

Protein and Allergen Analysis of Latex Mattresses

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Key Words

Allergy · ELISA · Latex allergen · Mattress · Western blot

Abstract

Background: Latex allergy is a well-recognized health problem. The wide use of latex in daily life raised the question whether latex articles could be a source of allergens. The present study was carried out to analyse various latex mattresses for protein and allergen characterization. **Methods:** Five latex foam mattresses were reduced to powder and proteins were extracted using either water or urea. The protein content of the extracts was analyzed by SDS-PAGE, IEF, 2-D gel electrophoresis and amino acid sequence analysis. The presence of specific IgE, directed toward mattress proteins found in human sera of latex-allergic patients, was tested using ELISA and immunoprints. **Results:** The results showed that no protein or allergen could be extracted from synthetic foam. However, depending on the type of mattress and extraction procedure, various quantities of proteins could be extracted from mattresses containing natural latex. The ELISA levels of various latex-allergic patients were always more significant against urea extracts than against water extracts, except for one mattress. The protein and allergen patterns were qualitatively similar for all mattresses containing natural latex. Hev b 1 was identified by N-terminal amino acid sequence analysis. **Conclusion:** Because the natural rubber of the mattresses contains latex allergens, these allergens are a potential source of sensitization and could constitute a risk, at least to allergic individuals.

Introduction

Latex allergy is now a well-recognized health problem. While its prevalence does not seem to exceed 1% in the general population [1], it can reach 7–12% for health care workers, especially surgeons, dentists and nurses [2–4]. The increased use of latex gloves in the late 1980s for protection against AIDS and other blood-borne viruses is certainly one of the main reasons for the increase in latex sensitivity. However, it seems that modifications in the manufacturing processes also play a role [5]. Moreover, some data suggest that the incidence of anti-latex IgE positive sera can reach 6% in a population of volunteer blood donors [6].

Latex products are widespread in the daily environment (e.g. balloons, baby pacifiers, shoes, mattresses). Therefore, the question whether such articles could be a source of allergic sensitization should be raised. In 1997, Baur et al. [7] examined mattresses among a series of various latex articles, including a great number of gloves, in use in the medical environment. They found proteins but no allergen in mattresses.

The aim of this study was to compare the proteins extracted from various natural and synthetic latex mattresses with a glove extract and a standardized latex extract used for skin prick tests, and to test for protein and allergen characterization.

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Materials and Methods

Mattress Origins

Five latex mattresses commonly used in households were studied: mattress M1 was exclusively composed of a synthetic foam; mattress M3 was 100% natural latex, and mattresses M2, M4 and M5 contained various proportions of natural rubber latex.

Human and Rabbit Sera

Human sera were obtained from latex-sensitive patients (9 women and 3 men) selected on the basis of positive values for serum-specific IgE (RAST class between 3 and 6). These sera have been used as immunochemical reagents to identify allergens upon their ability to bind to IgE.

The rabbit anti-latex serum was obtained from a classical hyperimmunization procedure using a standardized latex extract (Stallergènes, France) as an antigen.

Protein extraction and determination

Each mattress was reduced to powder after freezing and crushing in liquid nitrogen. One gram of each mattress powder was extracted for 30 min at 22°C either in 4 ml of distilled water or in 4 ml of 8 M urea containing 2% CHAPS (Sigma Biochemicals, USA). The extracted solution was filtered first with glass wool and then with a 0.45-µm pore size filter (Sartorius, Germany).

A latex examination glove (lightly powdered, hypoallergenic, Safeskin, Germany) was cut into small pieces, reduced to powder and extracted with urea-CHAPS according to the procedure described above.

The protein content of each sample was determined using a micro-BCA protein kit, following the manufacturer's instructions (Pierce, Rockford, USA). Bovine serum albumin (BSA; Sigma Biochemicals, USA) was used as the protein standard. This method was tested to give reliable results with BSA dissolved in 8 M urea and 2% CHAPS.

A latex extract used for skin prick tests was from Stallergènes (France) and was used as the standardized extract.

