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Identification of Hevamine and Hev b 1 as Major Latex Allergens in Taiwan

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Key Words
Latex allergy · Hev b 1 · Hevamine

Abstract
Background: Proteins from latex gloves have been documented to trigger occupational latex allergy among health care workers. Allergen characterization of latex glove extract has never been studied in Taiwan. This study aimed to identify allergenic proteins from latex gloves. ***Methods:*** Crude extracts of latex gloves were prepared with phosphate-buffered saline and 20 medical workers with a history of latex allergy were enrolled in this study. The specific IgE antibody was determined by the Pharmacia CAP system and in-house enzyme-linked immunoassay and immunoblotting. The target proteins were excised from two-dimensional PAGE and analyzed by electrospray ionization tandem mass spectrometry. ***Results:*** Immunoblotting of glove extracts revealed three IgE-binding proteins at a molecular mass of 45, 30 and 14 kDa. Peptide mass fingerprinting revealed that the protein at 45 kDa, which was recognized by 10% (2/20) of atopic sera tested, was an allergenic lipolytic esterase from *Hevea brasiliensis* (Hev b 13). The 30- and 14-kDa proteins, which were recognized by 55% (11/20) and 85% (17/20) of patients' sera, were found to be hevamine and rubber elongation factor (Hev b 1), respectively. ***Conclusions:*** Our results indicated that hevamine and Hev b 1 are the major allergens from latex gloves in Taiwan, which differs from the reports in Western countries.

Introduction

Natural rubber latex (NRL) is the milky sap obtained by tapping the bark of *Hevea brasiliensis* trees. It is a common component in various medical equipments and its allergic effect in humans has been widely studied [1]. Immediate allergy to latex gloves was first reported in 1979 [2] and numerous data on latex allergy were reported during the late 1980s due to the widespread increase in the utilization of latex gloves to reduce the risk of infection [3]. Nowadays, latex allergy has been recognized as a serious occupational disease in glove-wearing personnel, especially among health care workers [4–8]. A prevalence of 2–17% of latex allergy in Europe and the USA, and 6.8–8.6% in Taiwan among medical workers has been reported [4, 5]. To date, more than 30 significant allergens in NRL have been described [9], but only 13 of them have been cloned and named (Hev b 1 to Hev b 13) according to the World Health Organization and the International Union of Immunological Societies nomenclature [10–13]. More than half of the latex-sensitized subjects have evidence of concomitant food allergies, and this type of IgE cross-reactivity is called 'latex-fruit syndrome' [14–16]. It is conceivable that the structural similarity among proteins from diverse sources is the molecular basis of allergic cross-reactivity.

Recently, proteomics technology has been used to identify pollen and latex allergens [17, 18]. The mass spectrometer converts components of a mixture to ions and then analyzes them on the basis of their mass/charge

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(m/z) [19]. The data are automatically recorded by the data system and can then be retrieved for manual or computer-assisted interpretation. The electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis provides peptide mass fingerprints, which are compared with protein databases, and lead to protein identification. Previously, we had identified 30- and 42-kDa allergens from Indian jujube *(Zizyphus mauritiana)*, a frequently consumed tropical fruit in Taiwan, which are cross-reactive with latex allergens [20]. Recently, we reported the cloning of the 30-kDa Indian jujube allergen, Ziz m 1, and expressed recombinant protein in yeast *Pichia pastoris* [21]. Moreover, the computer-assisted homology search revealed that recombinant Ziz m 1 has a sequence similarity to hevamine from *Hevea brasiliensis.* Hevamine has been considered as a minor latex allergen in the Western countries. However, the present study identifies hevamine (30 kDa) as a major latex allergen using ESI-MS/MS among medical workers in Taiwan.

Subjects and Methods

Preparation of Crude Latex Glove Extracts

Proteins were extracted from eight different lots of lightly powdered, non-sterile latex gloves from three manufacturers (Protos, Indonesia; De Crown, Malaysia; Enchs, Indonesia) that are routinely used at Taichung Veterans General Hospital. Briefly, 100 g of gloves were cut into small pieces and mixed with 500 ml of sterile phosphate-buffered saline (PBS; pH 7.4) for 1 h at 37°C, prior to storage at 4°C overnight. Thereafter, the latex was removed by centrifugation at 4,000 *g* for 30 min, and the supernatant was filtered with a 0.45-μm filter (Millipore, Bedford, Mass., USA). The resulting extract was concentrated 150-fold using Amicon Ultra centrifugal filter devices with an exclusion size of 10 kDa (Millipore). Total protein contents were determined using the Bio-Rad Bradford assay (Bio-Rad, Hercules, Calif., USA). Bovine serum albumin (Sigma Biochemical, St. Louis, Mo., USA) was used as protein standard.

