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Molecular and Immunological Characteristics of Latex Allergens

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Abstract

Natural rubber latex proteins are a well-recognized cause of type-I allergic reactions that increasingly afflict health-care workers, housekeeping personnel, and other persons using latex gloves or latex products. More than a dozen individual latex allergens have been identified of which eight have received an international nomenclature designation. To study the biochemical and immunological properties in detail, it is desirable to clone and express each of these allergens. Proteins that are involved in rubber synthesis are most likely confined to latex whereas other enzymes, such as chitinases or glucanases, are also present in fruits and may account for the symptoms observed in the latex-fruit syndrome. The available data on the molecular characterization of latex allergens presented here illustrate the importance of this research in the production of better diagnostic tests and, perhaps, tools for immunotherapy.

Introduction

Natural rubber latex (NRL) is widely used in the manufacturing of medical devices and in a variety of everyday articles. Latex is the milky sap produced by the laticiferous cells of the tropical rubber tree *Hevea brasiliensis* of the family Euphorbiaceae. The liquid latex is used to produce dipped products such as gloves, condoms and balloons. In a dried form it is used for the manufacture of baby nipples, syringe plungers and vial stoppers. Over the last decade, immunoglobulin E-mediated allergy to NRL has been acknowledged as a major occupational problem. Latex allergy is frequently seen in health-care workers (HCWs), rubber industry workers, pollen-allergic patients and subjects undergoing multiple surgical procedures in early infancy [1, 2]. Clinical symptoms arise in sensitized patients from direct contact with an NRL product or from inhalation of airborne allergens bound to glove powder. Symptoms manifest as contact urticaria, rhinoconjunctivitis, asthma and mucosal swelling. Systemic reactions consist of generalized urticaria and anaphylactic shock.

The presence of specific IgE in NRL-allergic individuals may be verified by skin prick tests (SPTs) and/or by serological methods such as RAST and related techniques and, for research purposes, immunoblotting. The limited knowledge of the relevant NRL allergens has been a major hindrance in developing optimal tests for diagnostic and rubber product-monitoring purposes. It is a generally known fact that commercially available latex extracts vary significantly with respect to their allergen composition and allergen concentration.

The great majority of the latex literature deals with clinical evaluations and description of syndromes. Since the first report suggesting cross-reactivity between NRL and banana [3] cross-reactivities between NRL and various food allergens has been established as the 'latex-fruit syndrome'. Latex allergy has been reported to be associated with allergy to a variety of plant foods such as banana, avocado, kiwi, chestnut and potato [4]. In addition, cross-reactivity of latex allergens with pollen allergens from mugwort, ragweed and timothy grass pollen have been described [5]. The presence of common epitopes might in part explain the clinical symptoms observed in patients allergic to pollen on contact with latex.

Recombinant Allergens

As there are well over a dozen allergens present in NRL, only recombinant proteins will assure a uniform and standardized mix for diagnostic tests and for possible therapeutic applications. Recombinant allergens have been shown to be valuable reagents for experimental and diagnostic studies. The data produced from the study of the major birch pollen allergen Bet v 1 clearly illustrates the progress made possible by the application of molecular biology in allergology. The cDNA sequence of Bet v 1 was published in 1989 [6]. In 1993 the recombinant protein became available [7] and in 1995 the existence of Bet v 1 isoforms was discovered [8]. In 1996 the possible use of hypoallergenic isoforms of Bet v 1 for immunotherapy began to be investigated [9], and in 1998 the first Bet v 1 'designer allergen', which had been calculated by a computer algorithm to include all known characteristics of low IgE-binding capacity but with intact T-cell stimulatory ability, was published [10].

As has been shown for the tree pollen allergens, recombinant latex allergens will also prove useful for in vitro and in vivo diagnosis and for immunotherapy in the long run. They may also be used to spike natural extracts to compensate for the lack or low concentration of the natural counterparts which account for the frequently observed batch-to-batch variations. Comparatively few studies have been published with respect to the molecular biology of latex allergens. RNA is not readily available and must be isolated from latex at the site of tapping, i.e. straight from the *H. brasiliensis* tree on a rubber plantation. A specially designed protocol needs to be followed to obtain high-quality RNA. This RNA is the absolute prerequisite for cloning of full-length allergen-encoding genes and represents the basic reagent for anyone interested in producing recombinant latex proteins.

