



Unique and shared IgE epitopes of Hev b 1 and Hev b 3 in latex allergy

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Abstract

Of the several latex proteins cloned and expressed, the rubber elongation factor, Hev b 1, and the closely related Hev b 3, represent two major allergens associated with latex allergy. Although both allergens demonstrated IgE binding with sera from latex allergic patients, it was not known whether these two molecules shared any epitopes. Hence, in the present study using health care workers (HCW) and spina bifida (SB) patients with latex allergy, we investigated the IgE binding epitopes in Hev b 1 and Hev b 3. Recombinant Hev b 1 and Hev b 3 were expressed in a prokaryotic expression system, while overlapping decapeptides of Hev b 1 and Hev b 3 were synthesized on derivatized cellulose membrane. Eight IgE binding epitopes for Hev b 1 and eleven for Hev b 3 were identified using sera from latex allergic patients with SB. On further analysis of synthetic peptides encompassing these epitopes, similar IgE antibody reactivity was demonstrated with three Hev b 1 epitopes b1E3, b1E5, b1E6 and two Hev b 3 epitopes; b3E10 and b3E11. For Hev b 1, a unique IgE binding epitope was identified in the region of amino acid residues 16–25. In competitive ELISA, peptides b1E2 and b1E4 together inhibited 58% of IgE binding of Hev b 1, while b3E5 showed 22% inhibition in the IgE binding of Hev b 3. The results of the present study suggest that the understanding of linear and conformational IgE epitopes in the major latex allergens may provide better insight into the structure–function relationship of the allergens, and may lead to the development of better patient care and management strategies in latex allergy. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Natural rubber latex (NRL) is widely used in the manufacturing of medical devices and a number of domestic and industrial utility articles. Since the first clinical report of latex allergy, the IgE mediated type 1 allergy to NRL proteins has become an important medical and occupational problem (Nutter, 1979; Kelly et al., 1994; Kurup et al., 1995; Turjanmaa et al., 1996). Severe allergic reactions are frequently reported to occur perioperatively in children with spina bifida (SB). Recent studies have shown that as many as 8% of health care workers (HCW) and 28–67% of SB patients are sensitized to latex with an anaphylactic risk of over

500-fold compared to the general populations (Kelly et al., 1994; Slater, 1994; Turjanmaa et al., 1996).

Latex hypersensitivity reactions occur shortly after exposure to the allergen. The specific IgE antibodies bound to the high affinity FcεRI receptors on mast cells interact with allergens (through IgE binding epitopes) resulting in activation of mast cells and release of various pharmacological mediators (Abbas et al., 1997). These mediators, such as histamine, leukotrienes and prostaglandins could be responsible for the manifestation of the clinical symptoms of latex allergy (Breiteneder and Scheiner, 1998). Thus, the IgE binding epitopes of major latex allergens may have an important role in the immune mechanism of latex hypersensitivity (Chen et al., 1996). Some of these epitopes may also have potential possibilities in controlling the disease by immunotherapy (Kraft et al., 1998; Posch et al.,

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1998; Breiteneder and Scheiner, 1998). However, at the present time, our understanding of the structure–function relationship of the major latex allergens is not complete, although recent studies using overlapping synthetic peptides have provided information on the major B-cell epitopes for Hev b 1, Hev b 5 and Hev b 6 (Chen et al., 1996; Banerjee et al., 1997; Beezhold et al., 1997; Slater et al., 1999).

Hev b 1, identified as a 14.6 kD water insoluble protein, is associated mainly with large rubber particles (> 350 nm diameter) and is involved in rubber biosynthesis (Czuppon et al., 1993). On the other hand, Hev b 3, isolated from small rubber particles (> 70 nm), is highly unstable and frequently degrades into several polypeptides of lower molecular weight (from 24 to about 5 kD) even when stored at -20°C (Yeang et al., 1996). Previous studies have emphasized the involvement of these two latex proteins in both humoral and cell mediated immune responses in latex sensitized patients (Alenius et al., 1995, 1996; Lu et al., 1995; Chen et al., 1997; Yeang et al., 1998; Wagner et al., 1999; Johnson et al., 2000).