ELISA

The reactivity of 11 latex-sensitive patient sera (RAST class between 3 and 6) was tested using the ELISA method. Briefly, the microtest plates were coated with 10 µg/well of each sample (mattresses, gloves and a standard latex extract) in a 50 mM sodium carbonate buffer pH 9.6 overnight at 22°C. After blocking with 1% BSA in PBS-0.1% Tween 20 (PBS-BSA-Tw), the wells were successively incubated for 2 h at 37°C with 100 µl of the human serum dilution (1:10 in PBS-BSA-Tw) and 100 µl of alkaline-phosphatase-conjugated goat anti-human IgE (1:700 in PBS-BSA-Tw, Sigma Biochemicals, USA). The alkaline phosphatase activity was detected using *p*-nitrophenyl phosphate disodium (Sigma Biochemicals, USA) in 0.1 M Tris buffer pH 9.5. The wells were extensively rinsed with 0.9% NaCl-Tw after each incubation. A rabbit anti-latex serum was used as a positive control, diluted 1:1,000, followed by alkaline phosphatase-conjugated mouse anti-rabbit IgG (Sigma Biochemicals, USA) at 1:700.

SDS-PAGE and IEF

1-D SDS-PAGE of 10 µl mattress extracts were performed in either a 12% linear gel or an 8–18% gradient gel (ExcelGel, Amersham-Pharmacia Biotech, Sweden), using a flat-bed apparatus following the

manufacturer's instructions. The gels were silver-stained according to Blum et al. [8] or submitted to a Western blot.

An IEF analysis of the urea extracts was performed in a 4% polyacrylamide gel containing 5% v/v Servalyt® pH 2–11 (Serva, Heidelberg, Germany) on a flat-bed apparatus [9]. The gel was then fixed in trichloroacetic acid and silver-stained according to Blum et al. [8].

According to the results of IEF and SDS-PAGE, mattress M3 urea extract was further analysed by 2-D electrophoresis and immunoprinting.

2-D Electrophoresis

The M3 urea extract was submitted to an IEF as described above. Two 4-mm-wide strips of the focused gel were cut and submitted to a SDS-PAGE separation on an 8–18% gradient gel. One of the 2-D gels was silver-stained and the other one submitted to a Western blot.

Western Blot

After SDS-PAGE separation or 2-D electrophoresis, the proteins were electroblotted onto an activated nitrocellulose sheet (NC) (Schleicher & Schuell, Basel, Switzerland) according to De-meulemester et al. [10] with a semi-dry Novablot apparatus (Amersham-Pharmacia Biotech, Sweden) following the manufacturer's instructions (1 h, 1 mA/cm²). Just after blotting, the NC was dried and blocked with PBS-BSA-Tw for 1 h at 22°C. Then, the NC was incubated with either a rabbit anti-latex serum (1:1000 in PBS-BSA-Tw) or a human serum from a latex-sensitive patient (1:20 in PBS-BSA-Tw), overnight, 22°C. After extensive washing, the NC sheets were incubated for 2 h at 22°C with either alkaline phosphatase-conjugated mouse anti-rabbit IgG (1:700 in PBS-BSA-Tw; Sigma) or alkaline-phosphatase-conjugated goat anti-human IgE (1:700 in PBS-BSA-Tw; Sigma). The alkaline phosphatase activity was detected using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Sigma) in 0.1 M Tris buffer, pH 9.5.

