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Latex allergen database

Two-dimensional (2-D) electrophoresis followed by immunoblotting and *N*-terminal protein microsequencing were used to characterize and identify the IgE-reactive proteins of *Hevea* latex that are the main cause of the latex type I allergy affecting especially health care workers and spina bifida children. This approach generated a comprehensive latex allergen database, which facilitated the integration of most of the latex allergen data presented in the literature. The major latex allergens Hev b 1, Hev b 3, Hev b 6 and Hev b 7 have been localized on our 2-D maps. Moreover, we were able to identify six previously undescribed IgE-binding latex proteins, namely enolase, superoxide dismutase, proteasome subunit C5, malate dehydrogenase, triosephosphate isomerase and endochitinase. The generated latex 2-D maps will provide valuable information to develop strategies for the isolation of the novel IgE binding proteins in order to study the frequency of sensitization among both risk groups. Detailed knowledge of all proteins involved in latex allergy will allow better diagnosis of latex allergy and to monitor the success of prevention strategies that are needed to reduce the high prevalence of latex allergy among both risk groups.

1 Introduction

Since 1979, when natural latex allergy was first reported by Nutter [1], it has become a serious hazard especially for health care workers (HCWs) and patients with long-term hospitalization, *e.g.*, spina bifida (SB) children (for review see [2]). Natural rubber latex proteins are present in numerous latex products and both risk groups become sensitized via skin, mucosal and wound contact or inhalation of airborne allergens released from powdered latex gloves [3]. While the prevalence of latex sensitization among the general population is less than 1%, 3–18% of HCWs and up to 50% of SB patients are affected [4–6]. Enormous efforts have been undertaken to identify and characterize the causative latex allergens. However, in most previous studies one-dimensional (1-D) electrophoresis techniques combined with immunoblotting were applied [7]. This approach is in general not compatible with modern methods of protein analysis such as protein sequencing, amino acid composition analysis and mass spectrometry. Therefore we used high-resolution 2-D polyacrylamide gel electrophoresis with immobilized pH gradient (IPG-Dalt) for the establishment of a 2-DE latex protein database. The IgE-reactive latex proteins were first localized by probing 2-D latex blots with pooled sera of latex allergic patients and subsequently identified by *N*-terminal protein microsequencing or specific antibodies. Besides the identification of previously undescribed IgE-binding latex proteins we were able to integrate most of the latex allergen information presented in the literature into our latex protein database.

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Nonstandard abbreviations: CBB, Coomassie Brilliant Blue; **EAST,** enzyme allergosorbent test; **HCW,** health care worker; **IPG-Dalt,** two-dimensional polyacrylamide gel electrophoresis with immobilized pH gradient; **mAb,** monoclonal antibody; **RAST,** radio allergosorbent test; **REF,** rubber elongation factor; **SB,** spina bifida; **SPT,** skin prick testing

Keywords: Latex allergy / Two-dimensional polyacrylamide gel electrophoresis / Immunoblotting / Protein microsequencing

2 Materials and methods

2.1 Sera and antibodies

Sera were obtained from ten adult HCWs and five SB children who had clinical symptoms of the latex type I allergy and a positive serum IgE antibody level (RAST classes 3 or higher) as measured by the CAP system of Pharmacia (Uppsala, Sweden). Sera without latex-specific IgE from five persons of each patient group exposed to latex were used as controls. Latex particle proteins separated by 2-DE were characterized by two monoclonal antibodies (mAbs), termed II4G9 and II4F9, directed against purified rubber elongation factor (REF). Overlapping synthetic peptides covering the whole sequence of REF were produced by Chen *et al.* [8] to determine their binding characteristics. While the binding site of II4G9 was found to be at the *C*-terminus of REF (AA 121–137), II4F9 reacts with an epitope at the *N*-terminus (AA 46–64) of REF. A specific antiserum raised in rabbit against purified tobacco β-1,3-glucanase was used to detect the respective protein homolog in *Hevea brasiliensis* latex.

2.2 Sample preparation

Latex protein samples were prepared as previously published [9]. Briefly, non-ammoniated latex was diluted 1:1 with 50 mM Tris-HCl buffer, pH 8.0, containing 0.05% w/v Triton X-100 and centrifuged to separate the latex particles from the aqueous layer known as the C-serum. The top liquid layer containing the rubber particles was isolated and extracted using a 2% SDS solution in 50 mM Tris-HCl buffer, pH 8.0, containing 0.05% w/v Triton X-100. The protein solutions of both C-serum and latex particles were dialyzed against water and then

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lyophilized. For 2-DE the lyophilized latex protein extract was resuspended in lysis buffer (9 M urea, 0.8% w/v carrier ampholytes, pH 3–10, 1% w/v dithiothreitol and 2% w/v CHAPS) for 30 min (20°C) with occasional vortexing. The sample was then centrifuged for 1 h at 40 000 *g* (20°C). The clear supernatant was removed and aliquots were stored at −70°C until use.