Patients and Latex-Specific IgE

Skin prick test was performed using both commercial latex crude extract (100 IR/ml; Stallergenes SA, France) and laboratory-prepared latex glove extracts at a concentration of 100 μg/ml in PBS with 50% glycerol after informed consent as previously described [20]. The Institutional Review Board of Taichung Veterans General Hospital had approved the project. A total of 20 latex-sensitive hospital employees and 10 healthy non-allergic individuals were included in this study. Diagnostic measurement of allergen-specific IgE was performed with the CAP-FEIA system (Pharmacia Diagnostic, Sweden). Specific IgE to laboratory-prepared latex extracts was measured by using the enzyme allergosorbent test kit according to the manufacturer's instructions (Allergopharma, Reinbek, Germany). Sera were stored at –70°C.

SDS-PAGE and Western Blot Analysis

Glove extracts were subjected to 4–12% of SDS-PAGE (30 μg/lane) according to the method described by Laemmli [22]. For detection of IgE binding to the glove proteins, immunoblotting was performed with individual patient serum from 20 latex-allergic subjects and a sera pool of 10 normal subjects. The separated proteins on the gels were transferred onto polyvinylidene fluoride membrane [23]. Strips were blocked with 5% skimmed milk for 1 h and incubated overnight in patient's serum diluted 1:10 as primary antibody. After washing, alkaline phosphatase-labeled mouse anti-human IgE monoclonal antibodies (1:2,000; PharMingen, San Diego, Calif., USA) were used as secondary antibody. 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.

Two-Dimensional Gel Electrophoresis

Latex extract was subjected to two-dimensional (2D)-PAGE analysis with Bio-Rad protean II xi 2-D cell system as recommended by the manufacturer. Briefly, the first-dimension tube gels were cast in 14-cm (1.5 mm internal diameter) capillary tubes containing 1.5% CHAPS, 0.5% Nonidet P-40, 9.2 *M* urea, 4.5% acrylamide/bis (29.2:0.8) and 5% pH 3–10 carrier ampholytes (Bio-Rad). Following electrophoresis in the first dimension, the second-dimension SDS-PAGE was performed as described above. Immunodetection was performed as described above except that the development was performed using a chemiluminescent substrate solution (Applied Biosystem, Bedford, Mass., USA) and the signals were recorded by exposure to an X-ray film.

Mass Spectrometry

Proteins were visualized with silver stain after 2D-PAGE, and the target protein spots were excised for in-gel digestion using a Montage In-Gel Digest Kit (Millipore) according to the manufacturer's instructions. The tryptic peptide mixtures were analyzed by ESI-MS/MS (Finnigan MAT, San Jose, Calif., USA), which was performed by the National Cheng-Kung University Proteomic Research Core Laboratory, Tainan, Taiwan, and were searched with a Mascot computer program (Matrix Science).

Results

Characteristics of Latex-Allergic Patients and IgE Antibodies

Medical history, skin test results and IgE determinations of the 20 allergic subjects are summarized in table 1. In all cases in our study, positive skin tests to latex extracts were demonstrated. Either CAP-FEIA or enzyme allergosorbent test studies detected increased specific IgE levels to latex in all subjects. All of the non-allergic subjects showed a negative skin reaction and their sera contained no latex-specific IgE (<0.35 kU/l).

