

Arbeiten aus dem Paul-Ehrlich-Institut, Band 94
10th International Paul-Ehrlich-Seminar, Lübeck 2002
Druck- und Verlagshaus Sperlich

Latex: A New Target for Standardization

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Introduction

Natural rubber latex (NRL) IgE-mediated hypersensitivity has been recognized as an important health problem during the last 20 years. Manufactured products, such as surgical and household gloves, catheters, condoms, baby pacifiers, and toy balloons may contain allergenic proteins capable of eliciting type I allergic reactions and probably also causing sensitization of exposed subjects [1]. NRL exposure can take place either through direct skin/mucus membrane contact or via inhalation. Children with *spina bifida*, health care workers, persons with a history of multiple surgeries, workers in a rubber industrial company and persons with food allergy and atopy belong to a group with a higher risk to develop a NRL allergy.

The diagnosis of NRL allergy should be based on both a positive clinical history and a positive *in vivo* test result, the most reliable test being the skin prick test. Determination and quantification of NRL-specific IgE antibodies using *in vitro* tests are not sufficient alone, but may provide valuable additional support to NRL protein sensitization. Based on the specific symptoms (urticaria or acute erythema, respiratory symptoms ranging from allergic rhinitis to asthma) of the patient, suitable cutaneous or inhalation challenge tests have to be performed.

Standardized skin prick test extracts and well-characterized allergen mixtures for IgE determination are important prerequisites for a correct skin prick testing and IgE determination of specific IgE. Sources of NRL are of varying quality and difficult to standardize for diagnostic purposes. Therefore, the knowledge of the protein content and allergenicity of the extracts are important items. Although research resulted in obtaining purified natural allergens from natural rubber latex, up to now no agreement has been achieved concerning the selection of appropriate allergens for developing standardized reagents for reliable diagnostic of latex exists.

Allergens from NRL

The main constituent of latex after tapping the *Hevea brasiliensis* tree is the natural rubber, the polymeric hydrocarbon cis-polyisoprene. Only 1-2% of the fresh weight of *Hevea* latex are proteins, which are not homogeneously distributed in the latex sap [2]. After ultracentrifugation (53,000g) of the fresh latex sap Moir [3] distinguished as many as nine fractions of the *Hevea* latex, but basically three main fractions (rubber phase, the C-serum, and the bottom fraction (B-serum)) are easily discerned. The rubber phase comprises the rubber particles and two main, insoluble proteins which are extractable from the surface of the rubber particles. Most of the C-serum and B-serum proteins are water-soluble. Latex C-serum contains various proteins (more than 200 polypeptides) and some of them are enzymes associated with the rubber biosynthesis. In contrast, the B-serum contains a smaller numbers of proteins including hevein, which makes up more than 50% of the total soluble B-serum proteins. The composition of the latex serum obtained by centrifugation of the latex may be very variable concerning its protein content, depending on the treatment of latex after collection from the rubber tree as well as on variation in latex proteins due to genetic or environmental factors (e.g. significant seasonal differences concerning allergenicity of latex proteins from three of the most widely planted rubber clones: RRIM 600, GT 1, and PB 260) [2]. Twenty-five per cent of the proteins of all fractions bind IgE-antibodies from NRL-allergic patients [4].

Scientific efforts have resulted in the identification of several latex allergens. So far, 16 latex allergens have been included in the official allergen list of the IUIS Allergen Nomenclature Subcommittee [5] (Table 1). Depending on the patient groups studied significant differences concerning the IgE binding pattern to the different allergens are observable after SDS-PAGE and immunoblotting with patient sera. The IgE-reactivity of Hev b 1 and Hev b 3, both located on the surface of the rubber particles, has been found to be particularly associated with *spina bifida* patients [6,7], and especially Hev b 1 is also of intermediate relevance in latex-allergic health care workers (HCW) [8]. One of the most important NRL allergens for HCW appears to be the prohevein, a 20 kDa protein assigned by the WHO/IUIS as Hev b 6.01 [9]. Prohevein is the precursor of hevein (Hev b 6.02; 4.7 kA) and the C-terminal domain Hev b 6.03 (14 kDa). Hevein comprises the most important part of IgE-binding epitopes in the prohevein molecule. In addition, hevein shows homology to several chitin-binding lectins domains and may be responsible for certain cross-reactivity to several other plants and food. Other important allergens are Hev b 5 (acidic protein with unknown function located in the C-serum in very low concentrations in NRL) and Hev b 2 (β -1,3-glucanase; basic protein). Intermediate (20-50% positive IgE response) or minor allergens in all tested NRL allergic patients are Hev b 8 (profilin), Hev b 9 (enolase), Hev b 10 (manganese superoxide dismutase) and Hev b 11 (class I chitinase).

