

REVIEW

Latex allergens

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Introduction

Latex type I allergy has become a serious problem especially for health care workers (HCW) and patients undergoing repeated surgeries like spina bifida (SB) children since allergenic proteins of natural rubber latex (NRL) are present in a variety of latex products [1]. Sensitization occurs from skin, mucosal and wound contact or inhalation of airborne allergens released from powdered latex gloves [2]. Clinical symptoms of latex allergy range from urticaria, conjunctivitis and rhinitis to asthma and anaphylactic shock. While no data about the incidence of latex allergy are available, some prevalence studies have been performed in Europe and USA. It is reported that up to 18% of surgeons and nurses working in operating theatres or examination rooms are affected [3,4], and up to 50% of patients with spina bifida become sensitized to latex-made articles during hospital stays [5]. Turjanmaa *et al.* [6] and Moneret-Vautrin *et al.* [7] found that the prevalence of latex sensitization among the general population is less than 1%. A recent survey by Miguel *et al.* [8] indicated a potential risk for the general population as well. They detected a significant amount of latex allergens or latex crossreactive material in sedimented and airborne particulate material derived from tyre debris.

For reliable, unequivocal diagnosis and prevention of latex allergy standardized reagents containing the proteins related to allergic symptoms are essential [9]. Therefore, the causative allergens are intensively studied and characterized. Here we present a comprehensive data collection on latex allergens investigated at the molecular level in order to demonstrate their individual importance in latex allergy.

Natural latex allergens (Table 1)

Field latex contains about 1–2% proteins and can be fractionated into three zones by high speed centrifugation: rubber particles at the top, the yellowish C-serum and the bottom fraction (B-serum). About 25–30% of the total protein content are bound to the rubber particle surface. The C-serum contains aqueous proteins and proteins released from damaged luteoids [10] that comprise most of

the bottom fraction. For the characterization of latex proteins, electrophoretic methods in combination with IgE-immunoblotting have been widely applied [11,12]. In some of these studies discrepant results were obtained, probably due to different latex extracts used as demonstrated by Kekwick *et al.* [13]. They found that the most potent source of allergenic proteins both for skin-prick tests (SPT) and immunoblotting was the luteoid fraction of fresh, non-preserved latex compared with ammoniated latex.

Particle-bound proteins

Two major particle-bound latex proteins [14] exist which are rarely soluble in water and can be extracted by detergent-containing solutions. While the 14.6 kDa protein is mainly found on large rubber particles (> 350 nm), the 23 kDa polypeptide is located on small rubber particles (average diameter 70 nm).

Rubber elongation factor (Hev b 1) The 14.6 kDa latex protein represents the 'rubber elongation factor' (REF). According to Dennis and Light [15], REF is necessary for latex prenyltransferase to add multiple cis-isoprene units to rubber molecules. REF was the first latex allergen identified and termed Hev b 1; it was discovered and isolated from gloves by Czuppon *et al.* [16]. Recent studies by Chen *et al.* [17] indicate that 50% of latex-allergic HCW ($n=105$) and 80% of SB patients ($n=69$) are sensitized to this protein as investigated by skin-prick testing (SPT) and EAST measurements. For this comprehensive study, pure Hev b 1 preparations as determined by two-dimensional (2D) electrophoresis and mass spectrometry have been used. According to these results, Hev b 1 can be regarded as an important latex allergen for latex-sensitized SB patients and to a minor degree for HCWs. Alenius *et al.* [18] and Yeang *et al.* [14] only partially confirmed the allergenicity of Hev b 1 when examining latex-allergic SB patients. Alenius *et al.* found only in four out of 39 adult latex-allergic patients IgE antibodies to purified REF by ELISA. Yeang *et al.* were not able to detect Hev b 1 specific IgE antibodies within a group of 20 adult latex-allergic patients by recombinant derived REF in immunoblotting. The discrepant results on the sensitization frequency among HCWs have not been clarified yet; they are probably caused by different antigens,

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Table 1. Identified and characterized latex allergens of *Hevea brasiliensis*

