

Generation of Allergen-Enriched Protein Fractions of *Hevea brasiliensis* Latex for in vitro and in vivo Diagnosis

S. Wagner^a M. Bublin^a C. Hafner^b T. Kopp^c D. Allwardt^a U. Seifert^a S.A. Arif^d
O. Scheiner^a H. Breiteneder^a

^aDepartment of Pathophysiology, Center for Physiology and Pathophysiology, and Departments of Dermatology, Divisions of ^bGeneral Dermatology and ^cImmunology, Allergy and Infectious Diseases, Medical University of Vienna, Vienna, Austria; ^dBiotechnology and Strategic Research Unit, Malaysian Rubber Board, Kuala Lumpur, Malaysia

Key Words

Hevea brasiliensis latex • Latex allergy • Natural rubber latex

Abstract

Background: The latex of *Hevea brasiliensis* trees contains a complex proteome that includes a range of allergenic proteins. Current latex extracts that are used for the diagnosis of latex allergy still lack important allergens. We aimed to devise a production process for an improved reagent that would ideally contain the complete latex allergome. **Methods:** Latex C-serum was fractionated by ammonium sulfate precipitation, and B- and C-serum proteins were then separated by anion exchange chromatography. Proteins eluting within defined salt concentration ranges were pooled into six final fractions. Fractions were evaluated by two-dimensional electrophoresis and subsequent IgE immunoblot for their spectrum of allergens. The presence of the most important latex allergens in the fractions was checked by Western blot analyses. Each fraction was further evaluated by skin prick test (SPT). **Results:** Reproducibility of the preparation method was demonstrated with two batches of latex. Comparison of latex B- and C-serum to the six fractions showed a

remarkable increase in the number of detectable allergens in the fractions. The presence of the latex allergens Hev b 1–8 and Hev b 13 in the fractions was demonstrated. In SPTs, the fractions produced wheal-and-flare reactions comparable to commercial latex extracts. **Conclusions:** This method provides reproducible latex protein fractions of high allergen content for the diagnosis of latex allergy.

Copyright © 2007 S. Karger AG, Basel

Introduction

The latex allergy epidemic of the late 1980s and 1990s has been attributed to the drastic increase in glove usage in response to concerns regarding AIDS [1]. The problem of latex allergy is still not resolved but significant progress has been made in the prevention of natural rubber latex (NRL) allergy. Today, many hospitals and scientific research facilities in the US and Western Europe have comprehensive emergency treatment programs for latex-allergic patients including the use of latex-free gloves and the ban of powdered gloves. Recent data about the high prevalence of latex allergy in Japanese children [2] and in

people from fast-developing countries, e.g. Taiwan [3], Turkey [4] and Poland [5], show that latex allergy is still an existing problem, and the increased use of latex products may lead to the emergence of a new epidemic. Evaluation of any new emergence of latex allergy requires reliable surveillance and testing.

Specialized cells of the tropical tree *Hevea brasiliensis*, called laticifers, synthesize natural latex that serves as the raw material for the production of latex devices. NRL, which is rather complex, contains approximately 3% proteins. These proteins represent the proteome of these cells. A portion of these proteins are able to bind IgE and to elicit allergic reactions. From two-dimensional gel electrophoresis it is known that the latex allergome includes at least 50–60 different proteins [6, 7]. To date, 13 latex proteins (Hev b 1–13) have been officially accepted as latex allergens [8]. In several studies, the use of individual allergens for the diagnosis of latex allergy was evaluated. The results of studies using some of the characterized latex allergens as purified natural or recombinant proteins indicated that only a few proteins might be sufficient for the diagnosis of latex allergy [9, 10]. However, a recent study employing an extended panel of recombinant and natural latex allergens [11] and the concomitant characterization of Hev b 13 [12] have demonstrated that further proteins are indeed necessary for a comprehensive diagnosis.

At present, the skin prick test (SPT) is considered the most reliable test for the diagnosis of latex allergy [13–15] and is often used as the gold standard for the evaluation of other assays [14, 15]. Identifying an individual as sensitized to latex by SPT is nevertheless highly dependent on the allergen content of the protein extract used in the diagnostic test. The results regarding the diagnostic capacity of commercial serological assays, e.g. the Phadia CAP system, DPC AlaSTAT or Hycor HyTECH, are controversial. Such assays have been suggested to represent valid alternatives to SPTs [16, 17], good combinations with SPT [18], tests with acceptable sensitivities, specificities, and predictive values [19], but also tests that produce high rates of false-negative as well as false-positive results [20, 21]. The diagnostic performance and reproducibility of these assays is like the SPT highly dependent on the allergen content of the reagents used. Low diagnostic sensitivity was related to poorly represented and/or denatured allergens such as Hev b 5 [22], and as a consequence diagnostic extracts were spiked with recombinant Hev b 5 [23]. Although the quality of latex extracts has improved over recent years, there is definitely room for further improvement [24].

Based on the need for a reproducible diagnostic reagent we aimed to develop a process for the production of such a reagent that contains in large parts the latex allergome at sufficient concentrations for diagnosis of latex allergy.