Recombinant forms of latex allergens with homologues in fruits will be particularly important in properly diagnosing and delineating an individual's hypersensitivities as extracts of many fruits are not available in required qualities. Precisely diagnosing an allergic individual will allow the clinician to be very specific in recommendations on how to deal with the allergy, especially in advising which foods to avoid or in explaining where excessive restrictions in the diet are not necessary. Furthermore, the thorough characterization of NRL allergens at the molecular level will not only yield better diagnostic tests but perhaps also tools for immunotherapy. Information about the molecular features of latex allergens will also enable rubber manufacturers and governmental authorities to ensure that potentially harmful NRL gloves, medical devices, and consumer products are withheld from the market.

Latex Allergens with a Nomenclature Designation (table 1)

Hev b 1, Rubber Elongation Factor

Hev b 1 was the first latex allergen that was characterized on the molecular level. Czuppon et al. [11] isolated a 58-kD protein from raw latex and latex gloves. The allergen was a noncovalent homotetramer molecule, in which the 14.6-kD monomer was identified by amino acid composition and sequence homologies of tryptic peptides as the rubber elongation factor found in natural latex of the Malaysian rubber tree. Hev b 1 is a highly hydrophobic protein tightly bound to large rubber particles (diameter >350 nm) purified from *H. brasiliensis* latex and is necessary for prenyltransferases from a number of sources to add multiple cis-isoprene units to rubber molecules. Hev b 1 accounts for 10–60% of the total protein in whole latex but is absent in C serum, the supernatant fluid obtained when rubber particles are removed by centrifugation [12]. The amino acid sequence of Hev b 1 was determined and deposited in the database by Dennis et al. [13]. Comparison of the sequence of Hev b 1 with other known sequences did not reveal significant sequence similarities that would suggest an evolutionary relationship. The nucleotide sequence was determined by Attanayak et al. [14].

Alenius et al. [15] reported a relatively low frequency of IgE antibodies to purified Hev b 1 with the exception of spina bifida (SB) patients. In ELISA 4/39 (10.3%) latex-allergic patients had IgE antibodies to purified Hev b 1 and sera from 4/6 (67%) latex-allergic children with SB or other congenital abnormalities exhibited IgE antibodies to Hev b 1 [15]. Yeang et al. [16] produced and tested the recombinant Hev b 1. The authors concluded that it was not a major latex allergen, because its ability to bind IgE in immunoblots was confined to two serum pools from SB patients and not observed in 19 adults with clinical symptoms of latex allergy.

In contrast, Hev b 1 purified from latex sap induced lymphocyte proliferation responses in 12/23 (52%) latex-sensitized hospital employees indicating that Hev b 1 is a relevant allergen for HCWs [17]. Sera from 11 latex-allergic subjects including 3 from SB patients, 2 monoclonal antibodies raised against purified Hev b 1, and 3 rabbit antisera were employed to define B-cell epitopes on Hev b 1 [18]. The C-terminal portion of Hev b 1 and the region with amino acid residues 31–64 were determined as the most allergenic/antigenic. In both regions, the minimal IgE-binding epitope was almost identical to the IgG-binding epitope [18]. In a recent study, Chen et al. [19] suggested that Hev b 1 was not only a major allergen for SP patients, but also an important latex allergen for HCWs. 50/62 (81%) SB patients with IgE

Table 1. Latex allergens with a nomenclature designation

Hev b 1 , rubber elongation factor 137 aac, 14.590 kD, pI 5.04, EMBL: X56535, SWISS-PROT: P15252 One of the two latex allergens associated with spina bifida, minor/ major allergen
Hev b 2 , β -1,3-glucanase 374 aac, 41.306 kD, signal peptide: residues 1–36, C-terminal extension: residues 353–374, mature protein 35.088 kD, pI 9.5; EMBL: U22147, SWISS-PROT: P52407 Major and cross-reactive latex allergen
Hev b 3 , a protein homologous to Hev b 1 23–27 kD; pI 4.3–5.7, 5.1 (values vary as reported by several researchers) The second latex allergen associated with spina bifida
Hev b 4 , a microhelix component 50–57 kD; pI 4.5 The significance as an allergen has not been determined
Hev b 5 , acidic protein 152 aac, 16 kD, pI 3.5; EMBL: U51361, U42640 A major and probably a cross-reactive latex allergen (kiwi, buckwheat)
Hev b 6 , Prohevein/Hevein, 20 kD/4.7 kD 204 aac (21.859 kD), signal peptide 1–17, hevein 18–60 (43 aac, 4.7 kD, pI 4.9), C-terminal domain 61–204 (144 aac, 14 kD, pI 6.4–7.7), EMBL: M36986, SWISS-PROT: PO 2877 A major latex allergen
Hev b 7 , patatin-like protein 43–44 kD, EMBL: U80598 Minor latex allergen with potential cross-reactivity to proteins from members of the Solanaceae (potato, tomato) and avocado
Nucleotide and/or amino acid (aac) sequences where known are listed by their EMBL and/or SWISS-PROT accession numbers.

to latex allergens had Hev b 1-specific IgE, and 52/103 (50%) latex-allergic HCWs had IgE that bound to Hev b 1.

