On the allergenicity of Hev b 1 among health care workers and patients with spina bifida allergic to natural rubber latex

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Background: Recent studies have caused much controversy about the prevalence of IgE antibodies to Hev b 1 among health care workers (HCWs) and patients with spina bifida (SB) who are allergic to latex. This investigation was carried out to verify the results reported.

Method: Serum samples from 140 patients with SB as well as from 105 HCWs allergic to latex were tested by enzyme allergosorbest test (EAST) and EAST-inhibition assay to evaluate the rate and degree of sensitization to highly purified Hev b 1.

Results: Eighty-one percent of patients with SB who were allergic to latex had IgE antibodies against Hev b 1. The prevalence of anti–Hev b 1 antibodies among HCWs allergic to latex was 52.3%. In 15 of 33 serum samples from patients with SB that were randomly tested, the IgE binding to commercial latex allergens could be completely inhibited by Hev b 1; in only six cases was the maximum inhibition of IgE binding to latex by Hev b 1 less than 50%. Testing two monoclonal anti-Hev b 1 antibodies with extracts of five brands of latex gloves revealed a predominant presence of Hev b 1 protein as a monomer or its aggregates. Molecular analysis of human leukocyte antigen–D region genes DRB and DQB1 suggested no statistically significant correlation between the human leukocyte antigen alleles tested and IgE responsiveness to Hev b 1.

Conclusions: Our results indicate that Hev b 1 not only makes significant contributions to the IgE binding to latex, but it is also the unique sensitizer in about 45% of patients with SB who are allergic to latex. (J Allergy Clin Immunol 1997;100:684-93.)

Key words: *latex allergy, Hev b 1, IgE, spina bifida, rubber elongation factor*

Abbreviations used

2D-PAGE:	Two-dimensional polyacrylamide gel electrophoresis
DNA:	Deoxyribonucleic acid
EAST:	Enzyme allergosorbent test
ES-MS:	Electrospray mass spectrometry
HCW:	Health care worker
HLA:	Human leukocyte antigen
HPLC:	High-performance liquid chromatography
mAb:	Monoclonal antibody
NRL:	Natural rubber latex
SB:	Spina bifida
SDS:	Sodium dodecylsulfate
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SPT:	Skin prick test

IgE-mediated type I hypersensitivity to natural rubber latex (NRL) represents an important occupational problem, especially among the health care workers (HCWs) who have to use latex gloves regularly.[1-3] However, since 1989, when the possibility of severe allergy to NRL in children with spina bifida (SB) was first reported,[4] studies based on clinical history and immunologic findings (skin and serologic testing) have shown that between 18% and 73% of individuals with SB are sensitive to latex.[5-7] Therefore, patients with SB are at the highest risk for developing latex allergy. The type of allergic reactions to NRL can range from contact urticaria, rhinoconjunctivitis, and asthma to life-threatening anaphylactic shock. Exactly how this allergy develops is still not known, in particular the initial steps of sensitization. The unspecified proteins retained in the final latex products are thought to be the allergen in type I hypersensitivity reactions.[8]

The IgE binding reactivity of Hev b 1 (rubber elongation factor[9]) was first reported by Czuppon et al.[10] In that study, Hev b 1 was shown by competitive inhibition assay to react with serum IgE in serum samples from all 13 of their patients (HCWs) allergic to latex. However, recent studies[11-12] that involved an immunoblotting technique showed a far lower prevalence (about 20%) of anti–Hev b 1 antibodies especially among HCWs allergic to latex. Because the Hev b 1 protein is tightly associated with rubber particles[9] and getting an appropriate amount of purified Hev b 1 is very difficult, these results are so far not confirmed by a well-established detection method with a larger number of serum samples.

In our most recent studies[13] on the allergenicity of hevein in NRL, we showed that about 75% of HCWs with an allergy to latex had hevein-specific IgE antibod-

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ies; however, less than 30% of the patients with SB who had latex allergy showed IgE antibodies against hevein in their sera. These findings indicate that different patient groups may be susceptible to developing an IgE response to special latex allergen. Regarding the extremely high prevalence of latex allergy in patients with SB, the characterization and evaluation of the major responsible latex allergens are therefore of utmost importance, particularly for preparing reliable diagnostic reagents and in developing preventive measures to avoid the development of latex allergy.

In this study we first analyzed five brands of latex gloves that were most frequently used in our hospital and demonstrated that Hev b 1 is the predominant protein released in the aqueous extract. Using a new procedure with a combination of detergent solubilization and high-performance liquid chromatography (HPLC), we isolated and purified a considerable amount of Hev b 1 protein from rubber particles. By enzyme allergosorbent test (EAST) and immune inhibition assay, we evaluated the IgE binding reactivity of highly purified Hev b 1 in serum samples from 140 patients with SB and from more than 100 HCWs with allergy to latex. Our results demonstrate that Hev b 1 significantly contributes to the elicitation of allergic responses to latex and, in many cases, it may also be the unique sensitizer to cause the latex allergy in individuals with spina bifida. Moreover, studies of deoxyribonucleic acid (DNA) typing of human leukocyte antigen (HLA)-D genes in a group of 60 patients with SB were also performed to investigate the potential genetic propensity to develop an immediate-type immune response in such patients.

METHODS

Natural rubber latex

NRL was obtained from the Rubber Research Institute of Malaysia. It was collected from rubber trees *(Hevea brasiliensis)* of the clone RRIM 600 and was immediately ammoniated at 0.7% and shipped without freezing.

Latex glove extracts

Latex gloves were cut into about 5 × 5 mm^2 pieces and were extracted in phosphate-buffered saline containing 1% sodium dodecyl sulfate (SDS) at pH 7.2 by shaking for 2 hours at 37° C. Thereafter the rubber pieces were removed by centrifugation, the supernatant was dialyzed through a cellulose tube (molecular weight cutoff of 1500 Da) against distilled water for 48 hours at 4° C, and then lyophilized.