In view of the prevalence and severity of latex hypersensitivity reactions in both children and adults and involvement of B cell epitopes in the clinical manifestation of the disease, we focused our attention on defining the specific structure–function relationship of Hev b 1 and Hev b 3. Recombinant allergens were overexpressed and their structural and functional similarities were compared. Overlapping peptides were synthesized on derivatized cellulose membranes and the IgE binding epitopes of Hev b 1 and Hev b 3 were studied using sera from patients with SB. The identified SB serum reacting IgE binding epitopes were further evaluated using sera from HCW. The results indicate that the common IgE binding epitopes are located near the C-terminal regions of Hev b 3, while for Hev b 1 the specific epitope binding to SB patients were seen at the amino acid residues 16–25. Several shared and unique epitopes in Hev b 1 and Hev b 3 were identified and such information may be of value in the reliable diagnosis of latex hypersensitivity and in understanding the pathophysiology of the disease.

2. Materials and methods

2.1. Subjects

Serum samples from 20 subjects with latex allergy, ten HCW and ten SB patients, were used for the study. All of the latex allergic subjects were diagnosed by clinical history, immediate wheal and flare skin test reactivity to a latex glove extract, or to an allergen extract prepared from natural rubber latex (NRL) (Lu et al., 1995). A control group of ten subjects with no

clinical evidence of latex allergy or reactivity to Hev b 1 and Hev b 3 from HCW and SB groups were also included in the study.

2.2. Preparation of recombinant Hev b 1 and Hev b 3

Genes encoding Hev b 1 and Hev b 3 were amplified by PCR. The template DNA used was the latex cDNA library as previously described (Banerjee et al., 1997). Primers used for the amplification of full length Hev b 1 were: sense 5'ATGGCTGAAGACGAAGACAACC 3' and antisense 5'ATTCTCTCCATAAAACACCTTAGC 3' and that for Hev b 3 were 5'ATGGCTGAAGAGGTGGAGGAAG3' and 5'TTATGATGCCTCATCTCCAAACA3', respectively. The sense and antisense primers for Hev b 1 had *Bam* H 1 and *Xho* 1 at their 5' ends and Hev b 3 primers had *Bam* H 1 and *Hind* III restriction sites at their 5' ends respectively. The amplified PCR products were purified from gel and subcloned into PCR 2.1 vector (TA Cloning Kit; Invitrogen, San Diego, CA) and sequenced by the dideoxy-chain termination method (Sanger et al., 1977). The TA plasmid carrying the latex gene was digested with the appropriate restriction enzymes and ligated with similarly digested pET 23 b (+) vector (pET Expression System; Novagen, Madison, WI) and the cells expressing the recombinant latex allergens were processed and analyzed as previously described (Banerjee et al., 1999).

2.3. Purification of recombinant allergens

Recombinant Hev b 1 overexpressed in pET with six histidine tags at its C-terminal end was purified using a Ni^{2+} -nitrilotriacetic acid agarose column whereas rHev b 3 was electroeluted from preparative gel (Bio-Rad, Richmond, CA).

2.4. Cross-reactivity of Hev b 1 and Hev b 3 by inhibition ELISA

In order to study the cross reactivity between recombinant Hev b 1 and Hev b 3, an ELISA inhibition was carried out using sera from two HCW and two SB patients with latex allergy. Individual serum samples diluted 1: 10 were incubated with decreasing concentrations of 20 μg to 10 ng of Hev b 3. The preincubated sera were then added to the microtiter plates coated with Hev b 1. Similarly serum samples were preincubated with decreasing concentrations of Hev b 1 and added to Hev b 3 coated plates. Further steps in the assay were carried out as described earlier (Kurup et al., 1998).

