



Allergenic proteins of natural rubber latex

H.Y. Yeang,* Siti Arijia M. Arif, Faridah Yusof, and E. Sunderasan

Biotechnology and Strategic Research Unit, Rubber Research Institute of Malaysia, Malaysian Rubber Board,
P.O. Box 10150, 50908 Kuala Lumpur, Malaysia

Accepted 18 March 2002

Abstract

As the living cytoplasm of laticiferous cells, *Hevea brasiliensis* latex is a rich blend of organic substances that include a mélange of proteins. A small number of these proteins have given rise to the problem of latex allergy. The salient characteristics of *H. brasiliensis* latex allergens that are recognized by the International Union of Immunological Societies (IUIS) are reviewed. These are the proteins associated with the rubber particles, the cytosolic C-serum proteins and the B-serum proteins that originate mainly from the lutoids. Procedures for the isolation and purification of latex allergens are discussed, from latex collection in the field to various preparative approaches adopted in the laboratory. As interest in recombinant latex allergens increases, there is a need to validate recombinant proteins to ascertain equivalence with their native counterparts when used in immunological studies, diagnostics, and immunotherapy. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Allergen; Allergy; *Hevea brasiliensis*; Natural rubber latex; Protein

1. Proteins of natural rubber latex

Latex occurs in the plant kingdom in more than 12,000 species belonging to some 900 genera. Of these laticiferous plants, about 1000 species contain rubber. Commercial natural rubber is derived from only one cultivated plant: the rubber tree, *Hevea brasiliensis*, a plant that originates from Brazil but is cultivated mainly in Southeast Asia today. The terms *rubber* and *latex* are often used interchangeably in the literature and, hence, an early clarification is in order. Latex exudes from the rubber tree when it is tapped and its main constituent (other than water) is natural rubber, the polymeric hydrocarbon *cis*-polyisoprene. *Hevea* latex is regarded as the living cytoplasm of laticiferous cells and, accordingly, it is a rich blend of organic substances, including a mélange of proteins. A small number of these proteins have given rise to the problem of latex allergy.

Proteins make up about 1–2% fresh weight of *Hevea* latex [1–3]. Because natural rubber latex is not a homogeneous fluid, latex proteins are not homogeneously dispersed. Latex proteins are found in the latex sera and

are also associated with latex organelles that can be separated by high-speed centrifugation. About 70% of latex proteins are soluble, with the remaining being associated with membranes.

1.1. Proteins in the latex fractions

Although Moir [4] distinguished as many as nine fractions in *Hevea* latex following ultracentrifugation at 53,000g, there are basically three main fractions that are easily discerned [5]. These are the rubber phase, the C-serum, and the bottom fraction (Fig. 1). The rubber phase comprises the rubber particles that are packed centripetally by centrifugation. With the C-serum in the interstices between rubber particles removed, two main proteins are extractable from the surface of the rubber particles. Whereas most of the C-serum and B-serum proteins are water-soluble, those of the rubber particles are generally insoluble. About 11 mg of proteins is bound to each gram of rubber.

The C-serum refers to the aqueous medium in which all the latex organelles are suspended. Being the cytoplasm of the laticifer, latex C-serum contains a large variety of proteins associated with cellular metabolism, as might be expected. For example, all the enzymes of

* Corresponding author.

E-mail address: hyyeang@lrm.gov.my (H.Y. Yeang).

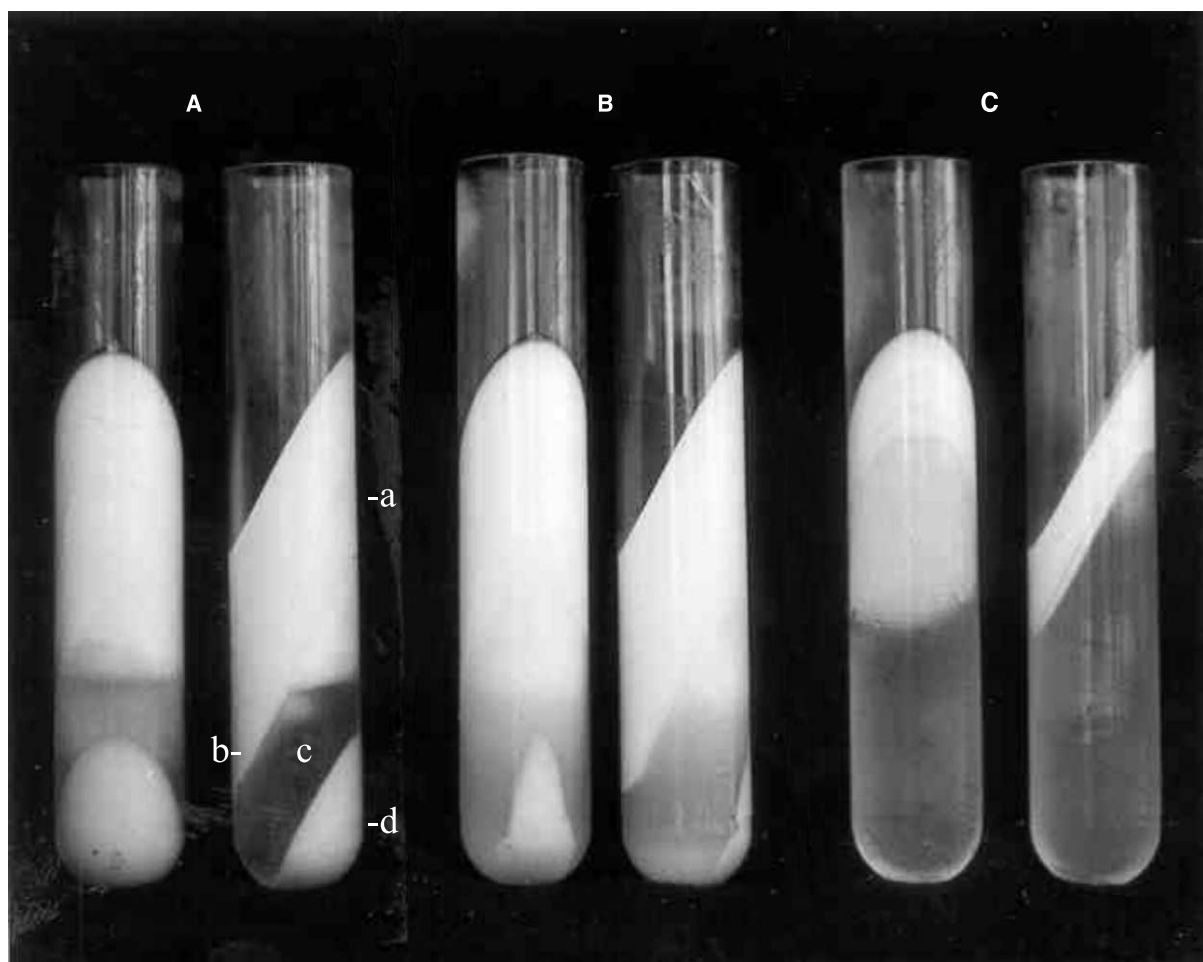


Fig. 1. High-speed centrifugation of natural rubber latex. (A) Fresh latex centrifuged at 44,000g in an angled rotor for 1 h shown in front and side perspectives of the centrifuge tube. The latex separates into three main fractions: the rubber cream (a,b), the C-serum (c), and the bottom fraction (d), which comprises mainly lutoids, the source of latex B-serum. Large rubber particles make up most of the rubber cream (a) while small rubber particles are found in Moir's zone 2 (b) of the rubber cream. (B) When ammonia is added to latex to a final concentration of 0.7%, the bottom fraction diminishes as the lutoids rupture and B-serum is released into the C-serum. On storage, the bottom fraction disappears completely. (C) When one part of latex is mixed with two parts of buffered glycerol ("Goodyear preservative") the bottom fraction similarly disappears.

the respiratory pathway are present. Various enzymes specific to latex, such as the enzymes associated with the rubber biosynthesis pathway, are also found in the latex C-serum. The C-serum proteins are therefore numerous, probably in the hundreds. A typical sample of C-serum contains about 12 mg proteins per milliliter.

While the latex bottom fraction comprises mainly the lutoids, other minor organelles (e.g., ribosomes, endoplasmic reticulum) are also present [6]. Hence, although the B-serum is commonly thought of as the lutoidic serum, it also contains minute constituents derived from the other minor organelles deposited in the bottom fraction. The latex B-serum is obtained by repeated freezing and thawing of the bottom fraction of centrifuged latex [7]. B-Serum typically contains about 24 mg proteins per milliliter. Compared with the C-serum, there are a smaller number of proteins (fewer than 20 major peptides), with a single protein, hevein, making up 50–70% of the total B-serum soluble proteins [8,9].

Whereas the rubber particle proteins and practically all the C-serum proteins are generally acidic proteins, B-serum has a mixture of acidic and basic proteins [10]. A typical distribution of proteins in the rubber particles, the C-serum, the B-serum, and the lutoid membranes is shown in Table 1. These figures are comparable to those given by Tata [3].

In the latex glove industry, the total proteins extractable from gloves are used as an indicator of their potential allergenicity [11,12]. In this connection, one noteworthy characteristic of the C-serum and B-serum proteins is their solubility in trichloroacetic acid (TCA). In preparing a protein for quantitation, for example, using the Lowry assay [13], TCA precipitation is often used as a rapid purification step to remove interfering substances in the test sample. Whereas practically all the C-serum proteins are precipitated by 5% TCA, only about 30% of B-serum proteins are similarly precipitated. The TCA-soluble proteins in B-serum can

Table 1
Typical protein distribution in the centrifuged latex fractions^a

Latex fraction	Protein concentration (mg/ml latex)	%
Rubber particle membranes	3.5	25
C-serum	6.0	43
B-serum	3.6	26
Bottom fraction membranes	0.9	6
Total	14.0	100

^a Protein in bottom fraction membranes based on Tata [3]. Other values from, H.Y. Yeang (unpublished).

nevertheless be precipitated by 0.2% phosphotungstic acid (PTA) [8,14]. Latex gloves may contain disproportionately larger amounts of B-serum proteins since 70% of a glove eluate was found to remain soluble in the presence of 5% TCA [14,15]. As with B-serum, the addition of 0.2% PTA precipitates the proteins effectively. From these findings, a combination of TCA and PTA is commonly adopted to precipitate proteins in latex glove eluates prior to protein quantitation [16].