Table 1. *Hevea brasiliensis*

IUIS Name

Hev b 1	R
Hev b 2	
Hev b 3	
Hev b 4	
Hev b 5	
Hev b 6.01	
Hev b 6.02	
Hev b 6.03	
Hev b 7.01	
Hev b 7.02	
Hev b 8	
Hev b 9	
Hev b 10	
Hev b 11	
Hev b 12	
Hev b 13	

Recombinant NR

The clear advantage of recombinant allergens is possible to produce large amounts of the proteins. However, the presence in allergenic recombinant Hev b 1 [10,11] in recombinant form. Most of the fact that they have been used in identification studies with native Hev b 6.02. Although IgE-reactivity between IgE-reactive Hev b 6.02 and the same IgE is unaltered in *E. coli*. Co-responsible for sucrose

Table 1. *Hevea brasiliensis* latex allergens.

IUIS Name	Description	MW (kDa)	Location in latex
Hev b 1	Rubber elongation factor (REF)	14.6	Rubber particles
Hev b 2	β -1,3-Glucanase	34-36	B-Serum
Hev b 3	Small rubber particle protein (SRPP)	24-27	Rubber particles
Hev b 4	Microhelix	50-57	B-Serum
Hev b 5	Acidic protein	16-24	C-Serum
Hev b 6.01	Prohevein	20	B-Serum
Hev b 6.02	Hevein	4.7	B-Serum
Hev b 6.03	C-Domain of prohevein	14	B-Serum
Hev b 7.01	Patatin homologue	42	B-Serum
Hev b 7.02	Patatin homologue	44	C-Serum
Hev b 8	Profilin	14	C-Serum
Hev b 9	Enolase	51	C-Serum
Hev b 10	Mn-superoxide dismutase	26	B-Serum
Hev b 11	Class I Chitinase (PR3)	32	B-Serum
Hev b 12	Lipid transfer protein	9	?
Hev b 13	Esterase (ENSP)	42	B-Serum

Recombinant NRL allergens

The clear advantage of recombinant proteins in contrast to native proteins is that it is possible to produce large-scale quantities in high and reproducible quality. Recombinant allergens also facilitate the study of the molecular basis of the immunoreactivity of the proteins. However, they have to be validated against natural proteins for equivalence in allergenic reactivity. Since the appearance of the first recombinant latex allergen Hev b 1 [10,11] more than a dozen of latex allergens have been produced in recombinant form. Most of the recombinant latex allergens are produced in *E. coli* due to the fact that they have none or no important posttranslational modification(s). In our validation studies we demonstrated a very good correlation between rHev b 6.02 and native Hev b 6.02 tested in the CAP assay (Figure 1). In contrast, the correlation between IgE-reactivity to rHev b 2 and native Hev b 2 was very poor (Figure 2). Although IgE from a large proportion of latex-allergic patients binds to native Hev b 2, the same IgE is unreactive, or only poorly reactive to the recombinant version (produced in *E. coli*). Conformational differences or posttranslational modifications may be responsible for such differences. Nevertheless, recombinant Hev b 2 synthesized in

yeast (which performs glycosylation) was similarly unreactive to IgE, although it was not clear if the yeast carbohydrate is similar to that of the native protein [2].