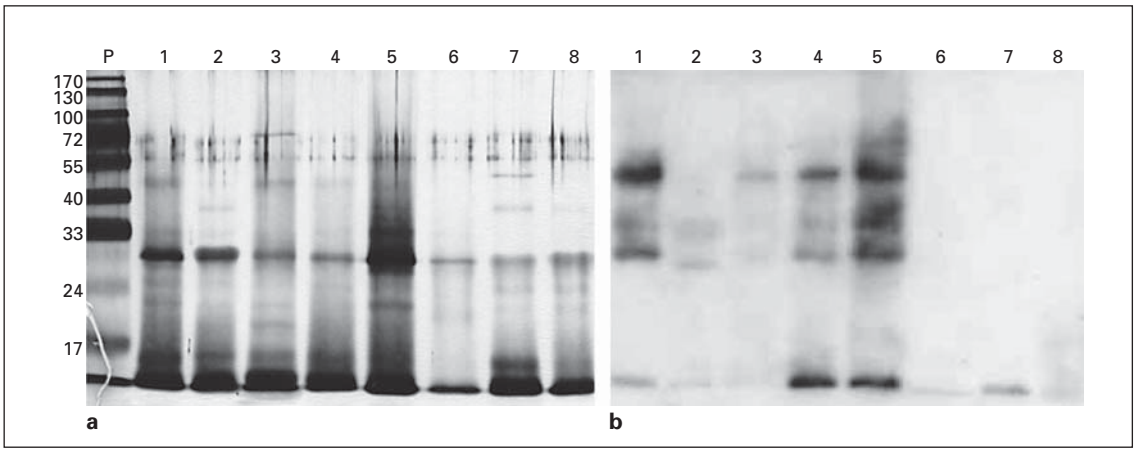

Fig. 1. Protein patterns by silver-stained SDS-PAGE (**a**) and IgE-binding allergenic proteins by immunoblotting (**b**) of crude latex glove extracts from eight lots of three different manufacturers. Lane P: molecular weight standards in kilo daltons; lanes 1–2: Protos; lane 3: De Crown; lanes 4–8: Enchs.

Table 1. Characteristics of latex-allergic patients

Patients	Age/sex	Occupation	Latex-specific IgE CAP/EAST, kU/l	Latex SPT crude/glove	Clinical symptoms
1	42/F	technician	1.5/1.09	3+/3+	AR, AC
2	31/F	nurse	27.4/38.4	4+/4+	A
3	36/F	dentist	99/49.2	4+/4+	anaphylaxis, A
4	37/F	technician	0.9/0.5	3+/2+	AR, AC
5	42/M	physician	12.9/25.7	3+/4+	AR, AC
6	36/F	dentist	4.7/0.8	4+/2+	A, AR
7	31/F	nurse	14.4/12.4	3+/4+	A, AR
8	26/F	lab assistant	23.1/13.7	3+/2+	A, AR, AD
9	45/F	technician	1.9/3.8	3+/2+	A, AR
10	32/F	nurse	20.2/0.7	2+/4+	AR, AC
11	35/F	technician	11.3/2.5	4+/4+	A
12	32/M	surgeon	0.35/0.8	2+/4+	AD
13	46/F	technician	1.9/0.4	4+/4+	A, AR
14	33/M	technician	16.2/56	2+/4+	AD
15	26/F	lab assistant	25/26.2	2+/3+	AR/AC
16	32/F	nurse	0.35/3.3	4+/3+	AR
17	31/M	physician	10.8/5.7	4+/2+	AC
18	24/F	lab assistant	0.9/20.9	+/3+	AR, AC
19	34/F	nurse	2.2/3.5	2+/2+	AR
20	54/M	surgeon	0.61/0.38	4+/4+	AR, AD

EAST = Enzyme allergosorbent test; SPT = skin prick test; AR = allergic rhinitis; AC = allergic conjunctivitis; A = asthma; AD = atopic dermatitis.

Latex Protein and Identification of Major Latex Allergens

Protein resolution of laboratory-prepared extracts from eight different lots of latex gloves by SDS-PAGE revealed many proteins, with molecular weights ranging from 13 to 71 kDa, which were visible following silver staining (fig. 1a). The respective patterns of proteins from various latex gloves with the same PBS extraction protocol were similar, and amounts varied from less than 25 to 145 μg per gram of latex glove. Immunoblotting dem-