Patient and control serum samples

Serum samples from all 140 patients with SB (70 males, 70 females) collected to date in the Children's Hospital of Cologne, Germany, were used in this study. The mean age of the patients was 9.7 years (range, 1.2 to 27.1 years). Two patients had never received an anesthetic, and the other 138 patients had a history of receiving one or more general anesthetics (range, 1 to 27). Based on the questionnaire and SX1-test in the CAP system, 46 (33%) patients were found to be atopic. Based on the CAP system, 69 (49%) patients had specific IgE antibodies to latex in their sera as determined by latex RAST. Of these 69 patients, 24 (33.3%) also showed hypersensitivity responses to latex products. The reactions included contact urticaria, rhinitis, bronchospasm, angioedema, and anaphylaxis ($n = 4$). For comparison, 105 serum samples from HCWs with latex allergy were studied. All patients in the HCW group were occupationally exposed to latex gloves. They showed a positive skin prick test (SPT) result to latex extract prepared in our laboratory and had specific IgE antibodies to latex in sera as determined by latex RAST with the CAP system. As negative controls, serum samples from 10 HCWs who had neither clinical symptoms nor IgE antibodies to latex proteins were used.

Production of monoclonal antibodies to Hev b 1

Two monoclonal antibodies (mAbs) directed against Hev b 1 protein used in the study were produced as previously described.[14] Both mAbs were isotyped as IgG-κ. Epitope mapping with the use of synthetic overlapping Hev b 1 peptides has revealed that one of the mAbs, indicated as II4G9, was specifically bound to the C terminal of Hev b 1 (residues 122 to 134)[14] and the other mAb, II4F9, recognized the peptide with amino acid residues of 46 to 54.

Protein purification and characterization

Hev b 1 was isolated from the NRL by a modification of a previously described procedure.[15, 16] Crude Hev b 1 protein was extracted from rubber particles that were freed from the latex serum proteins by a solution containing 2% SDS. To remove the residual SDS detergent in the extract, the lyophilized protein extract suspended in the Tris buffer (50 mmol/L) was loaded onto a column of detergent adsorber gel (5 ml, Boehringer Mannheim, Mannheim, Germany) previously equilibrated with the same buffer. The column was eluted at a flow rate of 1 ml/min and the fraction containing proteins was collected. For preparative purification of the Hev b 1 protein, the SDS-free protein solution was applied to a RESOURCE 3 ml reverse-phase column (6.4 mm id × 100 mm; Pharmacia Biotech) equilibrated with 40% solution B (0.06% trifluoroacetic acid in acetonitrile; solution A was 0.1% trifluoroacetic acid in water) and eluted by HPLC with a linear gradient from 40% to 70% solution B in 25 minutes at a flow rate of 2 ml/min. Elution of proteins was monitored by ultraviolet absorbance at 230 nm. The pooled fractions containing Hev b 1 protein, eluting with a retention time at about 15 minutes, were either lyophilized or concentrated with the use of a stirred pressure ultrafiltration cell with a YM3 membrane (Amicon). The purity of the Hev b 1 protein was verified by analytical HPLC on a column of Grom-Protein C4 using a gradient from 40% to 90% solution B in 25 minutes at a flow rate of 1 ml/min. For the mass determination, the purified Hev b 1 protein at a concentration of about 2.5 pmol/μl in acetonitrile/water (3:7 vol/vol) containing 10% acetic acid was injected into the electrospray ion source of a Finnigan TSQ 7000 triple quadrupole mass spectrometer at a flow rate of 3 μl/min. The spectra were recorded under unit-mass resolution in the positive ion mode.

Measurement of IgE antibodies

IgE antibodies to the whole latex allergens were determined by the CAP system using commercial solid-phase latex allergen (latex ImmunoCAP) from Pharmacia Biotech according to the manufacturer's instructions. Specific IgE antibodies against Hev b 1 were detected by EAST as described previously[14] with the use of paper discs coupled with HPLC-purified Hev b 1 protein (coupling concentration of 2 mg protein/ml). A Phadezym RAST kit (Phar-

macia) was used to estimate the specific IgE values. It should be noted that the measuring range of IgE antibodies in serum samples differs by these two methods. According to the manufacturer, the ImmunoCAP used as allergen carrier by the CAP system has a higher binding capacity than a corresponding paper disc. In addition, the CAP system utilizes IgE (Fc)–specific combinations of polyclonal and monoclonal anti-IgE antibodies that are labeled with β-galactosidase–generating fluorescence, and this reagent shows higher immunoactivity than that used in the Phadezym kit for EAST. Thus, the lower and upper cutoff points by the CAP system are 0.35 and 100 kU/L, while the measuring range by EAST is 0.35 to 17.5 kU/L. Serum samples with IgE concentration ≥0.35 kU/L were regarded as positive, as recommended by Pharmacia. All positive samples were assayed a second time to confirm the results. In addition, the samples with anti–Hev b 1 IgE results between 0.35 and 0.7 kU/L as determined by EAST were examined by an autoinhibition assay to verify the binding specificity. The results were then regarded as positive if the IgE binding was able to be completely inhibited by Hev b 1 solution.

Inhibition enzyme immunoassays

The relative ability of Hev b 1 protein to interact with IgE antibodies in patient serum samples was determined by competitive inhibition in modified CAP assays as follows: A 10 μl antigen solution at a concentration of 1 mg/ml in dilution buffer (phosphate-buffered saline containing 1% bovine serum albumin) was mixed with 50 μl patient serum. The antigen-antibody mixes were allowed to equilibrate overnight at 4° C. The mixes were then individually transferred into wells with solid-phase allergen latex ImmunoCAP, and the IgE antibodies binding to latex were determined by the CAP system as described above. As a control, the IgE concentration in the serum samples was predetermined with the same process by mixing 50 μl patient serum with 10 μl dilution buffer. To obtain analogous conditions, the serum samples with a specific IgE concentration greater than 15 kU/L were prediluted to give a final IgE concentration of about 5 to 10 kU/L. Results are expressed as the percent inhibition of the reaction of IgE in the absence of inhibitor.