Hev b 2, β -1,3-Glucanase

A group of proteins called pathogenesis-related (PR) proteins are synthesized by plants in response to microbial infection. Several PR proteins have been identified as β -1,3-glucanases. Sequences of several distinct tobacco β -1,3-glucanase isoforms, both acidic (extracellular) and basic (intracellular), have been reported and the regulation of the gn1 isoform has been studied [20]. Clones encoding a basic β -1,3-glucanase were isolated from a cDNA library prepared from the latex of *H. brasiliensis* using the *Nicotiana plumbaginifolia* cDNA clone gn1 as a probe. Nucleotide sequence analysis showed that a 1.2 kb *Hevea* cDNA encoding a basic β -1,3-glucanase had 68% nucleotide similarity to the

gn1 cDNA [21]. The presence of an N-terminal extension (residues 1–36) and a C-terminal extension (residues 353–374) suggested that it belongs to class-I β -1,3-glucanases. Genomic Southern analysis indicated the presence of a low-copy gene family encoding β -1,3-glucanases in *H. brasiliensis*.

Alenius et al. [22] purified a 36-kD latex protein to homogeneity by HPLC gel filtration and reversed phase chromatography. The sequences of three tryptic peptides showed similarities from 67 to 83% to several plant endo- β -1,3-glucanases. Sequences of the peptides SWVQK and YIAVGNEISPVNR were identical to portions of the sequence published by Chye et al. [21], whereas the peptide SYLNPIIR differed in two amino acid residues. The purified 36-kD protein bound IgE from 6/29 (21%) adult latex-allergic patients' sera, but did not react with IgE from 6 SB patients [22].

The basic β -1,3-glucanase isolated from the B-serum of NRL was first described as the allergen Hev b 2 by Sunderasan et al. [23]. Hev b 2 appeared as a doublet of 34 kD in SDS gels and both bands were recognized by a monoclonal antibody. IgE from one latex-allergic patient was shown to bind to Hev b 2. Isoelectric focusing of Hev b 2 revealed a single spot at pI 9.5. The N-terminus of Hev b 2 was blocked, but cyanogen bromide digests produced peptides which were sequenced. One 11 residue peptide FDENNXP-PEVE was 90% identical to the published sequence and another 13 residue peptide RNIHDAIRSAGLQ was identical [23]. Both peptide sequences also showed a good similarity to the published sequences of endo- β -1,3-glucanases from *N. plumbaginifolia*, *N. tabacum* and tomato.

Hev b 3

In the literature, two polypeptides, which are frequently recognized by IgE of SB patients with latex allergy but not by IgE of latex-allergic HCWs are described. Alenius et al. [24] examined sera from 15 SB children and 5 children with other congenital anomalies for IgE antibodies to NRL antigens. IgE antibodies in 10/12 (83%) US SB patients and in 2/3 Finnish SB patients recognized a previously undescribed 27-kD allergen. Two patients with other congenital anomalies also had IgE to the 27-kD allergen. IgE antibodies to the 27-kD allergen were not detected in other latex-allergic patients. Alenius et al. [25] purified the 27-kD protein from NRL by HPLC. As the N-terminus was blocked the protein was digested with trypsin and the purified tryptic peptides were subjected to sequence analysis. Six of the 14 sequenced peptides (numbers 3, 8, 11a and b, 13, and 14) showed sequence similarities to stretches within the first 130 amino acid residues of Hev b 1.

Similarly, Lu et al. [26] reported a polypeptide (MW 23 kD; pI 4.8) to be closely associated with hypersensitivity in SB patients. The 90 amino acids sequenced from this 23-kD protein showed 45% similarity with Hev b 1, and it shared identical amino acid sequence motifs with the 27-kD protein described by Alenius et al. [25]. As the data profiles of both proteins match each other well it can be assumed that both research groups investigated the same protein. The purified 23-kD polypeptide reacted with IgE from 13/17 (76%) latex-allergic SB patients, but from only 1/5 HCWs with latex allergy. Furthermore, all the sera of patients with anaphylaxis to latex showed reactivity with the 23-kD polypeptide.