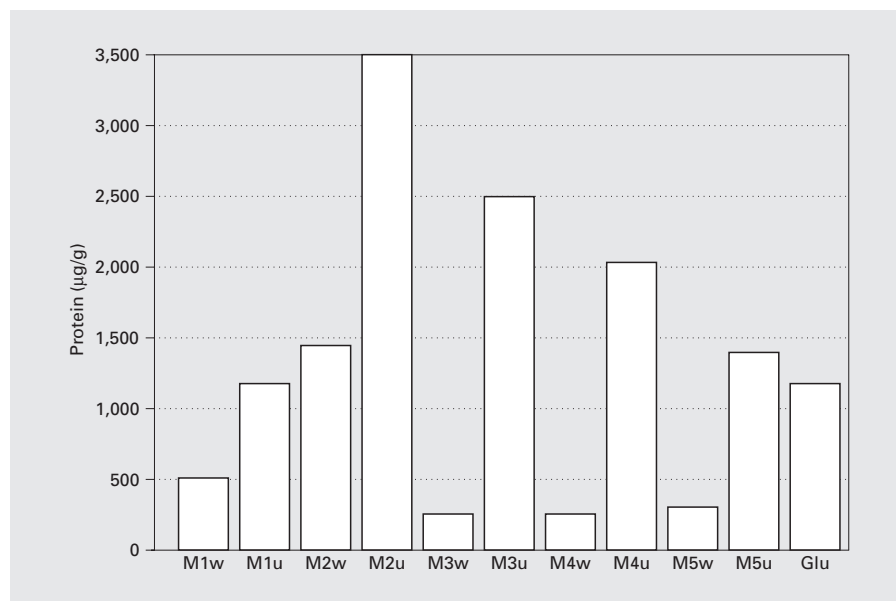
Screening by Immunoprinting

A single deposit (8 cm wide) of the M3 urea extract was submitted to an IEF as described above. The immunoprint was performed by laying the gel on a NC sheet for 1 h under 1 kg pressure. After printing, the NC was dried and cut into 3-mm-wide strips. Each strip was blocked with PBS-BSA-Tw for 1 h at 22°C in an Octaline disposable tray (Intermed, Denmark). Then, each strip was incubated overnight at 22°C with 0.5 ml of a single human serum diluted 1:10 in PBS-BSA-Tw. The presence of IgE was then detected as described for the Western blots.

Amino Acid Sequence Analysis

After an IEF separation, the proteins were electrotransferred overnight onto a ProBlott membrane (Applied Biosystems, Weiterstadt, Germany) in 50 mmol/l borate, 50 mmol/l Tris (pH 8.3). After Coomassie staining, the spot of interest was cut out. Microsequencing was performed by Edman degradation with an Applied Biosystems sequencer (model 473A). The N-terminal amino acid sequence was compared for homologies to already known proteins by using the Basic Local Alignment Search Tool (BLAST) algorithm [11] (PIR, Swiss-Prot and Genpept databases).

Fig. 1. Respective amounts of protein extracted from various latex samples evaluated by micro-BCA, using BSA as a protein standard. The water (w) or urea-CHAPS (u) extracts of five mattresses (M1–M5) were quantified along with a urea-CHAPS glove extract (Glu).



Results

Protein Extraction

The respective amounts of protein extracted from each mattress powder with either water or urea are shown in figure 1. The results show that water allowed the extraction of only very small amounts of protein (maximum 500 µg/g), except for one mattress (M2), which reached the value of 1,444 µg/g. Using urea, the quantity of proteins extracted increased from 2 to 10 times the amount obtained with water. Mattresses M3 and M4, which had the lowest level of water-extracted proteins, reached a level of 2,504 and 2,025 µg/g, respectively, with urea extraction. Mattress M2 contained the highest protein level with urea extraction, with a value of 3,480 µg/g. Mattresses M1 and M5 gave protein levels in the urea extracts analogous to the level of the glove extract (1,164, 1,386 and 1,154 µg/g, respectively).

ELISA Reactivity of Human Sera

Human sera from 11 patients allergic to latex were tested for reactivity against the various mattress extracts. The latex extract from Stallergènes recognized by a rabbit anti-latex serum served as the control. The results of the ELISA are presented in table 1. Among the 11 human sera, 1 serum (No. 29) reacted very strongly with the Stallergènes extract. Serum 29 also reacted very strongly with all the mattress urea extracts, except the M1 extract. Moreover, only the M2 water extract was recognized by serum 29. The optical densities obtained were similar with serum 29 and the rabbit serum for each extract. However, the reactivity of serum 29

was higher against mattress extracts than against the standardized latex extract.

SDS-PAGE and Immunoblotting

The SDS-PAGE separation of the various latex extracts is shown in figure 2. After SDS-PAGE separation and silver staining, the standardized latex extract displayed the classical pattern of latex with more than 15 protein bands. No protein band was identified in the M1 extracts (water and urea) but a smear was observed between 20 and 35 kD. The water and urea extracts of M2 and the urea extract of M3 displayed three major bands: two bands around 14–16 kD, and one band at 30 kD. An additional band was observed at 46 kD, mostly in the M2 extracts. M4 and M5 urea extracts showed a major band which corresponded to that of the high-molecular mass of the 14–16 kD doublet. A smear was visible in the M2, M4 and M5 extracts.

With the same urea extraction protocol, the latex glove pattern contained only two bands around 14–16 kD without a smear.

After Western blotting and incubation with human serum 29, no band was recognized in the M1 extract (fig. 3a, lane M1). However, it is clear that the 14–16 kD doublet and the two other bands (30 and 46 kD) were recognized by IgE antibodies in M2 and M3 extracts (fig. 3a, lanes M2u, M2w and M3). An additional protein band with a molecular mass less than 14.4 kD was seen in the M2 urea extract (fig. 3a, lane M2u, arrow). In M4 and M5 extracts (fig. 3a, lanes M4 and M5), the high 14–16 kD band was well recognized, the low 14–16 kD and the 30-kD bands were weaker.