2.3 Electrophoresis, blotting and immunostaining

IPG-Dalt was carried out according to Görg *et al.* [10] with minor modifications. Second-dimensional SDS-PAGE (12%T, 4%C) was carried out simultaneously on 4 slab gels without stacking (16 × 16 × 1.0 mm^3) at 15°C using the Laemmli buffer system [11]. Analytical 2-DE gels (40–80 µg protein load) were silver-stained according to Blum *et al.* [12], whereas micropreparative 2-DE gels (protein load 300–500 µg) were subjected to protein blotting according to Kyhse-Anderson [13] or Baker *et al.* [14]. The latter PVDF membranes were used for microsequencing, while the 2-DE gels blotted onto PVDF with the buffer system of Khyse-Anderson were subjected to immunostaining performed according to [15]. Briefly, the dried membranes were wetted in methanol for 5 s and washed 3 × 5 min in TBS. Blocking was performed with 1% BSA and 1% PVP-40 in TBS for 6–8 h. After being washed three times with TBS the blots were incubated overnight with pooled serum diluted 1:20 in TBST (TBS containing 1% Tween 20) from ten latex allergic patients. The membranes were then washed three times with TBST and incubated with alkaline phosphatase-conjugated anti-human IgE (1:1000 in TBST) for 4 h. After three washes with TBST, IgE binding was demonstrated by adding a BCIP/NBT solution (each 0.35 mM in 100 mM Tris-HCl, pH 9.5, containing 100 mM NaCl and 5 mM $MgCl_2$). To detect nonspecific IgE immunostaining, control experiments using sera from non-latex allergic persons occupationally exposed to latex were performed in parallel.

2.4 *N*-terminal sequence analysis

The blotted proteins were visualized with Coomassie Brilliant Blue R-250 (CBB) stain. Protein spots of inter-

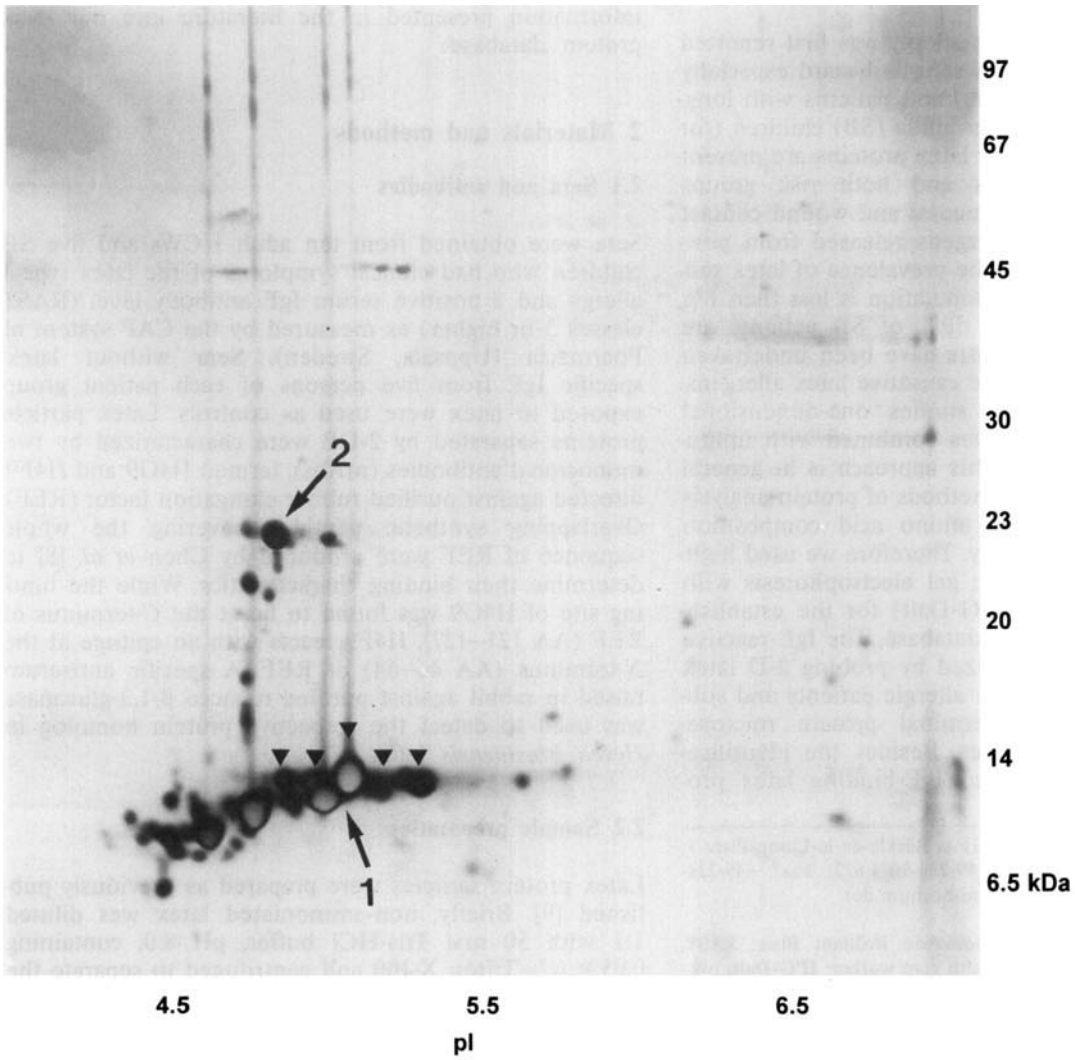

Figure 1. IPG-Dalt of latex (*Hevea brasiliensis*) particle proteins. First dimension: IPG 4–7. Second dimension: SDS-PAGE, 12%T; Silver stain. Spots marked with triangles reacted with mAb 1l4G9. For more details see Section 3.1 (1) Hev b 1; (2) Hev b 3.

est were excised from the blotting matrix and analyzed by an ABI Model 477A pulsed-liquid protein sequencer equipped with an on-line phenylthiohydantoin (PTH) amino acid analyzer (Model 120A). For each spot ten to fifteen Edman degradation cycles were performed. The *N*-terminal protein sequences were compared for homologies to already known proteins using the BLAST algorithm [16]. Theoretical isoelectric point (p*I**) and molecular weight (M_r*) of proteins described in the manuscript were computed using the Compute pI/Mw tool obtained *via* the ExPASy server (http://expasy.hcuge.ch; Geneva University Hospital, University of Geneva, Switzerland).

