-serum proteins are water-soluble. Latex C-serum contains various proteins (more than 200 polypeptides) and some of them are enzymes associated with the rubber biosynthesis. In contrast, the B-serum contains a

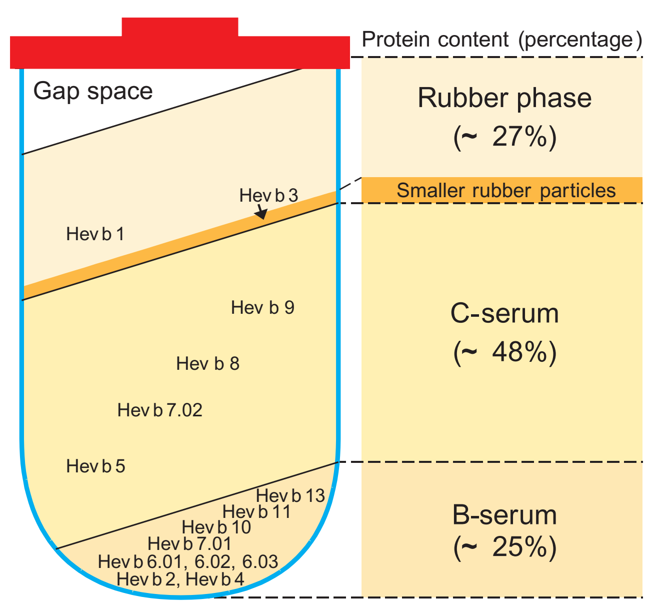

Figure 2: Distribution of protein content and localization of characterized allergens in non-ammoniated latex after centrifugation (53,000 g)

smaller number of proteins among which hevein is the most prominent and makes up more than 50% of the total soluble B-serum proteins. In general, the composition of the latex serum obtained by centrifugation may be very variable concerning its protein content, depending on the treatment of the latex after collection from the rubber tree as well as on variation in latex proteins due to genetic or environmental factors (e.g. significant seasonal differences in allergenicity of latex proteins from three of the most widely planted rubber clones: RRIM 600, GT 1 and PB 260) [21]. Approximately 25% of the proteins of all fractions bind IgE antibodies from NRL- allergic patients [23].

Research efforts have resulted in the identification of several latex allergens. Currently 16 allergens have been included in the latest nomenclature list of the International Nomenclature Committee of Allergens (IUIS) and assigned official numbers (http://www.allergen.org). Table 2 gives a short overview of the current status of characterized natural rubber latex allergens and displays selected data about their biochemical properties, biological function, and physiological role. Table 3 summarizes the immunological and clinical data.

Table 2: Overview of characterized natural rubber latex (NRL) allergens

Latex allergen*	Molecular mass (kDa)	Protein name, biological function or physiological role	Available as recombinant protein?	Reference
Hev b 1	14.6	Rubber elongation factor (REF)	Yes	25-27,64
Hev b 2	34-36	ß-1,3-glucanase	Yes	29
Hev b 3	24-27	Small rubber particle protein	Yes	30-35
Hev b 4	50-57	Beta-glucosidase (component of the microhelix complex)	No	29, 36
Hev b 5	16-24	Acidic structural protein	Yes	37-41
Hev b 6.01	20	Prohevein (hevein precursor)	Yes	42-46
Hev b 6.02	4.7	Hevein	Yes	43, 44
Hev b 6.03	14	C-domaine of prohevein	Yes	43
Hev b 7.01	42	Patatin-like protein (esterase) from latex B-serum	Yes	47-50
Hev b 7.02	44	Patatin-like protein (esterase) from latex C-serum	Yes	50
Hev b 8	14	Profilin (actin-binding protein)	Yes	51-53
Hev b 9	51	Enolase	Yes	54
Hev b 10	26	Manganese (Mn) superoxide dismutase (MnSOD)	Yes	55, 56
Hev b 11	32	Class I chitinase	Yes	57, 58
Hev b 12	9	Lipid transfer protein	Yes	59
Hev b 13	42	Esterase	In progress	60

*IUIS nomenclature

Table 3: Immunological and clinical properties of characterized latex allergens
LAP= latex allergic patients, HCW= health care workers, SB= spina bifida patients