1.2. Purified latex allergens

Ten latex proteins are recognized by the International Union of Immunological Societies (IUIS) to be allergenic (Table 2). In previous reviews that describe latex allergens [17–20], attention has been drawn to the fact that several latex allergens—Hev b 5, 7, 8, 9, and 10—have varying degrees of amino acid sequence homology

with known food, pollen, and mold allergens. Many of the *Hevea* latex allergens—Hev b 1, 2, 3, 4, 6, 7, and 10—are also proteins related to plant defense or stress [21–23]. Responses to stress and wounding vary. For example, wounding and the application of abscisic acid or (ethylene-releasing) ethephon results in accumulation of Hev b 6 RNA transcripts [24], while neither bark wounding nor ethephon treatment elicits an increase in Hev b 3 RNA transcripts [25]. The specific characteristics of latex allergens have served to group the proteins. For example, Blanco et al. [26] suggested the term “latex–fruit” syndrome for concurrent allergic reactions to natural rubber latex and various fruits. Breiteneder and Scheiner [19] proposed a latex–fruit/food allergen panel that included the latex proteins Hev b 2, 5, and 7 and a latex–mold panel encompassing Hev b 9 and 10. In this article, the latex allergens are grouped by their origin in natural rubber latex.

1.2.1. Rubber particle proteins

Hev b 1 and Hev b 3 are the two major proteins located on the surface of the rubber particles. Hev b 1 is found mainly on the large rubber particles (generally above 0.4 µm in diameter) whereas Hev b 3 is more abundant in the smaller rubber particles [27].

1.2.1.1. Hev b 1. The rubber particle protein, Hev b 1, was identified by Dennis and Light [28] who named it the “rubber elongation factor” (REF). The protein is thought to be involved in rubber biosynthesis, although the exact mechanism is still under investigation. Hev b 1 was the first named latex allergen [29]. Together with

Table 2
Latex allergens recognized by the International Union of Immunological Societies (IUIS)

IUIS code	Identity	pI ^a	Molecular mass (kDa) ^a	Location in latex
Hev b 1	Rubber elongation factor (REF)	4.9–5.3 ³⁶ , (5.0) ²⁵ , 8.5 ^b	{14.6} ²⁸ , 58 ^b	Rubber particles
Hev b 2	β-1,3-Glucanase	9.5 ⁷⁵ (9.8) ⁸⁰	[36] ^d , 36 ⁷⁶ , 34–36 ^{75,79} , (35) ⁴⁴	B-serum
Hev b 3	Small rubber particle protein (SRPP)	4.3–5.7 ^{27,38} , 4.8 ³⁹ , (4.6 ⁴³ –4.8 ²⁵)	[22–23] ²⁷ , (22) ^{25,43}	Rubber particles
Hev b 4	Microhelix, cyanogenic glucosidase	4.5 ⁷⁵	50–57 ^c	B-serum
Hev b 5	Acidic protein	3.5 ⁴⁷ , (3.9) ⁴⁷	[16] ⁴⁷ (16.0) ⁴⁷	C-serum
Hev b 6.01	Prohevein	(5.3) ²⁴	20 ⁹⁰ (19) ^c ²⁴	B-serum
Hev b 6.02	Hevein	4.7 ² (4.7) ²⁴	{4.7} ⁸⁴ (4.7) ²⁴	B-serum
Hev b 6.03	Prohevein C terminus	(6.0) ^c ²⁴	14 ⁹⁰ (13.3) ^c ²⁴	B-serum
Hev b 7.01	Patatin homolog, rubber biosynthesis inhibitor	4.4 ^d (5.0) ⁵⁸ (4.8) ⁵⁷	[44] ⁵⁶ (43) ^{57,58}	C-serum
Hev b 8	Profilin	(4.9) ⁶⁶	10.2, 14.2, 15.7 ⁶⁴ (14.0) ⁶⁶	C-serum
Hev b 9	Enolase	5.9, 6.0 ³⁶ (5.6) ⁷¹	51 ³⁶ , 48 ⁷¹ (48) ⁷¹	C-serum
Hev b 10	Mn-superoxide dismutase	4.3 ^d , 6.1 ³⁶ (5.7) ¹⁰² (6.3) ¹⁰³	45 ¹⁰¹ , 25 ³⁶ (23) ^{102,103}	B-serum

^a Values corresponding to the protein as predicted from the cDNA sequence are shown in parentheses. Molecular masses determined by mass spectrometry are shown in square brackets, and those determined by amino acid sequencing of the complete native protein are shown in curly brackets. Other values are estimates by SDS–PAGE for molecular mass and isoelectric focusing for pI.

^b Putative tetrameric form reported by Czuppon et al. [29].

^c Predicted mature protein processed from its translated precursor as described by Chye and Cheung [74] for Hev b 2 and by Soedjanaatmadja et al. [89] for Hev b 6.

^d Unpublished results of H.Y. Yeang, (for Hev b 2), F. Yusof, (for Hev b 7), and F. Yusof, and E. Sunderasan, (for Hev b 10).

^e Reduced form of Hev b 4. Unreduced form has a mass of about 100 kDa [75].

Hev b 3, sensitivity to Hev b 1 has been particularly associated with spina bifida [27,30,31], with latex-sensitive adults far less commonly sensitive to this protein [27,31,32]. These findings notwithstanding, more than half of the latex-allergic health care workers sampled in one study were found to be sensitized to Hev b 1 [33]. Although the 14.6-kDa protein occurs predominantly as a monomer in fresh latex, a presumed homotetramer of 58 kDa, in both native [29,34] and recombinant [35] forms, has also been reported. The actual structure of the putative tetramer may be complex since its experimental *pI* of 8.45 [29] is far removed from that of monomeric Hev b 1, which has an observed *pI* of 4.9–5.3 [36] and the value of 5.0 predicted from its protein and cDNA sequence [28,35]. Hev b 1, eluted from latex gloves may also occur in other molecular masses such as 8, 14, 30, and 42 kDa [33].

1.2.1.2. Hev b 3. A second latex allergen to which spina bifida patients are especially sensitive has been variously reported as a 27-kDa protein [37–39], a 23-kDa protein [40,41], and a 24-kDa protein [27,42]. Akasawa et al. [42] first showed that an allergenic 24-kDa protein reactive with IgE from spina bifida patients was derived from the rubber particles. This protein was subsequently identified by Yeang et al. [27] as Hev b 3, with its molecular mass revised to 22–23 kDa based on mass spectrometry analyses. The molecular mass as deduced from its cDNA clone is 22.4 kDa [25,43]. Oh et al. [25] coined the common name “small rubber particle protein” (SRPP) and presented evidence to suggest that the protein may also play a role in rubber biosynthesis, as in the case of Hev b 1. Although rubber particle proteins may be regarded as cytosolic peptides [25], Hev b 1 and Hev b 3 are closely bound to the rubber particles. Unlike the other latex allergens that are water-soluble, Hev b 1 and Hev b 3 are insoluble proteins. Nevertheless, Hev b 3 can be detected in the C-serum because the smallest rubber particles, in which Hev b 3 is abundant, do not cream on centrifugation and remain suspended in the serum [44]. A small amount of the protein solubilizes when latex is ammoniated to stabilize it [44,45].

The cDNAs of Hev b 1 [35,46] and Hev b 3 [25,43] have been cloned. The translated amino acid sequences of these proteins show a high similarity to each other and to the stress-related protein, PvSRP, of the French bean, *Phaseolus vulgaris*. Hev b 3 shows overall identities of 47% to Hev b 1 and 54% to PvSRP. When only the matching regions of the paired proteins are considered, amino acid identity increases to 72% between Hev b 1 and Hev b 3 and 68% between PvSRP and Hev b 3.

1.2.2. The C-serum proteins

Of the 10 IUIS-recognized latex allergens, four—Hev b 5, 7, 8, and 9—are from the latex C-serum, which is the

cytosol of the latex vessel. This number does not include the rubber particle proteins described above which are also considered to be of cytosol origin.

1.2.2.1. Hev b 5. With a *pI* of 3.5, Hev b 5 is known as the *acidic protein* for the reason that its function in *Hevea* latex is unknown and is hence wanting of a more appropriate common name [47,48]. It is heat-stable, being able to withstand autoclaving. The protein contains only 14 of the 21 naturally occurring amino acids and unusually large amounts of glutamic acid, threonine, alanine, and proline. The amino acid sequence of Hev b 5 has 47% sequence identity with an acidic protein (pKIWI501) of the kiwi fruit, *Actinidia deliciosa* [47]. Comparing the DNA sequences of these two species, the degree of identity is 80% over a region of 75 bp within its open reading frame [48]. The 16 kDa protein migrates anomalously when electrophoresed, with an apparent molecular mass of 25 kDa [47].

Since the cDNA of Hev b 5 was isolated by Akasawa et al. [47] and by Slater et al. [48], much of the research on Hev b 5 has employed the recombinant protein. Although the Hev b 5 RNA transcript does not appear to be uncommon in latex [48] the actual amount of mature Hev b 5 protein present in natural rubber latex is a matter of debate. Because of its molecular characteristics, this unusually acidic protein is difficult to isolate and can easily escape detection [47]. Although Akasawa et al. [47] reported that most brands of latex gloves contained Hev b 5, Beezhold et al. [49] was able to detect only very low levels of the protein in natural rubber latex. Chen et al. [50] made the same observation and raised the possibility that native Hev b 5 in natural rubber latex could be unstable.