Materials and Methods

Latex B- and C-Serum

Fresh *Hevea* latex was collected in chilled containers from rubber trees (*H. brasiliensis*, cultivar RRIM 600). Cultivar RRIM 600 is the most frequently used cultivar for the commercial collection of latex. The latex was centrifuged at 44,000 g at 4°C for 1 h resulting in three main fractions: a top fraction containing the rubber particles, an aqueous phase, the C-serum, and a bottom fraction containing lutoids. The clear aqueous C-serum was freeze dried and stored at –20°C. The bottom fraction was resuspended in 0.4 M mannitol, resedimented and subjected to repeated alternate freezing and thawing to rupture the lutoids. The fluid of the lutoids, the B-serum, was then recovered by centrifugation [25].

Fractionating of Latex C-Serum by Ammonium Sulfate Precipitation

One hundred milligrams of lyophilized latex C-serum were dissolved in 20 mM Tris/HCl, pH 7.5. $(\text{NH}_4)_2\text{SO}_4$ was added to a 25% saturation and stirred for 30 min at 4°C. The precipitated material was centrifuged at 12,000 g for 20 min at 4°C. The proteins in the supernatant were then precipitated by adding $(\text{NH}_4)_2\text{SO}_4$ to a 50% saturation. Precipitated proteins were recovered by centrifugation and $(\text{NH}_4)_2\text{SO}_4$ was added to the supernatant to a 75% saturation. The solution was again centrifuged and the proteins from the three precipitation steps stored at –20°C.

Anion Exchange Chromatography

Precipitates from the ammonium sulfate fractionation were dissolved in 20 mM Tris/HCl, pH 7.5, and desalted by a PD-10 column (GE Healthcare, Uppsala, Sweden). Each fraction was applied to a MonoQ HR5/5 column (GE Healthcare). In the same way, 100 mg lyophilized latex B-serum were dissolved in 20 mM Tris/HCl, pH 7.5, and applied to a MonoQ HR5/5 column. Unadsorbed proteins were eluted from the column with the same buffer, and adsorbed proteins were eluted using a linear gradient of 0–1 M NaCl in 20 mM Tris/HCl, pH 7.5. Fractions were analyzed by 12% SDS/PAGE and staining with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Richmond, Calif., USA). Protein concentrations were determined with a BCA protein assay using BSA as a reference protein (Pierce, Rockford, Ill., USA).

Two-Dimensional Gel Electrophoresis

Latex proteins (100 µg) were first precipitated by acetone. Cold acetone (–20°C) was added 3:1 and the solution incubated at –20°C for 30 min. After centrifugation at 15,000 rpm for 30 min at 4°C, precipitated proteins were washed with 90% cold acetone. After centrifugation for 10 min, the precipitated proteins were dried at room temperature and then dissolved in 125 µl isoelectric focusing sample buffer (7 M urea, 2 M thiourea, 2% CHAPS, 0.5% IPG buffer, pH 3–10, and 0.002% bromophenol blue). Samples

were then applied to gel strips (Immobiline™ Dry Strip, pH 3–10, 7 cm; Amersham Biosciences, Uppsala, Sweden), and strips rehydrated overnight in the isoelectric focusing buffer. Focusing of the strips was performed at 10,000 Vh, with a maximum of 7,000 V at 20°C using the Ettan™ IPGphor (Amersham Biosciences). Prior to the second dimension, strips were incubated in equilibration buffer (50 mM Tris/HCl, pH 8.8, 6 M urea, 2% SDS, 20% glycerol and 0.002% bromophenol blue) with 65 mM DTT for 15 min and then with 250 mM iodoacetamide in the same buffer without DTT. Protein separation was then performed by 12% SDS-PAGE, and the gels were used for either Coomassie brilliant blue staining or for Western blot analysis.

IgE Immunoblots with 2D Gels

Latex proteins separated by 2D gel electrophoresis were transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Membranes were treated with blocking buffer (40 mM Na₂HPO₄, 7 mM NaH₂PO₄, 0.5% BSA, 0.05% w/v sodium azide and 0.5% w/v Tween-20, pH 7.5) for 30 min. Membranes were then incubated overnight at 4°C with a serum pool consisting of 5 sera of latex-allergic patients diluted 1:5 in blocking buffer. After three washing steps, membranes were incubated with ¹²⁵I-labeled anti-(human IgE) IgG (IBL, Hamburg, Germany) overnight. Patients' IgE binding was visualized on Biomax™ MS film (Kodak, Rochester, N.Y., USA) with equal time of exposure of the films. A serum pool from 3 healthy atopic individuals with high IgE levels for house dust mite, but negative SPTs and negative CAP results to latex was used as negative control.

Natural and Recombinant Latex Allergens

Recombinant Hev b 1, rHev b 3, rHev b 5, rHev b 6.02, rHev b 7 and rHev b 8 were purchased from Biomay (Vienna, Austria). Recombinant Hev b 1, rHev b 5 and rHev b 6.02 were expressed as fusion proteins with the maltose binding protein (MBP). Natural Hev b 2, nHev b 4 and nHev b 13 were purified from fresh latex B-serum as described previously [12, 25].

Detection of Single Allergens in Dot Blot Experiments

One microgram of the latex allergens or a recombinant MBP protein (New England Biolabs, Ipswich, UK) was dotted onto a nitrocellulose strip. Three serum pools were divided each into seven parts. Six parts of the two pools were preincubated with one of the latex fractions B1, B2 and C1–C4 and one part remained untreated. After blocking, the nitrocellulose strips were incubated with the serum pools diluted 1:5 in blocking buffer overnight at 4°C. After three washing steps, membranes were incubated with ¹²⁵I-labeled anti-(human IgE) IgG and patients' IgE binding was visualized on Biomax™ MS. A serum pool from 3 healthy atopic individuals with high IgE levels for house dust mite, but negative SPTs and negative CAP results to latex was used as negative control.