Table 1. ELISA of latex-sensitive patients and a rabbit anti-latex serum tested with latex extracts of various origins

Serum	M1w	M1u	M2w	M2u	M3w	M3u	M4w	M4u	M5w	M5u	Stall	RAST
34	2	2	2	2	2	2	2	3	1	2	5	3/6.84
9	2	2	1	2	2	2	1	3	1	2	13	3/9.42
33	2	2	1	1	2	2	2	2	1	1	6	3/12.6
28	–	1	–	–	–	–	–	1	–	–	17	4/18.0
1	–	1	–	–	1	–	–	1	–	–	8	4/20.7
2	1	2	–	1	1	1	1	2	–	1	7	4/28.0
3	1	2	–	–	1	–	1	2	–	–	8	4/32.6
40	–	1	–	1	1	–	–	1	–	–	46	5/84.0
42	1	2	3	3	1	3	1	4	1	3	43	6/>100
8	2	2	1	1	2	2	2	2	1	1	25	6/>100
29	3	10	191	268	8	262	4	274	4	273	173	6/>100
62	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	6/>100
Rabbit	4	8	282	296	18	296	7	295	10	293	336	ND

Eleven sera from latex-sensitive patients were tested for their IgE reactivity to water (w) and urea (u) extracts from various mattresses (M1–M5) and a standard latex extract (Stall). A rabbit anti-latex serum was used as a positive control.

Values are optical densities $E \times 100$. RAST class/kU/l are indicated on the right. (–) = Optical densities below 0.005. ND = Not done.

The positive control of the latex nature of these proteins was done by the recognition of the same bands by the rabbit anti-latex serum (fig. 3b). The rabbit IgG recognized a great number of proteins in the Stallergènes extract. In the mattress extracts, the bands around 14–16, 30 and 43 kD were recognized by rabbit IgG, except for M1 for which no band was identified.

IEF and Immunoprints

The IEF separation of the various mattress urea extracts compared with the glove and the standardized Stallergènes extracts showed a similar pattern for gloves and mattresses (fig. 4a). As well as with SDS-PAGE analysis, no protein band was identified in the M1 extract and the patterns of M2 to M5 were equivalent in quality, with distinct quantities of proteins corresponding to the variations given by the micro-BCA. In M2, M3, M4 and M5, as well as in gloves, most of the proteins were acidic (pH < 4.75) but some isolated bands appeared between pH 6 and 9. Compared with this pattern, the Stallergènes extract contained a number of bands between pH 4 and 6.45 and 5–6 bands between pH 8.5 and 10.6.

The screening of 21 human sera by immunoprinting allowed us to identify three sera which contained IgE against the M3 urea extract. The pattern of recognition was identical for the three sera. Two out of these three sera compared with a negative one are presented in figure 4b. The result

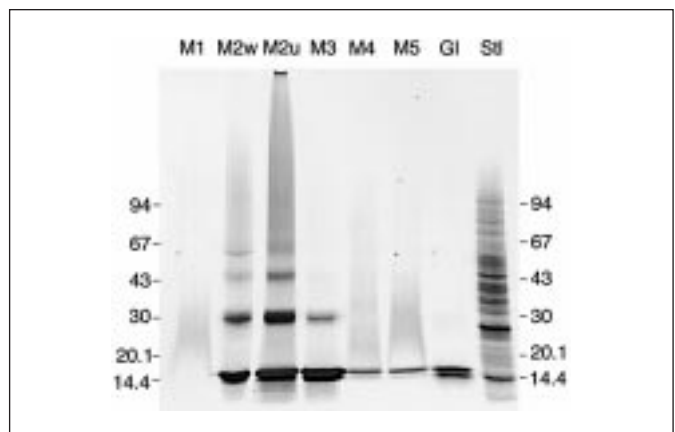


Fig. 2. Silver-stained SDS-PAGE of various latex products. Ten microliters of each sample were analyzed in a 8–18% polyacrylamide gradient gel. M1 = Mattress 1, urea extract; M2 = mattress 2; w = water extract; u = urea extract; M3 = mattress 3 (urea extract); M4 = mattress 4 (urea extract); M5 = mattress 5 (urea extract); Gl = gloves (urea extract); Stl = standard latex extract from Stallergènes.

shows that almost all the bands were recognized by both sera. The three bands with pHs around 6.3, 7.8 and 8.5, which were clearly recognized in M3 urea extract, do not seem to correspond to major bands in the Stallergènes extract.

