

Characterization and identification of latex allergens by two-dimensional electrophoresis and protein microsequencing

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Background: Proteins of natural rubber latex cause IgE-mediated sensitization in 3% to 18% of health care workers and in up to 50% of patients with spina bifida.

Objective: This study was aimed at the generation of a comprehensive latex protein database by two-dimensional electrophoresis (2-DE).

Methods: Proteins extracted from fresh *Hevea brasiliensis* latex were separated by 2-DE. IgE-reactive proteins were analyzed by immunoblotting with sera of health care workers with latex allergy. Protein microsequencing and monoclonal antibodies were used to identify the latex allergens.

Results: The latex C-serum 2-DE map was very complex and exhibited about 200 distinct polypeptides. The proteins eluted from the latex particles consisted primarily of two groups of acidic proteins located in the 8 to 14 kd and 22 to 24 kd areas of the 2-DE map. Major IgE-reactivity was detected with C-serum proteins in the 56, 45, 30, 20, 14, and <6.5 kd areas of the immunoblots. The 8 to 14 kd particle proteins exhibited distinct IgE reactivity, whereas the 22 to 24 kd proteins were not stained. Seven of the soluble IgE-reactive protein spots showed high homology with enolase, superoxide dismutase, triosephosphate isomerase, proteasome subunit, and chitinase and represent previously undescribed latex allergens; whereas nine protein spots corresponded to known latex allergens, namely prohevein, hevein, prohevein C-domain, and heveamine. As identified by monoclonal antibodies, the IgE-reactive latex particle proteins mainly represent the allergenic rubber elongation factor.

Conclusions: Two-dimensional electrophoresis, followed by immunoblotting and protein microsequencing, can rapidly identify a large number of IgE-binding latex proteins. The 2-DE latex maps generated will provide valuable information for the development of strategies to isolate the relevant latex allergens. Because the novel latex allergens are common plant enzymes, they may also act as cross-reacting proteins in various foods. (*J Allergy Clin Immunol* 1997;99:385-95.)

Key words: Natural rubber latex, latex allergy, IgE, two-dimensional electrophoresis, immunoblotting, protein microsequencing, latex proteins

Proteins of natural rubber latex, present in a variety of latex products, are the main cause of IgE-mediated latex

Abbreviations used

CHAPS:	3-[(3-Cholamidopropyl)dimethylammonio]-1-propane-sulfonate
IPG:	Immobilized pH gradient
IPG-Dalt:	Two-dimensional electrophoresis with immobilized pH gradients
mAb:	Monoclonal antibody
MW:	Molecular weight
pI:	isoelectric point
PVDF:	Polyvinylidene difluoride
REF:	Rubber elongation factor
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
TBS:	Tris-buffered saline
TBST:	TBS containing 1% Tween-20
2-DE:	Two-dimensional electrophoresis

hypersensitivity, which mainly affects health care workers and persons undergoing repeated surgeries, such as patients with spina bifida. For the unequivocal diagnosis of latex allergy, standardized reagents containing the proteins related to allergic symptoms are required. However, the thorough characterization and identification of proteins reacting with IgE antibodies of patients with latex allergy is a great challenge, because natural rubber latex contains a very complex protein mixture consisting of both soluble and particle-bound proteins.¹ Despite this fact, several investigators have succeeded in the identification of some proteins responsible for IgE-mediated latex hypersensitivity both in rubber raw materials and processed rubber products. The first major latex allergen was identified by Czuppon et al.,² using glove protein extracts, as rubber elongation factor (REF), a 14.6 kd latex protein tightly bound to rubber particles. Recently, Alenius et al.³ purified and identified three natural latex proteins by microsequencing, namely prohevein (20 kd), heveamine (30 kd), and a 36 kd protein exhibiting high homology to several plant endo-1,3- β -glucosidases. They demonstrated that prohevein is another major latex allergen, whereas the other two polypeptides seem to be of lesser importance. Beezhould et al.⁴ focused their interest on a 46 kd allergen in natural latex and found a sequence homology to patatin,⁵ a major storage protein of the Solanaceae. A polypeptide reacting significantly with IgE of patients

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with spina bifida and latex allergy was analyzed by Alenius et al.^{6,7} They purified a 27 kd protein, and microsequencing of the peptide fragments revealed a partial homology to REF. Similarly, Lu et al.⁸ described a 23 kd latex allergen with significant homology to REF as being characteristically recognized by IgE of children with spina bifida who are allergic to latex.

In most previous studies of latex allergens, one-dimensional techniques and immunoblotting have been used.⁹ Moreover, these studies were purely descriptive with no attempt to identify the immunoreactive proteins. Such one-dimensional methods are generally unable to resolve complex mixtures of proteins sufficiently for successful application of modern methods of protein analysis such as protein microsequencing, amino acid composition analysis, and mass spectrometry. In contrast, high-resolution two-dimensional electrophoresis (2-DE) is capable of resolving complex protein mixtures into a characteristic pattern of thousands of spots; each spot usually contains only a single protein. Despite these obvious advantages, only Kurup et al.¹⁰ and Akasawa et al.¹¹ have used 2-DE to obtain maximum information about the IgE reactivities of latex antigens, but they did not link 2-DE to protein analysis tools to identify the protein allergens.

In this study we have used micropreparative 2-DE with immobilized pH gradients (IPG-Dalt) for the analysis of both soluble and particle-bound natural rubber polypeptides. The 2-DE protein separations were then used to locate the IgE-reactive proteins by Western immunoblotting with the sera of health care workers with latex allergy and subsequently to identify them by N-terminal protein microsequencing or by probing with monoclonal antibodies (mAbs). This study generated a comprehensive 2-DE latex protein database, which has allowed integration of most of the latex allergen data presented in the literature and has resulted in increased knowledge of the polypeptides involved in latex allergy.