3 Results and discussion

Hevea latex is produced by specialized cells called laticifers and contains about 1–2% proteins. Three different fractions can be obtained by high-speed centrifugation, namely rubber particles at the top, the bottom fraction and the yellowish C-serum in between. While the C-serum contains soluble proteins and proteins released from the bottom fraction (preferably from damaged lutoids [17]), the particle-bound proteins can only be efficiently extracted by detergent-containing solutions [18].

3.1 Particle-bound latex proteins

The particle-bound protein fraction was analyzed in an IPG of 4–7 (Fig. 1). The silver-stained 2-D pattern showed two acidic protein species with molecular masses of 8–14 and 23 kDa, respectively. Figure 2 demonstrates the IgE reactivity of the latex particle-bound proteins using sera of both HCWs and SB patients. While most of the 8–14 kDa proteins exhibited IgE binding capacity with sera of both patient groups, the 23 kDa protein spots were exclusively stained with sera of SB children. Both protein spots of the 14 and 23 kDa area were subjected to protein sequencing, but no sequence information was obtained, probably due to blocked *N*-termini.

3.1.1 Rubber elongation factor (Hev b 1)

Two mAbs directed against the rubber elongation factor (p*I** 5.04, M_r* 14590), a major latex particle protein, were used to characterize our 2-D map. The mAb II4G9 that is directed against an epitope at the *C*-terminus of REF reacted with five IgE binding proteins in the 14 kDa area of the latex 2-D map (Fig. 1). In contrast, all proteins of the 8–14 kDa cluster were stained by the mAb II4F9 that exhibits specifity to an *N*-terminal located epitope in REF (results not shown). This indicates that the polypeptides with molecular masses smaller than 14 kDa are degradation products of REF lacking parts of the *C*-terminus. The allergenicity of REF was extensively studied by Chen *et al.* [19] by skin prick testing (SPT) and enzyme allergosorbent test (EAST) measurements. They found that 50% of HCWs (n = 105) and 80% of SB patients (n = 69) are sensitized to this protein. Alenius *et al.* [20] and Yeang *et al.* [21] confirmed the significance of REF for SB patients as major sensitizing protein, but according to their investigations the sensitization frequency among HCWs is much less. The incongruous results may be explained by different antigens, methods (EAST, ELISA, immunoblot), or patient groups with heterogeneous exposure and sensitization patterns used in the respective studies.

3.1.2 23 kDa rubber particle protein (Hev b 3)

The allergenicity of the 23 kDa latex particle protein termed Hev b 3 was first discovered by Alenius *et al.* [22]. The reason why this protein is characteristically recognized by sera from SB children is still unclear. Both Lu *et al.* [23] and Alenius *et al.* [24] obtained internal sequence information after enzymatic digestion that indicate a significant homology with REF, but no biochemical function was attributable until now.

3.2 C-serum proteins

In contrast to the particle proteins, the C-serum protein fraction consists of more than 200 distinct polypeptides when analyzed in an IPG of 4–9 (Fig. 3). The majority of the soluble proteins have p*I* values of 4–8 and molecular masses of 100 to less than 6.5 kDa. The IgE-binding latex proteins were identified by probing blots with pooled sera of ten latex allergic HCWs. The IgE-binding characteristics of the C-serum proteins is demonstrated

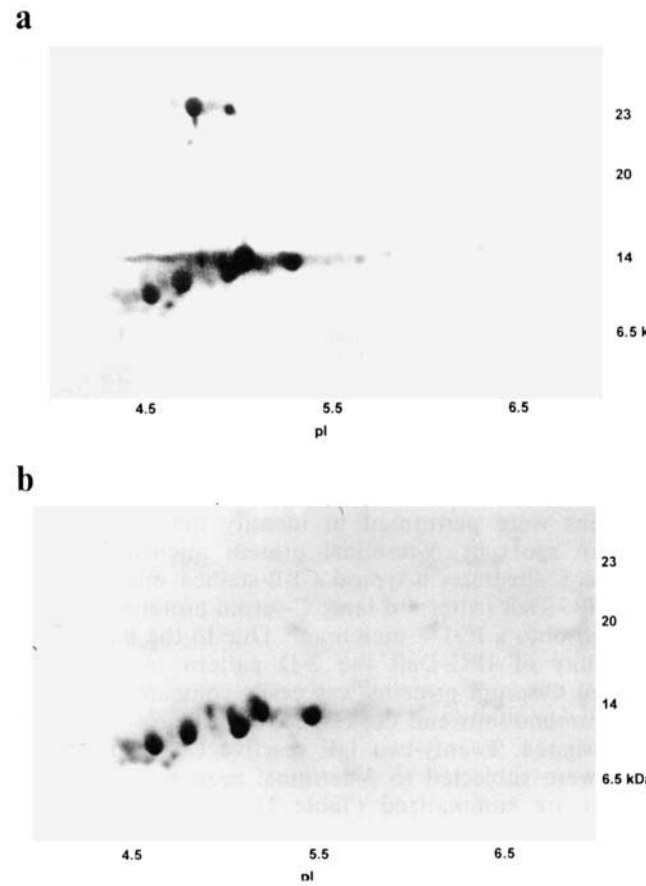

Figure 2. Latex (*Hevea brasiliensis*) particle proteins separated by IPG-Dalt (see Fig. 1) and electroblotted onto a PVDF membrane. Immunostaining with (a) pooled serum of latex-allergic spina bifida patients, (b) pooled serum of latex-allergic health care workers, followed by secondary alkaline phosphatase-labeled anti-human IgE.