2.5. Peptide synthesis on SPOTs membranes

Peptide synthesis was carried out on derivatized cellulose membrane using F-moc amino acids as specified by the manufacturer (Genosys Biotechnologies, Inc., The Woodlands, TX). Based on the published amino acid sequences of Hev b 1 and Hev b 3 (IUIS Allergen list <ftp://biobase.dk/pub/who-IUIS/allrgen.list>), decapeptides were synthesized at an offset of five amino acids encompassing the complete molecules. Twenty-six decapeptides for Hev b 1 and 39 for Hev b 3 were synthesized. Once the IgE binding regions of these allergens were identified, in order to detect the specific epitopes, decapeptides at an offset of one amino acid were synthesized. The selected synthetic peptides were then evaluated with sera from latex sensitized SB and HCW patients. All the identified epitopes of Hev b 1 and Hev b 3 were synthesized commercially with over 90% purity (Alpha Diagnostics, San Antonio, TX).

2.6. Antibody binding of the synthetic peptides

The cellulose membranes with synthetic peptides were blocked overnight before incubating with a 1:10 dilution of pooled serum from ten SB patients. Washing was carried out in between with 0.1% Tween 20 in Tris buffered saline (T-TBS). Biotinylated anti-human IgE (Vector Laboratories, Burlingame, CA) diluted 1:1000 in T-TBS was then added to the membranes and incubated for 1 h at room temperature. The membranes were then treated with streptavidin alkaline phosphatase at 1:10 000 dilution (Sigma, St. Louis, MO) for 45 min and washed as before. The color was developed by using nitroblue tetrazolium-5-bromo-4-chloro-3-indolylphosphate (Bio-Rad) as previously described (Banerjee et al., 1997). The intensities of the color reactions were analyzed by densitometric scanning of the SPOT membranes using an image acquisition and analysis instrument (AMBIS, Inc., San Diego, CA). Various peptides synthesized at an offset of one amino acid were similarly analyzed for their IgE antibody binding and the fine mapping of the antibody peptide interactions revealed eight IgE binding linear peptides for Hev b 1 and 11 for Hev b 3. These selected epitopes from Hev b 1 and Hev b 3 were further evaluated for IgE binding with sera from HCW using procedures similar to that described above.

2.7. Peptide specific antibody

An indirect ELISA was carried out using maleic anhydride activated polystyrene plates (Pierce, Rockford, IL). Peptides were dissolved in 0.1M phosphate, 0.15M NaCl, pH 7.2 at a concentration of 1 mg/ml. Wells were coated with 100 µl of peptide (12.5 µg/ml) and incubated at 37°C overnight. The strips were then

blocked with PBS containing 3% BSA and 0.05% Tween 20 for 1 h at 37°C. To study IgE antibody binding, sera diluted to 1:10 were added to the wells and incubated for 3 h at 37°C. The subsequent step included the addition of biotinylated anti-human IgE (1:500) to the respective wells and incubation for 1 h at 37°C. The enzyme conjugate streptavidin peroxidase was then added at a dilution of 1:1000 and incubated for 1 h at 37°C. The color development was carried out using the enzyme substrate *ortho*-phenylene diamine, as previously described (Banerjee et al., 1999).

2.8. Competitive ELISA for demonstrating cross-reactive epitopes in Hev b 1 and Hev b 3

To analyze the surface availability of linear peptides for antigen–antibody interaction, an inhibition ELISA was carried out using polystyrene plates coated with peptides b1E2 and b1E4 of Hev b 1 and b3E5 of Hev b 3. Wells of 96 well microtiter plates (Dynatech Laboratories, Chantilly, VA) were coated with recombinant Hev b 1 (5 µg/ml) and Hev b 3 (10 µg/ml) and incubated at 4°C overnight. Pooled serum samples from SB patients diluted 1:10 and mixed with peptides b1E2, b1E4 and b3E5 at various concentrations ranging from 500 ng to 50 µg were added to the wells of the second plate and incubated for another 2 h at room temperature. The reaction mixtures from the second plate were then transferred into the wells of the Hev b 1 and Hev b 3 coated plate and incubated for 2 h at room temperature. The binding of the free antibodies in preincubated sera to solid phase coated Hev b 1 and Hev b 3 were evaluated as described earlier (Banerjee et al., 1999).