1.2.2.2. Hev b 7. Studies on the latex protein that was later to be named Hev b 7 were carried out in the mid-1990s independently in three laboratories. In early latex allergy research, IgE-binding proteins of about 42–46 kDa had frequently been observed on Western immunoblots following polyacrylamide gel electrophoresis of natural rubber latex extracts [42,51,52]. From this, it was inferred that a major allergenic protein of that size was present in the latex. It was Beezhold et al. [53] who isolated and reported a 46-kDa latex allergen that had partial protein homology to patatin, the major storage protein of potatoes. This protein was assigned the WHO/IUIS name Hev b 7. Around the same time, Subroto et al. [54] isolated from the latex B-serum a 43-kDa protein that also had amino acid homology to patatin. About this time also, Yusof et al. [55,56] was investigating a 43.7-kDa proteinaceous rubber biosynthesis inhibitor isolated from the latex C-serum. Again, the protein showed amino acid sequences similar to those of patatin. Like patatin, this protein had lipolytic acyl hydrolase activity. When the proteins isolated by Sub-

roto and Yusof were shown to be allergenic, they were deemed to be essentially similar to Beezhold's Hev b 7.

When a recombinant version of Hev b 7 became available through the work of Kostyal et al. [57] and Sowka et al. [58], it was found to be reactive with IgE from latex-allergic patients. Like its native counterpart, recombinant Hev b 7 shows esterase activity [58]. Hev b 7 has 43% amino acid sequence identity with *Nicotiana tabacum* patatin and 39–42% identity with various patatins from the potato plant. The first seven amino acids are cleaved off in the mature native protein. However, this short peptide does not appear to be a typical signal peptide, pointing to the cytosolic origin of the native protein. This is consistent with the fact that native Hev b 7 is isolated from the latex C-serum. Whereas potato tuber patatins are N-glycosylated, contain a signal peptide, and are localized in the vacuoles [59,60], native Hev b 7 is unglycosylated even though its cDNA sequence encompasses two potential N-glycosylation sites [57,58]. Glycosylation of recombinant Hev b 7 prepared by Sowka et al. [58] was made possible by expressing the protein in yeast (*Pichia pastoris*). The recombinant product obtained was a doublet of about 43 kDa, with the heavier peptide glycosylated. However, allergenicity of this protein does not appear to be dependent on its glycosylation status [58]. Partial amino acid homology notwithstanding, cross-reactivity between Hev b 7 and patatin was not established by Sowka et al. [61]. Our own studies support this proposition, but Seppälä et al. [62] have found it to be true only among latex-allergic children, but not among latex-allergic adults among whom cross-reactivity occurs between patatin and Hev b 7.

1.2.2.3. Hev b 8. Latex profilin, Hev b 8, is a member of a group of plant allergens known to cause cross-reactivity between pollen and food of plant origin. *Hevea* profilin was first shown to be allergenic by IgE inhibition assays [63]. Native *Hevea* profilins that separated as peptides of 10.2, 14.2, and 15.7 kDa were described by Nieto et al. [64], who also isolated and cloned several full-length cDNAs encoding the protein. Further studies on purified Hev b 8 have been based on recombinant proteins translated from leaf RNA by Rihs et al. [65] and latex RNA by Ganglberger et al. [66]. Profilin expressed in *Hevea* latex has a predicted molecular weight of 14.0 kDa and a *pI* of 4.9 and has been shown in a study to be allergenic to latex-allergic patients who were also sensitized to pollen or plant food [66]. In particular, the similarity in structure between Hev b 8 and Bet v 2, the birch pollen profilin, renders cross-reactions between these two proteins common [66,67]. Vallier et al. [63] found profilin to be barely detectable in glove eluates and considered it questionable if this minor allergen was a significant factor in the sensitization of occupationally exposed patients to latex. It seems probable that pri-

mary sensitization to latex profilin in the majority of cases takes place via pollen or food profilins [66]. In skin prick tests, only one patient out of 31 has been reported to react to recombinant Hev b 8 [68].

1.2.2.4. Hev b 9. The minor latex allergen Hev b 9 is the enzyme enolase (EC 4.2.1.11). This enzyme, 2-phospho-D-glycerate hydrolase, which catalyzes the reversible conversion of 2-phosphoglyceric acid, to phosphoenolpyruvic acid, has been reported in the latex C-serum [69,70]. Wagner et al. [71] calculated a molecular mass of 47.6 kDa for the recombinant form of the protein, which is a functional enzyme. The native latex protein of approximately the same molecular size is believed to occur as a homodimer [71]. Being an enzyme of the carbohydrate catabolism pathway, enolase is ubiquitous among living organisms. Latex enolase has 72% overall amino acid sequence identity with human enolase. IgE from patients sensitized to enolases of the molds *Cladosporium herbarum* and *Alternaria alternata* cross-react with Hev b 9. Comparisons of protein sequences show that *Hevea* enolase displays about 60% sequence identity with the mold enolases. Its active enzyme catalytic site is well conserved [71].

1.2.3. The B-serum proteins

It is tempting to classify the extracytosolic proteins in the latex B-serum as lutoidic proteins since the B-serum is prepared from the "bottom fraction" which comprises mainly lutoids. Nevertheless, other organelles sedimented by centrifugation are also present in the bottom fraction and the possibility that some proteins in fact originate from a minor organelle cannot be disregarded. All of the recognized allergenic proteins located in the latex bottom fraction—Hev b 2, 4, 6, and 10—are plant defense proteins

1.2.3.1. Hev b 2. Latex β -1,3-glucanase (EC 3.2.1.39) has been investigated by several researchers in basic studies unrelated to allergy [54,72–74]. This protein, assigned the name Hev b 2, was identified by Sunderasan et al. [75] and Alenius et al. [76] as a frequently observed latex protein of 36 kDa that was recognized by IgE of latex-allergic patients. Since then, Hev b 2 has been shown to be one of the most allergenic among latex proteins, in both IgE binding [32,34,77] and skin reactivity [78]. Latex glucanase has been detected as several isoforms [72,73,79]. The molecular mass of one allergenic variant, determined by mass spectrometry, was 36.43 kDa (H.Y. Yeang, unpublished). When prepared from B-serum isolated from fresh latex, the protein appears on SDS–polyacrylamide gel electrophoresis as a doublet of 34–36 kDa [75], 32–35 kDa [73], or 35–38 kDa [79], the variation perhaps due to differences in molecular mass references used. Both the peptides of the doublet are recognized by a monoclonal antibody [75]. Yagami et al.

[79] reported that the apparent doublet of Hev b 2 could be further resolved into three glucanase isoforms of 35, 36.5, and 38 kDa by concanavalin A affinity chromatography. The two heavier peptides, which were glycosylated, were more allergenic than the unglycosylated light peptide. Yagami et al. [79] surmised that at least some reported IgE binding to Hev b 2 could be attributed to its carbohydrate moieties cross-reacting with the carbohydrate epitope of phospholipase A₂ (PLA₂). Unlike all the other recognized latex allergens that are acidic proteins, Hev b 2 is a basic protein with a pI of about 9.5 [75]. As in the case of Hev b 4, this protein solubilizes only in high ionic (salt) content, and precipitates out when the protein solution is diluted or dialyzed. The allergen therefore resists being washed away with water during the manufacture of latex gloves, but subsequently dissolves in the sweat of the glove user.

After the DNA encoding Hev b 2 was first published by Chye and Cheung [74], other isoforms were subsequently cloned and the recombinant protein was synthesized. The Hev b 2 recombinant fusion protein retains the functionality of the glucanase enzyme [80]. It tests positive against both the monoclonal and polyclonal antibodies originally developed against the native protein, showing that the epitope sites for these antibodies are unaltered in the recombinant protein. Whereas native latex glucanase is reactive with IgE antibodies from sera of latex-allergic patients, IgE does not, however, bind with the recombinant protein, even when precaution is taken to replicate the mature protein by omitting the N-terminal and C-terminal extensions deduced from the cDNA sequence [74,80]. It can therefore be inferred that the IgE-specific epitope of the native protein is altered during synthesis of the recombinant protein. As several isoforms of latex glucanase exist [72,73,79], an alternative explanation for the recombinant protein not being allergenic is that the cDNA cloned is not that of the allergenic isoform. Since native glucosidase is glycosylated, it is also possible that the IgE epitope resides in the carbohydrate moiety of the protein [79]. The lack of IgE binding in the recombinant protein may therefore be due to the bacteria-synthesized protein being unglycosylated. Nevertheless, recombinant Hev b 2 synthesized in yeast (which supports glycosylation) is similarly unreactive to IgE, although it was not clear if the yeast carbohydrate is similar to that of the native protein (H. Breiteneder, 1999, personal communication).

1.2.3.2. Hev b 4. Sunderasan et al. [75] described an IgE-binding glycosylated protein that comprised a triplet of allergenic peptides of molecular mass 50–57 kDa when separated electrophoretically under reducing conditions. In its unreduced form, a band of about 100 kDa was discernible. The three peptides making up Hev b 4 may have related biochemical functions in the latex and may

be components of a protein complex. They share a common characteristic in remaining soluble only in the presence of high ionic concentration (e.g., 0.2 M NaCl and higher). This protein complex dissociates into soluble subunits in the presence of salt. Removal of the salt by dilution or dialysis reforms the protein complex, leading to the precipitation of all three peptides of the complex. The Hev b 4 protein complex is known as the microhelix (or Hev b 4 is a component of the microhelix), and is visible under the electron microscope [75].