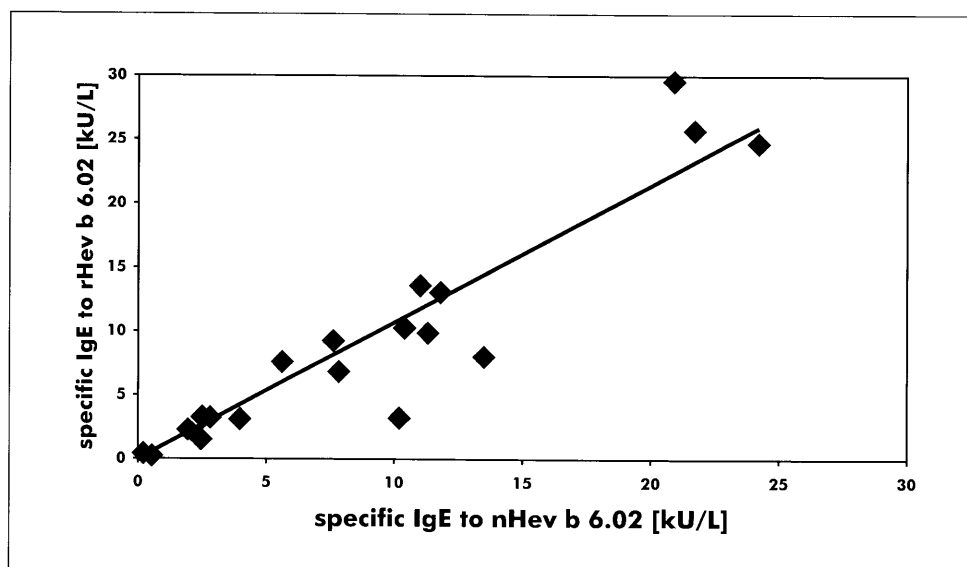


Fig. 1. Comparison of latex-specific IgE results obtained with nHev b 6.02 and rHev b 6.02. Both allergens were coupled on ImmunoCAP and the tests were performed with 20 sera of latex-allergic HCWs using the Pharmacia CAPsystem (correlation: $r=0.937$).

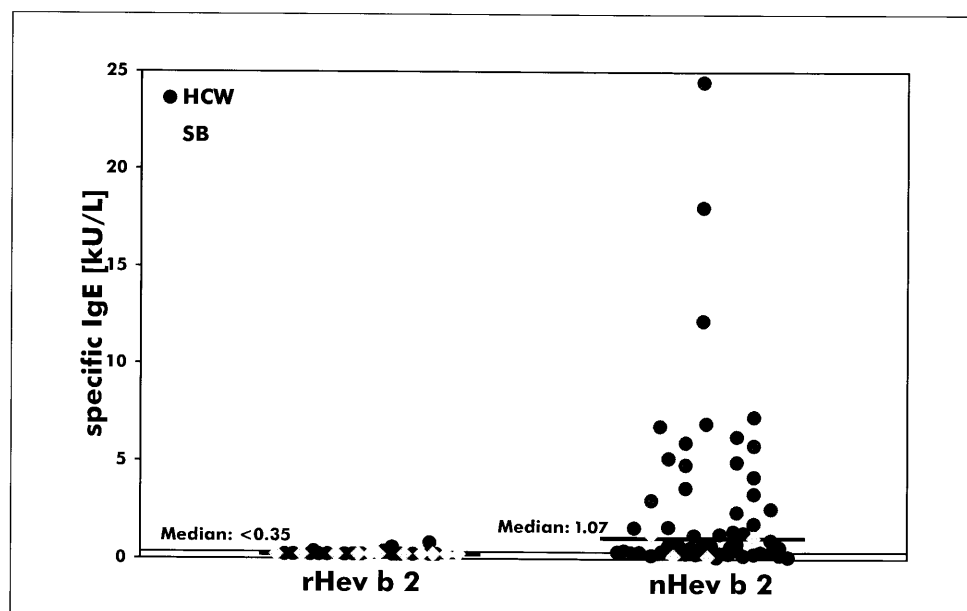


Fig. 2. Comparison of specific IgE values obtained with rHev b 2 and nHev b 2. Sera of 53 latex-allergic HCW and 8 latex-allergic spina bifida were tested. rHev b 2 was coupled on ImmunoCAP (measured by UniCAP) and nHev b 2 on paper discs (measured by EAST).

Diagnostic tools for latex allergy

Most NRL-allergic products, especially gloves, should be protein-free. However, using the gloves vary widely. In addition, latex products are not guaranteed to be ammoniated prior to use. Ammoniated NRL gloves are not guaranteed to be free of latex [12].