3. Results

3.1. Recombinant Hev b 1 and Hev b 3 share similar functional properties

Purified recombinant Hev b 1 and Hev b 3 overexpressed in pET system demonstrated proteins at the molecular weight regions of 14–15 kD and 23–25 kD respectively (Fig. 1). In ELISA, both proteins showed distinct IgE and IgG binding with sera from latex allergy patients with SB and HCW (data not shown). The competitive ELISA studies using individual sera from SB and HCW groups are shown in Fig. 2A and B. Three out of four sera preincubated with rHev b 3 at a concentration of 20 µg could inhibit more than 80% IgE binding reactivity of the solid phase coated Hev b 1. A dose dependent decrease in inhibition of IgE binding to Hev b 1 was observed with sera preincubated with a decreasing concentration of rHev b 3 from 20 µg to 10 ng and vice versa. No inhibition of the Hev

b 1 and Hev b 3 binding to the latex specific IgE was detected with an irrelevant allergen Asp f 2 from *Aspergillus fumigatus*.

3.2. Multiple continuous IgE binding regions are present in Hev b 1 and Hev b 3

In order to define IgE antibody binding regions of these allergens, 26 decapeptides at an offset of five amino acids for Hev b 1 and 39 for Hev b 3 were synthesized. Based on the densitometric scanning of the SPOT membranes, IgE binding regions of Hev b 1 were identified in the amino acid residues 1–55, 60–75 and 85–110 and the Hev b 3 antigenic regions were identified in the amino acid residues 1–70, 85–127 and 138–188 (data not shown).

The specificity of the IgE peptide interaction was further defined by synthesizing peptides at an offset of one amino acid from the selected regions of Hev b 1 and Hev b 3. Densitometric analysis of the reactions of synthetic peptides of Hev b 1 and Hev b 3 with IgE are shown in Fig. 3A and B. These results demonstrate six immunodominant IgE epitope regions in the amino acid residues 1–75 and two others near the C-terminal region from amino acid residues 90–108 for Hev b 1. Similarly, eleven IgE epitope regions have been identified along the entire length of Hev b 3. The identified IgE epitope regions of Hev b 1 and Hev b 3 and their sequence comparison are shown in Table 1. The amino acid sequence homology exhibited conserved sequences in Hev b 1 and Hev b 3 at the residues from amino acid 1–114. As shown in Table 1 the first four selected peptides (E1–E4) near the N-terminal regions of Hev b 1 and Hev b 3 exhibited highly conserved amino acid sequences.

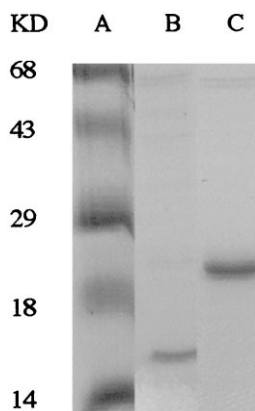


Fig. 1. Purified recombinant Hev b 1 and Hev b 3 (10 µg/lane) were analyzed by 12% SDS-PAGE. Lane A: molecular weight markers; Lane B: Hev b 1; Lane C: Hev b 3.