Identification/ homology	kDa	pI	Description/biological function	Recombinant available	Present in NRL products	Numbers of sensitized individuals ³	Methods	References
hevein Hev b 6.02	4.7	4.9	Homology to several chitin-binding proteins. Suggested to be involved in the coagulation of latex	+	+	48/64 (HCW) 3/11 (SB) 17/21 (HCW)	EAST EAST SPT	25, 30, 31
C-domain of prohevein Hev b 6.03	14	6.4, 7.7, 7.2	Homology to wound-inducible proteins, e.g., WIN1 of potato	+	unknown	11/52 (32A, 14C, 6SB)	ELISA	25, 29, 31
rubber elongation factor (REF) Hev b 1	14.6	4.9	Tightly associated with rubber particles. REF plays a role in rubber elongation	+	+	56/69 (SB) 55/105 (HCW) 10/12 (SB) 4/39 (31A, 8C) 0/20 (A)	EAST EAST SPT ELISA immunoblot	14, 17, 18
Hev b 5	16	3.5	Homologous with kiwi fruit protein pKIWI501, function unknown	+	+	12/13 (HCW) 32/57 (SB)	RAST RAST	37, 38
prohevein Hev b 6.01	20	5.6	Two-domains proteins that is processed into a N-terminal domain (hevein) and a C-terminal domain	+	unknown	17/20 A 4/6 SB	immunoblot immunoblot	29, 31
Hev b 3	23	4.8	Tightly associated with rubber particles. Homologous with REF; function unknown.	–	+	36/52 (32A, 14C, 6SB) 12/15 (SB) 13/17 (SB) 1/5 (HCW)	ELISA immunoblot ELISA ELISA	21, 22, 23
hevamine	30	8.7	Bifunctional enzyme with lysozyme/chitinase activity	–	unknown	1/29 (20A, 6SB, 3 C)	immunoblot	29
endo-1,3- β -glucosidase Hev b 2	36	9.3	Pathogenesis related protein class 2	–	+	6/29 (20A, 6SB, 3 C)	immunoblot	29, 35
patatin-like protein Hev b 7	46	5.3	Patatins are storage protein of the <i>Solanaceae</i>	–	unknown	9/40 (HCW)	immunoblot	39, 40
Hev b 4	50–57 ¹		4.5					
–	immunoblot	35	protein complex	Component of the B-serum microhelix			–	unknown

¹ reduced form in SDS-PAGE

² different isoforms in 2D electrophoresis

³ HCW, health care worker; SB, spina bifida; A, adult (profession not specified); C, children without congenital malformations.

different methods (ELISA, EAST, immunoblot) or patient groups with heterogeneous exposure and sensitization patterns. Chen *et al.* [17] investigated the sensitization frequency of Hev b 1 in HCWs occupationally exposed to latex, while Alenius *et al.* [18] and Yeang *et al.* [14] also used sera from adult patients with other professions.

Recently, also B-cell epitopes of REF have been determined by nine synthetic overlapping peptides (19mers) covering the whole sequence of REF. Chen *et al.* [19] found that the most allergenic/antigenic regions of Hev b 1 are located at the C-terminus and within amino acid residues 31–64. In addition, Hev b 1-induced lymphocyte proliferation responses in 52% of 23 latex sensitized HCWs showed that Hev b 1 is an important antigen in this patient group [20]. The antigen-specific proliferation response to Hev b 1 was not associated with the respective specific IgE antibody levels.

23 kDa rubber particle protein (Hev b 3) The IgE-binding capacity of this protein was first described by Alenius *et al.* in 1993 [21]. Interestingly, the allergenicity of this particle protein seems to be mainly restricted to SB patients (Table 1), but this outcome has to be confirmed by using a larger HCW patient group. Both Alenius *et al.* [22] and Lu *et al.* [23] purified this polypeptide and obtained partial sequence information that showed a significant homology with REF. No biochemical function of this allergen that fragments immediately on the addition of B-serum proteins has been described.

C-serum proteins

By using high resolution two-dimensional (2D) electrophoresis for the characterization of C-serum proteins we could identify about 200 distinct C-serum protein spots with molecular weights from ~100 kDa to less than 6.5 kDa by silver staining [24]. About 60 proteins spots showed IgE-binding capacity in immunoblotting using pooled serum of HCWs. Meanwhile, many IgE-binding C-serum proteins have been characterized at the molecular level and the prevalence of corresponding sensitization among both risk groups has been investigated.