METHODS

Apparatus and chemicals

Equipment and chemicals for 2-DE (power supply EPS 3500 XL, Multiphor II electrophoresis system, Immobiline Dry strip kit, vertical slab gel electrophoresis unit SE 600, Immoblines, Pharmalyte 3-10, and GelBond PAGfilm) were purchased from Pharmacia Biotech (Uppsala, Sweden). Protein blotting was performed with the semi-dry system (16 × 16 cm) of Roth (Karlsruhe, Germany). Immobilon P (pore size, 0.45 μm) polyvinylidene difluoride (PVDF) membranes were from Millipore (Bedford, Mass.), and Fluorotrans PVDF membranes (0.2 μm) were from Pall (Dreieich, Germany). Dialysis tubing (SPECTRA/POR 3, MWCO 3500), acrylamide (2× crystallized), N,N'-methylene-bis-acrylamide, dithiothreitol, sodium dodecylsulfate (SDS), Tris, glycine, bovine serum albumin (fraction V), molecular weight (MW) marker proteins, and alkaline phosphatase-labeled goat anti-mouse IgG were from Serva (Heidelberg, Germany). Urea, iodoacetamide, polyvinylpyrrolidone (PVP-40, MW, 40,000 d), 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate (CHAPS), Triton X-100, 5-bromo-4-chloro-3-indolyl phosphate, nitro blue tetra-

zolium, and alkaline phosphatase-labeled goat anti-human IgE were from Sigma (Deisenhofen, Germany). All other chemicals for electrophoresis, blotting, and staining were from Roth and Merck (Darmstadt, Germany).

Sera and mAbs

Sera were obtained from 10 adult health care workers who had clinical symptoms of latex type I allergy and a positive serum IgE antibody level (RAST class 3 or higher) as measured by the CAP system of Pharmacia. Five sera without latex-specific IgE from persons occupationally exposed to latex were used as controls.

Two mAbs, termed II4G9 and II4F9, directed against purified REF were used for the characterization of latex proteins separated by 2-DE. The antigenic binding sites of both mAbs have been determined by using overlapping synthetic peptides covering the whole sequence of REF.¹² Although 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.

Sample preparation

Fresh, nonammoniated natural rubber latex of *Hevea brasiliensis* (from Sri Lanka) containing 0.1% (wt/vol) sodium azide was kindly provided by Kautschuk GmbH (Frankfurt am Main, Germany).

To obtain both soluble and particle-bound protein extracts, fresh latex was diluted 1:1 with 50 mmol/L Tris-HCl buffer, pH 8.0, containing 0.05% (wt/vol) Triton X-100. The latex sample was then centrifuged for 1 hour (40,000 g, 10° C) to separate the latex particles from the aqueous layer known as the C-serum. The top liquid layer containing the rubber particles was removed and resuspended in a 2% SDS solution in 50 mmol/L Tris-HCl buffer, pH 8.0, containing 0.05% (wt/vol) Triton X-100 to solubilize the particle-bound proteins. This solution, which contained the latex particles, was sonicated for 15 minutes, then stirred for 1 hour. The protein solutions of both C-serum and latex particles were again centrifuged for 1 hour (40,000 g, 10° C) to remove residual latex particles. Before lyophilization, the pH of the protein extracts was adjusted to 7.0 with 10% acetic acid and dialyzed against water for 48 hours. The lyophilized protein extracts were stored at -70° C, and protein determination was performed according to a modified Lowry assay with ovalbumin as reference protein.¹³

For 2-DE, lyophilized latex protein extract was resuspended in lysis buffer (9 mol/L urea, 0.8% wt/vol carrier ampholytes, pH 3 to 10, 1% wt/vol dithiothreitol, and 2% wt/vol CHAPS) for 30 minutes (20° C) with occasional vortexing. The sample was then centrifuged for 1 hour at 40,000 g (20° C). The clear supernatant was removed, and aliquots were stored at -78° C until use.

Electrophoresis and blotting

Two-dimensional electrophoresis with immobilized pH gradients (IPG-Dalt) was carried out according to the method of Görg et al.¹⁴ with minor modifications. Polyacrylamide gels with immobilized pH gradients (IPG) of 4 to 7, 4 to 8, and 4 to 9 (4% T, 4% C; size, 250 × 130 × 0.5 mm³) were cast as described.^{15,16} The washed and dried IPG gels were then cut into 4 mm wide IPG strips, which were rehydrated overnight to a thickness of 0.4 mm with a solution consisting of 8 mol/L urea, 0.5% CHAPS, 0.2% dithiothreitol, and 0.2% Pharmalytes (pH 3 to 10). The rehydrated IPG gel strips were placed, side by side, on the cooling plate of a flatbed focusing chamber. Fifty to four hundred micrograms of protein was loaded onto each IPG gel

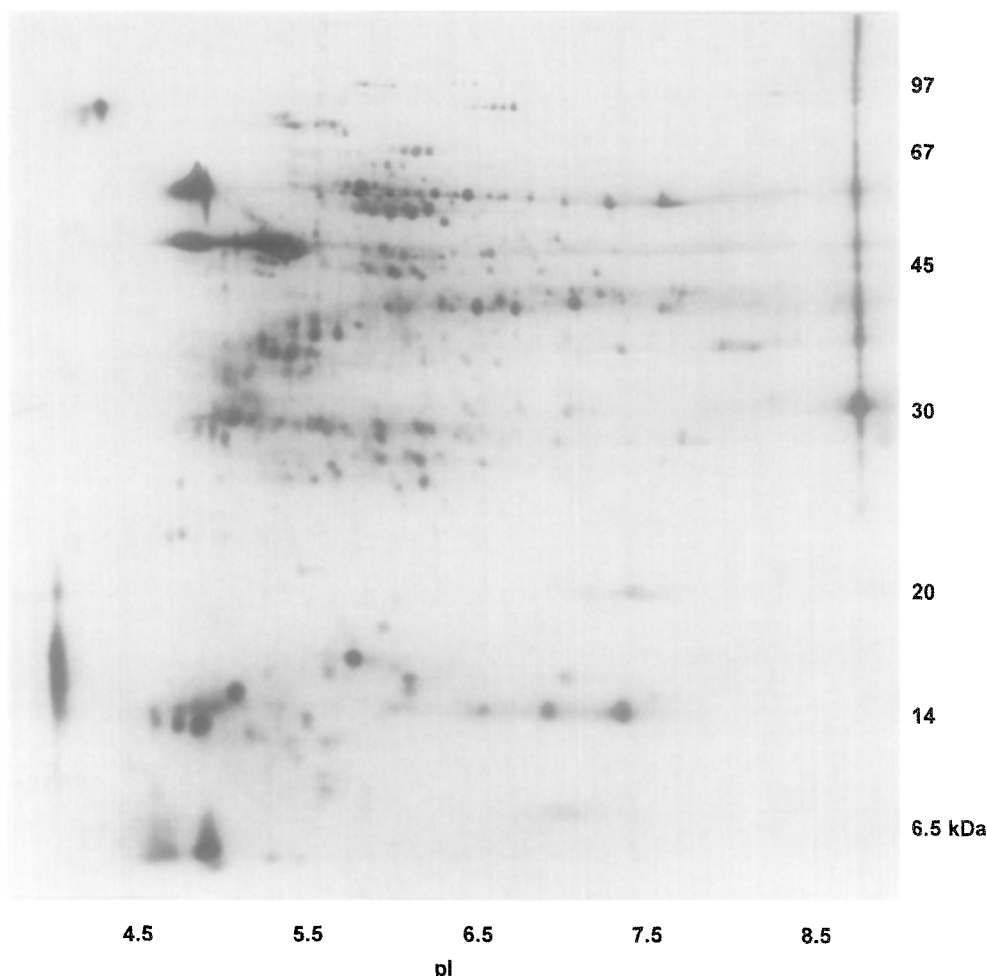


FIG. 1. IPG-Dalt of latex (*H. brasiliensis*) C-serum proteins (75 μ g). First dimension, IPG 4-9; second dimension, SDS-PAGE, T = 12% (silver stain).