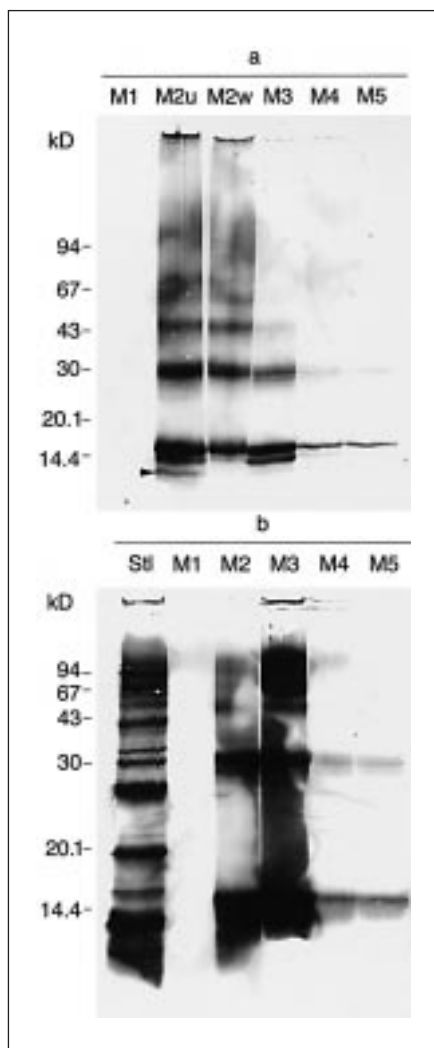


Fig. 3. Immunoblotting of various latex mattresses. **a** Mattress extracts were submitted to a SDS-PAGE in a 8–18% polyacrylamide gel gradient, transferred by Western blot on an NC sheet and revealed by IgE of a human serum from a latex-allergic patient (No. 29). **b** Mattress urea extracts were submitted to an SDS-PAGE in a 12% polyacrylamide gel, transferred by Western blot on an NC sheet and revealed by IgG of a rabbit anti-latex serum. Molecular masses (kD) are indicated for each gel. M1 = mattress 1 (urea extract); M2 = mattress 2 (w = water extract; u = urea extract); M3 = mattress 3 (urea extract); M4 = mattress 4 (urea extract); M5 = mattress 5 (urea extract); Stl = standard latex extract from Stallergènes.

2-D Electrophoresis

The 2-D electrophoresis of the M3 urea extract is presented in figure 5. The result shows that multiple spots were identified at a molecular mass corresponding to the lower molecule of the doublet. At least 4 spots were grouped in a zone of pH values less than 4.75, and 4 other spots were related to proteins of pH of approximately 5, 6.3, 7.8 and 8.5.

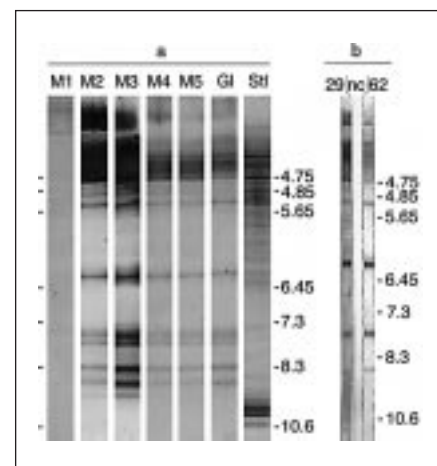


Fig. 4. IEF of various latex products. Mattress urea extracts were submitted to IEF in 4% polyacrylamide gel, 2–11 pH gradient. Isoelectric points are indicated on the sides. **a** silver-stained gel. Mattress urea extracts; M1 = mattress 1; M2 = mattress 2; M3 = mattress 3; M4 = mattress 4; M5 = mattress 5; Gl = glove (urea extract); Stl = standard latex extract from Stallergènes. **b** Immunoprints of an IEF of the M3 urea extract revealed by human IgE from latex-sensitive patients. 29 and 62: sera from latex-sensitive patients positive for mattress allergens. nc = Negative patient.