Yeang et al. [16] described a 24-kD protein found mainly on small rubber particles (average diameter 70 nm), which was recognized by IgE from latex-allergic SB patients, and named it Hev b 3. Hev b 3 has a tendency to fragment into several polypeptides of lower molecular weight (from 24 to about 5 kD) even when stored at -20°C . Hev b 3 fragments, resulting either from storage or by reacting with B serum, are electrophoretically discrete bands indicating that fragmentation occurs at predetermined sites on the molecule.

Hev b 4, a Microhelix Component

Hev b 4 was isolated from NRL B serum [23]. This acidic protein has a pI of 4.5 and appears under reducing conditions as a broad band of about 50–57 kD. One latex-allergic patient's serum was used in an immunoblot to show IgE binding to this band. Immuno-gold labelling with the monoclonal antibody USM/RB3 showed that Hev b 4 was a component of the microhelix protein complex of B serum. The luteoids, vacuole-like organelles, contain a fluid with several hydrolases and some pathogenesis-related proteins. Some highly confined proteins of luteoid B serum from complexes – the microfibrils and microhelices – that are large enough to be visible under an electron microscope. Amino acid sequencing of Hev b 4 revealed an N-terminal sequence of ELDEYLFSFGDGLYDAGNA, which did not match any previously published amino acid sequences. The significance of Hev b 4 as an allergen has not yet been determined.

Hev b 5

Hev b 5 was found by Akasawa et al. [27] as an acidic protein in extracts prepared from latex gloves, which were shown to elicit allergic reactions. From a latex cDNA library, the cDNA coding for this protein was isolated and sequenced. The deduced amino acid sequence showed a high degree of homology to an acidic protein identified in kiwi fruit *Actinidia deliciosa* var. *deliciosa*). The sequence homology (47% sequence identity) between these two acid-

ic proteins suggested a possible molecular explanation for the high frequency of allergic reactions to kiwi fruits in latex-allergic patients. However, the kiwi fruit protein appears in large quantities only during the early stages of fruit development.

Simultaneously, Slater et al. [28] isolated a cDNA clone by utilizing IgE from the serum of a latex-allergic HCW to screen a cDNA library from *Hevea* latex. The Hev b 5 cDNA encoded an open reading frame of 152 amino acid residues. The nucleotide and deduced protein sequences had significant similarity to an acidic (pI 3.5) 18.9-kD protein (SWISS-PROT P43393) from kiwi which is known to cause allergic reactions in some latex-allergic patients. Hev b 5 was expressed as the fusion protein rHev b 5/MBP. The uncleaved fusion protein bound IgE in RAST in 12/13 (92%) latex-allergic HCWs and in 32/57 (56%) of SB patients.

Hev b 6, Prohevein

A prohevein cDNA clone (HEV1) was isolated from a λ gt10 cDNA library [29]. HEV1 was 1,081 basepairs long and contained an open reading frame of 204 amino acids which coded for a putative signal sequence of 17 amino acids followed by a polypeptide of 187 amino acids. This polypeptide had two distinct domains, a 4.7-kD amino-terminal domain of 43 amino acids, corresponding to mature hevein, and a 14-kD carboxyl-terminal domain of 144 amino acids. The N-terminal region showed homology to wheat germ agglutinin (WGA) and several chitin-binding proteins and to the N-termini of wound-inducible proteins in potato and poplar. The C-terminal portion had 74–79% similarity to the C-terminal region of wound-inducible proteins of potato. Translocation experiments using an in vitro translation systems showed that the first 17-amino acid sequence encoded by the cDNA functions as a signal peptide [30]. Microsequencing data further suggested that the 4.7-kD amino-terminal domain and 14-kD carboxyl-terminal domain proteins were posttranslational cleavage products of the 20-kD polypeptide which corresponded to the preproprotein encoded by HEV1.

The primary structure of pseudo-hevein, a minor hevein component was determined [31]. Six differences with the sequence of the major hevein component were found. Analysis by ion-spray mass spectrometry showed that pseudo-hevein has a heterogeneous C-terminal extension of several glycine residues and that hevein itself also contains minor components with additional C-terminal amino acid residues. Sequence analysis of the C-terminal tryptic peptides of the C-terminal 14-kD domain of prohevein and comparison with the cDNA sequence indicated that they represent mature forms from which a C-terminal propeptide, possibly in-

volved in vacuolar targeting, has been removed [32]. The molar ratio of hevein to the C-terminal domain in the lutoid-body fraction of rubber latex is about 30:1. The lectin-like hevein, which is rich in cysteine and glycine, is involved in the coagulation of latex by bringing together rubber particles. This bridging involves a receptor glycoprotein which is localized on the surface of rubber particles [33]. The three-dimensional structure of hevein in aqueous solution obtained by NMR spectroscopy showed a close resemblance to the structures of the four domains of WGA [34].