Several commercial latex extracts have appeared to be highly standardized. The extract of *Hevea brasiliensis* (Hevea) is the most common. The liquid was stored at -20°C . SDS-PAGE analysis of the known IgE binding sites of the known IgE binding sites (22 $\mu\text{g}/\text{ml}$ protein = 93% specificity and a 93% positive predictive value) showed a non-standardized sensitivity. Certain components and effective skin prick tests by means of skin prick test reactions. The US-FDA is in the process of reviewing the FDA and should be [18,19].

In addition, it has been shown to induce systemic reactions. Compounds including Hev b 5, may have multiple molecules by multi-epitopes. It may be related to the cell membrane. Nevertheless,

In addition of using the application of skin prick tests and rHev b 8 as major allergens. His-tagged protein

IgE, although it was
protein [2].

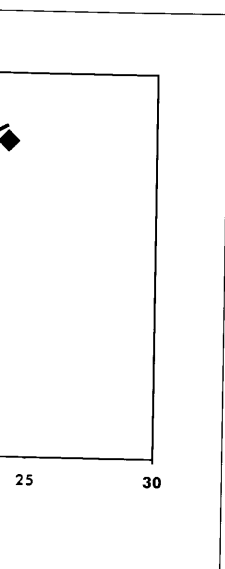


Fig. 6.02. Both
latex-allergic HCWs

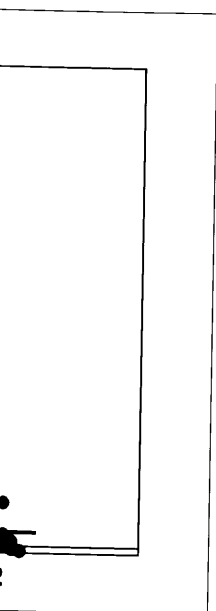


Fig. 6.03. Both
CAP (measured by

Diagnostic tools for skin prick testing – current situation

Most NRL-allergic patients would have been sensitized through the use of latex products, especially latex gloves. Thus, theoretically the ideal source of NRL allergens should be protein extracts and/or purified allergenic proteins from NRL gloves. However, using these materials many problems and difficulties arise because latex gloves vary widely in their protein content, qualitatively as well as quantitatively. In addition, latex proteins may be modified during glove production or during latex ammoniation prior to the manufacturing process. Standardization and reproducibility are not guaranteed if these materials were used as reference proteins. Therefore, non-ammoniated NRL is the preferred source of latex proteins from the practical point of view [12].

Several commercial preparations have been released in Europe and Canada that appeared to be highly specific and sensitive [13]. Stallergènes NRL preparation standardized extract is widely used in Europe and is manufactured from Malaysian rubber (*Hevea brasiliensis*, clone RRIM 600), frozen immediately after collection. After thawing the liquid was separated from the coagulate by ultracentrifugation (100,000g, 3 h) and the supernatant was filtered through 0.45 µm membranes, aliquoted and stored at -20°C. SDS-PAGE and immunoblot profiles of the extract were almost identical to an NRL standard (E8) provided by the US Food and Drug Administration containing all of the known IgE binding latex components. When using a 1:200 dilution of the extract (22 µg/ml protein = arbitrary index of reactivity (IR) 100) for skin prick testing a 100% specificity and a 93% sensitivity, as well as a negative predictive value of 100% and a positive predictive value of 96% were calculated [14]. Blanco et al. [15] described for several non-standardized commercially available NRL skin prick extracts also a very good sensitivity. Certain companies evaluate latex milk extracts for the production of a safe and effective skin prick test solution [16,17]. In the United States, confirmatory diagnosis by means of skin prick testing continues to be hindered by the lack of a standard skin prick test reagent [13]. The clearance of natural rubber latex skin test regions by the US-FDA is in progress. Two lots of NRL allergens that have been standardized with regard to the allergen and protein content (lots E5 and E8) are available from the FDA and should be a helpful tool in the production of a standardized skin test solution [18,19].

In addition, it has to be kept in mind that latex is the most common allergen to induce systemic reactions on skin testing, when compared with other allergens and compounds including drugs, foods and pollens. The exact molecular mechanism for this potency is not fully elucidated yet. It has been suggested that the major latex allergen Hev b 5, may have an enhanced ability to cross-link multiple effector cell-bound IgE-molecules by multivalency. Other possible reasons for high potency of latex allergens may be related to their bio-availability or enhanced ability to cross the mucus membrane. Nevertheless, little data are currently available.