SPTs

SPTs of patients with the use of purified Hev b 1 was approved by the Institutional Human Investigation Committee, University of Bochum and was completed with the consent of the parents of patients with SB. The purified Hev b 1 was dissolved in sterile saline solution containing 0.4% phenol (diluent) to give three final concentrations of 10, 50, and 100 μg/ml. For comparison, a latex solution containing total latex proteins (100 μg/ml) extracted from latex sap was used. A negative control solution (diluent) and a positive control solution (10% histamine) were applied to all patients. To avoid an unexpected overreaction, the subjects with positive IgE antibodies to latex or symptoms after exposure to latex products first underwent SPT with the Hev b 1 solution at the lower concentration. If no distinct reaction was observed, then the SPT was applied with more concentrated solutions. Skin reactions were read after 15 minutes and were considered positive when the mean wheal diameter was 3 mm or greater surrounded by an erythema and was at least half the size of the reaction to histamine as well.

Electrophoresis and immunoblotting

One-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblot analysis of the protein extracts from latex gloves were performed with the use of a 16% polyacrylamide gel under reducing conditions as described before.[17] The murine mAbs II4G9 and II4F9 (raised against Hev b 1) and alkaline phosphatase–conjugated goat anti-mouse IgG (Sigma Chemical Co., St. Louis, Mo.) were used to detect Hev b 1 protein in the glove extracts. A serum pool from five patients with SB and latex allergy and alkaline phosphatase–coupled goat anti-human IgE (ε-chain specific) antibodies (Sigma Chemical Co.) were used to detect the IgE binding activity to proteins in glove extracts.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) with immobilized pH gradients followed by immunoblotting with serum samples of patients allergic to latex or mAbs was performed as described previously.[15]

Deoxyribonucleic acid typing of human leukocyte antigen–D region genes DRB1, DRB3, DRB4, DRB5, and DQB1

To investigate the relationship between HLA class II alleles and the specific immune response to Hev b 1 in patients with SB and latex allergy, 30 blood samples from patients with SB who had specific IgE antibodies to Hev b 1 were studied by DNA typing of HLA-D genes DRB1, DRB3, DRB4, DRB5, and DQB1. DNA was extracted from the white blood cells of each subject by use of the DNA Easy-Prep kit (Lifecodes, Belgium). HLA-specific amplification and the determination of HLA class II phenotypes (DRB1, DRB3, DRB4, DRB5, and DQB1) were performed by polymerase chain reaction/sequence-specific oligonucleotide probe analysis, as described previously.[18] For comparison, blood samples from 30 patients with SB who were not allergic to latex were also studied. In addition, blood samples from 90 healthy subjects who showed no allergic responses by SPT with 20 standard allergens were used as controls. All subjects (patients and controls) are from the western part of Germany. This study was approved by the Committee on Medical Ethics of the Ruhr-University Bochum.

Statistical analysis

Statistical analysis was performed with the use of Fisher's exact test (two-sided) with a StatXact program (Cytel Software, Cambridge, Mass.) in all cases. With the determination of HLA class II phenotypes, the corrected p value (p_c) was calculated by multiplying the p value with the number of the alleles tested for each locus. The correction factor for DRB1 was 26, which corresponds to the 13 broad DRB1 antigens studied and the division into two subgroups (Hev b 1-positive and Hev b 1-negative). Similarly, the correction factors for other phenotypes were: DRB3 = 6, DRB4 = 2, DRB5 = 2, and DQB1 = 30.

RESULTS

Identification of Hev b 1 protein in gloves

To identify whether the Hev b 1 protein is also involved in the latex gloves and can be released by extraction with aqueous solution containing detergent, four brands of surgical and one brand of examination gloves regularly used in our medicine department were used for extraction of aqueous soluble proteins using 1% SDS solution. Protein analysis revealed that the soluble protein content in the different gloves ranged from 331 to 1033 μg protein per gram rubber. After dialysis and lyophilization, the protein extracts were analyzed by one-dimensional SDS-PAGE. As shown in Fig. 1, *A*,