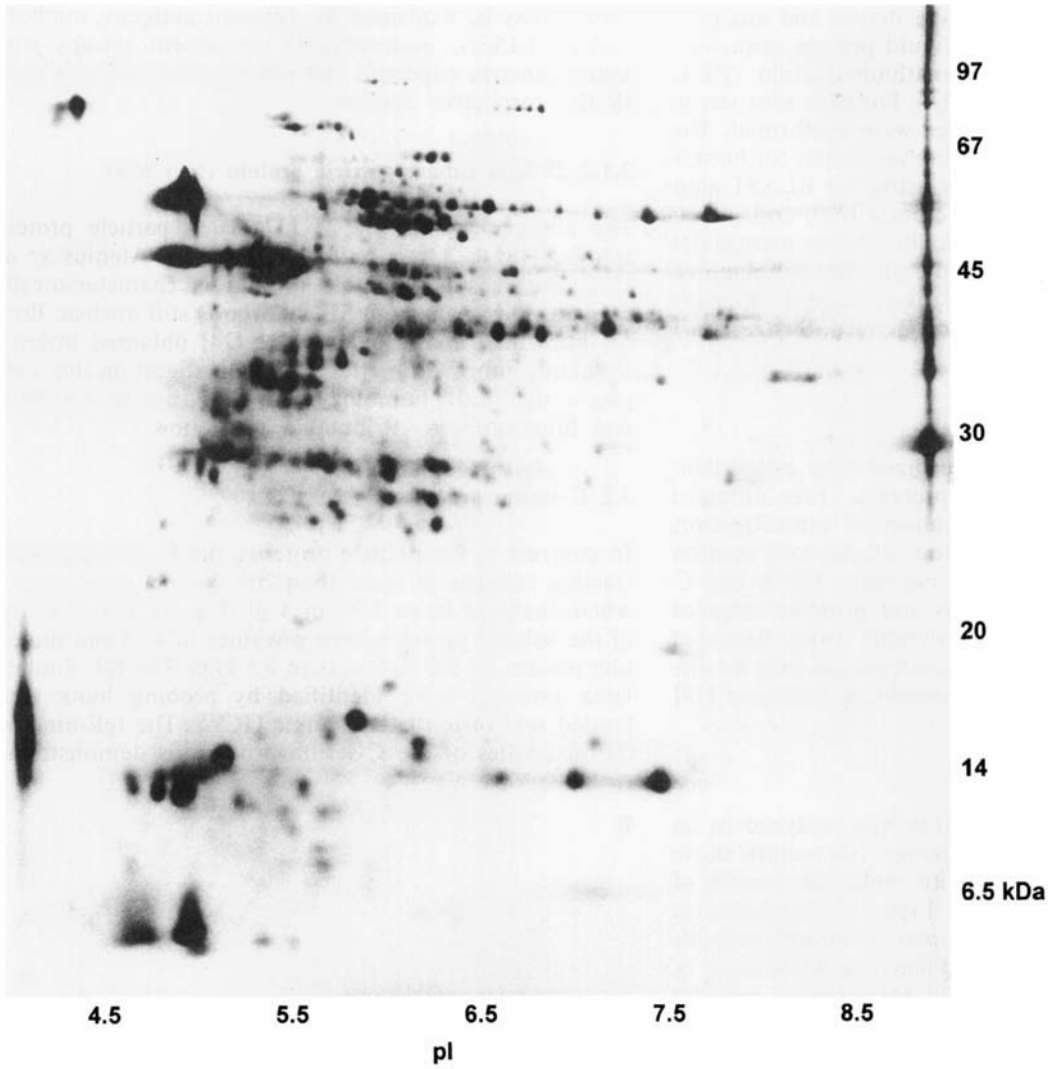

Figure 3. Two-dimensional electrophoresis with immobilized pH gradients (IPG-Dalt) of latex (*Hevea brasiliensis*) C-serum proteins. First dimension: IPG 4–9. Second dimension: SDS-PAGE, 12%T; Silver stain.

in Fig. 4. Distinct IgE binding was detected in the < 6.5, 14, 20, 30, 45 and 56 kDa area of the immunoblots and about 60 C-serum protein spots were stained in total. Micropreparative IPG-Dalt separations of the C- serum proteins were performed to identify the IgE reactive protein spots by *N*-terminal protein microsequencing. Figure 5 illustrates a typical CBB-stained micropreparative IPG-Dalt pattern of latex C-serum proteins (400 µg) blotted onto a PVDF membrane. Due to the high reproducibility of IPG-Dalt the 2-D pattern of the CBB-stained C-serum proteins was easily compared with the IgE immunoblots and the respective protein spots could be assigned. Twenty-two IgE reactive C-serum protein spot were subjected to *N*-terminal sequencing and the results are summarized (Table 1).