Among the constituent peptides of the Hev b 4 triplet, the middle peptide displays a partial amino acid sequence showing homology to *Arabidopsis thaliana* lipase/acyl hydrolase and *Brassica napus* myrosinase-associated protein. The heaviest and lightest peptides have partial matches to β -glucosidases of *Arabidopsis thaliana* and *Hordeum vulgare*. The 57-kDa Hev b 4 heavy peptide, in particular, has protein sequences similar to published sequences of several plant glucosidases, including those of cyanogenic glucosidases of cassava [81]. Enzymatic assays have confirmed that the protein possesses glucosidase activity, the enzyme activity being further demonstrated by isozyme staining of the protein after electrophoretic separation on native polyacrylamide gels. Enzymatic activity is also observed when linamarin is used as the specific enzymatic substrate, indicating that the protein is a cyanogenic glucosidase (linamarase) as suggested by the partial amino acid sequences. A partial cDNA sequence with homology to plant glucosidases has been isolated [81].

1.2.3.3. Hev b 6. Archer [82] estimated that water-soluble proteins made up approximately 20% of the dry matter in the “bottom fraction” of centrifuged latex. Of these soluble proteins, 70% could be attributed to hevein, a sulfur-rich, chitin-binding peptide. Hevein is the amino-terminal posttranslational cleavage product of prohevein its precursor. Prohevein (18.5 kDa), hevein (4.7 kDa), and the prohevein C domain (13.3 kDa) have been shown to be allergenic [76,83] and have been assigned the WHO/IUIS names Hev b 6.01, Hev b 6.02, and Hev b 6.03, respectively.

Hevein is heat-stable and soluble in trichloroacetic acid, a commonly used protein precipitating agent. It can be precipitated with phosphotungstic acid [3]. Hevein was the first latex protein to have its entire amino acid sequence determined. Walujono et al. [84] reported hevein to be a protein of 43 amino acids having a molecular mass of 4.729 kDa. Molecular masses of 4.718 [85] and 4.719 [83,86] have been obtained by mass spectrometry. The discrepancy between this value and the molecular mass of 10 kDa earlier reported by Archer [82] suggests that hevein may exist as a dimer under certain laboratory conditions although this putative dimer has not surfaced in more recent reports. Tata [87] observed a minor component in a hevein preparation

that was less anionic than the major component. This peptide, called pseudo-hevein, differs from hevein at six amino acid positions [85]. The small differences in amino acid sequences do not have a significant effect on IgE binding [88].

The cDNA encoding prohevein was cloned by Broekaert et al. [24] who deduced that the predicted amino acid sequence contained an N-terminal signal sequence of 17 amino acid residues. A further 14 residues are lost from the 187-amino-acid translational product to form mature prohevein [89]. During prohevein cleavage into hevein and the carboxyl domain, a propeptide of four to six residues linking the N and C domains is lost [89,90]. Although one molecule each of the N and C domains is derived from the cleavage of each molecule of prohevein, the molar ratio of hevein to the C terminus in B-serum is about 30:1. Soedjanaatmadja et al. [89] surmised that not only are the pre- and propeptides subject to proteolysis, but the C-domain is also catabolized, or otherwise removed, after mature hevein is formed.

Hevein shows homology to several chitin-binding lectins from wheat, barley, and rice. Its antifungal properties might be related to its affinity to chitin [91]. Hevein displays close homology to the wound-induced genes (*WIN1* and *WIN2*) of potato. The carboxyl terminal of prohevein is also homologous to the carboxyl-terminal region of proteins encoded by the *WIN* genes [24]. The main IgE-specific epitope(s) resides on the hevein (Hev b 6.02) domain while the carboxyl domain (Hev b 6.03) of prohevein is less allergenic in comparison [83,92]. Using synthetic peptides (decamers with two-amino-acid overlap), Beezhold et al. [93] found six B-cell IgE-reactive epitopes, two in the hevein domain and another four in the prohevein carboxyl terminal. A broad epitope on the hevein moiety showed nearly complete homology to wheat germ agglutinin (WGA). Using recombinant decamers of prohevein, Banerjee et al. [94] found 10 IgE-binding epitopes representing unique and shared epitopes from both the N and C domains. Despite strong evidence for the allergenicity of hevein, Slater and Chhabra [30] did not observe competition with IgE binding in competitive radioallergosorbent test (RAST) assays. As hevein has an inherent molecular affinity to other molecules, and may be subject to conformational differences [92,95], there is scope for further investigations into the peculiar allergenic characteristics of this protein.

1.2.3.4. Hev b 10. Besides enolase (Hev b 9), which is found in the latex C-serum, another enzyme ubiquitous among living organisms is manganese superoxide dismutase (MnSOD, EC 1.15.1.1.) from the B-serum. This enzyme is another minor latex allergen designated Hev b 10. Plant MnSOD are commonly found in mitochondria and peroxisomes [96,97]. Most of the laticifer mitochondria are not expelled with the tapped

latex [98,99], but the existence of the peroxisome has been suggested by the detection of catalase activity associated with a particulate body in the latex [100]. The possibility that Hev b 10 is located in peroxisomes present in the latex bottom fraction, and thence in the latex B-serum prepared from it, should not be discounted.

Yusof and Abdullah [101] purified native Hev b 10 from the latex B-serum and estimated its molecular mass to be about 45 kDa based on its migration on SDS-polyacrylamide gel. Genomic and cDNA clones of *Hevea* MnSOD were first isolated by Miao and Gaynor [102]. As the molecular mass of MnSOD predicted from its cDNA clone is 22.9 kDa [102,103], the native protein might be a homodimer that resists dissociation even under reducing conditions. MnSOD is a well-conserved protein, showing 49% sequence identity to MnSOD from *Aspergillus fumigatus* (Asp f 6) and 58% to human MnSOD. The higher identity of *Hevea* MnSOD sequences to human MnSOD sequences as compared with Asp f 6 is reflected in the results of cross-inhibition studies [103]. The Hev b 10 recombinant protein synthesized by Wagner et al. [103] was found to be enzymatically active.

1.2.4. Other latex allergens

Given the increased prevalence of latex sensitivity among atopic persons, significant numbers of assumed latex-allergic patients may not in fact be primarily sensitized to latex. These could be food-allergic individuals or people who suffer from pollen allergy [20]. Virtually any given protein is potentially allergenic to a small number of atopic patients when specific conditions are favorable, for example, exposure route, amount, frequency, and cross-reactivity [23]. Hence, latex proteins that have been reported as being allergenic to only small numbers of patients are not covered here.

Besides the 10 IUIS-recognized allergens, Hev b 1–Hev b 10, three recently isolated latex proteins are considered here. A new protein under review for IUIS designation is Hev b 11W. (The letter W denotes that the protein is under review.) This is a 33-kDa protein characterized only in its recombinant form [104], although an IgE-reactive protein spot in 2D electrophoresis has a sequence matching the protein [36,105]. This class I protein bears a chitin binding domain and the recombinant peptide binds IgE from latex-allergic patients. Hev b 11W displays sequence homology to hevein (Hev b 6.02), with 58% identity in the chitin binding domain. This homology may explain a large part of the protein's allergenicity. Nevertheless, the protein may also contain other IgE epitopes outside of the chitin binding domain [104].

Reports of a frequently encountered 42- to 46-kDa allergenic latex protein appeared resolved when a 43-kDa patatin homolog was assigned the name Hev b 7

[53] and the recombinant protein that became available confirmed the allergenicity of Hev b 7 [57,58]. However, patients who were sensitized to native or recombinant Hev b 7 were fewer than expected, and this did not reflect the frequent observation of 42- to 46-kDa IgE-reactive protein bands in Western blots [42,51,52,106,107]. It was therefore possible that another unknown latex allergen of that molecular mass existed. One such candidate is Hev b 7.02W, which is a 43-kDa protein similar in size to the originally described Hev b 7 (now designated Hev b 7.01). Unlike Hev b 7.01, which originates in the latex C-serum, Hev b 7.02W is located in the B-serum.

Even the inclusion of Hev b 7.02W may not adequately explain the 42- to 46-kDa latex allergen being so frequently encountered. We have recently isolated a new B-serum latex allergen of 42.98 kDa. This protein has been shown to be highly allergenic from the results of serologic assays and skin prick tests. The protein is N-terminal blocked, but internal peptide sequences show homology to the *early nodule-specific protein* (ENSP) of soya bean, *Glycine max*. No homology with patatin has been found, but, surprisingly, IgE binding by the ENSP homolog can nevertheless be inhibited by patatin prepared from potato tubers. It would therefore appear that the 42- to 46-kDa IgE-binding protein band commonly encountered in SDS-PAGE immunoblots is not explained by a single latex protein, but is attributed to two Hev b 7 isoforms and a third protein, the ENSP homolog.

2. Isolation and identification of latex allergens

The most common approach adopted to identify latex allergens has been to carry out an SDS electrophoretic separation of latex proteins and then to transfer the separated proteins by Western blotting onto nitrocellulose membrane. The allergenic protein bands are identified by incubating the membrane with serum of latex-allergic patients to detect IgE binding. Molecular weights of the proteins can be estimated from their migration on the gel. Further purification leads to the stage where their partial amino acid sequence can be determined. With the protein identified or the partial amino acid sequence of an allergenic protein known, attempts can be made to clone the DNA encoding the protein.

While identification of most latex allergens takes the above route, Hev b 5 is an example of a latex allergen that was not first identified by the purification of an IgE-reactive native latex protein. Slater et al. [48] employed IgE from the serum of latex-allergic patients to screen a cDNA library of *Hevea* latex. The positive cDNA clone was expressed in *Escherichia coli* to produce the latex allergen that was subsequently desig-

nated as Hev b 5. Although Akasawa et al. [47] isolated and characterized native Hev b 5 from latex serum and latex gloves, the recombinant Hev b 5 fusion protein is far more commonly used in latex allergy research today.