Prohevein (Hev b 6.01), hevein (Hev b 6.02) and prohevein C-domain (Hev b 6.03) Prohevein is a two-domain 20 kDa polypeptide, which is processed to a 4.7 kDa N-terminal (43 AA) and 14 kDa C-terminal domain (144 AA) [25–27]. The 4.7 kDa hevein is a small cysteine- and glycine-rich protein whose structure is homologous to lectins like wheat germ agglutinin and several endochitinases. Hevein can inhibit fungal growth due to its chitin binding capacity and is involved in the coagulation of latex [28]. The importance of prohevein as latex allergen was demonstrated by Alenius

et al. [29]. They found that 24/29 latex-allergic patients (including nine children) showed positive reactions in IgE-immunoblotting with purified prohevein. Recently, hevein was isolated by Chen *et al.* [30] and Alenius *et al.* [31], and both groups found that the IgE-reactivity of prohevein is mainly due to the hevein domain. About 80% of latex-allergic HCWs are sensitized to hevein, while the prevalence of sensitization of SB patients is about 30% [29]. In contrast, the sensitization frequency to the C-terminal domain of prohevein is 15–21% [31].

β -1,3-glucanase (Hev b 2) A 36 kDa IgE-binding protein was isolated by electroelution after SDS-PAGE and identified by protein sequencing to be homologous to several plant β -1,3-glucanases [29]. 6/29 latex-allergic patients were positive in IgE immunoblotting with purified allergen. β -1,3-glucanases (MW ~35 kDa) are pathogenesis-related (PR) proteins of class 2 and thought to be important plant defence-related products against fungal pathogens [32,33]. According to Brenton *et al.* [34], several isozymes of *hevea* β -1,3-glucanases exist, and they described both acidic and basic isoforms with pIs of 4.9, 5.5 and 9, 9.3, 10, respectively. Recently, Sunderasan *et al.* [35] demonstrated the allergenicity of a basic β -1,3-glucanase (pI 9.5; MW 34–36 kDa), but at the moment it is not clear whether the acidic isoforms exhibit IgE-binding capacity as well, and the pI-value of the IgE-binding β -1,3-glucanase described by Alenius *et al.* is still unknown.

Hev b 4 An acidic protein (pI 4.5) of the latex B-serum microhelix protein complex was identified by Sunderasan *et al.* [35] to be an IgE-binding protein (Hev b 4), but the frequency of respective sensitization among latex-allergic patients is unknown at present. This allergen appeared under reducing conditions of SDS-PAGE as a band of 50–57 kDa, while the molecular weight in its unreduced form was estimated to be about 100 kDa. In order to identify this allergen, N-terminal sequence analysis was performed, but the sequence alignment showed no homology to previously published sequence information.

Hev b 5 Akasawa *et al.* [36] identified a 25.6 kDa protein with a pI of 3.5 to react with IgE of sera of more than 50% adult latex-allergic patients ($n=29$) in immunoblotting. This allergen, termed Hev b 5 by Akasawa *et al.* [37], was purified by chromatographic means, and the molecular weight was reevaluated by mass spectrometry to be 16 kDa. Hev b 5 exhibited a high sequence homology to an acidic protein from kiwi fruit (pI ~3.7, MW 18.9 kDa) termed pKIWI501. This polypeptide appears in the early stage of fruit development, but its biological function is not known either. The allergenicity of Hev b 5 was further confirmed by dose-dependent histamine release using passively

sensitized human basophils. In an independent study, Slater *et al.* [38] screened a latex cDNA library with IgE of latex-allergic health care workers and identified an immunoreactive clone that was termed cDNA Hev b 5. Corresponding to Akasawa's findings, the deduced amino acid sequences of clone Hev b 5 showed a 46% homology with the kiwi fruit protein pKIWI501. In order to examine the IgE-reactivity more thoroughly, cDNA Hev b 5 was expressed as fusion protein rHev b 5/MBP. The uncleaved fusion protein exhibited significant binding of IgE in RAST in 12/13 latex-allergic HCWs and 32/57 SB patients.

46 kDa patatin-like allergen (Hev b 7) and other soluble allergens Beezhold *et al.* [39] reported that 9/40 latex-allergic HCWs recognized a 46 kDa latex protein in IgE immunoblotting. Recently, this protein was isolated and identified to be a patatin-like protein [40]. Patatins are storage proteins encoded by a multigene family found in plant families such as potato and tomato. They may have a dual role as a somatic storage protein and as an enzyme involved in host resistance.