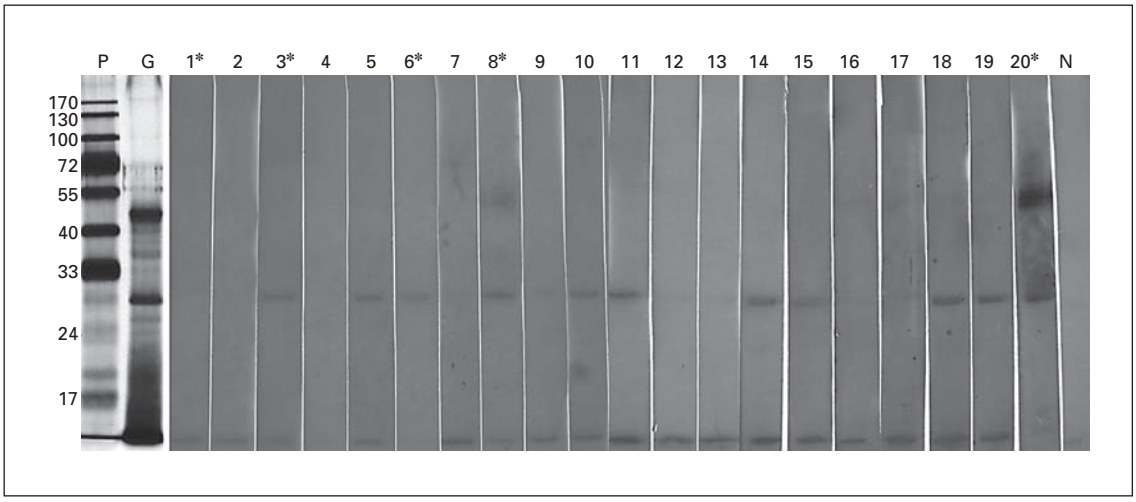

Fig. 2. Immunoblot patterns of a latex glove extract. Lane 1–20: patients 1–20 (table 1); lane N: a sera pool of non-allergic subjects; lane P: molecular weight standards in kilo daltons; lane G: latex glove extract by silver staining. Five representative sera used in 2D-PAGE analysis are denoted by an asterisk.

Table 2. Allergens from glove extract identified by ESI-MS/MS

Allergen (MW/pI)	Matching identity	GenBank access No.	MW/PI (GenBank)	Matching residue sequence	Sequence coverage %
45 kDa/5.2	Hev b 13, lipolytic esterase	GI: 30909057	43.3 kDa/5.0	^{49}AAAFYPLNPPYGETFFHR^{66} ^{116}LPTTIIPAHGGFSPFYLDVQYSQFR^{140} ^{151}ETGGIFAELVPEEYYFEK^{168} ^{271}DLPLATFVHVDIYSVK^{286} ^{287}YSLFSEPEK^{295}	21.0
30 kDa/5.2, 5.4, 6.0	hevamine, *H. brasiliensis*	GI: 234388	29.5 kDa/8.4	^{25}YSYVNIAFLNK^{35} ^{74}VMLSLGGGIGSYTLASQADAK^{94} ^{95}NVADYLWNNFLGGK^{108} ^{151}VYLTAAPQCPFPDR^{164} ^{214}IFLGLPAAPEAAGSGYVPPDVLISR^{238} ^{258}FYDDKNGYSSSILDSV^{273}	40
14 kDa/ 6.1, 6.4, 6.8	Hev b 1, REF	GI: 132270	14.6 kDa/4.9	^{2}AEDEDNQQGQGEGLK^{16} ^{100}DASIQVVSAIR^{110}	18.8

MW = Molecular weight.

onstrated that three predominant components were able to bind specifically to IgE (fig. 1b). Allergens of 14, 30 and 45 kDa bound 100% (8/8), 50% (4/8) and 50% (4/8) of the extracts tested. The most reactive latex extract (fig. 1, lane 5) was selected, and immunoblotting was performed using 20 latex-sensitive sera (fig. 2, lanes 1–20). Allergens of 14, 30 and 45 kDa bound 85% (17/20), 55% (11/20) and 10% (2/20) of the sera tested. The sera pool of 10 non-allergic subjects (fig. 2, lane N) did not display any IgE binding to any latex proteins.

2D-PAGE and ESI-MS/MS

Latex extract (fig. 1, lane 5) was used, and the isoelectric points (pI) of the 14-, 30- and 45-kDa latex allergens were 6.1–6.8, 5.4–8.7 and 5.2, respectively, as revealed by 2D-PAGE (fig. 3). The results of the immunoblotting