The observed high frequency of IgE antibodies to prohevein suggests that this protein is a major NRL allergen. In immunoblotting, the prohevein purified from ultracentrifuged bottom fraction of NRL-bound IgE antibodies from 24/29 (83%) sera of latex-allergic patients including positive results in 4/6 latex-allergic SB children or other congenital anomalies [22]. To analyze which region of the molecule carries the most significant IgE-binding epitope(s), Alenius et al. [35] purified prohevein and its C-terminal domain from NRL by gel filtration, reverse phase chromatography, and electroelution. In immunoblotting, prohevein bound IgE from 15/20 (75%), and the prohevein C-domain from 3/20 (15%) latex-allergic patients sera. In ELISA, 36/52 (69%) patient sera showed IgE binding to prohevein, whereas 11/52 (21%) sera had IgE to prohevein C-domain. Purified hevein inhibited 72% of IgE binding from pooled sera of NRL-allergic patients to solid phase glove extract and 45% of IgE binding to solid phase NRL. Of the 43 NRL-allergic patient sera tested, 56% showed IgE to purified hevein in ELISA. In skin prick testing, purified hevein elicited positive reactions in three quarters of the latex-allergic patients tested. These results indicated that the majority of prohevein's IgE-binding ability resides in its N-terminal hevein fragment.

Specific IgE antibodies to hevein were detected by enzyme-linked allergosorbent test in 48/64 (75%) sera from HCWs allergic to latex and in 3/11 (27%) sera from SB patients with hypersensitivity reactions to latex [36]. Skin prick testing showed a positive reaction to hevein in 17/21 (81%) patients with latex allergy.

Beezhold et al. [37] identified six IgE epitopes in the hevein preprotein by probing overlapping synthetic peptides of 10 amino acids with pooled sera from 10 latex-allergic patients. Two were located in the N-terminal 5-kD hevein domain and four were observed in the 14-kD C-domain. A broad epitope was located between the N-terminal amino acids 13–24. This epitope had nearly complete identity to WGA. Immunological cross-reactivity to WGA was confirmed by Western blot analysis with purified WGA. Four other epitopes had homologies to defense-related proteins

in potato and tobacco. The significant homology of these epitopes to a broad family of plant defense proteins further explains the increased prevalence of food allergies in latex-allergic individuals.

Hev b 7, Patatin-Like Protein

Beezhold et al. [38] and Tomazic et al. [39] detected an abundant 45- to 46-kD protein that reacted very intensely with IgE antibodies of HCWs with established latex allergy. Beezhold et al. [38] identified this protein as highly homologous to the patatin storage proteins encoded by a multi-gene family in potato and tomato and determined the N-terminal sequence as LTQGKKLTVFSID. 9/40 (23%) of latex-allergic HCWs recognized this 46-kD protein found in non-ammoniated latex.

In a further study, the intact 46-kD protein and the 30-kD cyanogen bromide fragment were analyzed for primary structure [40]. The N-terminal amino acid sequence of the 46-kD protein (Latex P46 LTQGKKITVLSID_{XG}) showed 60% sequence similarity to patatin, a protein from potato. Likewise, the 30-kD cyanogen bromide fragment (Latex P46 CNBr30 QAKDEKDFYLENCPKIF) had 59% sequence similarity to an internal region from exon 2 of patatin. A cytosolic phospholipase A₂ from potato tissues, which is involved in signal transduction in the resistance reaction induced in potato by inoculation with the late blight fungus, appeared to be patatin [41].

In addition, RT-PCR amplified a product from latex sap RNA that was 246bp in length was found to be 67% identical to the published patatin nucleotide sequence [42]. Northern analysis of latex leaf and latex sap RNA demonstrated the message to be most abundant in latex sap and weakly present in latex leaf. Recently, Hev b 7 was cloned and expressed in *Pichia pastoris* and not only shown to bind latex-allergic patients' IgE but also to possess esterase activity [43].

Latex Allergens without a Nomenclature Designation (table 2)

Hevamine

Hevamine is a 30-kD basic protein from the lutoids (vacuoles) of rubber latex and may play a role in plugging the latex vessels and cessation of latex flow. It is an enzyme with both lysozyme and chitinase activity and was isolated and purified from *H. brasiliensis* latex. The enzyme is homologous to certain PR proteins from other plants [44]. The primary structure of hevamine was determined and the positions of three disulfide bridges were identified [45]. The sequence had about 60% identity with that of a chitinase from