Latex allergen*	Significance as Latex allergen	Significance concerning cross-reactivity	IgE-binding prevalence of the allergen	Reference
Hev b 1	**High** (especially in spina bifida patients)	**Not observed yet**	HCW: 55/105 (52%) SB: 56/ 69 (81%)	27, 28
Hev b 2	**Medium**	**Medium**	HCW: 20/ 31 (65%) SB: 7/ 13 (54%)	63, 65
Hev b 3	**High** (especially in spina bifida patients)	**Not observed yet**	HCW: 13-20% SB: 76-78%	35, 61, 62
Hev b 4	**Not determined**	**Not observed yet**	No clear results	29, 60, 63
Hev b 5	**High** all risk groups: HCW, spina bifida, atopics	**Not observed yet** (structure homology with a kiwi fruit protein)	HCW: 68-92% SB: 33-56%	38, 61, 62
Hev b 6.01	**High** all risk groups: HCW, spina bifida, atopics	**High** (especially with banana, kiwi, avocado)	LAP: 15/20 (75%) LAP: 24/29 (83%)	42, 43
Hev b 6.02	**High** all risk groups: HCW, spina bifda, atopics	**High** (especially with banana, kiwi, avocado etc.; main IgE-binding epitope)	LAP: 24/ 43 (56%) HCW: 48/ 64 (75%) SB: 3/ 11 (27%)	43, 44
Hev b 6.03	**High** in context with Hev b 6.01	**High** (structure homology to plant stress proteins)	LAP: 3/20 (15%) LAP: 11/ 52 (21%)	44
Hev b 7.01	**Low-Medium**	**Unclear** structural homology to proteins from potato and tomato but no cross-reactivity with banana and avocado	LAP: 4/36 (11%) LAP: 17/ 35 (49%)	45, 49, 50
Hev b 7.02	**Medium only in SB**	**Unclear** see Hev b 7.01	SB: 15/38 (39.5%)	50, 66
Hev b 8	**Low**	**Medium** (profilin is a ubiquitous pan-allergen)	LAP: 2/19 (11%) HCW: 20-24% SB:6-12%	51-53
Hev b 9	**Low**	**Medium** Cross-reactivity with moulds	LAP: 16/110 (15%)	54
Hev b 10	**Low**	**Medium** Cross-reactivity with moulds	HCW: 0/20, SB: 2/20 LAP: 4/15 (27%)	55, 56
Hev b 11	**Low**	**High** Cross-reactivity with fruits and allergens especially hevein-like sequences	LAP: 10/57 (19%) LAP (53 HCW, 5SB): 17/58 (29%)	57, 58
Hev b 12	**Low**	**Medium** pan-allergen; cross-reactivity with fruits	LAP: 9/37 (24%)	59
Hev b 13	**High**	**Not determined yet**	HCWs by SPT: 39/62 (63%)	60

*IUIS nomenclature

Properties of individual latex allergens

Rubber particle proteins

The rubber particle proteins comprise the two allergens Hev b 1 and Hev b 3, both major allergens strongly associated with latex allergy in SB patients.

Dennis and Light [24] described a non-glycosylated protein comprising 137 amino acid residues with a molecular mass of 14.6 kDa and named it **rubber elongation factor** (REF). Czuppon *et al*. [25] discovered the IgE-binding reactivity of this protein, introduced it as the first major allergen of *Hevea brasiliensis* and named it **Hev b 1**. Hev b 1 is a latex-specific allergen without relevant homology to other plant proteins. In a study by Chen *et al*. [27], 81% of SB patients and 50% of HCWs with latex allergy showed IgE reactivity to this protein. In a multi-centre study, Kurup *et al*. [63] observed similar frequencies of IgE reactivity. 54-100% of SB patients with latex allergy reacted to Hev b 1 whereas a frequency of only 13-32% was observed in HCWs. Studies performed with recombinant Hev b 1 (rHev b 1) using the ImmunoCAP™ method showed that 16 out of 71 latex-allergic HCWs (23%) had IgE antibodies to rHev b 1 [28] and confirmed the results of studies performed with the native counterpart. Additionally, B- as well as T-cell epitopes in Hev b 1 have been identified [64]. In summary Hev b 1 is one of the major latex allergens in SB patients and is of intermediate relevance in the risk group HCW. rHev b 1 ImmunoCAP™ is now available for *in vitro* IgE diagnostics (Rk215).