Skin Testing

SPTs were performed with 4 latex allergic patients. The diagnosis of latex allergy was confirmed by a positive NRL glove challenge. The patients underwent SPT with commercial latex extracts (Soluprick® SQ, ALK-Abelló, Horsholm, Denmark; 500 µg/ml), latex fractions and saline as well as 0.1% histamine as negative and positive controls. Purified proteins of the latex fractions were adjusted to a concentration of 1 and 10 µg/ml in saline

solution. The test was evaluated after 20 min. SPTs with a wheal diameter ≥ 3 mm were defined as positive. Before enrollment into the study, written informed consent for skin testing was obtained from all patients.

Results

Latex Fractions

Proteins of latex C-serum were first separated by 25, 50 and 75% (NH₄)₂SO₄ precipitation. Proteins of the three precipitates and the remaining supernatant were then applied to 12% SDS/PAGE gels and blotted onto nitrocellulose membranes. IgE reactivity of the proteins was tested with a serum pool consisting of 10 sera of latex-allergic subjects. IgE-reactive proteins were detectable in the 50 and 75% (NH₄)₂SO₄ fractions (data not shown).

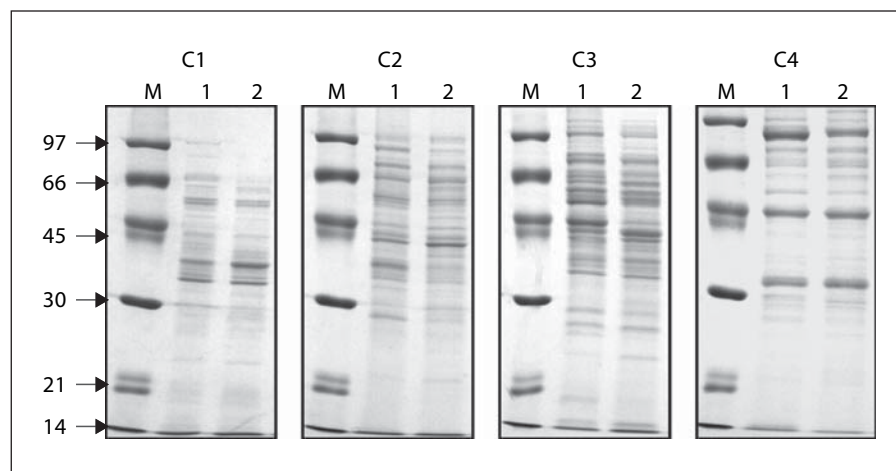
These (NH₄)₂SO₄ precipitates were then divided each into 20 fractions, and groups of peaks eluted at different concentrations of NaCl were pooled to yield the final fractions C1–C4. Fraction C1 contained proteins eluted with 150–200 mM NaCl, fraction C2 proteins eluted with 300–500 mM NaCl; both C1 and C2 were derived from the 50% (NH₄)₂SO₄ precipitate. Fraction C3 contained proteins eluted with 300–450 mM NaCl, and C4 with 450–550 mM NaCl; both C3 and C4 were derived from the 75% (NH₄)₂SO₄ precipitate. Fractions of two different latex C-serum batches were generated, and 5 µg of each fraction were separated by 12% SDS-PAGE and stained with Coomassie brilliant blue (fig. 1). Protein patterns appeared very similar, with small differences in the concentration of certain proteins (fig. 1, lanes 1 and 2).

Latex B-serum was directly applied to anion exchange chromatography yielding two fractions. Fraction B1 contained unadsorbed proteins and fraction B2 proteins eluted with 150–250 mM NaCl.

Two-Dimensional Electrophoresis and Immunoblotting

Proteins of latex B- and C-serum or latex B- and C-serum fractions were separated by 2D gel electrophoresis. One hundred micrograms of each protein extract were used, and gels stained with Coomassie brilliant blue or transferred onto nitrocellulose. Coomassie staining of latex B- and C-serum, latex B fractions B1 and B2, and latex C fractions C1–C4 showed that separation of proteins according to their surface charge resulted in higher concentrations of proteins in the fractions (fig. 2, 3, left panels). For detection of IgE binding proteins, the membranes were incubated with a serum pool consisting of 5 ran-

Fig. 1. SDS-PAGE results of the four latex C-serum fractions (C1–C4) obtained from two batches of latex C-serum (lanes 1 and 2). M = Molecular weight marker (kDa) as indicated.



domly chosen sera of latex-allergic patients. In latex B- and C-serum, several IgE-binding proteins were detectable (fig. 2, 3, right panels). The number of detectable IgE-binding proteins was increased in the latex B fractions (fig. 2, right panels) as well as in the latex C fractions (fig. 3, right panels). According to the general increase in the concentrations of proteins in the latex C fractions, visualized by Coomassie staining, a high number of proteins in the latex C fractions was recognized by IgE from the serum pool (fig. 3, right panels).