3.2.1 Prohevein, hevein and prohevein C-domain (Hev b 6.01, Hev b 6.02, Hev b 6.03)

Spots 9 and 10 of Fig. 5 correspond to mature prohevein, a two-domain protein that is post-translationally processed to the 4.7 kDa *N*-terminal fragment hevein (spots 3, 4) and the 14 kDa *C*-terminal domain (spots 7, 8), while spots 5, 6, 11 and 12 are supposed to be intermediate fragments of the prohevein cleavage. The 43 amino acid domain of hevein is found in several plant proteins, such as wheat germ agglutinin (WGA), endochitinases (EC 3.2.1.14) and wound-induced proteins from potato (win1, win2) that have a common binding specificity for *N*-acetylglucosamine. Besides its chitin binding capacity hevein is involved in the coagulation of latex [25]. The *C*-terminal domain of prohevein belongs to the barwin family, *i.e.*, barley seed proteins suspected of being involved in a defense mechanism in plants. The IgE-binding capacity of prohevein was first discovered by Alenius *et al.* [26]. In 24 out of 29 latex allergic patients (including nine children) prohevein-specific IgE antibodies were detected by immunoblotting. Chen *et al.* [27] and Alenius *et al.* [28] found that the allergenicity of prohevein is mainly due to the hevein domain, since about 80% of latex allergic HCWs are sensitized to this allergen while the prevalence to the *C*-terminal domain is about 15–21%. This data suggest that hevein is the major allergen for HCWs, but not for SB children, since

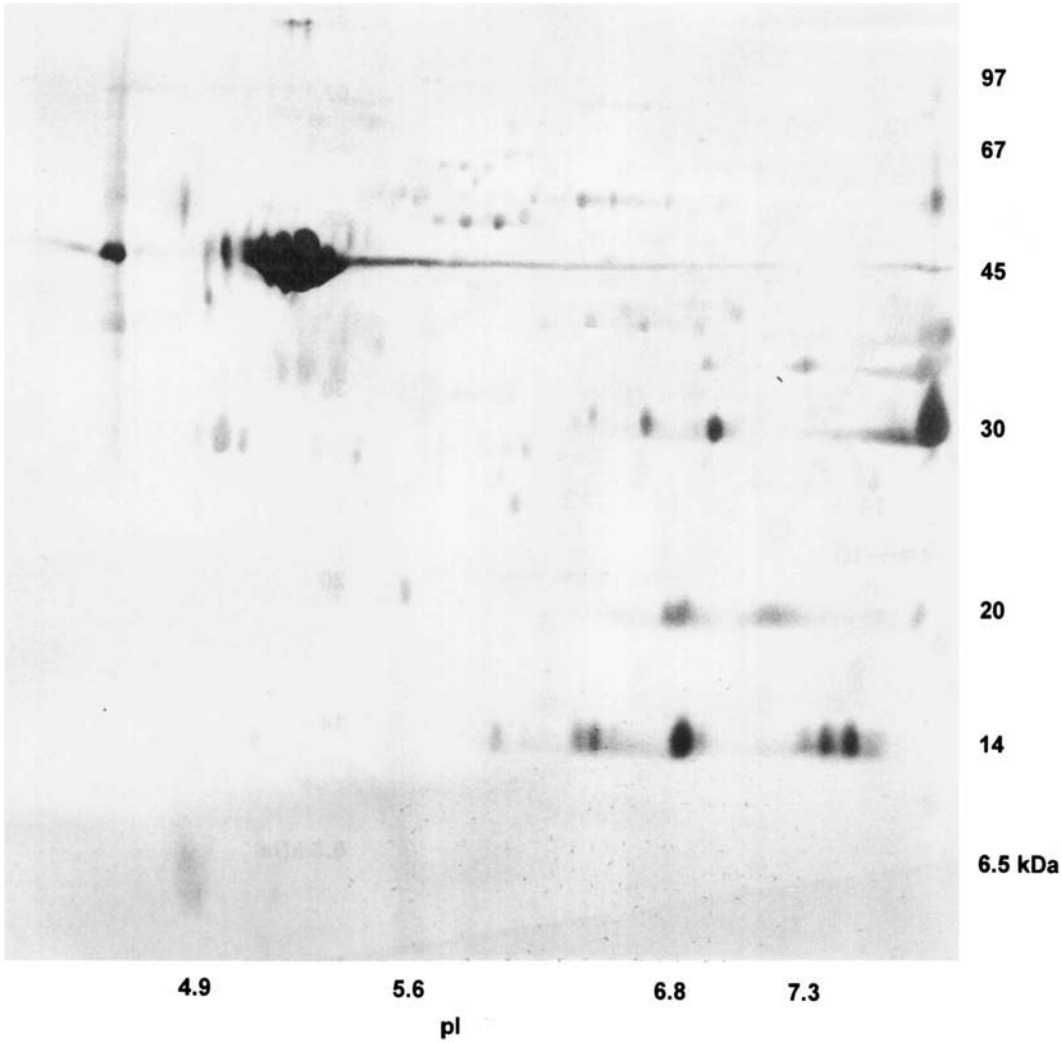

Figure 4. Latex (*Hevea brasiliensis*) C-serum proteins separated by IPG-Dalt (first dimension: IPG 4–8; second dimension: SDS-PAGE, 12%T) and electroblotted onto a PVDF membrane. Immunostaining with pooled serum of latex-allergic health care workers and secondary alkaline phosphatase-labeled anti-human IgE.

only about 30% exhibited detectable IgE antibody levels in EAST.