Hev b 3 (23 kDa), forms an integrated part of the **small rubber particles** and is free of posttranslational modifications like glycosylation. IgE-reactivity of this protein was first described by Alenius *et al*. [30]. Several studies with sera of latex-sensitized SB patients showed IgE reactivity frequencies of 67-83% [31,32]. The reason for these observed high frequencies may be due to stretches of high sequence homology between Hev b 3 and Hev b 1[33]. I contrast, the reactivity to Hev b 3 is less frequent among HCW. Recent studies by Kurup *et al*. [63] and Yip *et al*. [65] displayed a Hev b 3-specific skin prick test sensitivity in only 7% of the latex allergic HCWs tested. *In vitro* measurements with recombinant Hev b 3 (rHev b 3) coupled to ImmunoCAP™ (Rk217) revealed a comparable frequency of 12.5% in 40 latex allergic HCWs tested [61,62].

C-serum proteins

Four characterized latex allergens; Hev b 5, Hev b 7.02, Hev b 8, and Hev b 9, belong to the group of C-serum proteins, which are present in the cytosol fraction of the latex.

The most important allergen of this subgroup is **Hev b 5, an acidic (pH 3.5) and heat-stable 16-24-kDa protein**, rich in glutamatic acid as well as proline residues. Its physiological function is unknown. Native Hev b 5 was characterized by Akasawa *et al*. [37], whereas the first recombinant Hev b 5 (rHev b 5) was described by Slater *et al*. [38]. Hev b 5 protein displays 46% homology to an acidic protein of the kiwi fruit (pKIWI 501) and is present only in small amounts in NRL. Therefore, most of the research was performed with the recombinant form rHev b 5. In serological tests 92% of latex-allergic adult HCWs and 56% of the SB patients showed Hev b 5-specifc IgE antibodies in their sera [38]. Furthermore it was recently shown that rHev b 5 could be used as a complement reagent to enhance the quantitative performance of latex ImmunoCAP™ for specific IgE measurement [41]. A significant number (16%) of serum samples became more strongly positive to the improved k82 (spiked with rHev b 5) than to the regular k82 latex ImmunoCAP™, and a rather small number of previously negative serum samples became positive [41]. Hev b 5 may be the missing allergen to fill the diagnostic gap for some allergic patients with clear clinical latex allergy but with negative serological reactivity. rHev b 5 ImmunoCAP™ (Rk218) is now available as a single allergen for IgE *in vitro* diagnostics.

Hev b 7.02 is a 44-kDa allergen in the C-serum [50], which is also present as a 42 kDa-protein in the B-serum (Hev b 7.01) [47]. Both Hev b 7 isoforms are non-glycosylated although their cDNAs show two potential glycosylation sites [49]. Both also have comparable properties and sequence homologies (42 and 39%) with the potato storage protein patatin [50] but there exists no evidence for cross-reactivity with neither patatin nor proteins from banana or avocado [50]. Nevertheless Hev b 7 was named the **patatin-like protein**. Furthermore Hev b 7 displays esterase activity in both its native and recombinant forms. The latter (rHev b 7) is available from several laboratories [48-50]. In a recent study, Wagner *et al*. [66] demonstrated that rHev b 7.02 has the same properties as its native counterpart and is associated with latex allergy in SB patients. 15 out of 38 SB patients (39.5%) showed Hev b 7.02-specific IgE antibodies. Several other studies in latex-allergic adults revealed Hev b 7-specific IgE antibody frequencies between 11 and 39% [47,49,65].