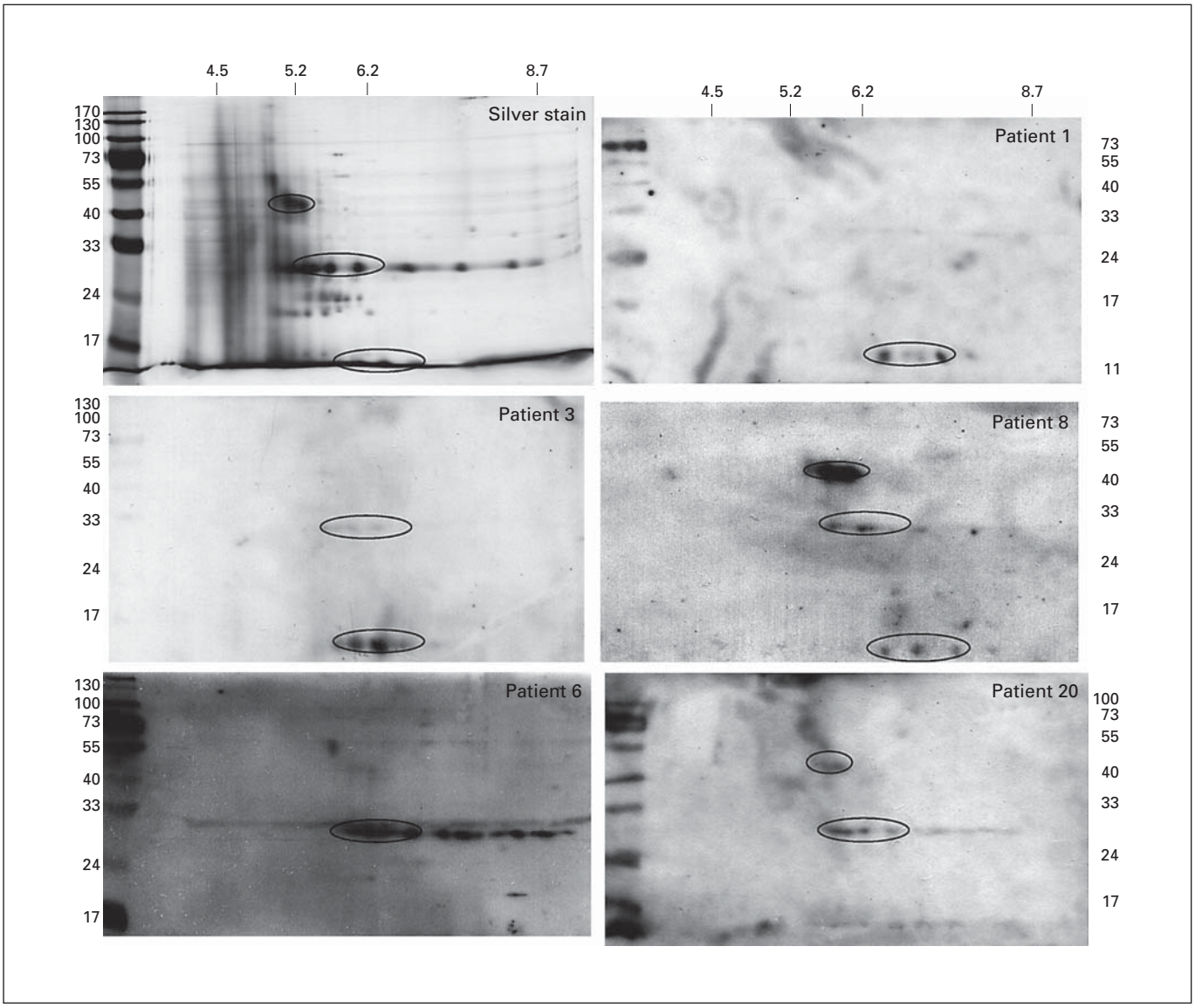

Fig. 3. Two-dimensional proteomics map of latex glove extract by silver nitrate staining and immunoblotting of 5 representative sera. Molecular weight standards are shown at left and right, and pI on the top. Black circles indicate proteins recognized at 45, 30 and 14 kDa, for which ESI-MS/MS was performed.

using sera from 5 representative patients (patient 1, 3, 6, 8 and 20) are shown in fig. 3. The stable signals at 810.9 and 580.0 m/z obtained from tryptic peptide mixture of the 14-kDa allergen were analyzed by ESI-MS/MS and identified as the tryptic peptides ^{2}AEDEDNQQGQGEGLK^{16} and ^{100}DASIQVVSAIR^{110} from the rubber elongation factor (REF) protein (14.6 kDa, pI 4.9) of *H. brasiliensis* (Hev b 1; GenBank GI: 132270). By using the same technique, 6 tryptic peptides, ranging from 666.5 to 1,255.8 m/z, obtained from the 30-kDa allergen, and 5 tryptic peptides, ranging from 550.1 to 1,061.2 m/z, obtained from the 45-kDa latex allergens, exhibited sequence identity with hevamine (29.5 kDa, pI 8.4; GenBank GI: 234388) and Hev b 13 (43.3 kDa, pI 5.0; GenBank GI: 30909057) allergens, respectively. These results are summarized in table 2.