strip near the anode with the help of sample application cups (Pharmacia DryStrip kit) placed on the surface of the IPG gel strips. Protein solutions containing SDS were applied at the cathode. Isoelectric focusing was performed for 20,000 to 40,000 volt hours, depending on the IPG gradient and protein load¹⁷ at 20°C.¹⁸ After isoelectric focusing, the IPG gel strips were immediately stored in a plastic bag at -78°C. Before SDS-polyacrylamide gel electrophoresis (SDS-PAGE), the IPG gel strips were equilibrated for 2 $\times$ 10 minutes in 2 $\times$ 10 ml equilibration solution (0.05 mol/L Tris-HCl buffer [pH 8.8] containing 6 mol/L urea, 30% wt/vol glycerol, 2% wt/vol SDS, and a trace of bromophenol blue). Dithiothreitol (1% wt/vol) was added to the first equilibration solution, and iodoacetamide (260 mmol/L) was added to the second. Two-dimensional SDS-PAGE (12% T, 4% C) was carried out simultaneously on four slab gels without stacking (16 $\times$ 16 $\times$ 1.0 mm³) at 15°C by using the Laemmli buffer system.¹⁹ Proteins of analytical 2-DE gels were silver-stained according to the method of Blum et al.,²⁰ whereas micropreparative 2-DE gels were subjected to protein blotting according to the method of Kyhse-Andersen²¹ or Baker et al.²² The latter PVDF membranes were used for microsequencing, whereas the 2-DE gels blotted onto PVDF with the buffer system of Kyhse-Andersen²¹ were subjected to immunostaining. After blotting, the membranes were rinsed

three times for 5 minutes in Tris buffered saline (TBS: 150 mmol/L NaCl in 50 mmol/L Tris-HCl, pH 7.4), air-dried for 1 hour, and stored at 4°C in sealed plastic bags.

Immunostaining

The sera of health care workers with latex allergy and mAbs directed against REF were used for immunostaining. Briefly, the dried membranes were wetted in methanol for 5 seconds and washed three times for 5 minutes in TBS. Blocking was performed with 1% bovine serum albumin and 1% PVP-40 in TBS for 6 to 8 hours. After being washed three times with TBS, the blots were incubated overnight with pooled serum diluted 1:20 in TBS containing 1% Tween-20 (TBST) from 10 patients with latex allergy or hybridoma supernatant (1:100 in TBST). The membranes were then washed three times with TBST and incubated with alkaline phosphatase-conjugated anti-human IgE (1:1000 in TBST) or alkaline phosphatase-conjugated anti-mouse IgG (1:10,000 in TBST) for 4 hours. After three washes with TBST, IgE and IgG binding, respectively, were demonstrated by adding a 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium solution (each 0.35 mmol/L in 100 mmol/L Tris-HCl [pH 9.5], containing 100 mmol/L NaCl and 5 mmol/L MgCl₂).

To detect nonspecific IgE immunostaining, control experi-

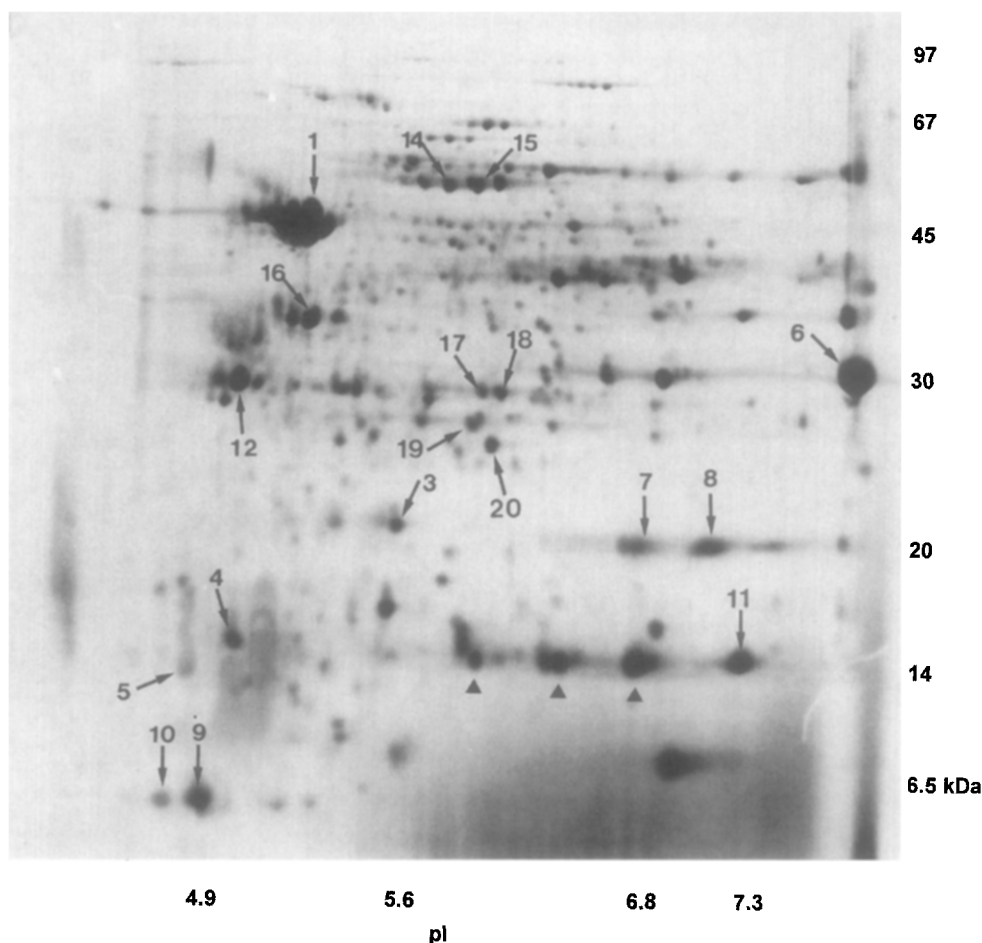


FIG. 2. Latex (*H. brasiliensis*) C-serum proteins (400 μ g) separated by IPG-Dalt (first dimension, IPG 4-8; second dimension, SDS-PAGE, T = 12%) and electroblotted onto a PVDF membrane (Coomassie Brilliant Blue stain). N-terminal-sequenced latex proteins are indicated by numbers (see Table I). For more details see text.

ments with sera of persons not allergic to latex but occupationally exposed to latex were performed in parallel. Similarly, an mAb directed against the allergen Chi t 1.01 was used in control experiments to ensure the specificity of mAbs II4G9 and II4F9.