In addition of using extract of latex sap or latex glove extract Yip et al. [20] described the application of six single recombinant latex allergens (rHev b 2, rHev b 5, rHev b 6, and rHev b 8 as maltose-binding protein fusion proteins, and rHev b 3 and rHev b 7 as His-tagged proteins) in skin prick testing. In their patient group (31 latex-allergic

patients) a combination of recombinant latex allergens rHev b 5, Hev b 6, and rHev b 7 was suitable to diagnose latex allergy with 93% sensitivity and 100% specificity.

Serological assays for a latex-specific IgE determination

Alternatively serological assays for detection of anti-latex IgE may be used. Comparison of the two major serologic assays (Pharmacia ImmunoCAP-system and AlaSTAT) using for natural rubber latex-specific IgE antibodies detection demonstrated different results of specificity and sensitivity dependent upon the cut-off values used [15,17]. For the AlaSTAT assay with 0.54 kU/L as the 95th percentile threshold value in nonatopic control subjects, sensitivity was 100% and specificity was 83%. According to the threshold value of 0.35 kU/L, a sensitivity of 97% and a specificity of 83% for the ImmunoCAP assay and a sensitivity of 100% and a specificity of 33% for the AlaSTAT assay were observed [17]. In addition, results of these and other studies indicate that IgE test systems gave false-negative results in 24 to 27% of skin test positive subjects [18]. One reason for these discrepancies may be that in various systems the allergens are in a denatured form while bound to a solid-phase surface and not all epitopes are equally accessible. On the one hand, in some cases no NRL-specific IgE antibodies can be detected despite severe allergic reactions to NRL, such as bronchial obstruction and positive workplace-related challenge test occur [21]. On the other hand, the false-negative serologic results may be also due to the absence of some allergens among the capture antigen used in the assay. Indeed, by addition of rHev b 5 to

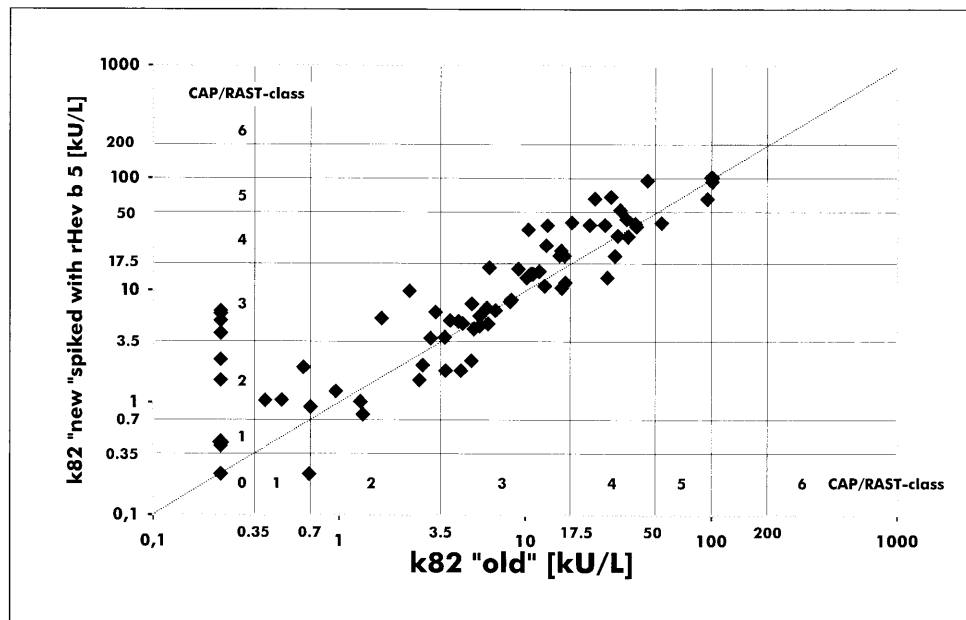


Fig. 3. Comparison of latex ImmunoCAP results between k82 ("old") and k82 spiked with rHev b 5 ("new"). 70 patient (latex-allergic HCW and spina bifida patients) were tested. A good correlation between the results of k82 ("old") and k82 spiked with rHev b 5 was observed. 8 negative serum samples tested with the "old" k82 yielded positive results with k82 spiked with rHev b 5 ("new").

the diagnostic repertoire and negative latex-specific IgE. Hev b 5 may play a role. Based on these results, a combination of k82 latex vials were retested: a significant number of previously negative samples suggest that recombinant Hev b 5 may improve *in vitro* diagnosis. All steps required for the assay were added as a stable reagent. Since spring 2000, the assay is commercially available.