Discussion

This study presents the first examination of latex allergen profiles among medical workers in Taiwan. The crude protein profiles of extracts from eight lots of three different manufacturers routinely used in our Medical Center on SDS-PAGE seemed consistent among all of the glove extracts. However, the IgE-binding patterns revealed significant differences that may be due to the lot variation and protein concentration of extracts. Although the total protein level from the latex glove extract has been used to estimate the 'allergenicity' of latex products, we know from previous reports that protein levels can vary from less than 75 to 7,500 μg protein per gram of latex glove [24]. The manufacturing process may lead to denaturing of proteins in raw latex, and it has been reported that the allergen profiles differed between finished products and the raw materials [25, 26]. Previous studies also documented that the total protein level does not necessarily correlate with the allergen content [24, 27, 28]. There was no known 'safety margin' for the level of total latex protein with regard to the allergenicity of latex gloves, unless we measure the true IgE-binding allergen directly.

Latex allergy is a well-characterized occupational problem. Many reports have extensively studied allergenic proteins responsible for this immediate-type hypersensitivity. Hev b 2, Hev b 5, Hev b 6.01, Hev b 6.02 and Hev b 13 have been reported to be the major allergens relevant to the latex-sensitized adult [12]. Bernstein et al. [29] reported that Hev b 13 elicited positive skin response in 63% of subjects and appeared to be the major NRL allergen in medical workers. In this study, immunoblotting identified two major (14 and 30 kDa) and one minor (45 kDa) IgE-binding proteins from latex extracts that were subsequently identified by ESI-MS/MS as the known latex allergens REF (Hev b 1), hevamine and lipolytic esterase (Hev b 13). The 14-kDa allergen (Hev b 1) and 30-kDa allergen (hevamine) bind more than 50% of our latex-allergic sera, suggesting that these two proteins are major allergens in Taiwanese medical workers. However, only 10% of our studied subjects were reactive to the 45-kDa (Hev b 13) latex allergen by immunoblotting. Some allergens could be more or less important according to the origin of the sensitization [30].

The REF was the first protein identified as a latex allergen; it was named Hev b 1 in 1993 [10]. Hev b 1 has previously been described as a protein of 14.6 kDa with pI between 4 and 5 [31]. Our 2D-PAGE results showed that the 14-kDa latex allergen with pI of 6.1–6.8 was detected. Chardin et al. [32] reported that Hev b 1 displayed more basic pI, which varied from 5.7 to more than 8.3 in latex mattresses, and concluded that Hev b 1 might be the last protein to be washed out from latex products. Our results also suggested that Hev b 1 is the predominant water-soluble protein from latex gloves and is also a major sensitizer (85%) among medical workers in Taiwan.

Hevamine is a 30-kDa protein with lysozyme and chitinase activity isolated from the lutoids of *H. brasiliensis* latex [33]. The enzyme is considered to be a minor allergen as it bound to IgE in only 1/29 latex-allergic sera tested in one report [34]. Recently, we reported the cloning of a major 30-kDa Indian jujube *(Z. mauritiana)* allergen, identified as a latex hevamine, cross-reacting with latex allergen [20, 21]. In this study, hevamine was abundantly present in glove extract and could be recognized by 55% of tested sera. Thus, the 30-kDa latex allergen is a candidate for a major *H. brasiliensis* allergen and designated as Hev b 14 (at least 50% according to the World Health Organization and the International Union of Immunological Societies) [35].

In summary, three allergens from latex gloves were identified by immunoblotting using patients' IgE. Our results clearly indicate that hevamine (a 30-kDa allergen) and Hev b 1 (a 14-kDa allergen) are important latex allergens and may help to determine the allergenicity of the latex gloves in Taiwan. An efficient method to evaluate the allergenicity of latex products is essential for the successful reduction in the exposure to potentially allergenic proteins.

Acknowledgements

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