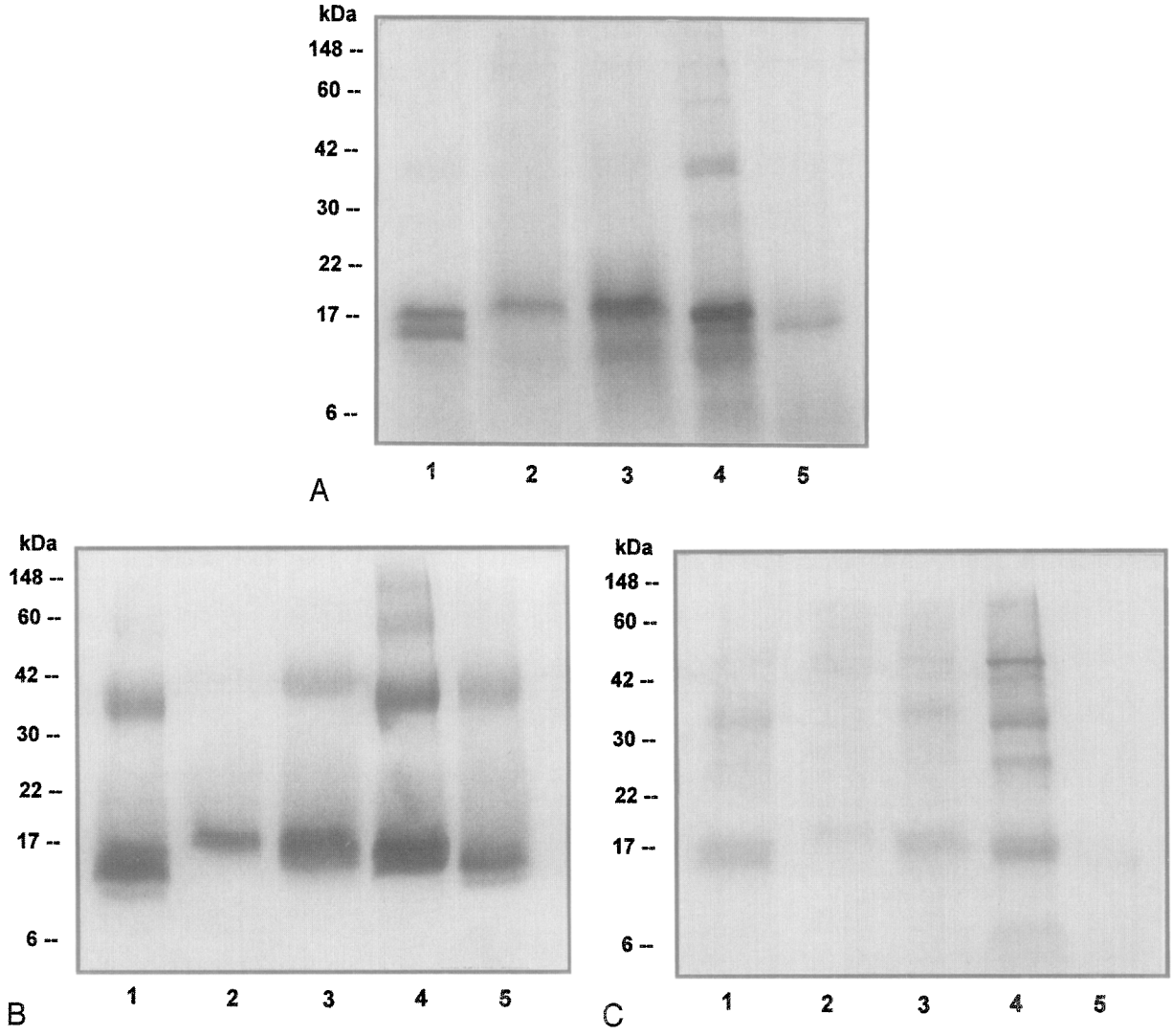

FIG. 1. SDS-PAGE analysis and immunoblot results of protein extracts from latex gloves. **A**, SDS-PAGE analysis of protein extracts (10 μg protein each) from four brands of surgical (lanes 1 to 3 and 5) and one brand of examination (lane 4) gloves by use of a 16% gel and Coomassie brilliant blue staining. **B**, Immunostaining of the glove extracts by the use of mAb II4G9 directed against Hev b 1 residues 122 to 134. **C**, Immunostaining of the glove extracts using a serum pool from five patients with SB and latex allergy with an IgE value of 13.3 kU/L against Hev b 1.

most of the soluble proteins from the latex gloves appeared in the 15 kd area. In samples with nos. 1 and 4, protein bands with molecular weights of 40 kd or more could be also observed. Results of immunoblot analysis with mAb II4G9 (Fig. 1, *B*) revealed that at least three bands in the extracts from three surgical gloves (lanes 1, 3, and 5) and up to six bands in the extract of the examination glove (lane 4) were recognized by the mAb. Only one sample extract of surgical glove (lane 2) showed a single band with the mAb reactivity.

Monoclonal antibody II4G9 is specifically recognized by Hev b 1 protein with the C terminal residues of 122 to 134[14] and showed no cross-reaction to any proteins in both soluble and particle-bound protein fractions of NRL by immunoblotting as described previously.[15] Thus, it can be concluded that the protein bands with molecular weight other than 14 kd on SDS-PAGE represent the aggregate products with Hev b 1 fragment, which probably happened during production of the gloves. Immunoblotting with the second mAb II4F9 gave a identical binding profile, except that additional smeared bands appeared in the 8 to 10 kd area (data not shown). This indicates the presence of the Hev b 1 fragments by loss of some C terminal amino acids, because the mAb II4F9 binds specifically to the Hev b 1 residues 46 to 54. Immunoblotting with the use of IgE antibodies in a serum pool from five patients with SB who were allergic to latex with an IgE value of 13.30 kU/L against Hev b 1 showed a similar reaction pattern (Fig. 1, *C*). The negative control of unrelated mAbs and