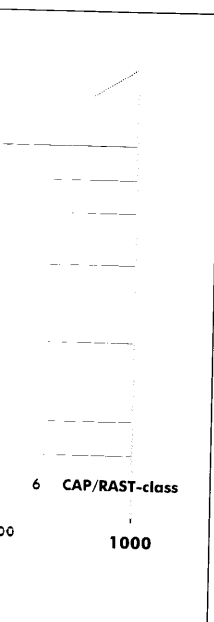
In addition to the single latex allergen, the assay can determine latex allergy.

Assays for the quantification of latex-specific IgE

One important step in the diagnosis of latex allergy is the quantification of latex-specific IgE. Especially in the aim to control the allergic reaction, as well as to reduce the risk of severe reactions, it is necessary to quantify the protein levels of latex-specific IgE. The D5712 modification (ASTM) for the quantification of NRL-specific IgE is effective in reducing the number of false-positive results with rabbit antisera for the quantification of latex-specific IgE. More consistent measurements can be achieved with monoclonal antisera measuring the immune response. Measurement of latex-specific IgE in ELISA inhibits the non-specific binding of latex proteins. It has a number of disadvantages: it does not display the same reactivity. In addition, the sera recognize different epitopes of latex proteins. Hev b 1 and Hev b 5 are the most important. Moreover, when a poor quality of latex proteins is used, displaying the same reactivity. Another disadvantage is the use of antibodies for a few years.

Hev b 6, and rHev b 7
% specificity.

IgE may be used.
ImmunoCAP-system and
detection demon-
on the cut-off values
percentile threshold
specificity was 83%.
and a specificity of
specificity of 33% for
se and other studies
of skin test pos-
in various systems
surface and not all
NRL-specific IgE
L, such as bronchial
[21]. On the other
sence of some aller-
mon of rHev b 5 to



ed with rHev b 5
of correlation
negative serum samples

the diagnostic repertoire of latex allergens, 8 out of 16 subjects with clear latex allergy and negative latex-specific IgE value using Pharmacia CAP system showed significantly elevated IgE levels to Hev b 5. These findings demonstrate that latex allergen Hev b 5 may play a special role in diagnostic tests showing false-negative IgE results. Based on these results, ImmunoCAP (Pharmacia Diagnostics, Sweden) with the preparation of k82 latex was spiked with rHev b 5 (K82 plus-spiked with rHev b 5") and sera were retested: a significant number (16%) of positive serum samples become more strongly positive to the "k82 plus" than to the regular k82 latex, and a rather small number of previously negative serum samples became positive [22] (Figure 3). These results suggest that recombinant allergens may be suitable to develop a new approach to improve *in vitro* diagnosis of latex allergens, if relevant allergens are too labile to survive all steps required for the production of a standardized allergen preparation they can be added as a stable recombinant protein to the allergen preparation during the production. Since spring 2002 the new "k82 spiked with rHev b 5" latex ImmunoCAP are commercially available to improve the *in vitro* diagnosis of latex allergy.

In addition to the use of single recombinant latex allergens (as well as purified native single latex allergens) in *in vitro* tests (e.g. coupled on ImmunoCAP) it was possible to determine latex allergen sensitization profiles in different latex allergic patient groups [8].