2.1. Sourcing allergenic latex proteins

Many patients acquire latex allergy indirectly through cross-reactions with other allergens (e.g., food or pollen proteins) to which they are already allergic. Apart from these, most latex-allergic patients would have been sensitized through the use of latex products, principally latex gloves. Hence, the ideal purified latex allergens for research and clinical applications should theoretically be proteins isolated and purified from latex gloves. There are nevertheless considerable problems in sourcing latex allergens from gloves because they vary widely in their protein content, both qualitatively and quantitatively [108–110]. Hence, standardization and reproducibility become major concerns if they were to be used as reference proteins. Moreover, latex proteins may be modified (denatured, fragmented, aggregated, etc.) during glove production or during latex ammoniation prior to the manufacturing process [111,112], and this would lead to further complexity in the interpretation of research data [30,108]. RAST inhibition [108] and ELISA inhibition [113] data and immunoblot studies [113] suggest that protein preparations from ammoniated and nonammoniated latex are immunologically comparable. Therefore, even though ammoniated latex and latex glove extracts contain more complete immunoreactive repertoires for detecting IgE antibodies [113], non-ammoniated latex is the preferred source of latex proteins from the practical viewpoint [30,114].

2.2. Latex collection and preparation for protein analysis

Latex is used as the test reagent in the diagnosis of latex allergy and in *in vitro* tests (e.g., in IgE inhibition assays) relating to latex allergy. As the rubber that is present in latex is unstable (being prone to coagulation), it is usually the latex serum, rather than whole latex, that is used for these purposes. The latex serum obtained by centrifugation of the latex can be very variable in protein content, depending on how the latex is treated after collection from the rubber tree. The latex bottom fraction (where Hev b 2, 4, 6, and 10 are located) is intact in latex freshly collected on a fine day. If the latex is diluted (experimentally or from rainwater contamination), damage to the osmotically sensitive luteoids occurs and some B-serum from damaged luteoids is released or leaks into the C-serum. A degree of luteoid damage will also ensue if latex is allowed to stand over an extended period.

In the latex industry, the most common method of stabilizing latex for manufacturing use is by the addition of ammonia and other adjuvants. However, ammoniation of latex causes extensive breakage of the lutoids and consequent loss of the bottom fraction (Fig. 1). If ammoniated latex is centrifuged, the serum that is obtained is a mixture of C-serum and B-serum. The protein profile of this latex serum changes with time [112]. A small amount of the rubber particle membrane proteins will be solubilized into the serum in the presence of ammonia [44,45,112], but this preparation is generally still deficient in the rubber particle proteins. In some clinical and research applications, the use of nonammoniated latex (NAL) is preferred over ammoniated latex. One common way to obtain NAL (strictly NAL serum) is to mix the collected latex into “Goodyear preservative,” which is a formulation of buffered glycerol [42,114–116]. The high concentration of glycerol serves to reduce degradation of the latex by proteolytic enzymes [42] and latex serum is later recovered by centrifugation. Even when stabilized in this formulation, the bottom fraction gradually diminishes as the lutoids break down and release their contents of B-serum (Fig. 1). Here again, the serum obtained by centrifugation is mainly a mixture of B-serum and C-serum. A method to obtain a latex protein extract that includes the soluble serum proteins together with the normally insoluble rubber particle membrane proteins is to treat whole latex with detergents (e.g., Triton X-100 and/or SDS) prior to centrifugation [27,45]. The treatment solubilizes the rubber particle membrane proteins and, at the same time, ruptures the lutoids. The latex serum that is obtained by centrifugation contains C-serum, B-serum, and rubber particle membrane proteins. However, such a preparation is unsuited to applications where the presence of detergents is undesirable.

It can be seen from the above that C-serum is obtained only from fresh, unpreserved latex where the intact lutoids can be separated as the bottom fraction by centrifugation. Once the lutoids break, the latex serum obtained after centrifugation is actually a mixture of mainly B-serum and C-serum, notwithstanding the fact that such mixtures are very often erroneously termed “C-serum” in the literature.

Besides differences that can arise from handling the latex, variation in latex proteins may also be due to genetic or environmental factors. In a study using IgE-competitive inhibition assays and IgE Western immunoblots, significant seasonal differences were observed in allergenic latex proteins from three of the most widely planted rubber clones: RRIM 600, GT 1, and PB 260 [117]. Agronomic practices may also alter the levels of allergens in latex. Stimulation of latex flow by ethephon increases RNA transcripts of various latex proteins [118], including hevein (Hev b 6.02) [24] and MnSOD (Hev b 10) [102].

2.3. Approaches to protein purification

Irrespective of which latex protein is being isolated, the task would be made easier if as much of the other irrelevant proteins as possible were removed from the outset. If the starting material for protein isolation were fresh latex, then the first step is to centrifuge the latex to separate out the latex fractions. Depending where the targeted protein is located (Table 2), the rubber cream, C-serum, or bottom fraction is recovered as the source of the protein. To isolate the B-serum proteins, the bottom fraction is subjected to repeated freezing and thawing to rupture the lutoids and thus release their fluid content [7]. If ammoniated latex or latex collected in buffered glycerol is used as the latex protein source, most of the lutoids would have already been damaged and the B-serum and C-serum would have mixed.

For protein isolation and purification in general, purified latex protein allergens can be prepared by a combination of various chromatographic approaches such as ion exchange chromatography, hydrophobic interaction chromatography, affinity chromatography, gel filtration, and reverse-phase chromatography. Mass spectrometry and various related novel techniques are being increasingly used to characterize proteins in terms of their mass spectra, molecular mass, and amino acid sequence. As these methods are well established, they are not described further.

Besides the above standard procedures, some latex allergens have unique or uncommon characteristics that may facilitate their purification by taking advantage of these special properties. For example, Hev b 6.01 (prohevein) and Hev b 6.02 (hevein) are chitin-binding proteins and purification through a chitin column can be adopted [90].

The rubber particle proteins, Hev b 1 and Hev b 3, are water-insoluble and they need to be solubilized with detergent to extract them from the rubber particle surfaces [27]. A mixture of 1% SDS and 0.1% Triton X-100 performs this function well. As there are only two main allergens of rubber particles, the detergent extract containing solubilized Hev b 1 and Hev b 3 can be efficiently separated by preparative electrophoresis using the Bio-Rad Prep Cell (H.Y. Yeang, unpublished results).

Hev b 2 and Hev b 4 are known to be only sparingly soluble in pure water, but dissolve readily in a salt solution [75]. Hence, these proteins can be lost during laboratory manipulations when dialyzed against water or diluted excessively in water. Hev b 4 remains completely soluble when the sodium chloride concentration is 0.1 M and above, whereas the threshold for Hev b 2 is 0.2 M (H.Y. Yeang, unpublished results). Hence, Hev b 2 or Hev b 4 should not be dissolved, diluted or dialyzed in sodium chloride (or an equivalent salt) of less than 0.2 M.

Hev b 5 is another example of a latex protein that requires particular care in its purification. The 16-kDa

native protein migrates like a 25-kDa peptide on an SDS electrophoresis gel [47] while the recombinant protein resembles a 36-kDa protein in its migration [48]. This anomaly is due to the high proline content and acidity of the protein [48]. Hev b 5 should be stained with silver since the protein lacks aromatic amino acids and does not stain readily with Coomassie blue. The absence of aromatic amino acids tyrosine, phenylalanine, and tryptophan also implies that the protein may not be readily detected if eluted fractions from chromatographic columns are checked for UV absorbance at 250–290 nm. The fractions should be read at 205–220 nm to detect the amide bond. If electroblotting of the protein is attempted, this protein is liable to pass through the nitrocellulose membrane and passive blotting is therefore recommended [47].

With new advances in technologies for proteomics, novel approaches become available for protein discovery and characterization. By the mid-1990s, 2D electrophoresis could be coupled to protein microsequencing that enabled IgE-positive protein spots on the gel to be N-terminal sequenced. The latex proteins Hev b 9 (enolase) and Hev b 10 (MnSOD) were identified as allergens using this approach [36,105]. Latex allergens that are N-terminal blocked pose problems as no reading is obtained. Moreover, given the large number of peptides present in latex, overlapping can still occur even after separation in the second dimension. This may give rise to identification error if the allergen is N-terminal blocked since the unblocked coinciding proteins are sequenced by default. N-Terminal-blocked latex allergens are not uncommon, and in fact, 5 of the 10 IUIS-recognized latex allergens fall into this category: Hev b 1, 2, 3, 5, and 7.

3. Recombinant latex allergens

Recombinant proteins are increasingly used in pharmaceutical and other clinical applications. The clear advantage of recombinant proteins over native proteins is that they are highly reproducible and independent of the biologic vagaries that sometimes plague natural sources of the proteins. Recombinant allergens also facilitate the study of the molecular basis of the immunoreactivity of the proteins. These advantages notwithstanding, recombinant proteins need to be validated against native proteins for equivalence in allergenic reactivity before they can be more widely adopted in use.

3.1. Production of recombinant proteins

Since the appearance of the first recombinant latex allergen, Hev b 1 [27], all 10 IUIS latex allergens, save Hev b 4, have been produced in recombinant form.

Synthesis of recombinant proteins is typically initiated by generating cDNA encoding the target protein by the polymerase chain reaction (PCR), using a *Hevea* latex cDNA library as the template. Alternatively, reverse transcription is coupled to PCR (RT-PCR) to generate the cDNA from messenger RNA. The DNA is then recloned into a suitable expression vector. Most of the recombinant latex allergens—Hev b 1, 2, 3, 5, 6, 8, 9—have been successfully synthesized by overexpression in bacteria (*Escherichia coli*). Hev b 7 was expressed in a yeast (*Pichia pastoris*) that facilitated the production of a glycosylated protein. A more novel approach was adopted to synthesize immunologically intact hevein (Hev b 6) as an avidin fusion protein in baculovirus-infected insect cells [119]. The protocols for molecular manipulation in the laboratory are not described in detail here since in most cases, proprietary kits are employed and the vendor's instructions are followed. The recombinant protein allergen obtained is usually in the form of a fusion protein comprising the allergen linked to a tag protein that facilitates affinity purification. The purified fusion protein can be used in this form or, alternatively, the tag protein can be cleaved off by an enzymatic reaction to give the free protein.