3.2.2 β-1,3-glucanase (Hev b 2)

Alenius *et al.* [26] detected a 36 kDa IgE binding protein to be homologous to several plant β-1,3-glucanases by protein sequencing. Six out of 29 latex-allergic patients were positive in IgE immunoblotting using the electro-eluted protein as target antigen. Similarly, Sunderasan *et al.* [29] demonstrated the allergenicity of a basic β-1,3-glucanase (p*I* 9.5; 34–36 kDa). According to Brenton *et al.* [30], several isozymes of *Hevea* β-1,3-glucanases exist, and they described both acidic (p*I*s of 4.9, 5.5) and basic (p*I*s of 9, 9.3, 10) isoforms. We have not been able to precisely localize the allergenic, basic *Hevea* β-1,3-glucanases, since no preparative IPG gradients in the range > pH 8 were applied. However, we assume a relationship of the basic (p*I* > 8) protein spot 25 in the 36 kDa area of the latex 2-D map presented in Fig. 5 because it reacted specifically with an antiserum produced against a basic tobacco β-1,3-glucanase.

3.2.3 Microhelix protein complex (Hev b 4)

Sunderasan *et al.* [29] identified an acidic protein (p*I* 4.5) of the latex B-serum microhelix protein complex to bind IgE from one latex allergic patient. The sequence alignment for the *N*-terminal amino acid residues showed no homology to previously published sequence information. The molecular weight of this protein was estimated to be 50–57 in the reduced and 100–110 kDa in the unreduced form by SDS-PAGE. Since the frequency of respective sensitization among latex allergics was not investigated, the meaning of this protein in latex allergy cannot be assessed. In our latex 2-D map no polypeptide that binds IgE and matches the p*I* and molecular mass characteristics given by Sunderasan *et al.* [29] could be detected.

3.2.4 Hev b 5

Hev b 5 is the synonym for an acidic (p*I* 3.5) 16 kDa allergen that reacted with IgE of sera of more than 50% of adult latex-allergic patients ($n = 29$) in immunoblotting. Akasawa *et al.* [31] isolated and sequenced the cDNA coding for this protein from a latex cDNA library.