Detection of Single Latex Allergens

The presence of the latex allergens Hev b 1, 2, 3, 4, 5, 6.02, 7, 8 and 13 was demonstrated in IgE dot blot analysis. Recombinant and natural proteins were dotted onto nitrocellulose strips that were then incubated with serum pools each consisting of 4 sera previously tested in ELISA to display IgE to the respective allergens. Serum pool 1 was used for detection of Hev b 1, 3 and 5, serum pool 2 for the detection of Hev b 2, 4, 6.02 and 13 and serum pool 3 for the detection of Hev b 7 and 8. Serum pool 1 and 2 were also tested on MBP as several allergens were used as MBP fusion proteins. All serum pools were preincubated with the six latex fractions (B1, B2 and C1–C4), and decreased IgE binding is shown in figure 4. IgE binding to rHev b 1 and 3 was inhibited by fraction C2 and weaker by fraction C4. Binding to natural Hev b 2, nHev b 4, rHev b 6.02 and nHev b 13 was strongly inhibited by fraction B2 and slightly by fraction B1. Fraction C4 was able to inhibit IgE binding to rHev b 5, and fractions C1–C4 abolished binding to rHev b 7 and 8. There was an IgE binding observable to nHev b 2 by the buffer control and the control serum, but this was much weaker than the one

from the immune serum. There was no binding to MBP observable as illustrated with the result of serum pool 1 (fig. 4, lane MBP).

Skin Testing

The ability of the different fractions to induce reactions in vivo was tested by SPTs with two concentrations of the six fractions on 4 patients with NRL allergy. All patients had positive SPTs to at least two of the fractions and to a commercial latex extract that had a 50–500 times higher protein concentration than the fractions (table 1). Patients 1 and 2 had positive results to both latex B fractions. Wheal diameters of the single reactions were smaller than to the commercial extract. Patient 4 showed reactions to three latex C fractions and patient 3 to one latex B fraction and to three latex C fractions (table 1). Wheal diameters of some of the reactions were comparable to the one obtained with the commercial extract. Reactions to the lower concentration were obtained for latex B2 and C3 fractions; in both cases the wheal diameters were smaller than the ones observed with the higher concentration.

Discussion

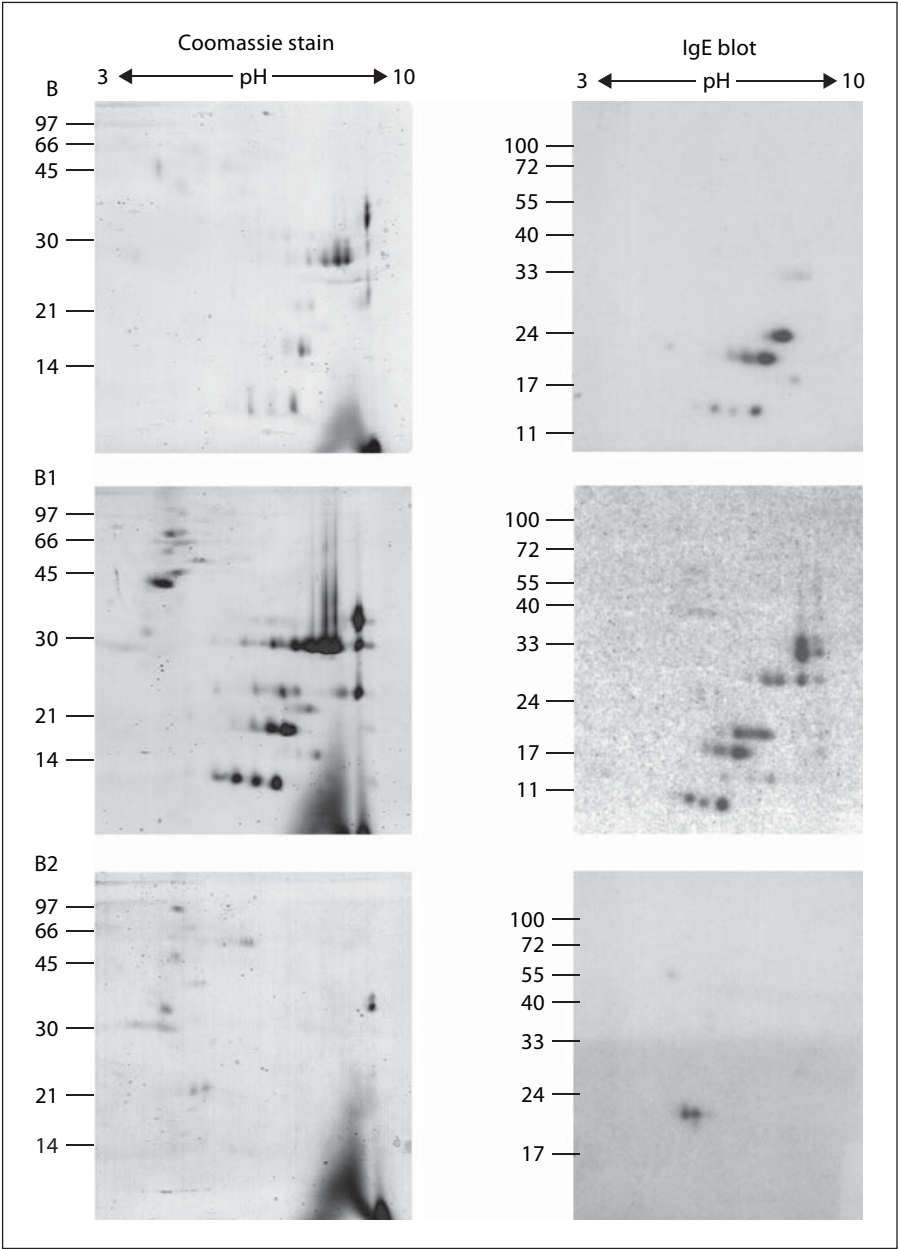
Latex allergy is an immune disease manifesting as immediate-type allergic symptoms following contact with latex or latex products in sensitized individuals. Correctly identifying individuals who have become sensitized and are likely to suffer from symptoms upon repeated exposure to latex products is a major goal of the prevention of allergic reactions. The quality of any allergy diag-