Table 2. Latex allergens without a nomenclature designation

Hevamine 273 aac, 29.550 kD, SWISS-PROT: P23472 A minor latex allergen
Class-I chitinases 32 kD, contain chitin-binding motif at N-terminus Cross-reactive (banana, avocado) and very likely major latex allergens
Class-II chitinases 30 kD, do not contain a chitin-binding motif at the N-terminus Cross-reactive and very likely major latex allergens
Mn-superoxide dismutase (MnSOD) 233 aac, 25.839 kD, transit peptide 1–27, EMBL: L11707, SWISS-PROT: P35017 The significance is not yet determined, possible cross-reactive allergen between latex and moulds
Enolase (2-phosphoglycerate dehydratase) 46 kD, undetermined significance, a putative cross-reactive allergen between latex and moulds
Profilin 14 kD, a minor/major cross-reactive latex allergen
Latex lysozyme 27 kD, pI 9.5, undetermined significance, cross-reactivity with homologues from fig and papaya possible
Triosephosphate isomerase 27 kD, significance not determined
Proteasome subunit 25.3 kD, significance not determined
Nucleotide and/or amino acid (aac) sequences where known are listed by their EMBL and/or SWISS-PROT accession numbers.

cucumber and 95% with the N-terminal sequence of the lysozyme/chitinase of *Parthenocissus quinquefolia* [45]. The three-dimensional structure of hevamine was refined at 1.8-Å resolution and the final model consists of all 273 amino acid residues and 206 ordered water molecules [46]. The purified hevamine bound IgE from only 1/29 latex-allergic sera tested [22].

Class-I Chitinases

Nearly all plant chitinases isolated to day are endochitinases, i.e. they hydrolytically degrade chitin from within the polymer rather than at its terminus [47]. Class-I chitinases are basic and contain a cysteine-rich N-terminal domain with chitin-binding properties. The first indication of a 30-kD cross-reactive allergen between latex, avocado and banana was given by Lavaud et al. [48]. Allergens of natural

latex, latex gloves, avocado pear, and banana extracts were investigated by immunoblotting sera from patients with latex and fruit allergies. In sera from patients with latex and fruit allergy, prominent IgE binding was revealed at about 30kD with latex and fruit extracts. Cross-inhibition of immunoblotting confirmed that this major allergen was linked to a common epitope present in latex and fruits.

Akasawa et al. [49] isolated and identified an avocado chitinase (approximately 30kD) that reacted with IgE antibodies from 14/22 (64%) patients allergic to latex. The full cDNA sequence of a major allergen of avocado, its IgE-binding properties and its cross-reactivity with latex allergens were reported by Vanek-Krebitz et al. [50]. The cDNA clone obtained from a differential cDNA library coded for a class-I endochitinase. In immunoblotting, hevein-immunized rabbit serum identified a 33-kD banana protein which also bound IgE-antibodies from 7/16 latex-allergic patient sera [51]. In immunoblot inhibition, purified hevein completely inhibited IgE-binding to the 33-kD banana protein. These results suggested that cross-reacting IgE-binding epitopes are shared by hevein and a 33-kD banana protein.

Class-II Chitinases

Class-II chitinases are acidic, lack the N-terminal cysteine-rich domain and have a high sequence similarity to class-I chitinases within the catalytic domain [47]. A 19-residue N-terminal amino acid sequence obtained by two-dimensional immunoblot and protein microsequencing was published by Posch et al. [52]. A sequence alignment search for the peptide DIGSIISKSTFEEFLKXGN obtained from 'spot 12' exhibited a high sequence homology to several plant chitinases.

Mn-Superoxide Dismutase

An 11 residue N-terminal amino acid sequence obtained by two-dimensional immunoblot and protein microsequencing was published by Posch et al. [52]. A sequence alignment search for the peptide QTXSPDLPYD obtained from 'spot 20' showed identity to a matching portion of the MnSOD from *H. brasiliensis*. A genomic clone encoding manganese-containing superoxide dismutase (MnSOD) was isolated from a *H. brasiliensis* genomic library by using a heterologous cDNA probe of MnSOD from *N. plumbaginifolia* [53]. The full-length cDNA was cloned by PCR from total mRNA. Of the two distinct cDNAs of MnSOD isolated, MnSOD-A has a perfect match with exons of the nuclear gene, while MnSOD-B has a 90.2% homology and is 6 nucleotides longer than MnSOD-A in the putative transit peptide region. The nuclear gene comprises 6 exons and 5 introns, giving a total length of 3,211 bp.

Southern analysis of genomic DNA from *Hevea* probed with a genomic fragment indicated there are at least two genes of MnSOD in *Hevea*.