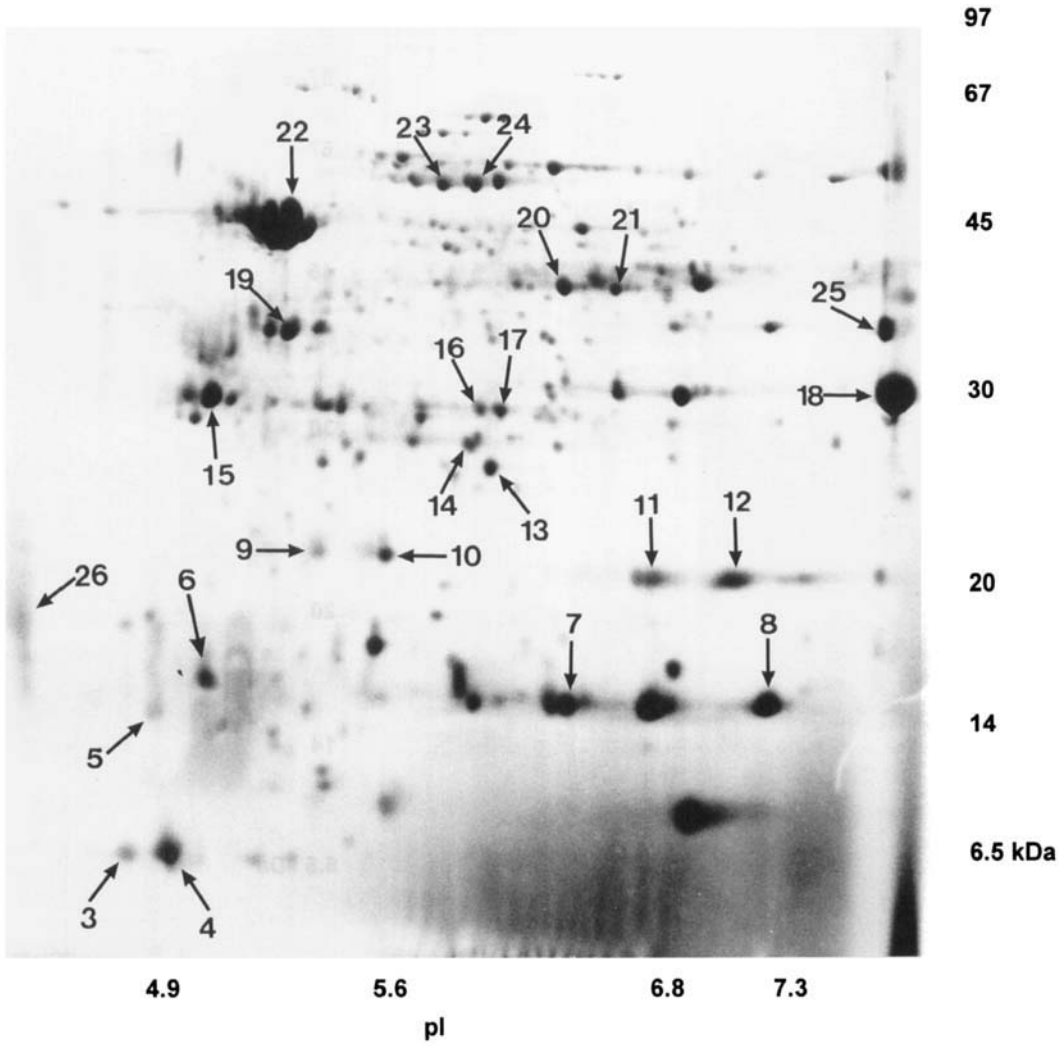

Figure 5. Latex (*Hevea brasiliensis*) C-serum proteins (400 µg) separated by IPG-Dalt (see Fig. 4) and electroblotted onto a PVDF membrane. Coomassie stain. Characterized and/or identified IgE-binding latex proteins are indicated by numbers (see Table 1).

The deduced primary structure of this allergen showed a high degree of homology (47%) to an acidic kiwi fruit protein with unknown function termed pkIW1501 (p*I* ~ 3.7, 18.9 kDa). Similar results were obtained by Slater *et al.* [32]. They screened a latex cDNA library and found an IgE-reactive clone that was termed Hev b 5. Slater *et al.* [32] found that 46% of the deduced Hev b 5 sequence is identical to the aligned pKlW1501 sequence, with greatest homology at the *N*- and *C*-termini. Sixty-five percent of latex allergic SB patients and 92% of HCWs exhibited significant binding to recombinant-produced Hev b 5, indicating that this protein can be regarded as major latex allergen. We assume a relationship of spot 26 with Hev b 5, since this polypeptide shows similar p*I* and M_r values (p*I* < 4, 16 kDa). However, no detectable IgE reactivity was observed in our immunoblotting experiments using sera of latex-allergic HCWs.

3.2.5 46 kDa patatin-like allergen (Hev b 7)

The IgE-binding protein spot 22 was identified by protein sequencing to be significantly related to patatin, which is a storage protein encoded by a multigene family found in plant families such as potato and tomato. It may have a dual role as a somatic storage protein and as an enzyme involved in host resistance. Beezhold *et al.* [33, 34] reported that 9/40 latex-allergic HCWs recognized this 46 kDa patatin-like latex allergen, termed Hev b 7, in IgE immunoblotting.

3.2.6 Hevamine and other soluble IgE-binding proteins

The *N*-terminus of spot 18 was identified as being highly homologous with hevamine A (29.5 kDa), a bifunctional enzyme with lysozyme/chitinase activity. Two basic isoforms are known, namely hevamine A, which is most abundant, and hevamine B. No official allergen name was assigned to this minor allergen, because only 1/29 latex-allergic patients reacted in IgE immunoblotting experiments performed by Alenius *et al.* [26]. The sequence alignment searches for the IgE-binding protein spots 13, 14, 15, 16, 19, 20, 21, 23 and 24 exhibited high sequence homologies (see Table 1) to superoxide dismutase, proteasome component C5, endochitinase, triose-

Table 1. *N*-terminal sequence alignment of IgE-reactive latex polypeptides (see Figs. 1 and 5)