3.2. Validating recombinant proteins

The successes with recombinant latex allergens achieved so far notwithstanding, the recombinant protein is not invariably interchangeable with its native counterpart as the IgE-mediated allergic reaction relies on more than a reproduction of the linear amino acid sequence. Specific interaction between a protein and another molecule (in this case the latex protein antigen and the interacting IgE) is dependent on the fitting of surfaces of the molecules in three dimensions. IgE recognition of the allergen takes into consideration the folding of the protein, as well as other posttranslational modifications. An example of the latter is protein glycosylation, and yet the binding of IgE to carbohydrate does not always elicit a clinical allergic response [120]. Recombinant proteins, and perhaps more so the fusion proteins where the allergen is linked to a proportionately large vector peptide, may not exhibit a conformation similar to that of native proteins. Hence, it is not improbable that the use of recombinant allergens in diagnostics can give rise to false-negative results. An example of a false-negative result arising from a recombinant latex protein is in the case of Hev b 2. Although IgE from a large proportion of latex-allergic patients binds to native Hev b 2, the same IgE is unreactive, or poorly reactive, to the recombinant version [34,68]. Conformational differences in the recombinant protein may explain this difference [68].

Besides false-negative test results, a recombinant protein may also give rise to false-positive outcomes.

This may occur when a latex protein shares linear epitope sequences with another protein that happens to be allergenic. The native latex protein may nevertheless be nonallergenic because it differs in molecular conformation. The recombinant latex protein, on the other hand, may not have the benefit of such structural dissimilarity. If the linear epitope displayed by the recombinant latex protein matches that of an allergen, it is possible that the recombinant protein may be allergenic even if the native equivalent is itself quite innocuous. Given the homology of latex allergens with many food, pollen, and mold allergens, false-positive results arising from recombinant proteins may not be uncommon. We have encountered patient IgE that is reactive with the recombinant MBP–Hev b 6.01 fusion protein, but unreactive with equimolar native Hev b 6.01, native 6.02, and native 6.03.

Another example of recombinant protein reactivity that is inconsistent with its native counterpart is found in Hev b 5. The fusion MBP–Hev b 5 protein which is highly allergenic has been observed to lose IgE recognition once the MBP domain is excised [34,121]. In this situation, allergenicity is lost despite the presumed presence of the IgE-specific linear epitopes. (In fact, no fewer than six IgE-specific sites have been identified [121].) The above examples show that fidelity of linear protein epitope sequences in recombinant proteins does not necessarily elicit the predicted IgE reactivity.

Recombinant latex allergens may in time be commonly incorporated into a spectrum of research and clinical applications [122]. However, a thorough validation needs to be undertaken before recombinant peptides can substitute for the native proteins. Comparisons of other allergens in native and recombinant forms have shown that they are not always equivalent. For example, van Ree et al. [123] compared the natural and recombinant pollen allergens, Bet v 1 and Bet v 2, by means of RAST and RAST inhibition. Whereas Bet v 1 demonstrated an excellent match with its native counterpart, the IgE-binding capacity of recombinant Bet v 2 was more than twofold lower. With recombinant latex allergens, most studies that have been completed so far only indicate whether or not they bind IgE or if they induce allergic reactions. Systematic comparisons of equivalence in allergenicity between recombinant and native latex proteins have yet to be actively pursued.

4. Next steps

Identification of the major latex allergens is a crucial step toward the understanding of latex allergy and the means to resolve many of the outstanding health-related problems associated with it. Among the next important objectives to address with respect to the problem of latex allergy are the use of purified proteins in the treatment of latex allergy (immunotherapy) and its prevention and

management (allergy diagnostics; assays to regulate and standardize allergen levels in latex products).

The protein and allergen levels in latex gloves have steadily decreased in recent years in response to consumer demand for low-allergen products [124,125], but sustained vigilance is called for so that high standards of quality control by manufacturers are maintained. In the West where most complaints of latex allergy originate, patients are unlikely to encounter latex; their problems arise primarily from latex products such as latex gloves. Despite their obvious importance, little is known about the allergen profiles of commercial gloves because suitable immunoassays for individual latex allergens are only beginning to emerge. While the allergenicity of latex products can be estimated from total latex proteins and latex antigens [11,12,124,126,127], immunoassays based on purified latex allergens might perform best. Such latex immunoassays require purified proteins for antibody development and to use as controls and calibration standards.

In the diagnosis of latex allergy, unpurified latex has practical utility as a test reagent and has generated useful research information [114,128,129]. There are, however, limitations in adopting its use as a reference material. Variation in allergen content in the latex due to genetic, environmental, and agronomic factors has been mentioned above. Even for a given batch of latex, the different allergens in the latex vary in their relative quantities and in their allergenicities. One allergen could be present at a level tens or hundreds of times that of another. A patient who is only marginally allergic to a protein that is present in abundance might have a strongly positive test response to a whole-latex preparation, whereas a patient who is allergic to an allergen that is underrepresented in latex might show a negative test response. In this connection, a test reagent formulated from judicious proportions of purified allergens has the advantages of test sensitivity and reproducibility. Where it is practical, diagnosis for sensitivity or reactivity to individual latex allergens may be carried out to construct an allergenic protein profile for the patient.

Finally, purified latex proteins have a role to play not only in the prevention and management of latex allergy, but also in its treatment through immunotherapy. Because of the complexity and variability of natural rubber latex, crude latex preparations may not be best suited for therapy aimed at mitigating the symptoms of latex allergy. On the other hand, the availability of pharmaceutical grade latex proteins will facilitate well-regulated, safe, and systematic treatment.

References

- [1] B.L. Archer, A.I. McMullen, in: *Proceedings, Nat. Rubb. Conf.* 1960, Kuala Lumpur, Rubber Research Institute of Malaya, Kuala Lumpur, 1961, pp. 787–795.