N-terminal sequence analysis

The blotted proteins were visualized with Coomassie Brilliant Blue R-250 stain (Roth, Germany) (0.1% in destaining solution; 2 minutes). After destaining in water/methanol/acetic acid (45:45:10), the blots were rinsed for 15 minutes in deionized water and air-dried. Protein spots of interest were excised from the blotting matrix and placed directly into a New Blot cartridge from Applied Biosystems (ABI) fitted to an ABI model 477A Pulsed-Liquid Protein Sequencer equipped with an online phenylthiohydantoin amino acid analyzer (model 120A). Routinely, 10 to 15 Edman degradation cycles were performed for each spot. The partial protein sequences were compared for homologies to already known proteins by using the Basic Local Alignment Search Tool (BLAST) algorithm.²³ Theoretic isoelectric points (pIs) and MWs of proteins described in this study were computed by means of the Compute pI/MW tool obtained through the ExPASy server (<http://expasy.hcuge.ch>, Geneva University Hospital, University of Geneva, Switzerland).

RESULTS

Two-dimensional electrophoresis of latex proteins

Both the soluble and particle-bound proteins were first subjected to 2-DE with a wide (4 to 9) IPG gradient to determine their pI and MW profiles. Fig. 1 shows a typical 2-DE map of latex C-serum proteins at an analytical protein load (approximately 75 μ g). About 200 distinct C-serum protein spots with MWs from approximately 100 kd to less than 6.5 kd were visible by silver staining. The majority of the soluble proteins had pI values ranging from 4 to 8, with the exception of a basic 30 kd protein spot (pI $\geq$ 8.7) and some acidic 14 to 20 kd polypeptides exhibiting pI values with a pH less than 4. Therefore an IPG of 4 to 8 was chosen for micropreparative IPG-Dalt separation of latex C-serum proteins to be analyzed by IgE immunostaining and N-terminal protein sequencing experiments. A typical Coomassie Brilliant Blue-stained micropreparative IPG-Dalt pattern of latex C-serum proteins (protein load, approximately 400 μ g) blotted onto a PVDF

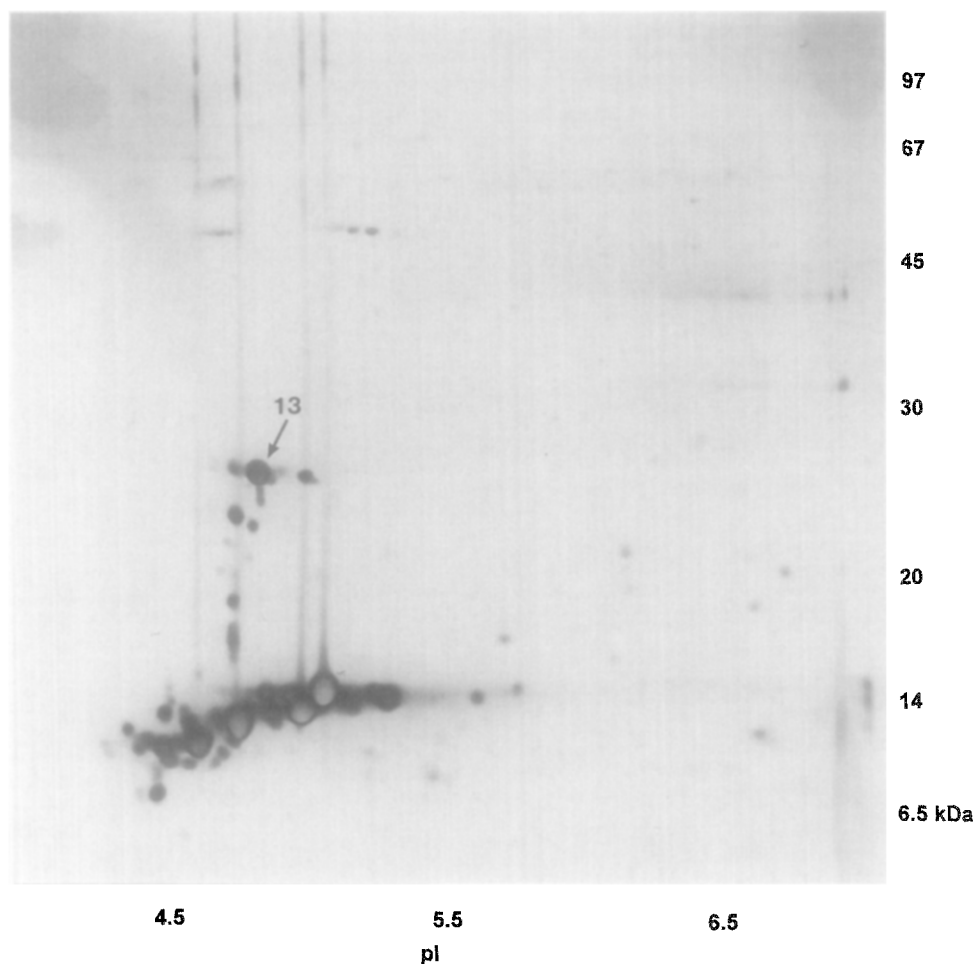


FIG. 3. IPG-Dalt of latex (*H. brasiliensis*) particle proteins. First dimension, IPG 4 to 7; second dimension, SDS-PAGE, T = 12% (silver stain). N-terminal-sequenced latex proteins are indicated by numbers (see Table I).

membrane is shown in Fig. 2. Additional latex proteins, especially in the 20 kd area, were visualized by Coomassie Brilliant Blue dye, because silver staining (Fig. 1) produced several negatively stained protein spots.