The highest molecule of the doublet displayed a wide spot with an acidic pI lower than 4.75. The molecule identified at 30 kD was also very acidic (pH < 4.75). The molecule with a molecular mass lower than 14 kD corresponded to 3 spots at pH around 6.3, 7.9 and 8.6.

The Western blot of the 2-D gel showed that all these spots were recognized by the IgE of a sensitive patient (data not shown).

Amino Acid Sequence Analysis

N-terminal amino acid sequence analysis of the allergen shown in figure 5 (arrow) was performed. The sequence of the 10 N-terminal residues was determined as GEGLKYL-GFV. This sequence showed 100% identity with peptide 11–20 of Hev b 1 [12].

Discussion

In this study, we used two protein extraction solutions, distilled water and urea-CHAPS, to analyse latex allergens. Both solutions were chosen for their compatibility with 2-D electrophoretic analysis [13]. Micro-BCA, a sensitive and widely used commercially available protein test was chosen for its compatibility with urea-CHAPS extraction.

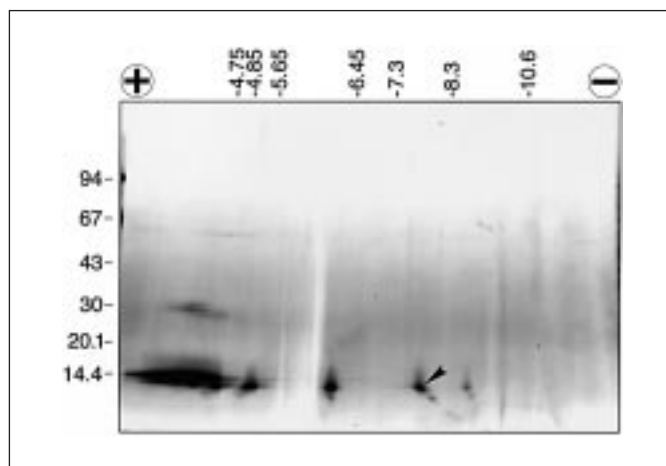


Fig. 5. Silver-stained 2-D electrophoresis of M3 urea extract. First dimension: IEF in 4% polyacrylamide gel, pH 2–11. Second dimension: 8–18% SDS-PAGE. Arrow indicate the protein submitted to N-terminal amino acid sequence analysis. Molecular masses (kD) and isoelectric points are indicated.

Distilled water extraction resulted in very low levels of protein from the various types of mattresses studied, except for one: M2. But extraction with urea allowed us to obtain 1,154 µg protein/g of glove powder and quantities varying from 1,164 to 3,480 µg/g of mattress powder depending on the mattress type. Some authors have used phosphate buffered saline as a protein-extracting medium for latex gloves [14–17]. With this type of extraction, Yunginger et al. [14] obtained protein levels varying from less than 75 to 5,700 µg/g, according to glove type, whereas Paluoso et al. [17] obtained values between 7 and 1,640 µg/g. With Safe-skin hypoallergenic examination gloves, Yunginger et al. [14] obtained the value of 1,725 µg/g. Therefore, it can be assumed that latex mattresses release a quantity of protein comparable to levels released by some powdered gloves. Previous studies have stated that the protein content does not directly correlate with allergen content [15, 17]. However, a recent classification [16] has shown that the more allergenic gloves were also those containing most protein (with only one or two exceptions out of twenty).

The protein pattern of the glove extract analyzed by SDS-PAGE, presented here corresponded well with that presented by Alenius et al. [18], with two major bands corresponding to the 14- to 16-kD doublet.

In the M1 extract, no silver-stained band was observed by SDS-PAGE. Using micro-BCA, based on a BSA standard curve, the M1 urea extract was found to be rich in protein (1,164 µg/g of powder). Thus, we cannot exclude the possibility that an extractable material is interfering with

the micro-BCA method, leading to an elevated 'protein-like' content. Moreover, in this sample, a smear ranging from 20 to 35 kD was clearly visible by SDS-PAGE, but was not recognized by Western blot. This smear was also found in the extracts of the mattresses that contained synthetic latex, but it was absent from the M3 and the glove extracts made of pure natural latex. Thus, the 'protein-like' extractable material could also be present in M2, M4 and M5 mattresses. In that case, the 'true' protein content should be that measured by us minus the value corresponding to the synthetic rubber. Even if the micro-BCA, as well as any of the protein tests we know of, does not give absolute values, the respective amounts of protein evaluated by our technique corresponded well with the SDS-PAGE results and the previous published glove values. This means that mattresses containing natural latex can release more allergens than gloves, in the same conditions of extraction. It is difficult to evaluate how these proteins could be released from the foam and get into contact with skin or spread into the air in normal conditions of use. Various factors such as the resistance of the foam to mechanical wear and the thickness and quality of the covering which isolate the foam from the skin could greatly influence the possibilities of release and, as a consequence, the risk of exposure and sensitization.