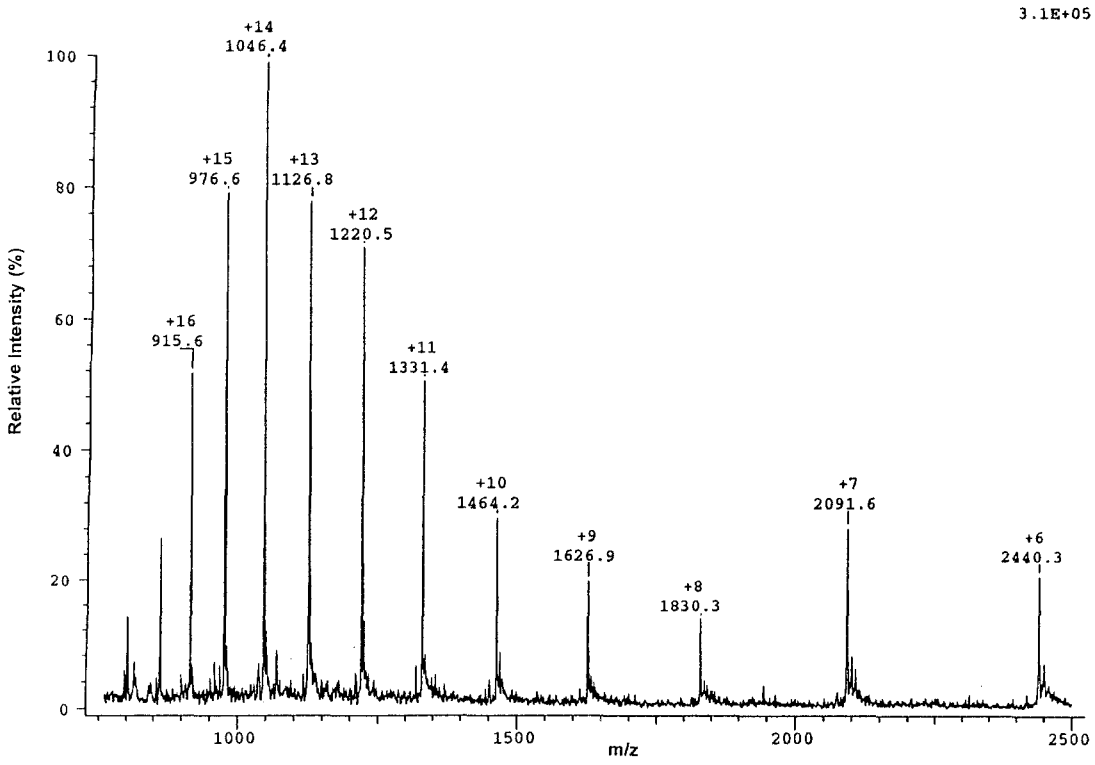

FIG. 2. Mass analysis of HPLC-purified Hev b 1 by ES-MS. The mass spectroscopy spectrum of Hev b 1 protein released from rubber particles by SDS treatment and purified by reverse-phase HPLC revealed a series of multiply charged protein ions. Deconvolution of these protein ions indicated the presence of a single species with a mass of 14634.0 Da, with a S.D. of 1.1 Da, which is consistent with the calculated mass of Hev b 1 in the SWISS-PROT database (14,632 Da). Further examination of this data set (results not shown) did not identify any other components.

latex-negative serum pool showed no binding reactivity of proteins in the glove extracts (data not shown).

Isolation and characterization of Hev b 1 protein in latex

As already shown in our previous work,[15] 2D-PAGE analysis of the crude extract obtained by extraction of rubber particles by SDS solution showed that the particle-bound proteins consisted primarily of two groups of proteins appearing in the 14 and 24 kd areas with an isoelectric point value of about 4.7 and 5.1, respectively. Immunoblotting analysis of the particle-bound proteins with mAbs II4G9 and II4F9 revealed that only proteins in the 14 kd area were recognized by the mAbs, indicating that they were proteins with Hev b 1 determinants (data not shown). After elution through a column containing detergent adsorber gel to remove the residual SDS, the crude protein extract was separated by preparative reverse-phase HPLC. The collected HPLC fractions containing Hev b 1 protein were pooled and further characterized by 2D-PAGE, HPLC, and electrospray mass spectrometry (ES-MS). As shown in Fig. 2, analysis of HPLC-purified Hev b 1 by ES-MS gave a single species with a molecular mass of 14,634.0 Da with a system error of 1.1 Da, which is in good agreement with the theoretical mass of Hev b 1 in the SWISS-PROT database (14,632 Da). No contaminant protein could be detected. Analysis of the purified protein by analytical HPLC on a C-4 reverse-phase column also revealed a single peak (data not shown). All these results indicate that the purification protocol produced a highly purified product. One hundred milliliters of NRL yielded about 11 mg of Hev b 1 protein.

Hev b 1-specific IgE antibodies in patient sera

Of 140 serum samples from patients with SB studied, 69 (49.3%) had increased levels of specific IgE antibodies to latex tested according to the CAP system. By EAST with the use of Hev b 1 protein-coupled discs as target allergen, 56 (81%) of the 69 serum samples from patients with a positive latex reaction also showed IgE antibodies directed to purified Hev b 1 protein. Of 71 latex-negative serum samples, three had borderline values of IgE binding to Hev b 1 (0.36 to 0.42 kU/L). The occurrence of IgE antibodies to latex as determined by CAP and to Hev b 1 by EAST is shown in Fig. 3.

In HCWs allergic to latex, the rate of seropositivity for anti–Hev b 1 IgE antibodies was significantly lower compared with that in patients with SB. As determined by EAST, 55 of 105 HCW patients had an elevated Hev

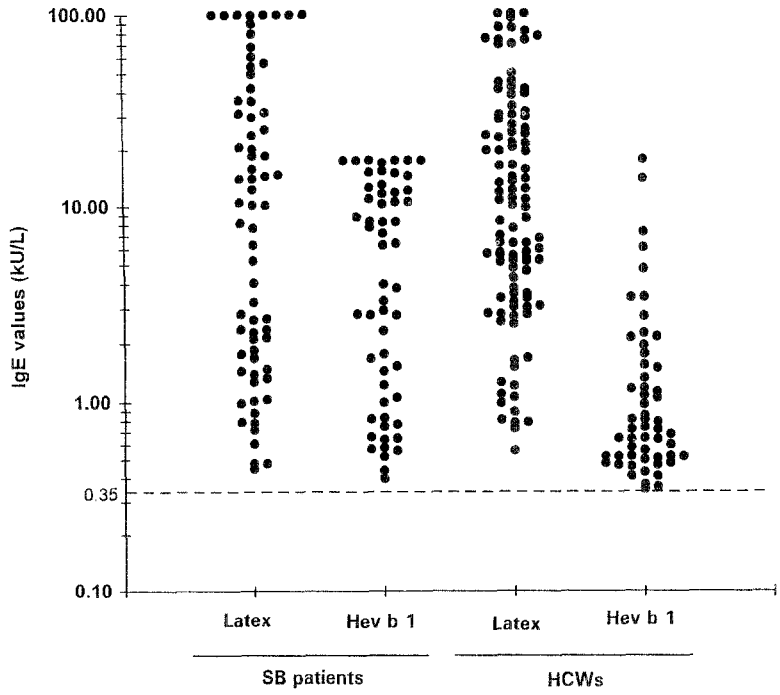

FIG. 3. Occurrence of IgE antibodies binding to latex and purified Hev b 1 in serum samples from patients with SB and HCWs allergic to latex. The IgE values to latex allergens were determined by the CAP system; values less than 0.35 kU/L were regarded as negative and in both cases are not indicated. All the latex-positive serum samples were tested with purified Hev b 1; 81% (56 of 69) of the samples from patients with SB and 52% (55 of 105) of those from HCWs showed positive IgE Ab to Hev b 1.