In contrast to the heterogeneous 2-DE pattern of the soluble latex proteins, the proteins eluted from the particles exhibited exclusively acidic properties and consisted primarily of two groups of proteins (Fig. 3). The first group of about 25 polypeptides (MW range, 8 to 14 kd) had pIs from 4.3 to 5.3, and the second group of about six proteins (MW range, 22 to 24 kd) had pIs of approximately 4.8.

Immunoprobings with pooled serum of health care workers with latex allergy

Micropreparative IPG-Dalt gels of soluble and particle-bound proteins were blotted onto PVDF membranes and incubated with pooled serum from 10 health care workers with latex allergy. About 60 C-serum polypeptides analyzed in an IPG of 4 to 8 showed IgE reactivity (Fig. 4); and distinct IgE binding was detected in the 56, 45, 30, 20, and 14 kd areas of the immunoblots. Additionally, a low MW

protein (pI, approximately 4.9; MW, <6.5 kd) was stained for IgE binding. The 2-DE IgE-stained pattern of latex C-serum proteins was easily compared with the silver-stained and Coomassie Brilliant Blue-stained gels, and the respective polypeptides could be assigned. Control experiments were performed to investigate the specificity of the detected IgE binding. They revealed some background staining independent of probing with sera of patients not allergic to latex as a result of nonspecific reactions of anti-human IgE antibodies with latex proteins, but the degree of color deposition in the control experiments was clearly lower, indicating the presence of specific IgE antibodies directed toward the respective protein spots in patients' sera. However, the IgE reactivity of three spots (marked with triangles in Fig. 4) could not be assessed because they showed similar staining intensity in the control experiments.

Fig. 5 demonstrates the IgE reactivity of the particle-bound latex proteins, which were investigated in an IPG of 4 to 7. Although most of the 8 to 14 kd proteins exhibited a distinct specific IgE reactivity, the 22 to 24 kd proteins were not stained. All reactions observed in immunoblotting

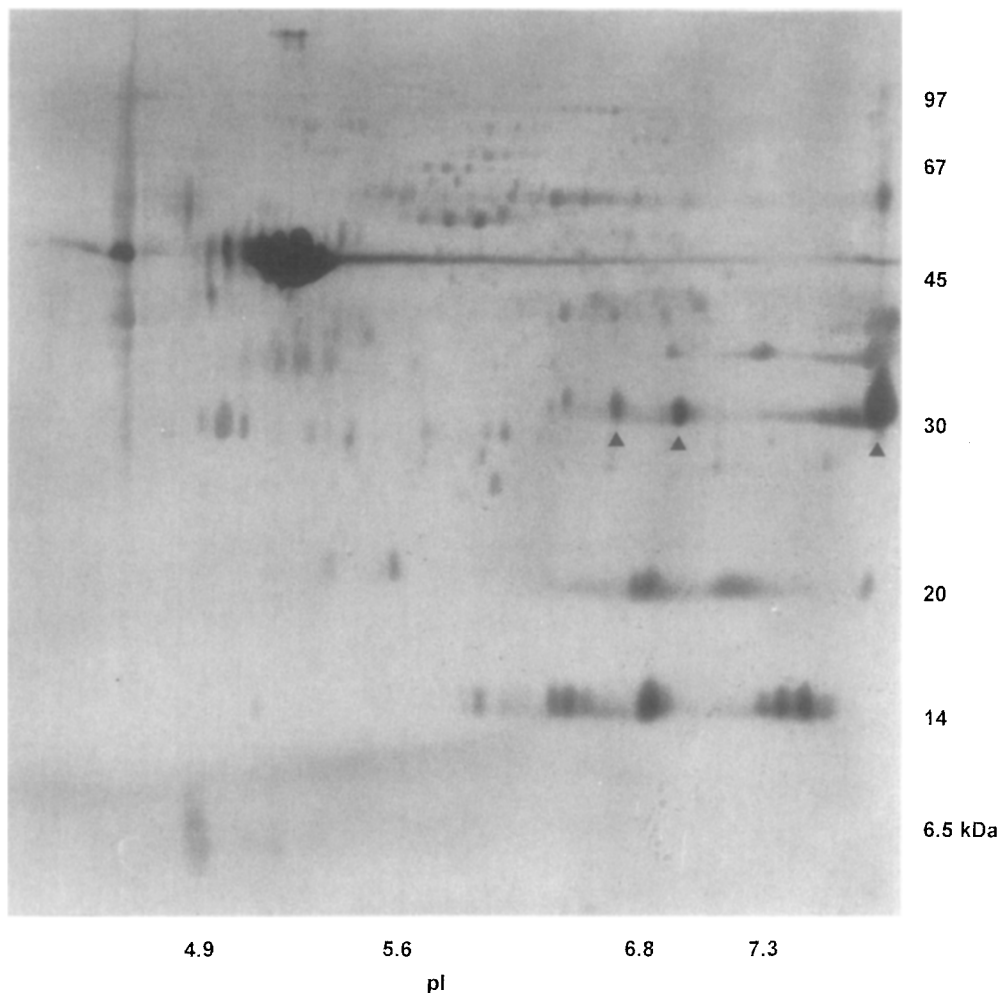


FIG. 4. Latex (*H. brasiliensis*) C-serum proteins separated by IPG-Dalt (cf. Fig. 2) and electroblotted onto a PVDF membrane. Immunostaining with pooled serum of health care workers with latex allergy and secondary alkaline phosphatase-labeled anti-human IgE. Nonspecific stained protein spots are marked with triangles.

with latex particle proteins were specific, because no color development was detected in the respective areas of the 2-DE immunoblots in the control experiments.

Identification of latex allergens by protein microsequencing and mAbs

To identify the soluble IgE-reactive polypeptides in latex, 18 C-serum immunoreactive protein spots were excised from the Coomassie Brilliant Blue-stained blot (Fig. 2) and subjected to N-terminal amino acid microsequencing. Sixteen protein spots yielded N-terminal sequence information presented in Table I, whereas protein spots 1 and 18 provided no sequence information and must therefore be blocked at the N-terminus. Sequence alignments of protein spots 3, 4, 5, 7, 8, 9, 10, and 11 revealed high sequence homology to hevein precursor (pI, 5.64; MW, 20,087 d), a two-domain protein that is posttranslationally processed to hevein (pI, 4.96; MW, 4727 d), and its C-terminal domain (pI, 6.43; MW, 14,891 d). Spot 3 (pI, 5.6; MW, approximately 21,000 d) probably represents the

mature hevein precursor and spots 9 and 10 (pI, 4.7 and 4.9; MW < 6.5 kd) hevein according to their experimental pI and MW data. Spots 4, 5, 7, and 8 are likely to be partially processed products of prohevein, whereas spot 11 (pI, 7.5; MW, approximately 14 kd) and its more acidic neighbors (marked with triangles in Fig. 2) are assumed to be identical with the C-terminal domain protein of hevein precursor.