Hevamine (29.5 kDa), a bifunctional enzyme with lysozyme/chitinase activity, is a defence-related protein of the rubber tree that can be classified into the PR-group 3. Two basic isoforms exist, namely hevine A which is most abundant and hevine B. According to studies of Alenius [29], hevine is not regarded as important allergen, since only 1/29 latex-allergic patients reacted in IgE-immunoblotting experiments. Yagami *et al.* [41] demonstrated that a 27 kDa protein (pI 9.5) with lysozyme activity extracted from ammoniated latex and latex gloves is an allergen. In a related study they showed the IgE-binding activity of a latex esterase [42], but only two sera of latex-allergic patients were used in both studies.

Allergens in NRL products

It is obvious that the individual latex proteins have different implications for latex-allergic HCWs and SB patients. About 80% of HCWs have hevein-specific IgE antibodies while only 27% of SB patients are sensitized to this allergen [30]. On the other hand, Hev b 1 and Hev b 3 are the major allergens for SB patients, but they have a secondary meaning for adult latex patients [17,18,22,23]. This phenomenon of different major allergens in both risk groups may be explained by several reasons like age (SB children usually have frequently latex contact from birth onwards), genetic factors [43] or main route of exposure and sensitization via mucosa, wounds, skin or airways. Additionally, qualitative and quantitative differences may exist in the latex protein profiles both risk groups are exposed to. Hamilton *et al.* [44] speculated that preferably the soluble proteins like Hev b 2 and Hev b 4 adhere to the cornstarch donning powder of the

gloves while the particle-associated allergens Hev b 1 and Hev b 3 are believed to stick to the latex matrix of the gloves. Up to now only few studies with limited information about the allergen profiles of latex gloves were performed and virtually nothing is known about the quantitative aspects of individual latex allergens in finished latex products [45]. Latex allergens present in latex products are suspected to be heat-stable polypeptides, since during manufacturing harsh conditions at high temperatures are applied. The allergenicity of individual latex proteins will be altered during that processes in two ways. Many IgE-binding epitopes of the proteins involved in latex allergy will probably be destroyed, but there is evidence that new ones exhibiting unpredictable immunogenic characteristics will be generated. In this context, Chen *et al.* [17] investigated the protein profile of five NRL gloves by REF-specific MoAbs. He found that REF represents the major allergenic component and that it forms high molecular weight aggregates, mainly 40-60 kDa, during glove production and speculated that the allergenicity of REF are enhanced due to an adjuvant effect. Besides high molecular weight aggregates of REF, 8-10 kDa fragments were also detected. The IgE-binding capacity of all Hev b 1 derivatives was confirmed by a serum pool derived from five SB patients.

Similarly, Lu *et al.* [23] developed a MoAb specific for Hev b 3 and they demonstrated that this 23 kDa particle-associated allergen was present in latex glove powders and on natural rubber latex gloves. However, in immunoblotting using glove extracts as target antigens, the MoAb failed to show strong reactivity while in ELISA distinct signals were obtained. These experimental findings indicate that this allergen is cleaved during preservation and/or manufacturing, but its epitopes are still preserved in an active form.

The IgE-binding capacity of hevein that contains four disulfide bridges was demonstrated by Alenius *et al.* [31]. They isolated six hydrophilic peptides from one highly allergenic latex glove by chromatography. They were identified to be 100% homologous to mature hevein by protein sequencing and mass spectrometry. Glove-derived hevein was able to inhibit 72% of IgE-binding from pooled sera of latex-allergic patients to solid phase glove extract indicating that the allergenicity of hevein was not significantly altered during glove manufacturing. According to Akasawa *et al.* [37], Hev b 5 is also present in most of the glove extracts investigated and it seems to be a heat-stable polypeptide. A latex extract containing Hev b 5 was subjected to an autoclaving procedure simulating a condition used in the manufacture of latex gloves. While most of the other proteins precipitated near the sample loading zone during IEF, the electrophoretic mobility of Hev b 5 was not altered demonstrating that this polypeptide was unaffected by autoclaving. Yagami *et al.* [46] verified the existence of latex esterase, chitinase, lysozyme and β -1,3-glucanase in

both surgical and household gloves by measuring their specific hydrolytic activities. This study demonstrates the enormous but admittedly unexpected heat-stability of these enzymes. Recent investigations demonstrated that these enzymes are involved in latex allergy.