The IgE-binding reactivity of the 14 kDa-protein **Hev b 8**, the **profilin** of *Hevea brasiliensis*, was initially identified by Vallier *et al*. [51]. Subsequently, recombinant isoforms of Hev b 8 with marginal differences in the amino acid sequence were described [52,53]. These differences obviously have no influence on the IgE-binding properties of the rHev b 8 isoforms. The observed frequencies of Hev b 8-specific IgE antibodies in sera of latex-allergic patients in different risk groups range between 6 and 24% [51-53]. Since profilin is a pan-allergen and the cross-reactivity between pollen and food is intensively studied, it seems that in the majority of cases the sensitization occurs via profilins of pollen or food origin [53]. However, its relevance in the latex-fruit allergy syndrome has to be clarified in further studies. rHev b 8 ImmunoCAP™ (Rk221) can now be used for *in vitro* diagnostics.

Hev b 9 is the **enolase** of *Hevea brasiliensis*, an enzyme with a molecular mass of 47.6 kDa [23]. Enolase is a ubiquitous protein, which is involved in the carbohydrate catabolism pathway. A sequence identity of about 60% was reported between the enolase of *Hevea brasiliensis* and the enolases of moulds [54]. Using a recombinant Hev b 9 (rHev b 9), IgE-binding reactivity was observed in 16 out of 110 latex-allergic adults (14.5%). Further, IgE-inhibition experiments with the enolases of *Cladosporium herbarum* and *Alternaria alternata* confirmed the cross-reactive potential of Hev b 9 [54]. In a recent study we found only one out of 40 HCWs tested and no patient with SB to have Hev b 9-specifc IgE antibodies [62]. rHev b 9 ImmunoCAP™ (Rk222) is now available as a single allergen for IgE-binding tests.

B-serum proteins

The B-serum currently includes a group of nine characterized latex allergens (Hev b 2, Hev b 4, Hev b 6.01, Hev b 6.02, Hev b 6.03, Hev b 7.01, Hev b 10, Hev b 11, Hev b 13) and comprises the extracytosolic proteins. With the exception of Hev b 7.01, all belong to the group of plant defence proteins. A tenth allergen belonging to this group is Hev b 12, a lipid transfer protein that was recently characterized in its recombinant form [59]. Its location in latex is still unknown.

Hev b 2, a **ß-1,3-glucanase**, is a basic enzyme with IgE-binding properties in 20-61% of latex-allergic patients, depending on the analysis method used [67]. Although IgE from a large proportion of latex-allergic patients binds to native Hev b 2, the same IgE is much less reactive to the recombinant version (produced in *E. coli*). Conformational differences or posttranslational modifications may be responsible. Nevertheless, recombinant Hev b 2 synthesized in yeast (which performs glycosylation) is similarly unreactive to IgE, although it was not clear if the yeast carbohydrate is similar to that of the native protein [21]. rHev b 2 ImmunoCAP™ (Rk216) is now available for IgE antibody analysis.

Hev b 4 was initially described by Sunderasan *et al*. [29] as a glycosylated polypeptide component of a triplet complex (**microhelix protein**), with a molecular mass of 50-57 kDa under reducing conditions. So far, the function and properties of Hev b 4 are not fully understood, although a recent study suggests that this protein might be a cyanogenic glucosidase [36]. A recombinant form has so far not been reported. A single IgE-binding study with the native Hev b 4, which is only soluble in the presence of high ionic concentrations, revealed IgE binding in 65% of the HCWs and 77% in the SB patients tested [63]. Nevertheless, since also control subjects without NRL allergy showed Hev b 4-specific IgE-binding, further studies are necessary to elucidate the role of Hev b 4 in latex allergy [67]. Positive skin prick tests with Hev b 4 were reported in 39% of 62 latex-allergic patients tested [60].

One of the most important NRL allergens for HCW appears to be **prohevein**, a 20 kDa precursor protein designated by the WHO/IUIS as Hev b 6.01. Posttranslational cleavage generates two further proteins, the 4.7-kDa **hevein** (**Hev b 6.02**) and the 14-kDa **C-terminal domain Hev b 6.03**. All three allergens exist in the plant, although the ratio between Hev b 6.01 and Hev b 6.03 is about 30:1 [21]. Hevein comprises the most important part of IgE-binding epitopes in the prohevein molecule. In addition, hevein shows homology to several chitin-binding lectins domains [23,46] and may be responsible for certain cross-reactivity with several other plants and foods [21]. Both rHev b 6.01 (Rk219) and rHev b 6.02 (Rk220) are now available as ImmunoCAP™ tests and in a recent study helped to characterize the cause of an anaphylactic reaction to apple juice containing acerola [70].