The N-terminus of spot number 6 was identified as being highly homologous with hevine A, a latex protein with lysozyme-chitinase activity; and the sequence alignment search for spot 12 exhibited a high sequence homology to several plant chitinases. Protein spots 14 and 15 corresponded to enolase from *Ricinus communis*, whereas database searches for the polypeptides 16 and 17 demonstrated homologies to triosephosphate isomerases. Sequence alignment for spot 19 revealed several proteasome subunits, and protein spot 20 corresponded to superoxide dismutase from *H. brasiliensis*.

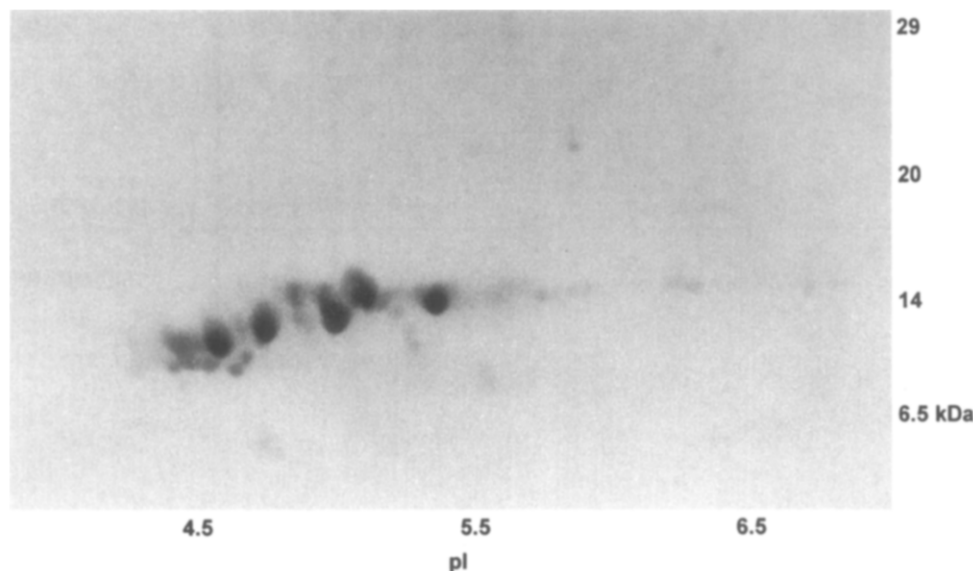


FIG. 5. Latex (*H. brasiliensis*) particle proteins separated by IPG-Dalt (cf. Fig. 3) and electroblotted onto a PVDF membrane. Immunostaining with pooled serum of health care workers with latex allergy and secondary alkaline phosphatase-labeled anti-human IgE.

Latex particle proteins were characterized by immunostaining with two mAbs directed against REF (pI, 5.04; MW, 14,590 d), a well-described latex allergen termed Hev b 1, which is tightly bound to rubber particles. The mAb II4F9 reacted specifically with all IgE-binding proteins of the 8 to 14 kd cluster (Fig. 6), except for three polypeptides, indicating that REF is the major component of this particular area of the 2-DE map shown in Fig. 3. In contrast, the mAb II4G9 that is specific for the C-terminus of REF reacted very strongly with four distinct polypeptides (pI range, 4.9 to 5.3) of identical MW of about 14.5 kd. Spot 13, which showed no IgE reactivity in immunoblotting with sera of health care workers, was subjected to N-terminal protein sequencing, but no sequence information was obtained because of blockage of the N-terminus.

DISCUSSION

In this study we applied IPG-Dalt to the analysis of both soluble and particle-bound natural rubber polypeptides. The resulting high-resolution 2-DE maps were then used to localize the IgE-binding proteins by immunoblotting with the sera of health care workers with latex allergy and subsequently to identify the latex allergens by N-terminal protein microsequencing.

This high-resolution 2-DE technique demonstrated very impressively the complexity of the latex protein fractions with very high quality concerning resolution and reproducibility, even at micropreparative protein loads. The protein patterns of both protein fractions differed markedly from each other. Although the latex C-serum fraction showed a very complex composition, exhibiting proteins with a broad pI range and MWs ranging from approximately 5 kd to approximately 100 kd, the proteins extracted from the latex particles re-

vealed two acidic protein clusters of approximately 8 to 14 kd and approximately 24 kd, which were exclusively present in this protein fraction.

The latex polypeptides of both protein fractions were analyzed separately for their IgE reactivity by probing the respective blots with pooled serum of 10 patients allergic to latex. About 60 distinct IgE-reactive protein spots of latex C-serum were observed. Recently, Kurup et al.¹⁰ analyzed the soluble latex proteins by 2-DE and reported 56 soluble latex proteins reacting with IgE antibodies of patients allergic to latex. However, their 2-DE maps cannot be compared with our results, because they published only a schematic presentation of their 2-DE IgE immunoblots. Moreover, they did not analyze the latex antigens exhibiting pI values above 7 and MWs smaller than 14 kd.

Because of the high reproducibility of spot positions generated by IPG-Dalt, the 2-DE IgE immunoblot pattern was easily compared with the silver-stained, as well as with the Coomassie Brilliant Blue-stained, 2-DE gels; and the respective polypeptides could be localized on the 2-DE maps. Eighteen protein spots of latex C-serum were subjected to N-terminal protein microsequencing, and 16 spots were identified by database searching, whereas two spots were blocked at the N-terminus. Seven IgE-reactive polypeptide spots represent previously undescribed latex allergens showing high sequence homologies to chitinase (spot 12), enolase (spots 14 and 15), triosephosphate isomerase (spots 16 and 17), proteasome subunit (spot 19), and superoxide dismutase (spot 20). The role of the novel IgE-binding proteins with respect to latex allergy cannot be assessed at the moment, because pure allergens are required for detailed studies in vitro and in vivo. However, because those proteins are common plant enzymes, they may

TABLE I. N-terminal sequence analysis and sequence alignment of IgE-reactive latex polypeptides (cf. Figs. 2 and 3)