- [2] B.L. Archer, D. Barnard, E.G. Cockbain, P.B. Dickenson, A.I. McMullen, in: L. Bateman (Ed.), *The Chemistry and Physics of Rubber-like Substances*, Maclaren and Sons/Wiley, London/New York, 1963, pp. 43–72.
- [3] S.J. Tata, *J. Rubb. Res. Inst. Malaysia* 28 (1980) 77–85.
- [4] G.F.J. Moir, *Nature (London)* 184 (1959) 1926–1928.
- [5] A.S. Cook, B.C. Sekhar, *J. Rubb. Res. Inst. Malaya* 14 (1953) 163–168.
- [6] S. Hamzah, J.B. Gomez, *J. Rubb. Res. Inst. Malaysia* 31 (1983) 117–129.
- [7] R.C.H. Hsia, *Trans. Inst. Rubb. Ind.* 34 (1958) 267–290.
- [8] S.J. Tata, *Studies on the lysozyme and components of microhelices of Hevea brasiliensis latex*, Ph.D. thesis, University of Malaya, 1980.
- [9] B.L. Archer, B.G. Audley, G.P. McSweeney, C.H. Tan, *Rubb. Res. Inst. Malaya* 21 (1969) 560–569.
- [10] G.F.J. Moir, S.J. Tata, *J. Rubb. Res. Inst. Malaya* 16 (1960) 155–165.
- [11] E. Yip, K. Turjanmaa, K.P. Ng, K.L. Mok, *J. Nat. Rubb. Res.* 9 (1994) 79–86.
- [12] E. Yip, G.L. Sussman, *J. Rubb. Res.* 3 (2000) 129–141.
- [13] O.H. Lowry, N.R. Rosebrough, A.L. Farr, R.J. Randall, *J. Biol. Chem.* 193 (1951) 265–275.
- [14] H.Y. Yeang, F. Yusof, L. Abdullah, *Anal. Biochem.* 226 (1995) 35–43.
- [15] H.Y. Yeang, F. Yusof, L. Abdullah, *Anal. Biochem.* 265 (1998) 381–384.
- [16] American Society for Testing and Materials (ASTM), *Standard Test Method for the Analysis of Aqueous Extractable Protein in Natural Rubber and its Products Using the Modified Lowry Method*, ASTM D 5712–5799, 1999.
- [17] K. Turjanmaa, H. Alenius, S. Mäkinen-Kiljunen, T. Reunala, T. Palosuo, *Allergy* 51 (1996) 593–602.
- [18] A. Posch, Z. Chen, M. Raulf-Heimsoth, X. Baur, *Clin. Exp. Allergy* 28 (1998) 134–140.
- [19] H. Breiteneder, O. Scheiner, *Int. Arch. Allergy Immunol.* 116 (1998) 83–92.
- [20] A. Nel, C. Gujuluva, *Ann. Allergy Asthma Immunol.* 81 (1998) 388–398.
- [21] T. Yagami, M. Sato, A. Nakamura, *J. Nat. Rubb. Res.* 10 (1995) 100–107.
- [22] T. Yagami, M. Sato, A. Nakamura, T. Komiyama, K. Kitagawa, A. Akasawa, Z. Ikezawa, *J. Allergy Clin. Immunol.* 101 (1998) 379–385.
- [23] T. Yagami, *Recent Res. Dev. Allergy Clin. Immunol.* 1 (2000) 41–64.
- [24] W. Broekaert, H. Lee, A. Kush, N.H. Chua, N. Raikhel, *Proc. Natl. Acad. Sci. USA* 87 (1990) 7633–7637.
- [25] S.K. Oh, H. Kang, D.H. Shin, J. Yang, K.S. Chow, H.Y. Yeang, B. Wagner, H. Breiteneder, K.H. Han, *J. Biol. Chem.* 274 (1999) 17132–17138.
- [26] C. Blanco, T. Carrillo, R. Castillo, J. Quilraltae, M. Cuevas, *Ann. Allergy* 73 (1994) 309–314.
- [27] H.Y. Yeang, K.F. Cheong, E. Sunderasan, H. Samsidar, N.P. Chew, H. Sharifah, R.G. Hamilton, M.J. Cardosa, *J. Allergy Clin. Immunol.* 98 (1996) 628–639.
- [28] M. Dennis, D. Light, *J. Biol. Chem.* 264 (1989) 18608–18617.
- [29] A.B. Czuppon, Z. Chen, S. Rennart, T. Engelke, H.E. Meyer, M.S. Heber, X. Baur, *J. Allergy Clin. Immunol.* 92 (1993) 690–697.
- [30] J.E. Slater, S.K. Chhabra, *J. Allergy Clin. Immunol.* 89 (1992) 673–678.
- [31] H. Alenius, N. Kalkkinen, E. Yip, H. Hasmin, K. Turjanmaa, S. Mäkinen-Kiljunen, T. Reunala, T. Palosuo, *Int. Arch. Allergy Immunol.* 109 (1996) 362–368.
- [32] V.P. Kurup, H.Y. Yeang, G.L. Sussman, N.K. Bansal, D.H. Beezhold, K.J. Kelly, D.R. Hoffman, B. Williams, J.N. Fink, *Clin. Exp. Allergy* 30 (2000) 359–369.
- [33] Z. Chen, R. Cremer, A. Posch, M. Raulf-Heimsoth, H.P. Rihs, X. Baur, *J. Allergy Clin. Immunol.* 100 (1997) 684–693.
- [34] H.Y. Yeang, K.S. Chow, F. Yusof, S.A.M. Arif, N.P. Chew, Y.H. Loke, *J. Invest. Allergol. Clin. Immunol.* 10 (2000) 215–222.
- [35] E. Goyvaerts, M. Dennis, D. Light, N.H. Chua, *Plant Physiol.* 97 (1991) 317–321.
- [36] A. Posch, Z. Chen, M.J. Dunn, C. Wheeler, M.J. Dunn, M. Raulf-Heimsoth, H.P. Rihs, X. Baur, *Electrophoresis* 18 (1997) 2803–2810.
- [37] H. Alenius, T. Pauloso, K. Kelly, V. Kurup, T. Reunala, S. Mäkinen-Kiljunen, K. Turjanmaa, J. Fink, *Int. Arch. Allergy Immunol.* 102 (1993) 61–66.
- [38] H. Alenius, V. Kurup, K. Kelly, T. Palosuo, K. Turjanmaa, J. Fink, *J. Lab. Clin. Med.* 123 (1994) 712–720.
- [39] H. Alenius, N. Kalkkinen, M. Lukka, K. Turjanmaa, T. Reunala, S. Mäkinen-Kiljunen, T. Palosuo, *Int. Arch. Allergy Immunol.* 106 (1995) 258–262.
- [40] L.J. Lu, V.P. Kurup, D.R. Hoffman, K.J. Kelly, P.S. Murali, J.N. Fink, *J. Immunol.* 155 (1995) 2721–2728.
- [41] L.J. Lu, V.P. Kurup, K.J. Kelly, J.N. Fink, *Allergy* 50 (1995) 545–550.
- [42] A. Akasawa, L.S. Hsieh, Y. Lin, *J. Allergy Clin. Immunol.* 95 (1995) 1196–1205.
- [43] B. Wagner, M. Krebitz, D. Buck, B. Niggemann, H.Y. Yeang, K.H. Han, O. Scheiner, H. Breiteneder, *J. Allergy Clin. Immunol.* 104 (1999) 1084–1092.
- [44] H.Y. Yeang, M.A. Ward, A.S.M. Zamri, M.S. Dennis, D.R. Light, *Allergy* 53 (1998) 513–519.
- [45] E.G. Cockbain, M.W. Philpott, in: L. Bateman (Ed.), *The Chemistry and Physics of Rubber-like Substances*, Maclaren and Sons/Wiley, London/New York, 1963, pp. 73–95.
- [46] D.P.S.T.G. Attanyaka, R.G.O. Kekwick, F.C.H. Franklin, *Plant Mol. Biol.* 16 (1991) 1079–1081.
- [47] A. Akasawa, L.S. Hsieh, B.M. Martin, T. Liu, Y. Lin, *J. Biol. Chem.* 271 (1996) 25389–25393.
- [48] J.E. Slater, T. Vedvick, A. Arthur-Smith, D.E. Trybul, R.G.O. Kekwick, *J. Biol. Chem.* 271 (1996) 25394–25399.
- [49] D.H. Beezhold, V.L. Hickey, J.E. Slater, G.L. Sussman, *J. Allergy Clin. Immunol.* 103 (1999) 1166–1172.
- [50] Z. Chen, H.P. Rihs, J.E. Slater, E.J. Paupore, E.M. Schneider, X. Baur, *J. Allergy Clin. Immunol.* 105 (2000) S83 (Abstract).
- [51] D.H. Beezhold, G.L. Sussman, D.A. Kostyal, N.S. Chang, *Clin. Exp. Immunol.* 98 (1994) 408–413.
- [52] V.J. Tomazic, T.J. Withrow, R.G. Hamilton, *J. Allergy Clin. Immunol.* 96 (1995) 635–642.
- [53] D.H. Beezhold, Paper presented at International Conference on Latex Protein Allergy: The Latest Position, Paris, France, 11 January 1995.
- [54] T. Subroto, G.A. Van Koningsveld, H.A. Schreuder, M.S. Soedjanaatmadja, J.J. Beintema, *Phytochemistry* 43 (1996) 29–37.
- [55] F. Yusof, J.M. Walker, B.G. Audley, M. Ward, Paper presented at The Protein Society, First European Symposium, Davos, Switzerland, 28 June–1 July, 1995.
- [56] F. Yusof, M.A. Ward, J.M. Walker, *J. Rubb. Res.* 1 (1998) 95–110.
- [57] D.A. Kostyal, V.L. Hickey, J.D. Noti, G.L. Sussman, D.A. Beezhold, *Clin. Exp. Immunol.* 112 (1998) 355–362.
- [58] S. Sowka, S. Wagner, M. Krebitz, S.A.M. Arif, F. Yusof, T. Kinaciyan, R. Brehler, O. Scheiner, H. Breiteneder, *Eur. J. Biochem.* 255 (1998) 213–219.
- [59] B. Kirschner, H. Hahn, *Planta* 168 (1986) 386–389.
- [60] U. Sonnewald, D. Studer, M. Rocha-Sosa, L. Willmitzer, *Planta* 178 (1989) 176–183.
- [61] S. Sowka, C. Hafner, C. Radauer, Focke, R. Brehler, J.D. Astwood, S.A.M. Arif, A. Kanani, G.L. Sussman, O. Scheiner, D. Beezhold, H. Breiteneder, *J. Allergy Clin. Immunol.* 104 (1999) 1302–1310.