b 1-specific IgE value in their sera (52.3% vs 81%, $p < 0.0001$). In addition, most of them had only low IgE levels to Hev b 1 (Fig. 3). Thus, we found that 29 of the 55 HCWs (52.7%) had a Hev b 1-specific IgE value between 0.35 and 0.7 kU/L and 11 (19.6%) had a Hev b 1-specific IgE value greater than 2 kU/L. In the patients with SB, the concentrations of anti–Hev b 1 IgE Ab in 45 (80.3%) of the 56 serum samples were greater than 0.70 kU/L ($p = 0.0004$), and 36 (64%) had an anti–Hev b 1 IgE value greater than 2 kU/L ($p < 0.0001$).

In the 18 serum samples from HCWs who underwent SPT, nine (50%) serum samples had an increased specific IgE value to Hev b 1, even though 17 patients showed a positive SPT to the Hev b 1 solution (see below).

Immune inhibition assay

As already mentioned in the Methods section, due to the different binding capacity of two types of allergens used in the CAP and EAST tests, the absolute IgE concentrations measured by the two methods cannot be compared directly. Therefore, to show to what extent the IgE concentration determined by the CAP system contributed to the binding with the Hev b 1 protein in the patients with SB, 33 serum samples with strongly positive IgE antibodies (>1 kU/L) to Hev b 1 were randomly selected for a RAST inhibition assay in the CAP system. Hev b 1 solution (0.35 mg/ml) was used as an inhibitor and the patient's serum sample was incubated with Hev b 1 solution (10 μl) before the reaction with solid-phase latex allergens bound to ImmunoCAP. The inhibition results shown in Table I indicate that in 15 serum samples (45.4%), the IgE antibodies binding to latex could be completely inhibited by Hev b 1. This suggests that the sensitization to latex in these 15 patients was caused exclusively by IgE binding epitopes present in the Hev b 1 protein. Similarly, IgE binding to latex in the

TABLE I. The ability of purified Hev b 1 to inhibit binding of IgE antibodies to latex in 33 serum samples from patients with SB who had different IgE concentrations

	Samples with IgE levels to latex (CAP class)*					
	II	III	IV	V	VI	Total
Inhibiton (%)						
100	6	6	2	1	0	15
75-95	0	1	6	0	1	8
52-74	0	0	0	0	4	4
<50	1	1	1	2	1	6
Total	7	8	9	3	6	33

Inhibition assays were performed by CAP with latex ImmunoCAP as target antigen and Hev b 1 as inhibitor.

* Criteria for the CAP classes: I, 0.35 to 0.7 kU/L; II, 0.7 to 3.5 kU/L; III, 3.5 to 17.5 kU/L; IV, 17.5 to 50 kU/L; V, 50 to 100 kU/L; VI, >100 kU/L.

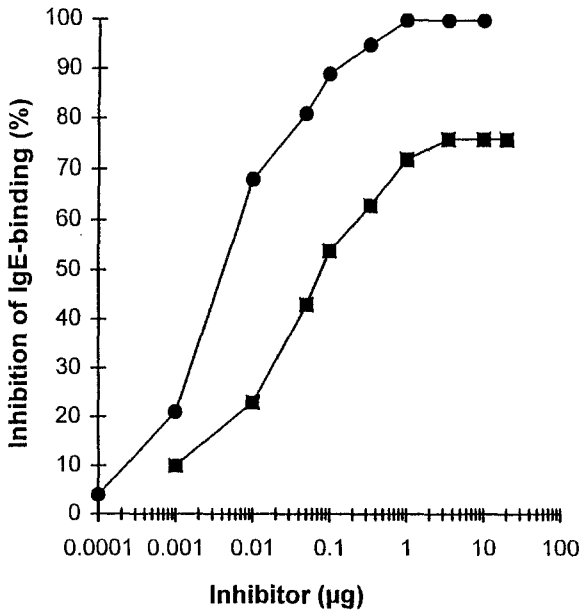

FIG. 4. Dose-related inhibition assay performed to confirm binding specificity of serum IgE antibodies to solid-phase Hev b 1 in EAST (●) and inhibition of IgE binding to solid-phase latex allergens in CAP system (■) by different concentrations of purified Hev b 1 (inhibitor) in a serum pool.

other serum samples could be considerably inhibited (52% to 95%) by preincubation with Hev b 1 solution. Maximum inhibition below 50% by Hev b 1 was found only in six (18%) cases. In the HCW group, 10 serum samples were randomly tested in the RAST inhibition assay. In none of them could the IgE antibodies to latex be completely inhibited by preincubation of serum with Hev b 1 solution.

An autoinhibition assay was also performed to assess the binding specificity of IgE antibodies to Hev b 1. The serum pool from five patients with SB and latex allergy was used in this test. As shown in Fig. 4, the inhibition was dose dependent, and the IgE binding to Hev b 1 was completely inhibited by 1 μg of Hev b 1; in fact, 50% inhibition was achieved by less than 10 ng of Hev b 1 protein. In addition, by using the same serum pool as the IgE source, up to 75% of the IgE binding to latex allergens (measured by the CAP system) could be inhibited by preincubating the serum sample with 3.5 μg Hev b 1 (Fig. 4). Increasing the amount of inhibitor up to 20 μg resulted in no additional inhibition of IgE binding.