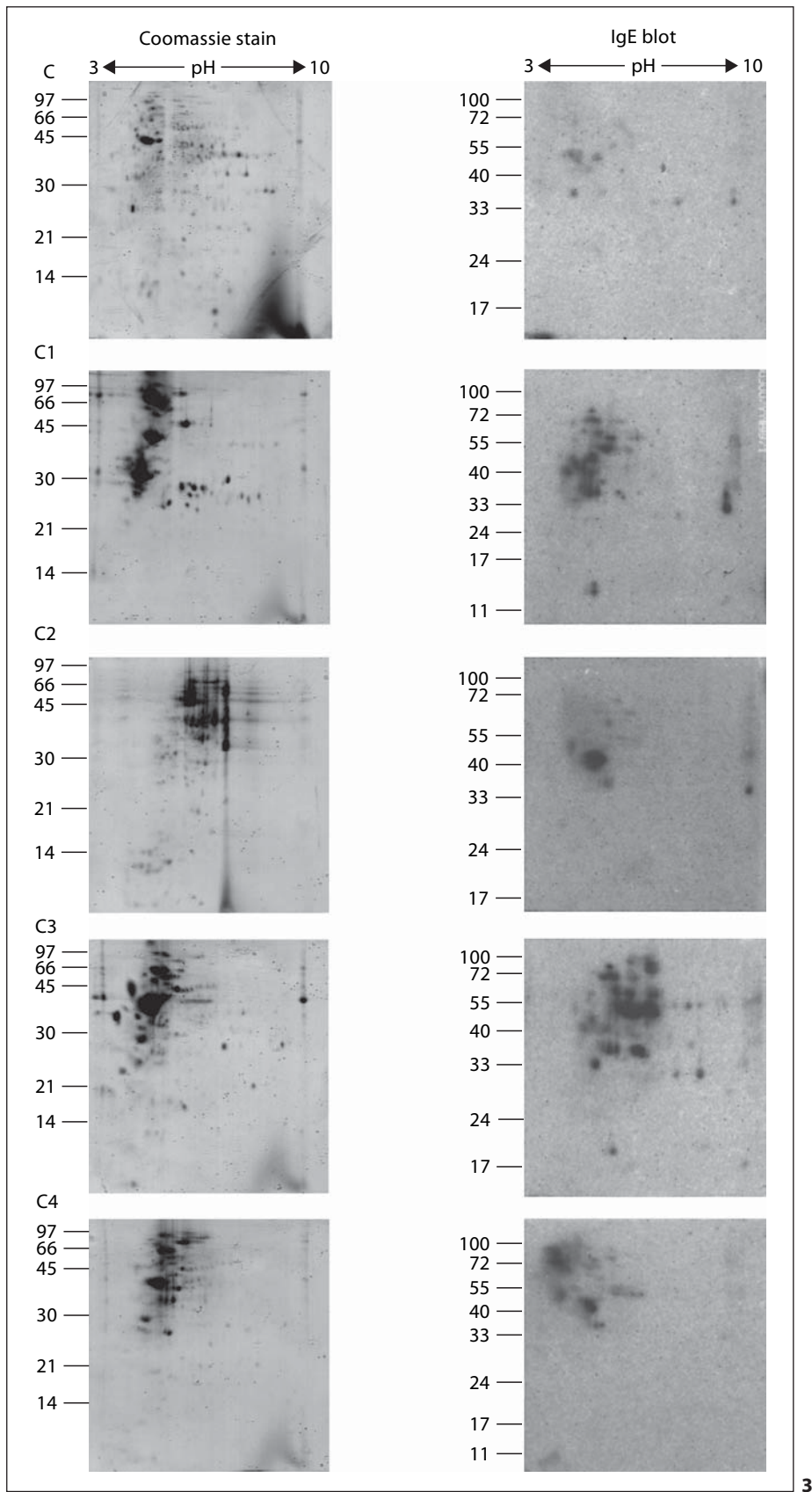
nostic test highly depends on the allergen source material and preparation methods. We have developed a method for the generation of latex protein extracts with reproducible protein content and high amounts of IgE-binding proteins including the described major allergens.