Spot No.	Expt. pl/ MW		Sequence alignment	Identification/description	pI/MW (SWISS-PROT)	SWISS-PROT accession number
1	5.3/45000	Blocked				
3	5.6/21000	Query:	1 EQCGRQAXGGXK 12 EQCGRQA G+++	HEVE_HEVBR/Hevein precursor (major hevein)	5.64/20087	P02877
		Sbjct:	1 EQCGRQAGGKLC 12			
4	5.0/14000	Query:	1 EQCGXXX 7 EQCG	HEVE_HEVBR/Hevein precursor (major hevein) (fragment)	5.64/20087	P02877
		Sbjct:	1 EQCGRQA 7			
5	4.8/13000	Query:	1 EQCGRQAGGX 10 EQCGRQAGG	HEVE_HEVBR/Hevein precursor (major hevein) (fragment)	5.65/20087	P02877
		Sbjct:	1 EQCGRQAGGK 10			
6	8.7/30000	Query:	1 GGIAIYXG 8 GGIAIY G	CHLY_HEVBR/Hevamine A [chitinase (EC 3.2.1.14); lysozyme (EC 3.2.1.17)]	8.44/29550	P23472
		Sbjct:	1 GGIAIYWG 8			
7	6.8/20000	Query:	1 EQCGRQAGGKL 11 EQCGRQAGGKL	HEVE_HEVBR/Hevein precursor (major hevein) (fragment)	5.64/20087	P02877
		Sbjct:	1 EQCGRQAGGKL 11			
8	7.1/20000	Query:	1 EQCGRQAGGKLCP 13 EQCGRQAGGKLCP	HEVE_HEVBR/Hevein precursor (major hevein) (fragment)	5.64/20087	P02877
		Sbjct:	1 EQCGRQAGGKLCP 13			
9	4.9/<6500	Query:	1 EQCGRQAGGK 10	HEVP_HEVBR/Pseudo-Hevein (minor hevein)	4.96/4727	P80359
		Sbjct:	1 EQCGRQAGGK 10			
10	4.7/<6500	Query:	1 EQXGRQXXX 9 EQCGRQ	HEVP_HEVBR/Pseudo-hevein (minor hevein)	4.96/4727	P80359
		Sbjct:	1 EQCGRQAGG 9			
11	7.3/13500	Query:	1 GLSASNVLATYHLY 14 G+SASNVLATYHLY	HEVE_HEVBR/Hevein precursor (major hevein) (C-domain)	6.43/14891	P02877
		Sbjct:	67 GGSASNVLATYHLY 80			
12	5.0/29000	Query:	1 DIGSIISKSTFEEFLKXGN 19 D+GSIIIS S F++ LK N	CHI2_TOBAC/Endochitinase B precursor (EC 3.2.1.14)	8.31/31468	P24091
		Sbjct:	60 DLGSIISSEMFQMLKHRN 78			
13	4.8/24000	Blocked				
14	5.9/51000	Query:	1 AITIXSVRARQIF 13	ENO_RICCO/ENOLASE (EC 4.2.1.11) (2-phosphoglycerate dehydratase)	5.56/47912	P42896
		Sbjct:	2 AITI SVRARQIF 14			
15	6.0/51000	Query:	1 AITIVSVRA 9	ENO_RICCO/ENOLASE (EC 4.2.1.11) (2-phosphoglycerate dehydratase)	5.56/47912	P42896
		Sbjct:	2 AITIVSVRA 10			
16	5.3/35000	Query:	1 RKVDVDVXVPYT 13 +KVDVAV VP+T	TPIS_CORGL/Triosephosphate iso- merase (EC 5.3.1.1)	4.8/27195	P19583
		Sbjct:	37 EKVDVAVTVPFT 48			
17	6.1/29000	Query:	1 ARKFFVGG 8 ARKFFVGG	TPIS_SCHPO/Triosephosphate iso- merase (EC 5.3.1.1)	6.16/27118	P07669
		Sbjct:	1 ARKFFVGG 8			
18	6.2/29000	Blocked				
19	6.0/26000	Query:	1 ANWSPYDNNGG 11 ANWSPYDNNGG	PRC5-ARATH/Proteasome component C5 (EC 3.4.99.46)	7.83/25349	P42742
		Sbjct:	13 ANWSPYDNNGG 23			
20	6.1/24500	Query:	2 QTXSPDLPYD 12	SODM_HEVBR/Superoxide dismutase precursor (EC 1.15.1.1)	5.89/23000	P35017
		Sbjct:	29 QT S PDLPYD 39			

Amino acid residues are given in one-letter notation. Gaps in the sequence are denoted by "X."

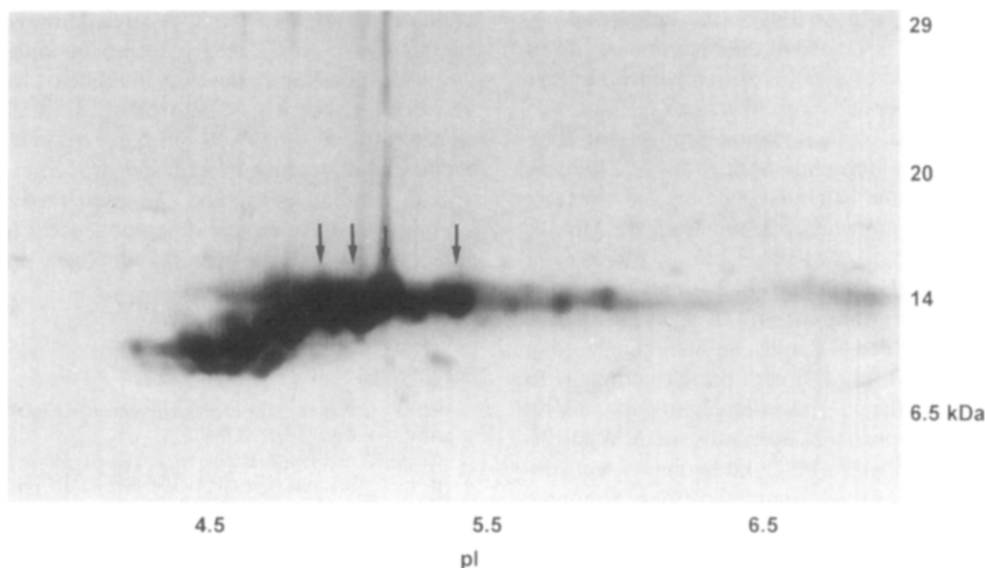


FIG. 6. Latex (*H. brasiliensis*) particle proteins separated by IPG-Dalt (cf. Fig. 3) and electroblotted onto a PVDF membrane. Immunostaining was done with mAb II4F9 specific for N-terminus of REF and secondary alkaline phosphatase-labeled anti-mouse IgG. Latex particle proteins stained by mAb II4G9, which reacts with an epitope at the C-terminus of REF, are marked by arrows.

represent potential cross-reacting proteins present in various foods. It is well known that there is an association between allergic reactions to latex and to several fruits such as banana, kiwi fruit, avocado, potato, and chestnut.^{5, 24} Recently, Akasawa et al.²⁵ isolated and identified an avocado chitinase (approximately 30 kd) that reacted with IgE antibodies from 14 of 22 patients allergic to latex.