Spot No.	Expt. pI/Mr	Sequence alignment	Identification/description	pI/M_r (Swiss-Prot)	Swiss-Prot accession number
1	4.9-5.3/14500	blocked (identified by mAbs)	REF_HEVBR /RUBBER ELONGATION FACTOR (REF) ALLERGEN HEV B 1	5.04/14590	P15252
2	4.8/23000	blocked (identified by similarity)	Homology to REF/ ALLERGEN HEV B 3	-	-
3, 4	4.7, 4.9/<6500	Query: 1 EQCGRQAGGK 10 EQCGRQAGGK Sbjct: 1 EQCGRQAGGK 10	HEVP_HEVBR/PSEUDO-HEVEIN (MINOR HEVEIN) ALLERGEN HEV B 6.02	4.96/4727	P80359
5	4.8/13000	Query: 1 EQCGRQAGGX 10 EQCGRQAGG Sbjct: 1 EQCGRQAGGK 10	HEVE_HEVBR/HEVEIN PRECURSOR (MAJOR HEVEIN) (fragment)	5.64/20087	P02877
6	5.0/14000	Query: 1 EQCGXXX 7 EQCG Sbjct: 1 EQCGRQA 7	HEVE_HEVBR/HEVEIN PRECURSOR (MAJOR HEVEIN) (fragment)	5.64/20087	P02877
7	6.4/13500	Query: 1 GGSASNVLATYHL 13 GGSASNVLATYHL Sbjct: 67 GGSASNVLATYHL 79	HEVE_HEVBR/HEVEIN PRECURSOR (MAJOR HEVEIN) (C-domain) ALLERGEN HEV B 6.03	6.43/14891	P02877
8	7.3/13500	Query: 1 GLSASNVLATYHLY 14 G+SASNVLATYHLY Sbjct: 67 GGSASNVLATYHLY 80	HEVE_HEVBR/HEVEIN PRECURSOR (MAJOR HEVEIN) (C-domain) ALLERGEN HEV B 6.03	6.43/14891	P02877
9	5.4/21000	Query: 1 EQCGGQAGGK 10 EQCG+QAGGK Sbjct: 1 EQCGRQAGGK 10	HEVE_HEVBR/HEVEIN PRECURSOR (MAJOR HEVEIN) ALLERGEN HEV B 6.01	5.64/20087	P02877
10	5.6/21000	Query: 1 EQCGRQAXGGXK 12 EQCGRQA G+++ Sbjct: 1 EQCGRQAGGKLC 12	HEVE_HEVBR/HEVEIN PRECURSOR (MAJOR HEVEIN) ALLERGEN HEV B 6.01	5.64/20087	P02877
11	6.8/20000	Query: 1 EQCGRQAGGKL 11 EQCGRQAGGKL Sbjct: 1 EQCGRQAGGKL 11	HEVE_HEVBR/HEVEIN PRECURSOR (MAJOR HEVEIN) (fragment)	5.64/20087	P02877
12	7.1/20000	Query: 1 EQCGRQAGGKLCP 13 EQCGRQAGGKLCP Sbjct: 1 EQCGRQAGGKLCP 13	HEVE_HEVBR/HEVEIN PRECURSOR (MAJOR HEVEIN) (fragment)	5.64/20087	P02877
13	6.1/24500	Query: 2 QTXSXPDLPYD 12 QT S PDLPYD Sbjct: 29 QTFSLPDLPYD 39	SODM_HEVBR/SUPEROXIDE DISMUTASE PRECURSOR (EC 1.15.1.1)	5.89/23000	P35017
14	6.0/26000	Query: 1 ANWSPYDNNGG 11 ANWSPYDNNGG Sbjct: 13 ANWSPYDNNGG 23	PRC5_ARATH/PROTEASOME COMPONENT C5 (EC 3.4.99.46)	7.83/25349	P42742
15	5.0/29000	Query: 1 DIGSIISKSTFEEFLKXGN 19 D+GSIIS S F++ LK N Sbjct: 60 DLGSIISSSMFDQMLKHRN 78	CHI2_TOBAC/ENDOCHITINASE B PRECURSOR (EC 3.2.1.14)	8.31/31468	P24091
16	6.1/29000	Query: 1 ARKFFVGG 8 ARKFFVGG Sbjct: 1 ARKFFVGG 8	TPIS_SCHPO/TRIOSEPHOSPHATE ISOMERASE (EC 5.3.1.1)	6.16/27118	P07669
17	6.2/29000	blocked			
18	8.7/30000	Query: 1 GGIAIYXG 8 GGIAIY G Sbjct: 1 GGIAIYWG 8	CHLY_HEVBR/HEVAMINE A [CHITINASE (EC 3.2.1.14); LYSOZYME (EC 3.2.1.17)]	8.44/29550	P23472
19	5.3/35000	Query: 1 RKVDVDVXVPYT 13 +KVDVAV VP+T Sbjct: 37 EKVDVAVTVPFT 48	PIS_CORGL/TRIOSEPHOSPHATE ISOMERASE (EC 5.3.1.1)	4.8/27195	P19583
20, 21	6.4, 6.6/41000	Query: 2 KETVRVLVTGAA 13 K+ VRV VTGAA Sbjct: 2 KKPVRVAVTGAA 13	MDH_ACIDE/MALATE DEHYDROGENASE (EC 1.1.1.37) (FRAGMENT).	-	P80540
22	5.3/45000	Query: 1 ATQGKKITVLFE 12 +TQGKKITVL++ Sbjct: 8 LTQGKKITVLSI 19	HBU80598/LATEX PATATIN HOMOLOG ALLERGEN HEV B 7	-	-
23,24	5.9, 6.0/51000	Query: 1 AITIXSVRARQIF 13 AITI SVRARQIF Sbjct: 2 AITIVSVRARQIF 14	ENO_RICCO/ENOLASE (EC 4.2.1.11) (2-PHOSPHOGLYCERATE DEHYDRATASE)	5.56/47912	P42896

phosphate isomerase, malate dehydrogenase and enolase. The identified proteins represent previously undescribed latex allergens; their role with respect to latex allergy cannot be assessed at the moment, because isolated allergens are needed for comprehensive *in vitro* and *in vivo* studies. Since IPG-Dalt is a time-consuming

method compared to 1-D electrophoresis techniques, the latex 2-D blots cannot be used for routine screening of single serum samples to investigate the sensitization frequency among latex allergics. Nevertheless, the latex 2-D maps provide valuable information (p*I* and M_r values) for the development of purification schemes, *e.g.*, by preparative electrophoretic methods and/or chromatographic means.

4 Concluding remarks

To our knowledge this is the first time that a 2-D protein database has been established for latex allergy (or any other allergy). This database provides not only a comprehensive data collection on individual latex allergens investigated at the molecular level by different research groups but also adds new ideas about additional IgE-binding proteins that may be involved in latex allergy. For the unequivocal diagnosis of latex allergy and to monitor the success of prevention strategies (*e.g.*, inactivation of latex allergens during the manufacturing process of latex products) knowledge about all proteins related to allergic symptoms is required. This database may also be useful to start to investigate the observed cross-reactivity between latex and botanically unrelated foods liks banana, kiwi, chestnut, potato, tomato and avocado. In the near future we intend to (i) enlarge the 2-D database with respect to acidic and basic latex protein in order to characterize latex allergens like Hev b 2 and Hev b 5 more precisely, (ii) establish the IgE reaction profile of latex C-serum proteins for latex-sensitized SB children, (iii) study the meaning of the previously undescribed latex allergens after their isolation, and (iv) to set up this comprehensive data collection on our internet server (http://www.bgfa.ruhr-uni-bochum.de/).

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