- [62] U. Seppälä, L. Ylitalo, T. Reunala, K. Turjanmaa, N. Kalkkinen, T. Palosuo, *Allergy* 55 (2000) 266–273.
- [63] P. Vallier, S. Balland, R. Harf, R. Valenta, P. Deviller, *Clin. Exp. Allergy* 25 (1995) 332–339.
- [64] A. Nieto, A. Mazón, F. Estornell, M. Boquete, F. Carballada, A. Martínez, J.A. Asturias, M. Aguirre, J. Martínez, R. Palacios, *J. Allergy Clin. Immunol.* 101 (1998) S207 (Abstract).
- [65] H.P. Rihs, Z. Chen, P. Rozynek, X. Bair, M. Lundberg, R. Cremer, *Allergy* 55 (2000) 712–717.
- [66] E. Ganglberger, C. Radauer, S. Wagner, G. O’Riordain, D.H. Beezhold, R. Brehler, B. Niggemann, O. Scheiner, E. Jensen-Jarolim, H. Breiteneder, *Int. Arch. Allergy Immunol.* 125 (2001) 216–227.
- [67] M.I. Díez-Gómez, C. Sánchez-fernández, G. Baz, F.J. Moradiallos, A. Martínez, *Allergy* 54 (1999) 951–961.
- [68] L. Yip, V. Hickey, B. Wagner, G. Liss, J. Slater, H. Breiteneder, G. Sussman, D. Beezhold, *Int. Arch. Allergy Immunol.* 121 (2000) 292–299.
- [69] J. d’Auzac, J.L. Jacob, *J. Rubb. Res. Inst. Malaya* 21 (1969) 417–444.
- [70] F.J. Bealing, *J. Rubb. Res. Inst. Malaya* 21 (1969) 445–455.
- [71] S. Wagner, H. Breiteneder, B. Simon-Nobbe, M. Susani, M. Krebitz, B. Niggemann, R. Brehler, O. Scheiner, K. Hoffmann-Sommergruber, *Eur. J. Biochem.* 267 (2000) 7006–7014.
- [72] F. Breton, M. Coupé, C. Sanier, J. d’Auzac, *J. Nat. Rubb. Res.* 10 (1995) 37–45.
- [73] N. Churngchow, A. Suntaro, R. Wittisuwannakul, *Phytochemistry* 39 (1995) 505–509.
- [74] M.L. Chye, K.Y. Cheung, *Plant Mol. Biol.* 29 (1995) 397–402.
- [75] E. Sunderasan, H. Samsidar, H. Sharifah, M.A. Ward, H.Y. Yeang, M.J. Cardoso, *J. Nat. Rubb. Res.* 10 (1995) 82–99.
- [76] H. Alenius, N. Kalkkinen, M. Lukka, T. Reunala, K. Turjanmaa, S. Mäkinen-Kiljunen, E. Yip, T. Palosuo, *Clin. Exp. Allergy* 24 (1995) 659–665.
- [77] R.G. Hamilton, S.A.M. Arif, F. Yusof, K.S. Chow, H.Y. Yeang, *J. Allergy Clin. Immunol.* 107 (2001) S320 (Abstract).
- [78] R. Biagini, D.I. Bernstein, R. Karnani, K. Murphy, C. Bernstein, B. Berendts, J.A. Bernstein, C. Abbott, H.Y. Yeang, S. Arija, R.G. Hamilton, *J. Allergy Clin. Immunol.* 109 (2002) S257.
- [79] T. Yagami, Y. Haishima, A. Nakamura, H. Osuna, Z. Ikezawa, T. Komiya, K. Kitagawa, *J. Allergy Clin. Immunol.* 104 (2000) S239 (Abstract).
- [80] H.Y. Yeang, K.S. Chow, Paper presented at the Symposium of the International Rubber Research and Development Board, Montpellier, France, 25–26 September 2001.
- [81] E. Sunderasan, K.S. Chow, M.A. Ward, H.Y. Yeang, *J. Allergy Clin. Immunol.* 109 (2002) S33.
- [82] B.L. Archer, *Biochem. J.* 75 (1960) 236–240.
- [83] Z. Chen, A. Posch, C. Lohaus, M. Raulf-Heimsoth, H.E. Meyer, X. Baur, *J. Allergy Clin. Immunol.* 99 (1997) 402–409.
- [84] K. Walujono, R.A. Scholma, J.J. Beintema, A. Mariano, A.M. Hahn, in: *Proceedings, Int. Rubb. Conf. 1975, Kuala Lumpur, vol. II, Rubber Research Institute of Malaysia, Kuala Lumpur, 1976*, pp. 518–531.
- [85] U.M.S. Soedjanaatmadja, J. Hofsteenge, C.M. Jeronimus-Stratingh, A.P. Bruins, J.J. Beintema, *Biochim. Biophys. Acta* 1209 (1994) 144–148.
- [86] H. Alenius, N. Kalkkinen, T. Reunala, K. Turjanmaa, T. Palosuo, *J. Immunol.* 156 (1996) 1618–1625.
- [87] S.J. Tata, in: *Proceedings, Int. Rubb. Conf. 1975, Kuala Lumpur, vol. II, Rubber Research Institute of Malaysia, Kuala Lumpur, 1976*, pp. 499–517.
- [88] J.H. Mikkola, R. Hänninen, J. Helin, N. Kalkkinen, K. Turjanmaa, T. Reunala, T. Palosuo, *J. Allergy Clin. Immunol.* 104 (2001) S244 (Abstract).
- [89] U.M.S. Soedjanaatmadja, T. Subroto, J.J. Beintema, *FEBS Lett.* 263 (1995) 211–213.
- [90] H.I. Lee, W.F. Broekaert, N.V. Raikhel, *J. Biol. Chem.* 266 (1991) 15944–15948.
- [91] J. van Parijs, W.F. Broekaert, I.J. Goldstein, W.J. Peumans, *Planta* 183 (1991) 258–264.
- [92] H. Alenius, N. Kalkkinen, T. Reunala, K. Turjanmaa, T. Palosuo, *J. Immunol.* 156 (1996) 1618–1625.
- [93] D.H. Beezhold, D.A. Kostyal, G.L. Sussman, *Clin. Exp. Immunol.* 108 (1997) 114–121.
- [94] B. Bannerjee, X. Wang, K.J. Kelly, J.N. Fink, G.L. Sussman, V.P. Kurup, *J. Immunol.* 159 (1997) 5724–5732.
- [95] H.D. Chen, C.L. Chen, S.W. Huang, H.F. Kung, H.C. Chen, *J. Biomed. Sci.* 5 (1998) 421–472.
- [96] J.A. Baum, J.G. Scandalios, *Arch. Biochem. Biophys.* 206 (1981) 249–264.
- [97] M.J. Droillard, A. Paulin, *Plant Physiol.* 94 (1990) 1187–1192.
- [98] P.B. Dickenson, in: L. Mullins (Ed.), *Proceedings, Nat. Rubb. Prod. Assoc. Jubilee Conf., Cambridge, 1964, McLaren and Sons, London, 1965*, pp. 52–56.
- [99] J.B. Gomez, in: *Proceedings, Int. Rubb. Conf. 1975, Kuala Lumpur, vol. II, Rubber Research Institute of Malaysia, Kuala Lumpur, 1976*, pp. 143–164.
- [100] M. Coupé, S. Pujarniscle, J. d’Auzac, *Physiol. Veg.* 10 (1972) 459–480.
- [101] F. Yusof, L. Abudullah, Paper presented at the Ninth National Biotechnology Seminar, Penang, Malaysia, 23–26 November 1997.
- [102] Z. Miao, J.J. Gaynor, *Plant Mol. Biol.* 23 (1993) 267–277.
- [103] S. Wagner, S. Sowka, C. Mayer, R. Cramer, M. Focke, V.P. Kurup, O. Scheiner, O. Breiteneder, *Int. Arch. Allergy Immunol.* 125 (2001) 120–127.
- [104] G. O’Riordain, C. Radauer, S. Wagner, C. Blanco, K. Hoffmann-Sommergruber, O. Scheiner, C. Ebner, H. Breiteneder, *J. Allergy Clin. Immunol.* 107 (2001) S57 (Abstract).
- [105] A. Posch, Z. Chen, M.J. Dunn, C.H. Wheeler, A. Petersen, G. Leubner-Metzger, X. Baur, *J. Allergy Clin. Immunol.* 99 (1997) 385–395.
- [106] V.P. Kurup, H. Alenius, K.J. Kelly, L. Castillo, J.N. Fink, *Int. Arch. Allergy Immunol.* 109 (1996) 58–67.
- [107] D.H. Beezhold, G.L. Sussman, G.M. Liss, N.S. Chang, *Clin. Exp. Allergy* 26 (1996) 416–422.
- [108] K. Turjanmaa, K. Laurila, S. Mäkinen-Kiljunen, T. Reunala, *Contact Dermatitis* 19 (1988) 362–367.
- [109] J.W. Yunginger, R.T. Jones, A.F. Fransway, J.M. Kelso, M.A. Warner, L.W. Hunt, *J. Allergy Clin. Immunol.* 93 (1994) 836–842.
- [110] H. Alenius, S. Mäkinen-Kiljunen, K. Turjanmaa, T. Palosuo, T. Reunala, *Ann. Allergy* 73 (1994) 315–320.
- [111] L.J. Lu, V.P. Kurup, K.J. Kelly, J.N. Fink, K.J. Kelly, *J. Lab. Clin. Med.* 126 (1995) 161–168.
- [112] H. Hasma, M.Y. Amir-Hashim, *J. Nat. Rubb. Res.* 12 (1997) 21–32.
- [113] A. Akasawa, L.S. Hsieh, Y. Lin, *J. Allergy Clin. Immunol.* 97 (1996) 1116–1120.
- [114] R.G. Hamilton, N.F. Adkinson Jr., *J. Allergy Clin. Immunol.* 98 (1996) 872–883.
- [115] D.J. Siler, K. Cornish, *Phytochemistry* 32 (1993) 1097–1102.
- [116] M.R. Woolhiser, A.E. Munson, B.J. Meade, *Toxicol. Sci.* 55 (2000) 343–351.
- [117] E. Unver, H.Y. Yeang, D. Hovanec-Burns, M. Lopez, A.S. El Shami, *Allergy Clin. Immunol. Int. Suppl.* 4 (1997) 34 (Abstract).
- [118] A. Kush, E. Goyvaerts, M.L. Chye, N.H. Chua, *Proc. Natl. Acad. Sci. USA* 87 (1990) 1787–1790.
- [119] K.J. Airene, O.H. Laitinen, H. Alenius, J. Mikkola, N. Kalkkinen, S.A. Arif, H.Y. Yeang, T. Palosuo, M.S. Kulomaa, *Protein Exp. Purif.* 17 (1999) 139–145.
- [120] R. van Ree, *J. Allergy Clin. Immunol.* 103 (1999) 1000–1001.
- [121] D.H. Beezhold, V.L. Hickey, J.E. Slater, G.L. Sussman, *J. Allergy Clin. Immunol.* 103 (1999) 1166–1172.
- [122] H. Breiteneder, *Allergy Clin. Immunol.* 10 (1998) 101–109.

- [123] R. van Ree, W.A. van Leeuwen, J.H. Akkerdaas, R.C. Aalberse, *Clin. Exp. Allergy* 29 (1999) 848–855.
- [124] T. Palosuo, S. Mäkinen-Kiljunen, H. Alenius, T. Reunala, E. Yip, K. Turjanmaa, *Allergy* 53 (1998) 59–67.
- [125] K. Turjanmaa, H. Reinikka-Railo, T. Reunala, T. Palosuo, *J. Allergy Clin. Immunol.* 104 (2000) S373 (Abstract).
- [126] D.H. Beezhold, M. Swanson, B.D. Zehr, D. Kostyal, *Ann. Allergy Asthma Immunol.* 76 (1996) 520–526.
- [127] American Society for Testing and Materials (ASTM), Standard Test Method for the Immunological Measurement of Antigenic Protein in Natural Rubber and its Products. ASTM D6499-00.
- [128] R.G. Hamilton, N.F. Adkinson Jr., and the Multi-Center Latex Skin Testing Study Task Force, *J. Allergy Clin. Immunol.* 102 (1998) 482–490.
- [129] J.W. Yunginger, *J. Allergy Clin. Immunol.* 102 (1998) 351–352.