Different preparations of latex allergens from various sources have been used for SPTs or serological assays. Ammoniated latex (AL) used for the manufacture of latex products may seem the best starting material for a diag-

nostic reagent. However, besides avoiding coagulation and bacterial contamination of latex, ammonium also causes unpredictable pH changes and losses of allergenic activity during storage [26]. Consequently, non-AL (NAL) was proposed as the reagent of choice as it is a reproducible and stable source of latex allergens [26]. NAL preparations provided good diagnostic sensitivity and specificity in SPTs [13, 27, 28]. In a previous study, Posch et al. [6] described that latex C-serum from NAL contained the important latex allergens Hev b 1, 3, 6 and 7. We decided

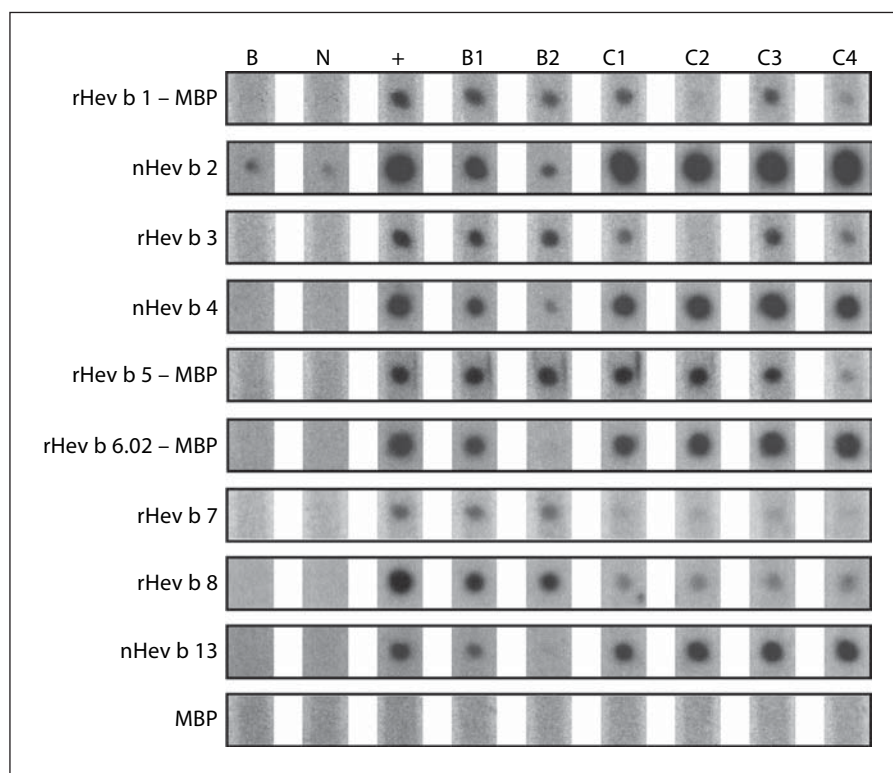
Fig. 2, 3. 2D gel images and IgE reactivity of proteins in latex B-serum and latex B-serum fractions B1 and B2 (2) and latex C-serum and latex C-serum fractions C1–C4 (3). Proteins were separated by 2D gel electrophoresis and transferred to nitrocellulose. Membranes were incubated with a serum pool consisting of 5 sera of latex-allergic patients. Molecular weight in kDa as indicated.





3

Fig. 4. For the detection of allergens, natural (n) or recombinant (r) allergens were dotted onto nitrocellulose and incubated with buffer (B), a normal human serum pool (N) or immune serum pools (+). The serum pools were also preincubated with the single fractions (B1, B2, C1–C4), and IgE binding was determined subsequently.



to separately use latex C- and B-serum obtained from NAL for the production of allergen-enriched latex fractions.

NRL from *H. brasiliensis* contains a great variety of proteins, of which at least 50–60 display IgE-binding properties [6, 7]. In our experience, it was almost impossible to produce one single protein extract from such a complex protein source that includes all latex allergens in sufficient amounts to form the basis of an accurate diagnostic reagent. Therefore, we developed a protocol to separate the NRL into several fractions in order to enrich the allergens under non-denaturing conditions.

In a first step, proteins from latex C-serum were separated into fractions by precipitation with ammonium sulfate as it protects most proteins from denaturation. Two of the four obtained fractions (50 and 75% ammonium sulfate saturation) contained IgE-binding proteins and were selected for further separation (data not shown). In a second step, proteins were separated by anion exchange chromatography using a MonoQ column. This column ensures highly reproducible separations both over time and from column to column due to the stability of the column matrix. Reproducibility of our method was shown with two different batches of latex C-serum (fig. 1).

Table 1. SPT results of 4 patients with the latex fractions (1 or 10 µg/ml) and a commercial latex extract (500 µg/ml)

	Patient 1	Patient 2	Patient 3	Patient 4
B1, 10 µg/ml	+	+	–	–
B1, 1 µg/ml	–	–	–	–
B2, 10 µg/ml	+	+	++	–
B2, 1 µg/ml	–	–	+	–
C1, 10 µg/ml	–	–	–	–
C1, 1 µg/ml	–	–	–	–
C2, 10 µg/ml	–	–	+	+
C2, 1 µg/ml	–	–	–	–
C3, 10 µg/ml	–	–	++	++
C3, 1 µg/ml	–	–	+	+
C4, 10 µg/ml	–	–	+	+
C4, 1 µg/ml	–	–	–	–
ALK 500 µg/ml	++	++	++	++

+ = A positive reaction: wheal diameter ≥3 mm; ++ = a positive reaction: wheal diameter ≥5 mm; – = a negative reaction.

As latex B-serum contains fewer proteins than latex C-serum, separation of latex B-serum proteins was only performed by anion exchange chromatography.