Superoxide dismutases are candidates for playing a role in autoimmunity as has been shown for an MnSOD from *Aspergillus fumigatus* [54]. A cDNA encoding a 26.7-kD MnSOD was isolated from an *A. fumigatus* cDNA library displayed on the surface of filamentous phages with serum IgE from *A. fumigatus*-sensitized individuals. The mould protein shared 51.5% identity and 67.2% similarity with the corresponding human enzyme. The *Aspergillus* and the human MnSODs were produced as recombinant proteins and were both recognized by IgE antibodies from 60 subjects allergic to the *A. fumigatus* MnSOD. The recombinant MnSODs also elicited specific immediate-type allergic skin reactions in these individuals. These observations provide evidence for autoreactivity to the human MnSOD in allergic persons sensitized to an environmental allergen from *A. fumigatus* who share a high degree of sequence homology to the corresponding human enzyme.

Enolase (2-Phosphoglycerate Dehydratase)

Posch et al. [52] characterized several additional latex allergens by two-dimensional electrophoresis and protein microsequencing. Protein spots '14c' and '15' (N-terminal sequence AITIVSVRARQIF) shared over 90% sequence identity with the N-terminus of *Ricinus communis* enolase (SWISS-PROT P42896). *Ricinus* belongs to the family Euphorbiaceae as does *Hevea*. The identity of the latex enolase sequence to the N-terminal sequence of a tomato enolase (SWISS-PROT P26300) was over 70%.

Interestingly, the allergen Cla h 6 (47kD, SWISS-PROT P42040) of the mould *Cladosporium herbarum* also is an enolase [55]. The cDNA of Cla h 6 encodes 441 amino acids. Cla h 6 shares 73% amino acid identity and 83% amino acid similarity with *Saccharomyces cerevisiae* enolase 1. Cla h 6 is a typical minor allergen of *C. herbarum* as it is recognized by IgE of 20% of all patients with a positive *Cladosporium* immunoblot. The *Ricinus* enolase shares 74.5% amino acid identity and 61% amino acid similarity with the *Cladosporium* enzyme. Sequence similarities in this range may well be the cause for cross-reactivities between the *Cladosporium* and the *Ricinus* as well as the latex enolase.

H. brasiliensis Profilin

Profilin was frequently identified as an allergen in many plant species. Using IgE-binding inhibition by purified profilins it was demonstrated that profilin is an IgE-binding component in the cytosolic fraction of natural latex, and, to a lesser extent, in the rubber fraction [56]. Thirty-five of 36

sera containing IgE to ragweed-profilin reacted with profilin from latex, indicating structural homologies between profilins from latex and ragweed. Because profilin is also present in banana extract, it is likely to be involved in cross-sensitivity to banana and latex. In a group of 19 individuals allergic to latex only two had anti-profilin IgE antibodies. Profilin was barely detectable on glove extract immunoblots [56].

The presence of cross-reactive IgE-binding components in the different latex extracts, extracts from mugwort, ragweed, timothy grass pollen, and recombinant birch pollen allergens (Bet v 1 and the birch profilin Bet v 2) was studied by immunoblot inhibitions and quantitative competition experiments [5]. The cross-reactive allergen profilin was purified from latex as an IgE-binding component, but was only present in small amounts in latex extracts. Recently, Nieto et al. [57] purified profilins (10.2, 14.2 and 15.7kD) from latex and skin prick tested 24 SB patients. They found positive reactions in all patients. SPTs were negative in atopic patients with fruit allergy. A group of 17 adults allergic to latex had positive SPT reactions in 95% of the cases tested. cDNA sequences of latex profilin were obtained by PCR and the allergen tentatively designated Hev b 12 [57]. At the moment, the significance of latex profilin for the clinical manifestation of latex allergy is not clear. Very recently, the IUIS Allergen Nomenclature Subcommittee has decided to apply the designation Hev b 8 to latex profilins.

Additional Latex Allergens

Lysozyme. Lysozymes were eluted from latex gloves or extracted from ammoniated latex and detected with a cell suspension-clearing test [58]. A chromatographically separated lysozyme (27kD, pI 9.5) was investigated for its physicochemical and enzymatic properties and allergenicity. The purified lysozyme was enzymatically very similar to lysozymes of fruit such as fig, or papaya and was recognized by IgE from sera of 2 latex-allergic individuals.

Papain. Baur et al. [59] studied the cross-reactivity of IgE antibodies recognizing epitopes of latex allergens and papain. 8/24 latex-sensitized individuals had IgE to purified papain and 6/12 papain workers with IgE antibodies to papain revealed IgE to latex allergens. Each patient group had not previously been exposed to the allergens from the other source. These results indicate the presence of common epitopes of papain and latex allergens.