Hev b 7.01 is the variant of the **patatin-like protein** found in the B-serum [47] and has properties comparable to its C-serum counterpart Hev b 7.02 (for details see Hev b 7.02). There is some evidence that Hev b 7 may be associated with not yet characterized particles in the cytoplasm, which either sediment with lutoid-bodies (Hev b 7.01) or remain in the cytosol fraction (Hev b 7.02) depending on centrifugation conditions [72].

Hev b 10, manganese-superoxide dismutase (MnSOD) is a 22.9-kDa-enzyme showing sequence identity to human MnSOD as well as that of *Aspergilus fumigatus* (Asp f 6) [56]. Therefore, Hev b 10 is the second *Hevea* allergen with cross-reactivity to moulds. IgE-binding studies of 20 latex-allergic HCW and 20 latex-sensitive SB patient sera revealed the presence of Hev b 10-specifc IgE antibodies in two SB sera [55]. A further study using selected sera of patients with latex and *Aspergillus fumigatus* allergy or ones with *Aspergillus fumigatus* allergy alone showed Hev b 10-specific IgE antibody frequencies of 27% (4/15) and 50% (3/6), respectively [56].

Hev b 11 is a 32-kDa **class I chitinase** recently identified by O´Riordain *et al*. [57]. It shows cross-reactivity with fruits such as avocado, banana and kiwi, and is involved in the latex-fruit syndrome. The reason for the cross-reactivity with fruit chitinases is a 43 amino acid residue long hevein domain showing a 56% identity with that of rHev b 6.01 [46]. IgE-binding studies performed with a recombinant Hev b 11 (rHev b 11) isoform revealed Hev b 11-specific reactivity in 17 of 58 (29%) sera of latex-allergic patients [58]. Recombinant rHev b 11 ImmunoCAP™ (Rk224) is now available.

The 9-kDa **lipid transfer protein** (LTP) of *Hevea brasiliensis* **Hev b 12** was recently identified by Beezhold *et al*. [59] using recombinant DNA techniques. LTPs belong to the PR-14 family of pathogenesis-related proteins and have been identified as allergens in numerous plants, especially of the Rosaceae family, including fruits like apple, peach apricot and plum. LTPs are in general highly conserved and characterized by eight cysteine residues, forming four disulfide bridges, which are responsible for the stable protein structure with respect to temperature changes and ionic strength. IgE-binding studies in 97 latex-allergic individuals revealed Hev b 12-specifc IgE in 24% [59]. Other studies utilized a recombinant Hev b 12 (rHev b 12) coupled to ImmunoCAP™ and determined the Hev b 12-specifc IgE in sera of 48 peach-allergic patiens, who in part also presented symptoms of latex allergy. 6 out of 48 (12.5%) sera showed IgE antibody values $\geq 0.35\ kU_A/L$ indicating a cross-reactive potential of this allergen especially in fruit-allergic individuals (Rihs *et al*., unpublished). So far, the natural form of Hev b 12 has not been isolated.

Hev b 13 is an **esterase** with a molecular mass of 43 kDa. It has been shown that some homology exists with the early nodule specific protein (ENSP) of *Glycine* max and Hev b 13 has therefore also been named ENSP-like protein [21]. Skin prick tests with the native Hev b 13 revealed positive results in 45% of 62 latex-allergic patients tested [60]. So far Hev b 13 is not available as a recombinant protein. Analysis of the Hev b 13 amino acid sequence revealed three potential glycosylation sites. Whether these sites carry attached glycans and play a role in IgE binding is uncertain and the subject of current research [71].