Nine of the 16 sequenced protein spots correspond to known latex allergens, namely prohevein (spots 3, 4, 5, 7, and 8), hevein (spots 9 and 10), prohevein C-domain (spot 11), and hevine A (spot 6). Prohevein (spot 3), a protein with two domains that is processed into hevein and prohevein C-domain, is a major latex allergen and has been well characterized. Palosuo et al.²⁶ found IgE antibodies to prohevein in 78% of 40 patients allergic to latex, whereas positive reactions to prohevein C-domain (spot 11) occurred in only 28% of the patients. Extended studies by Alenius et al.²⁷ and Chen et al.²⁸ clearly indicated that the 4.7 kd hevein (spots 9 and 10) is the primary reason for the IgE binding to prohevein. Protein spots 4, 5, 7, and 8 are likely to be partially processed products of prohevein. Interestingly, spots 4 and 5 were not consistently stained in our IgE-immunoblotting experiments, and at the moment no interpretation of this phenomenon is available.

Protein spot 6 was identified as hevine A, a bifunctional enzyme with lysozyme-chitinase activity. Because of nonspecific reactions in immunoblotting, the IgE reactivity of this protein could not be assessed, but according to Alenius et al.³ this polypeptide has little importance for the sensitization of patients allergic to latex and is not regarded as an important latex allergen.

No sequence information for the IgE reactivity of

C-serum proteins 1 and 18 was obtained. To better characterize and identify these allergens, deblocking of the N-terminal amino acid and/or internal sequence analyses must be performed. Internal sequences may also yield homologies with proteins already described. Spot 1 belongs to a protein cluster at ~45 kd present in high abundance in C-serum and exhibited strong IgE reactivity in immunoblotting. Similarly, Beezhold et al.⁴ and Tomazic et al.⁹ found, in SDS-PAGE, an abundant 45 to 46 kd protein that reacted very intensely with IgE antibodies of health care workers with latex allergy. Beezhold et al.⁵ identified this protein as highly homologous with patatins, which are storage proteins encoded by a multigene family found in plant families such as potato and tomato. Because no other abundant protein in the 45 kd area is present on the 2-DE C-serum protein map, we therefore assume a relationship of spot 1 with this important latex allergen described by Beezhold et al.⁵

The proteins eluted from the latex particles showed different IgE-binding characteristics. Although almost all 8 to 14 kd proteins were stained distinctly, the polypeptides with MWs of 20 to 24 kd showed no detectable reaction in IgE immunoprobings. The 8 to 14 kd proteins were identified as REF, a well-known and well-characterized latex allergen, by immunostaining with two specific mAbs directed against this allergen. The mAb II4G9, which is specific for the C-terminus of REF, reacted with four distinct polypeptides of different pIs (4.9 to 5.3) but identical MWs of about 14.5 kd, representing the mature REF. Because these proteins were extracted with an SDS-containing buffer, it is not clear whether the appearance of these isoforms is caused artificially during isoelectric focusing or genetically con-

trolled. In contrast to mAb II4G9, the epitope recognized by mAb II4F9 is located at the N-terminus, and all IgE-reactive proteins of 8 to 14 kd were stained by this antibody. The polypeptides with MWs smaller than 14.5 kd are suggested to be degradation products of REF lacking parts of the C-terminus. Spot 13 (MW, 24 kd; pI, approximately 4.8) and its close neighbors did not react with IgE of health care workers who participated in this study. In the literature, two polypeptides, which react significantly with IgE antibodies of patients with spina bifida and latex allergy but not with those of health care workers, are described. Alenius et al.^{6,7} analyzed a protein (MW, 27 kd; pI, 4.8) with partial homology to REF, which was a characteristic allergen in patients with spina bifida and latex allergy. Recently, the MW of this protein was determined to be 23 kd by mass spectrometry (T. Palosuo, personal communication). Similarly, Lu et al.⁸ reported a polypeptide (MW, 23 kd; pI, 4.8) to be closely associated with hypersensitivity in patients with spina bifida. The 90 amino acid residues sequenced from this 23 kd protein showed a 45.6% homology with REF, and it shared an identical amino acid sequence motif with the 23 kd protein described by Alenius et al.^{6,7} as well. Because the data profiles of both proteins match each other very well, it can be assumed that both research groups have investigated the same polypeptide. Moreover, the characteristics of spot 13 suggest that this protein is identical, or at least related to, the spina bifida allergen discussed above.

In conclusion, the results presented demonstrate the applicability of 2-DE in combination with IgE immunoprobings and N-terminal microsequencing to rapidly identify a large number of latex allergens that are involved in the sensitization of health care workers. The IgE reaction profiles obtained for health care workers with latex allergy may be different from those of patients with spina bifida and therefore cannot be generalized. However, our approach resulted in the identification of five previously undescribed IgE-reactive latex proteins. In addition, we have been able to correlate the most significant information on identified latex allergens in the literature with the protein patterns obtained from our 2-DE separations, except for one polypeptide. Alenius et al.³ reported that a 36 kd latex allergen with high homology to several plant endo-1,3- β -glucosidases reacted with six of 29 sera from patients with latex allergy, but this has not yet been localized on our 2-DE map.

Nevertheless, the high-resolution 2-DE latex protein separations presented in this study are not only an ideal starting point for allergen identification, but also provide valuable information (MW and pI data) for the development of strategies for their isolation (e.g., by chromatographic methods). Pure allergens are needed to analyze the overall frequency of patients in whom IgE antibodies to individual allergens develop in order to assess their importance with regard to sensitization frequency. Because of the higher serum consumption compared with one-dimensional electrophoresis techniques, IPG-Dalt cannot be used to screen single serum

samples of patients allergic to latex. However, because allergens have been identified by the methods used here, it will be possible to develop immunoassays to detect antibodies to these specific proteins. In addition, polyclonal antisera or mAbs to proteins separated by 2-DE can be raised by using protein spots cut from 2-DE gels. Specific antibodies can then be used to screen "latex allergens" present in very different sources (e.g., gloves and food) to analyze their immunologic properties in relation to latex allergy.

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