Co-sensitization and crossreactivity of latex allergens

Several case reports showed that latex allergy is often accompanied by sensitization to foods like banana, avocado, chestnut and kiwi. In a comprehensive study, Beezhold *et al.* [40] investigated the co-sensitization between latex and food suspected to be associated with latex allergy by skin-prick testing. Fifty-three per cent of 47 latex-allergic patients showed positive skin reactions to avocado, followed by potato (40%), banana (38%), tomato (28%), chestnut (28%) and kiwi (17%). The sensitization frequency among latex allergics was significantly increased when compared with 46 control persons. However, it has to be considered that the majority of positive food skin tests was not associated with clinically relevant symptoms.

While great efforts have been undertaken to identify and characterize the proteins responsible for latex allergy, only few information are simultaneously available on their crossreactivity behaviour. Extensive studies are missing using isolated latex allergens for inhibition experiments to analyse common features with IgE-binding food proteins. On the other hand, allergy to fruit like avocado, kiwi and banana are quite uncommon, and the causative allergens are poorly tagged. In most cases, SDS-PAGE followed by immunoblotting has been applied for the characterization of the crossreacting food allergens [47–49]. However, rarely no attempts have been made to isolate and identify the proteins which could be responsible for food intolerance in patients allergic to latex. Akasawa *et al.* [50] identified an avocado chitinase as one of the crossreacting proteins using the sera of patients with latex allergy. Fourteen out of 22 patients reacted to the 30 kDa avocado chitinase. Yagami *et al.* [46] speculated that the IgE-binding pathogenesis-related latex proteins like chitinase and β -1,3-glucanase are potential crossreacting proteins, since they are present in different plant families but show similar amino acid sequences and are serologically related. Hevein is a potential candidate as well, since this 43 amino acid motif has been found in plant lectins (e.g. wheat germ agglutinin) and various plant endochitinases. Recently, Mikkola *et al.* [51] demonstrated by immunoblot inhibition that hevein and a 33 kDa IgE binding banana protein share crossreacting epitopes. Patatins are storage proteins of the *Solanaceae*, and a patatin-like latex allergen (Hev b 7) was identified by Beezhold [40]. This may help to explain the unexpected sensitization to potato and tomato in some latex-allergic patients. Hev b 5 is highly homologous to the kiwi fruit

protein pKIWI501, but the immunological relationship of both proteins is unknown yet.

We could confirm co-sensitization between latex and food [52]. Sixty-eight per cent of our 31 adult latex allergic HCWs (19 atopics) showed immediate-type SPT reactions to avocado, followed by banana (35%), sweet pepper (35%), potato (32%), kiwi (30%), and/or tomato (16%). In 17 out of 21 subjects with SPT-positive reactions to avocado also specific IgE could be detected. Soluble latex C-serum proteins were able to completely inhibit avocado specific IgE in pooled sera of eight patients using avocado as target antigen. On the other hand, 20% cross-inhibition was achieved using soluble avocado proteins as inhibitor solution when measuring latex specific IgE. The IgE-inhibition experiments demonstrate that some latex allergenic structures are also shared with avocado proteins.

Concluding remarks

Since 1979, when natural rubber latex allergy was first reported by Nutter [53], enormous progress has been made in the identification of NRL allergens. This is a challenging task because NRL contains a very complex protein mixture consisting of both soluble and particle-bound proteins that may be additionally altered during preservation and production processes. Among the more than 200 polypeptides present in NRL, about 60 were found to bind IgE from sensitized patients. Nevertheless, two major latex allergens have been identified, i.e. soluble hevein for latex-allergic HCWs and particle-bound REF for SB patients. Meanwhile some more latex proteins (Table 1) have been investigated at the molecular level, and their sensitization potency has been determined for both risk groups. Additionally, some of the main latex allergens have also been detected in finished latex products like gloves, but no data exist about their qualitative and quantitative allergen profiles. The development of specific antibodies directed against purified key allergens would be a convenient way to perform quantitative immunoassays in order to screen latex allergens in very different sources.

This information may be useful to explain the different sensitization patterns of HCWs and SB patients. In summary, the role of many polypeptides in latex allergy is now well understood and has allowed better diagnostic tests and to monitor the success of prevention strategies (e.g. inactivation of IgE-binding proteins during production) that are needed to decrease the high prevalence of NRL allergy.

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