Skin prick testing using Hev b 1

To study the allergic reactions caused by Hev b 1 in vivo, 14 patients with SB and 18 HCWs, who had previously shown positive latex SPT responses and exhibited specific IgE antibodies to latex, underwent SPT with purified Hev b 1 protein. Results showed that 10 of 14 patients with SB had strongly positive SPT reactions to Hev b 1 solution, 8 of whom had these responses with diluted Hev b 1 solution (10 μg/ml), 2 with the solution of 50 μg/ml. Specific IgE antibodies binding to Hev b 1 could be detected in 9 of these 10 patients with skin reactions to Hev b 1. In comparison, 15 patients with SB who had a negative latex skin response showed neither IgE antibody nor SPT response to Hev b 1.

In the HCW group, 16 of 18 subjects had a strong Hev b 1 SPT response. However, in 13 of these 16 patients, the skin responses were elicited by more concentrated Hev b 1 solution (100 μg/ml), and only 3 had a response with Hev b 1 solution at a concentration of 10 μg/ml. Specific IgE antibodies binding to Hev b 1 were found in serum samples of eight patients with positive SPT responses to Hev b 1 solution. The concentration of the IgE antibodies to Hev b 1 ranged from 0.36 to 6.06 kU/L

Association between human leukocyte antigen and allergy to Hev b 1 in patients with spina bifida

To elucidate the possible pathogenic factors involved in the sensitization to Hev b 1 protein in patients with SB and latex allergy, blood samples from 30 randomly selected German patients with SB who had IgE antibodies to Hev b 1 were examined for HLA class II phenotypes (DRB1, DRB3, DRB4, DRB5, and DQB1) by DNA typing with polymerase chain reaction/sequence-specific oligonucleotide probe analysis. The frequencies of HLA class II alleles in the 30 patients were compared with findings in 30 patients with SB without IgE antibodies to Hev b 1 and in 90 healthy unrelated controls. According to statistical analysis, only the phenotype frequencies of DRB1*0701 (DR7) and DQB1*0201 (DQ2) in the patients with IgE antibodies to Hev b 1 were slightly elevated when compared with the patients without IgE antibodies to Hev b 1 (DR7: 40% vs 7%, $p < 0.005$, p_c = not significant; DQ2: 63% vs 27%, $p < 0.01$, p_c = not significant) or with the healthy control (DR7: 17%, $p < 0.02$, p_c = not significant; DQ2: 34%, $p < 0.006$, p_c = not significant). No significant difference in frequencies of HLA DRB1, DRB3, DRB4, DRB5, and DQB1 alleles between patients with Hev b 1 sensitization and control subjects was observed.

DISCUSSION

The current study was performed to verify the allergenicity of Hev b 1 as a major allergen in patients allergic to latex. The major strengths of this study are the large number of serum samples drawn from the subjects and the isolation of a great amount of highly purified Hev b 1 protein, which enabled us to perform objective measurements of anti–Hev b 1 IgE antibodies by use of a well-established in vitro test method (EAST) and to evaluate the allergenicity of Hev b 1 in vivo by SPT. Results can be summarized as follows.

First, greater than 80% of patients with SB and latex allergy (56 of 69) were seropositive for IgE antibodies against purified Hev b 1 as determined by EAST. The Hev b 1 protein also elicited positive skin responses in 10 of 14 (71%) patients with SB who showed skin reactions

to latex. These results strongly suggest that the Hev b 1 protein in NRL is the most important allergen responsible for the latex allergy in patients with SB.

Second, the binding of the IgE antibodies to latex proteins in patients with SB is largely attributed to the binding with Hev b 1. In many cases, Hev b 1 is also the monosensitizer in patients with SB who have latex allergy. This conclusion is supported by the finding that the purified Hev b 1 completely inhibited the binding of the IgE antibodies to latex in 15 of 33 serum samples (45.4%) from patients with SB and latex allergy, and in the other 12 serum samples (36.4%), the inhibition of the IgE binding ranged from 52% to 95%. Only in six serum samples (18.2%) was the inhibition of IgE binding to latex less than 50%.

Finally, the rate of sensitization and degree of the IgE antibody response to Hev b 1 differ significantly between latex-allergic patients with SB and HCWs. Among 105 serum samples of HCWs allergic to latex investigated, 55 (52%) had elevated anti–Hev b 1 IgE antibodies. Their IgE level in sera was statistically lower when compared with the patients with SB: in 29 (52%) of these 55 serum samples, the concentration of anti–Hev b 1 IgE antibodies ranged from 0.36 to 0.7 kU/L (SB group: 19.6%, $p = 0.0004$); only 11 (20%) of them showed anti–Hev b 1 IgE antibodies greater than 2 kU/L (SB group: 64%, $p < 0.0001$). However, a corresponding prevalence of positive SPT responses to Hev b 1 was observed among HCWs allergic to latex compared with patients with SB and latex allergy (88.8% vs 71.4%). Nevertheless, the fact that more than 50% of HCWs allergic to latex were seropositive for anti–Hev b 1 IgE antibodies clearly indicates that the Hev b 1 protein is a significant latex allergen for patients with occupational exposure to latex.