Utility of recombinant latex allergens

A clear advantage of the recombinant proteins in general is the possibility to produce large-scale quantities at highly reproducible quality. Since the appearance of the first recombinant latex allergen Hev b 1 [26,28], more than a dozen latex allergens have been produced in recombinant form. Most of the recombinant latex allergens are produced in *E. coli* due to the fact that they contain no or minor posttranslational modification(s). Recombinant allergens also facilitate the study of the molecular basis of the immune-reactivity of the proteins, but they have to be validated against native proteins for equivalence in allergenic reactivity before they can be more widely adopted for clinical use. With respect to validation, our data demonstrate a very good correlation between recombinant and native Hev b 6.02 tested with the ImmunoCAP™ assay (Fig. 3). In contrast, the correlation between IgE-reactivity to recombinant and native Hev b 2 is strongly restricted although the missing glycosylation of the recombinant Hev b 2 (rHev b 2) seems not to be the deciding factor.

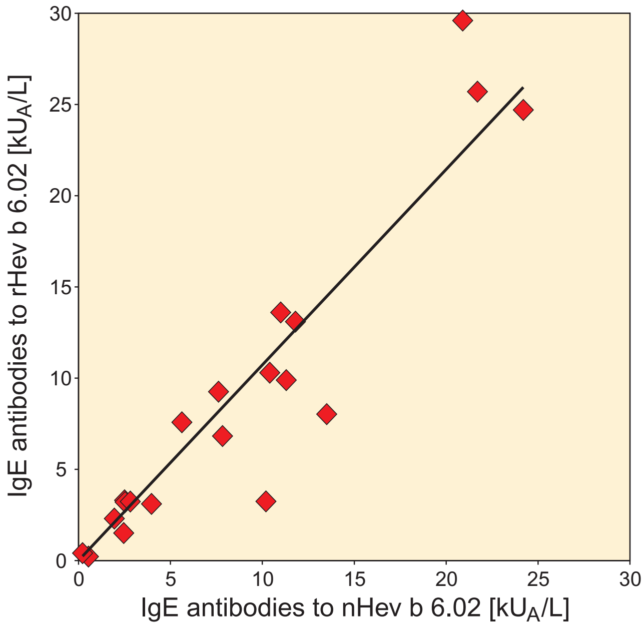

Figure 3: Correlation of IgE antibody values to rHev b 6.02 and to nHev b 6.02 determined by ImmunoCAP™

Serological assays for latex-specific IgE determination

The ability of specific IgE assay methods to detect relevant sensitization to latex has been examined in a number of studies. Pharmacia CAP System™ has been shown to exhibit a diagnostic sensitivity of 97% and a specificity of 84%, using the conventional detection limit of 0.35 kU$_A$/L [68].

Other studies have demonstrated cases of disagreement between results of latex-specific IgE analysis and SPT with latex extract [69]. This phenomenon may be explained by the occurrence of non-specific skin reactivity to the allergen extract, failure of *in vitro* tests to detect certain latex-specific IgE antibodies, or (most likely) a combination of both.

False negative IgE antibody test results may be due to the absence or shortage of some allergen components in the allergen extract used for antibody capture [40]. Indeed, 8 out of 16 subjects with clear latex allergy but negative IgE test results showed IgE reactive to rHev b 5. This finding suggested that latex allergen Hev b 5 might play a special role in diagnostic tests showing false negative IgE results.

Drawing from these results, a latex ImmunoCAP™ with rHev b 5 added to the latex extract was developed and used to re-test a panel of serum samples of confirmed latex allergics. With the rHev b 5-complemented test, a significantly higher IgE antibody level was obtained for a proportion of the sera (16%) and a smaller number of sera converted from negative to positive test results [41].

These results indicate a new approach to the improvement of allergen extracts used for *in vitro* tests: if a relevant allergen component is found to be present in suboptimal amount in a natural allergen extract, that component can be added as a stable recombinant protein to the extract preparation during production. Since the autumn of 2001, a new latex ImmunoCAP™ ("k82 spiked with rHev b 5") is commercially available as an improved *in vitro* test for latex sensitization.

Another new development is the availablility of ImmunoCAP™ tests for individual latex allergens, which may be used to easily determine allergen sensitization profiles in different groups of latex allergic patients [61,62].

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New Horizons – Allergy –

Published by Phadia AB,
P O Box 6460, SE-751 37 Uppsala, Sweden
Editor: Kerstin Wall
Phone: +46 18 16 50 00,
Fax: +46 18 16 63 23
E-mail: marketing.diagnostics@phadia.com