Two-dimensional gel electrophoresis was used to optimally visualize the protein and allergen diversity in the starting material and in the fractions. Separation of proteins into fractions increased the number of proteins that could be detected in polyacrylamide gels by Coomassie staining. Consequently, IgE immunoblots of the latex C-serum fractions detected a much higher number of IgE binding proteins compared to the original latex C-serum extract. It could be assumed that many of the protein spots represented closely related isoforms, glycosylated variants or even degradation products. Nevertheless, the number of IgE-binding protein spots detected in the latex C-serum fractions (fig. 2) were at least as many as the approximately 60 described by Posch et al. [6]. Even if a part of these IgE-binding spots is due to the presence of glycosylated allergens such as Hev b 2, 4 and 13 [12, 29, 30] and glycosylated IgE-binding non-allergens [31], the actual number of allergenic proteins present in latex certainly exceeds the 13 officially accepted latex allergens. In general, a well-balanced concentration between IgE-binding proteins in diagnostic assays is desirable. In unfractionated protein extracts, overrepresented IgE-binding proteins could mask or impair the detection of IgE binding to equally important allergens present only in lower amounts [32]. We conclude that the use of several fractions with an equalized allergen concentration is superior to the use of a total latex protein extract with rather diverse concentrations of individual allergens.

One major reason for the low sensitivity of the available serological diagnostic assays is the result of poorly represented and/or denatured allergens in the allergen-containing reagents. Especially, the allergens Hev 2, 4, 5, 6 and 7b, nowadays known as Hev b 13 [12], were described as totally absent or having denatured epitopes [22, 33]. Interestingly, the latex allergens Hev b 2, 5, 6, 7 and 13 are also described as the most important allergens [9–11]. Recently, Yeang et al. [32] demonstrated differences in mRNA and protein expression of several latex allergens in latex preparations and concluded that the use of latex serum may fail to detect latex-specific IgE reactivity in subjects. Therefore, we investigated whether our fractions contained the most important latex allergens. In our fractions Hev b 1, 2, 3, 4, 5, 6.02, 7, 8 and 13 were detected by the ability of one or more of the fractions to inhibit IgE binding to the correspondent recombinant or native allergen (fig. 4). The uniqueness of the fractions was thereby demonstrated as the IgE binding to some

proteins was inhibited by only one or two fractions (fig. 4). Based on the knowledge of the presence of the individual allergens in these fractions, a future quality control could be based on the detection of these lead allergens in newly prepared reagents. After we had demonstrated that the most important allergens were present in the fractions, the *in vivo* performance of the latex fractions was investigated with SPTs. Fractions B2 and C3 elicited similar wheal diameters than the commercial extract used in a 50 times higher concentration (table 1). Reactions to the other fractions were lower compared to the commercial extract, but each patient showed a positive reaction to at least two different fractions. This indicated that fractionation did not influence the allergenic capacity of the proteins. A prospective study on the diagnostic capacity of the fractions is underway.

During recent years first investigations using purified and recombinant allergens to diagnose latex allergy have been performed [9–11]. Routine use of recombinant allergens in latex allergy diagnosis is a desirable goal but is not satisfying unless a recombinant Hev b 2 is equivalent to the natural one [34], and the remaining important latex allergens are characterized and available as recombinant proteins. The ideal diagnostic reagent is therefore still not available. The data presented here indicate that our fractions contain the important latex allergens in their native conformation and in sufficient concentrations. They therefore represent an excellent tool for *in vitro* but also for *in vivo* diagnosis of latex allergy. In general, the strategy to separate and concentrate proteins of an allergenic source into fractions may also be applicable to other sources that contain a large variety of allergens such as molds.

Acknowledgments

We thank Dr. H. Y. Yeang from the Rubber Research Institute of Malaysia for providing latex B- and C-serum. This work was supported by the Austrian Science Fund Grant SFB F018-02 and by Biomay AG.