As has been already discussed by Alenius et al.,[11] different immunoassays with varying sensitivities used in the literature may be the critical reason giving rise to controversial results. Moreover, the different collection of patients studied and the usage of allergen materials with different degrees of purity may also play a role in getting distinct results. Recently, other proteins in latex (especially the minor protein of 23 to 25 kd [Hev b 3] located in latex particle and with sequence homology to Hev b 1) were found to be important allergens for patients with SB.[19-21]

The extremely high prevalence of Hev b 1 sensitivity in patients with SB and latex allergy may be associated with different factors. Because the exposure to latex in the patients with SB is mainly due to the contact with latex gloves worn by personnel during surgical procedures, the Hev b 1 content in gloves is likely to correlate with IgE responsiveness in patients with SB. By immunoblotting with mAbs raised against Hev b 1, we demonstrate for the first time that Hev b 1 is not only the predominant component in the protein extracts of latex gloves, but it can also appear in different molecular weights of about 8, 14, 30, or 42 kd or higher. These results indicate that the Hev b 1 molecule can be cleaved into small fragments or form high-molecular-weight aggregates during the manufacturing process. These results confirm those of Czuppon et al.,[10] who identified a 58 kd protein from a latex glove as Hev b 1, and also the findings of Todt et al.,[22] who suggested that the different latex protein bands in SDS-PAGE (12.7 to 48 kd) may all be derived from the Hev b 1 molecule, based on the results of amino acid analysis of these protein bands. The high Hev b 1 content in the latex gloves suggests that patients with SB may be more intensely exposed to Hev b 1 than to other latex proteins.

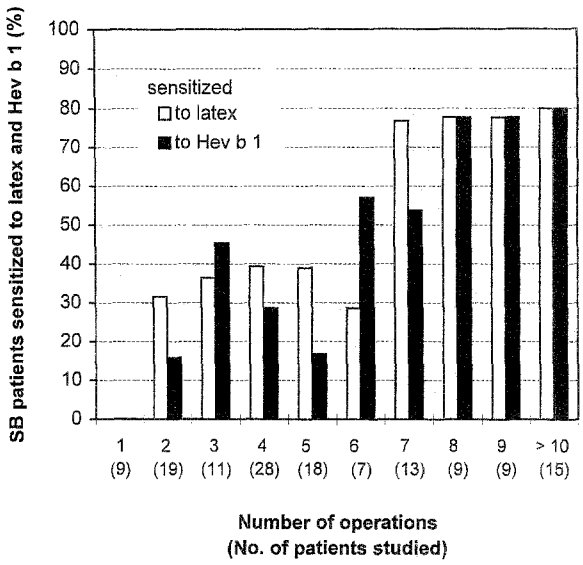

FIG. 5. Relationship between the number of operations and the prevalence of anti-latex and anti–Hev b 1 IgE antibodies in 138 patients with SB.

The frequency of surgical procedures for patients SB is likely to play an important role for the IgE response to latex and Hev b 1. During surgery, patients are not only exposed to latex gloves worn by multiple personnel, but also latex proteins may be released from some devices used during anesthesia and surgical incision, such as latex injection ports in intravenous lines, tubing, and bags; latex balloon-tipped barium enema catheters; and the latex plungers on syringes used to administer medication intravenously.[7] We have studied the relationship between the number of operations and the rate of IgE response to latex and Hev b 1 in 138 patients with SB. The results shown in Fig. 5 revealed that none of the nine subjects with one operation had positive IgE antibodies against latex or Hev b 1. In patients with SB for whom the number of operations ranged from two to six ($n = 83$), the rate of IgE responses was 28.6% to 39.3% to latex and 15.8% to 57.1% to Hev b 1, respectively. Patients undergoing 7 or more operations showed a quite similar prevalence of latex sensitivity of about 80%. Furthermore, all patients with eight or more operations who had a positive IgE response to latex had Hev b 1-specific IgE antibodies. These results not only clearly demonstrate that the number of operations in

patients with SB is related with the IgE response to latex and Hev b 1, they also confirm and extend the most recent findings by Porri et al.,[23] who described that the number of surgical procedures rather than SB per se is related to sensitization to latex.

The early exposure to latex may be an another reason patients with SB preferentially to produce IgE antibodies to Hev b 1. Surgery to close the back of patients with SB is generally performed within 24 hours after birth. Therefore, these patients have contact with latex soon after birth during the operation. Although information about age of exposure to allergen for sensitization in humans is still limited, studies by Konz et al.[24] revealed that patients with spinal cord injury (a condition accompanied by multiple operations) were at far lower risk of developing latex allergy. Compared with the population of patients with SB, the patients with spinal cord injury have statistically comparable numbers of operations and a similar route of exposure to latex; the main difference is that the contact with latex began later in life in the group with spinal cord injury. This result suggests that the age of exposure to latex allergen is likely to play a critical role in developing IgE antibodies to Hev b 1 in patients with SB.

The genetic propensity to develop an immediate-type immune response has been thought to be responsible for induction of the allergic state.[25] More recently, we demonstrated that the IgE responsiveness to hevein, a major latex allergen with a molecular weight of 4.7 kd in HCWs, is strongly associated with HLA alleles DR4 and DQ8 in subjects with latex allergy.[18] The high rate of IgE response to Hev b 1 in the patients with SB thus provides an excellent model for the molecular genetic studies. However, our present results obtained by DNA typing of HLA-D genes in a group of 60 patients with SB, 30 of whom had Hev b 1-specific IgE antibodies, suggest that there is no statistically significant correlation between HLA alleles tested and IgE responsiveness to Hev b 1.

In conclusion, our data suggest that the Hev b 1 protein is the most important allergen in latex for patients with SB. Although other latex allergens were also found in protein extracts of latex gloves, many patients with SB and latex allergy are apparently sensitized only to Hev b 1. Different factors may be involved in the pathogenesis of allergy to Hev b 1 in patients with SB. The high content of Hev b 1 in glove extracts and the exposure to latex in early life are likely to be the primary causes of sensitization to Hev b 1 protein. Additionally, as shown in our SDS-PAGE analysis of the glove extracts, Hev b 1 protein is able to form aggregates by binding either with itself or with other proteins or rubber polymers. Due to an adjuvant effect, the mucosal absorption of these "complexed Hev b 1 particulates" is thus suspected to be very active in stimulating the IgE Ab response. Further studies are required to clarify why patients with SB preferentially produce Hev b 1-specific IgE antibodies after exposure to latex.

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