Assays for the quantification of allergens

One important step in standardization includes the measurement of antigen/allergen content. Especially for monitoring of the latex content in NRL products e.g. gloves with the aim to control the protein levels of the finished products, to lower the exposure as well as to reduce the sensitization rate to NRL, different methods were developed to quantify the protein and/or the allergen level in latex products. The establishment of the D5712 modified Lowry assay (according to the American Society for Testing and Material, ASTM) for total protein measurement in 1995 was the first step in standardization of NRL content measurement. Standard total protein assays were initially effective in reducing the high protein levels in general, but these assays are plagued with false-positive reactions and limited sensitivity [23]. An inhibition ELISA using rabbit antisera for antigenic protein has been standardized and promises to provide a more consistent measurement of the proteins (ASTM-ELISA D6499). The use of polyclonal antisera meets the objective of measuring all latex proteins capable of inducing an immune response. Nevertheless, not all latex proteins do appear to be allergens. Measurement of allergenic proteins by using human sera from latex-allergic individuals in ELISA inhibition assays or in RAST inhibition assays although obviously ideal, has a number of difficulties. Since the comparison of two pools of patients' sera will not display the same reactivity for latex allergens, it is difficult to standardize the serum reactivity. In addition, the IgE repertoire of human serum is variable and different pool sera recognize different allergens, therefore (e.g. sera of *spina bifida* patients recognize Hev b 1 and Hev b 3 much more often than sera from health care workers). Furthermore, when a pool of sera is exhausted it would be difficult to establish a new pool displaying the similar reactivity concerning the heterogeneity of human reactivity to latex proteins. Another approach is to test for specific allergens using monoclonal antibodies for a few "representative" antigens [24,25]. Currently, one commercial test has

become available for measuring individual NRL allergens (Hev b 1, Hev b 3, Hev b 5 and Hev b 6.02) by capture ELISA-based assays using monoclonal antibodies and purified or recombinant allergens. An international comparison study for validation of the commercial available monoclonal antibodies based ELISA-assays and other methods for measuring latex proteins, antigens, and allergens is in progress.

Concluding remarks

In the last years many efforts have been undertaken to decline the incidence of latex allergy. For this purpose identification of the major latex allergens, their recombinant production and the determination of specific sensitization profiles for different patient groups (dependent of the various routes of exposure) are useful steps toward the understanding of the latex allergy and their management. The use of well-defined and standardized NRL extracts and/or purified proteins are the next objectives. In the diagnostic of NRL allergy, unpurified latex has practical usefulness as a test reagent and has generated useful research information. However, there are limitations in adopting its use as a reference material, because variations of allergen content in latex due to different factors was described. In this context, a test reagent formulated from judicious proportions of purified allergens (native or evaluated recombinant latex allergens) has the advantages of high test sensitivity and reproducibility. Determination of an allergenic protein profile may be a valuable strategy to enhance the diagnostic repertoire in certain cases. Spiking important but labile recombinant single allergens to native allergen preparations to enhance their amounts has been shown to improve diagnostic sensitivity. Assays based on specific antibody reagents against most of the important NRL allergens known so far have been produced and are under validation. First steps of standardization were performed and standardized extracts have to be evaluated in further applications and studies.

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Discussion

Chapman: Have you found any latex proteins, because...

Raulf-Heimsoth: Yes, however, you find many problems were reported...

Pauli: Which groups with *Ficus* and with...

Raulf-Heimsoth: Yes. In our hands we have found food or *Ficus* allergy, you look in pollen...

1. Hev b 3, Hev b 5 antibodies and purification for validation of the and other methods

the incidence of latex allergies, their recombinant for different patient steps toward the of well-defined and objectives. In the diagnosis a test reagent and variations in adopting in latex due to differences from judicious production of allergens) has the of an allergenic repertoire in certain to native allergen the diagnostic sensitivity the important NRL nation. First steps of to be evaluated in

rubber latex. Methods

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ization and identification sequencing. J Allergy

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Discussion

Chapman: Have you used any different expression vectors to express the recombinant latex proteins, because it seems that most of them cannot be expressed in *E. coli*.

Raulf-Heimsoth: We only have used one special expression vector. In the literature, however, you find some work done with yeast, and especially with the Hev b 2 the same problems were reported that we have with *E. coli*.

Pauli: Which group of allergens did you use for detecting the allergens cross-reacting with *Ficus* and with fruit?

Raulf-Heimsoth: We used the proheveins Hev b 6.01 and Hev b 6.02 and also chitinase. In our hands we had no positive responses to profilins. For the cross-reactivity with food or *Ficus* allergens only endochitinase and the hevein are very important. But when you look in pollen allergics, you see some cross-reactivity with the profilin Hev b 8.