References

- Ownby DR: A history of latex allergy. *J Allergy Clin Immunol* 2002;110:S27–S32.
- Kimata H: Increased incidence of latex allergy in children with allergic diseases in Japan. *Public Health* 2005;119:1145–1149.
- Chen YH, Lan JL: Latex allergy and latex-fruit syndrome among medical workers in Taiwan. *J Formos Med Assoc* 2002;101:622–626.
- Ozkan O, Gokdogan F: The prevalence of latex allergy among health care workers in Bolu (Turkey). *Dermatol Nurs* 2003;15:543–547, 554.
- Dudek W, Walusiak J, Wittczak T, et al: Natural rubber latex allergy: antigen specific IgE in Polish blood donors, prevalence and risk factors – preliminary data. *Int J Occup Med Environ Health* 2005;18:35–42.
- Posch A, Chen Z, Dunn MJ, et al: Latex allergen database. *Electrophoresis* 1997;18:2803–2810.
- Yagami T, Haishima Y, Tsuchiya T, et al: Proteomic analysis of putative latex allergens. *Int Arch Allergy Immunol* 2004;135:3–11.
- Wagner S, Breiteneder H: *Hevea brasiliensis* latex allergens: current panel and clinical relevance. *Int Arch Allergy Immunol* 2004;136:90–97.
- Kurup VP, Yeang HY, Sussman GL, et al: Detection of immunoglobulin antibodies in the sera of patients using purified latex allergens. *Clin Exp Allergy* 2000;30:359–369.
- Yip L, Hickey V, Wagner B, et al: Skin prick test reactivity to recombinant latex allergens. *Int Arch Allergy Immunol* 2000;121:292–299.
- Bernstein DI, Biagini RE, Karnani R, et al: In vivo sensitization to purified *Hevea brasiliensis* proteins in health care workers sensitized to natural rubber latex. *J Allergy Clin Immunol* 2003;111:610–616.
- Arif SA, Hamilton RG, Yusof F, et al: Isolation and characterization of the early node-specific protein homologue (Hev b 13), an allergenic lipolytic esterase from *Hevea brasiliensis* latex. *J Biol Chem* 2004;279:23933–23941.
- Ebo DG, Stevens WJ, Bridts CH, et al: Latex-specific IgE, skin testing, and lymphocyte transformation to latex in latex allergy. *J Allergy Clin Immunol* 1997;100:618–623.
- Palosuo T, Makinen-Kiljunen S, Alenius H, et al: Measurement of natural rubber latex allergen levels in medical gloves by allergen-specific IgE-ELISA inhibition, RAST inhibition, and skin prick test. *Allergy* 1998;53:59–67.
- Kim KT, Safadi GS, Sheikh KM: Diagnostic evaluation of type I latex allergy. *Ann Allergy Asthma Immunol* 1998;80:66–70.
- Lundberg M, Wrangsjo K, van Hage-Hamsten M: Diagnosis of latex allergy. *Allergy* 1997;52:1042–1043.
- De Swert LF, Van Laer KM, Verpoorten CM, et al: Determination of independent risk factors and comparative analysis of diagnostic methods for immediate type latex allergy in spina bifida patients. *Clin Exp Allergy* 1997;27:1067–1076.
- Nieto A, Mazon A, Estornell F, et al: The search of latex sensitization in spina bifida: diagnostic approach. *Clin Exp Allergy* 2000;30:264–269.
- Ownby DR, Magera B, Williams PB: A blinded, multi-center evaluation of two commercial in vitro tests for latex-specific IgE antibodies. *Ann Allergy Asthma Immunol* 2000;84:193–196.
- Blanco C, Carrillo T, Ortega N, et al: Comparison of skin-prick test and specific serum IgE determination for the diagnosis of latex allergy. *Clin Exp Allergy* 1998;28:971–976.
- Hamilton RG, Biagini RE, Krieg EF: Diagnostic performance of Food and Drug Administration-cleared serologic assays for natural rubber latex-specific IgE antibody. The Multi-Center Latex Skin Testing Study Task Force. *J Allergy Clin Immunol* 1999;103:925–930.
- Chen Z, Rihs HP, Slater J, et al: The absence of Hev b 5 antigen may cause false-negative results in serologic assays for latex-specific IgE antibodies (abstract). *J Allergy Clin Immunol* 2000;106:83.
- Lundberg M, Chen Z, Rihs HP, et al: Recombinant spiked allergen extract. *Allergy* 2001;56:794–795.
- Hamilton RG, Peterson EL, Ownby DR: Clinical and laboratory-based methods in the diagnosis of natural rubber latex allergy. *J Allergy Clin Immunol* 2002;110:S47–S56.
- Sunderasan E, Samsidar H, Sharifah H, et al: Latex B-serum β -1,3-glucanase (Hev b 2) and a component of the microhelix (Hev b 4) are major latex allergens. *J Nat Rubber Res* 1995;10:82–99.
- Hamilton RG, Adkinson NF Jr: Natural rubber latex skin testing reagents: safety and diagnostic accuracy of nonammoniated latex, ammoniated latex, and latex rubber glove extracts. *J Allergy Clin Immunol* 1996;98:872–883.
- Turjanmaa K, Palosuo T, Alenius H, et al: Latex allergy diagnosis: in vivo and in vitro standardization of a natural rubber latex extract. *Allergy* 1997;52:41–50.
- Hamilton RG, Adkinson NF Jr: Diagnosis of natural rubber latex allergy: multicenter latex skin testing efficacy study. Multicenter Latex Skin Testing Study Task Force. *J Allergy Clin Immunol* 1998;102:482–490.
- Yagami T, Osuna H, Kouno M, et al: Significance of carbohydrate epitopes in a latex allergen with beta-1,3-glucanase activity. *Int Arch Allergy Immunol* 2002;129:27–37.
- Sunderasan E, Bahari A, Arif SA, et al: Molecular cloning and immunoglobulin E reactivity of a natural rubber latex lecithinase homologue, the major allergenic component of Hev b 4. *Clin Exp Allergy* 2005;35:1490–1495.
- Mari A: IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. *Int Arch Allergy Immunol* 2002;129:286–295.
- Yeang HY, Hamilton RG, Bernstein DI, et al: Allergen concentration in natural rubber latex. *Clin Exp Allergy* 2006;36:1078–1086.
- Hamilton RG, Biagini R, Mackenzie B, et al: FDA-cleared immunoassays for latex-specific IgE are missing allergenic epitopes from multiple Hev b allergens (abstract). *J Allergy Clin Immunol* 2002;109:S259.
- Raulf-Heimsoth M, Rihs HP, Bruning T: Latex: a new target for standardization. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf AM*, 2003, pp 107–115.