Posch et al. [52] characterized several additional latex allergens by two-dimensional electrophoresis and protein microsequencing. Peptides 16 (RKVDVDVXVVPYT) and 17 (ARKFFVGG) demonstrated identities between 50 and 66% of spinach, rice and tomato triosephosphate isomerases. Peptide 17 showed high identity to the N-termini of sev-

eral triosephosphate isomerases from plants including *Ara-bidosia thaliana* and rice. Sequence alignments for spot 19 (ANWSPYDNNGG) revealed similarities to several pro-teasome subunits.

Discussion

It is obvious from the data presented that the molecular biology of latex allergens is an area that is still developing. Of the seven latex allergens that have a nomenclature designation, only four have been successfully cloned (Hev b 1, 2, 5 and 6). The sequences of three additional latex allergens without a nomenclature designation are known (MnSOD, hevamine, and profilin). More detailed information is clearly needed about Hev b 3, the second latex allergen that is associated with SB. Hev b 1 and Hev b 3 would be essential for the presurgical diagnosis of latex allergy of patients afflicted with SB. Furthermore, the availability of recombinant Hev b 1 and Hev b 3 would be a great help in clarifying the ongoing discussion about the connection of latex allergy and SB. These purified recombinant allergens would be needed to answer the question whether it is exposure or genetic predisposition that links SB to latex allergy.

It is known that latex allergy is often combined with allergy to certain fruits. To clearly diagnose a patient as latex-fruit allergic, a combination of latex allergens that have counterparts in several fruits are needed. The most likely candidates are chitinases, profilins, Hev b 2, Hev b 5 and Hev b 7. Sequences are known for Hev b 2 and 5, and will soon be published for latex profilins and Hev b 7. But this represents only the first step towards developing diagnostic reagents. These latex allergens will have to be available as highly purified recombinant proteins. This poses a great challenge to molecular biologists as the different allergens will require different expression systems and it is not possible to predict their characteristics in heterologous expression systems (e.g. yield of the recombinant protein, solubility, correct folding, glycosylation patterns).

It is desirable to differentiate a patient who is allergic only to latex from a latex-fruit-allergic patient. Whereas the latex-fruit-allergic patient will have to be more restricted in his diet, no such recommendations need to be given to the latex-allergic individual. Proteins involved in the synthesis of rubber seem to be confined to *Hevea* latex. These include Hev b 1, 3, 4 and 6. As recombinant allergens they would constitute the reagents necessary to diagnose an individual with allergy to latex only (table 3).

Based on sequence similarities it is tempting to postulate the existence of a latex mould syndrome. The enzymes

Table 3. Panels of recombinant *H. brasiliensis* allergens for differential diagnosis

'Latex only' panel

Hev b 1, rubber elongation factor
Hev b 3, small rubber particle protein
Hev b 4, microhelix component
Hev b 6, prohevein/hevein

'Latex-fruit syndrome' panel

Hev b 2, β -1.3-glucanase
Hev b 5, acidic protein
Hev b 7, patatin-like protein
Class I and class II chitinases
Profilins

'Spina bifida' panel

Hev b 1, rubber elongation factor
Hev b 3, small rubber particle protein

'Latex-mould' panel

Mn-superoxide dismutase
2-Phosphoglycerate dehydratase (enolase)

MnSOD and enolase are well conserved proteins and have been described as important allergens in moulds [54, 55]. They were also described as allergens identified in latex [52]. Once these recombinant latex allergens are available, this hypothesis can be tested using mould-allergic patients (table 3).

For the reasons mentioned above we suggest that the combination of recombinant latex allergens, once available, into certain panels can be used for differential diagnosis (table 3). It will be interesting to see what information can be obtained by these subgroups of latex allergens and how they influence the information given to each individual patient. These panels could also be used as standards to which batches of natural latex extracts could be compared.

Commercial latex extracts are total protein extracts with a varying composition of allergenic and non-allergenic proteins. Protein composition varies from batch to batch, and allergens that easily degrade, such as Hev b 3, may be completely absent from the diagnostic solutions. Recombinant allergens may be used to adjust the content of each single allergen present in the extract. However, the differential diagnosis of certain patient groups, e.g. latex allergics versus latex-fruit allergics, will not be possible using standard latex extracts as they contain all allergens belonging to each of the subclasses.

Specific immunotherapy can only be envisioned with the use of recombinant latex allergens as the physicochemical properties of the extracts used for diagnosis today are un-

suitable for in vivo application. Single recombinant latex allergens will be needed to determine the allergenic profile of a patient. The results will enable defining a mixture of recombinant latex allergens for specific immunotherapy strictly according to an individual's needs.

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