These results illustrate a significant diversity in the protein content of the commercial latex mattresses we studied. However, isoelectric focusing shows that mattresses containing natural latex (M2 to M5) displayed a very similar pattern of proteins with variable intensities. This common pattern is also very similar to the glove pattern and quite different from the Stallergènes latex extract. A concentration of acidic proteins as well as bands around pH 7.3 and 8.5 was observed in the mattress extracts and was not so clearly identified in the Stallergènes extract. The manufacturing processes could probably play a critical role in the quantity and nature of the releasable protein.

The allergenic patterns of mattress proteins were studied by immunoblotting using a human serum previously selected by ELISA in a series of 11 sera from latex-sensitive patients. None of the sera reacted against the M1 extracts, even the rabbit anti-latex serum. For the other extracts, one human serum reacted more strongly by ELISA against the mattress extracts than against the latex standardized extract from Stallergènes. This serum was used for the identification of the allergens by Western blot. A later screening by immunoprints following IEF allowed to identify a second and a third human serum which recognized latex mattress allergens. A third serum displayed a RAST anti-latex class 6 and IgE titers > 100. Thus, it seems that recognition of mattress allergens by latex-sensitive patients is not so rare, but

limited to patients with high IgE titers. A more extensive study could be needed to assess the real incidence of mattress allergen recognition.

In mattresses M2 and M3 three main protein bands were observed with molecular masses around 14–16 kD (doublet) and 30 kD, and one minor band at 46 kD. All these bands were recognized by IgE antibodies and shared electrophoretic migration characteristics with well-known latex allergens.

Rubber elongation factor, Hev b 1, and Hev b 5 were recognized as major latex allergens with respective molecular masses of 14.6 and 16 kD [19–21]. Both proteins were identified in natural rubber products [22]. At a molecular mass around 14 kD, Posch et al. [21] demonstrated by 2-D electrophoresis that multiple isoforms of Hev b 1 are present in a pH zone of less than 4.5–5.5, and various isoforms of the C-domain of prohevein, Hev b 6.03, in a pH range of 5.6–7.3. By 2-D gel electrophoresis, we obtained proteins of a molecular mass around 14 kD and basic isoelectric points around 7.8 and 8.5. The N-terminal amino acid sequence analysis of the band at a pH around 7.8 was done to identify this basic protein. The results showed that this band displayed 100% identity with peptide 11–20 of Hev b 1 [12]. Thus it can be concluded that this protein is an isoform or a degradation product of Hev b 1. The lack of the 4 strongly acidic amino acids situated in position 2–5 can explain the isoelectric point around 7.8.

Hevamine, a 30-kD protein, is not considered to be a major allergen, but has been shown to be recognized by some anti-latex sera [18, 23, 24]. Patatin-like protein, Hev b 7, is

a 46-kD protein which has been described as a major allergen in health care workers [25]. This molecule has recently been cloned and produced as a recombinant allergen [26].

A major band corresponding to the molecular mass of Hev b 5 was present in M4 and M5 extracts, together with a 30-kD band corresponding to the molecular mass of hevamine.

An additional band with a molecular mass below 14 kD was recognized in the M2 urea extract. This band could be related to other molecules ranging from 10 to 11 kD which have previously been shown to be potential latex allergens [18, 23].

In conclusion, mattresses containing natural rubber products (M2–M5) in various proportions contain extractable protein. When synthetic rubber was used (M1), no protein or allergen could be evidenced. Among the 21 latex-allergic patient sera used to detect allergens, only 3 recognized the allergens extracted from the mattresses but, in 1 mattress, these proteins were water-extractable. If latex mattresses are not yet considered to be potential sources of allergic sensitization, the potential release of allergens from rubber foam could constitute a risk, at least to latex-allergic individuals. The manufacturing processes of the various foams clearly influence the quantity and variety of the extractable allergens, as shown in this study.

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