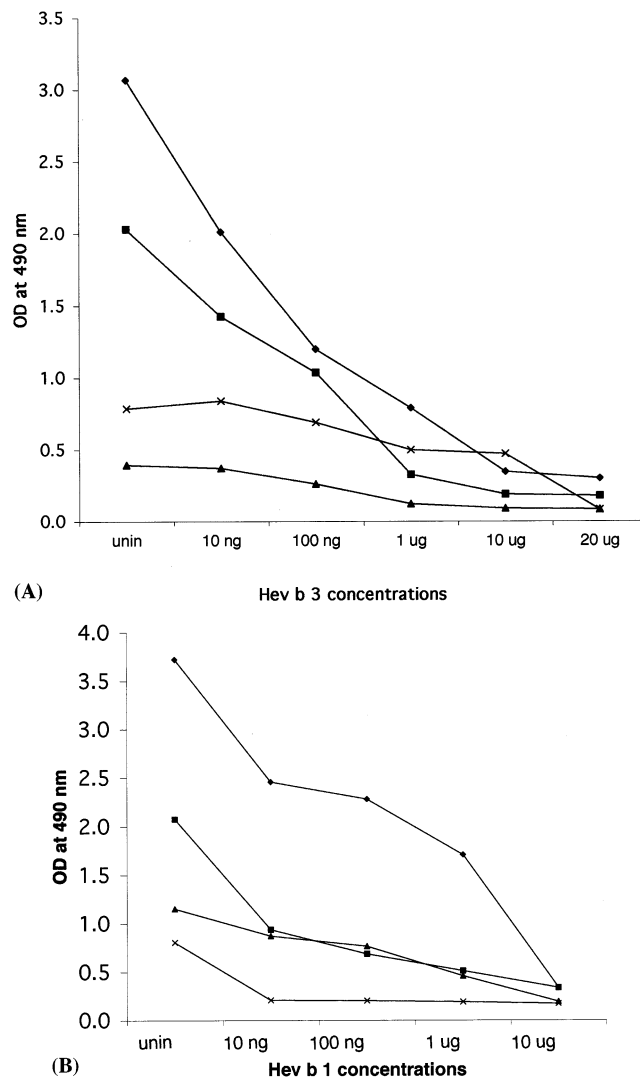


Fig. 2. (A) Inhibition of IgE binding to Hev b 1 by individual serum from two spina bifida (▲/■) and two HCW with latex allergy (×/◆) in competitive ELISA using Hev b 3. Various concentrations of rHev b 3 used for inhibition study were indicated on the 'abscissa' axis. ELISA absorbance (OD₄₉₀) value of inhibited and uninhibited serum samples are shown on the 'ordinate' axis. (B) Inhibition of IgE binding to Hev b 3 by same serum samples in competitive ELISA using various concentrations of Hev b 1 (see Fig. 2A for details).

3.3. Unique and shared IgE and IgG binding epitope regions of Hev b 1 and Hev b 3

In order to define the unique and shared IgE epitopes of Hev b 1 and Hev b 3, the peptides representing epitope regions of these proteins were evaluated further with sera from HCW for IgE binding (Fig. 4). Out of eight selected peptides from Hev b 1, six (E1–E6) exhibited significant IgE binding with sera from SB patients (Fig. 4a and b), while sera from HCW patients demonstrated IgE binding only with peptides E3, E5 and E6. However, the interaction of epitope and antibody reveals that the peptide KYLGfVQDAA (16–25) near the N-terminus is a specific IgE binding epitope recognized by sera from SB patients.

As shown in Fig. 4c and d, two peptides representing epitopes E10 (159–168) and E11 (179–188) of Hev b 3 near the C-terminal region of the protein demonstrated IgE antibody binding with HCW sera. Hence, E10 and E11 represented common IgE binding epitopes of Hev b 3, whereas, amino acid residues in E1–E9 were specific IgE epitopes reacting only with sera from SB patients. As the C-terminal region of the protein carrying common IgE epitopes for both HCW and SB

groups, the IgE binding specificity is residing mainly near the N-terminal region of Hev b 3.

3.4. Synthetic peptides of Hev b 1 and Hev b 3 showed specific IgE and IgG binding epitopes

Decapeptides representing identified epitopes for Hev b 1 and Hev b 3 were synthesized using F-moc amino acids. The results of ELISA for individual peptide

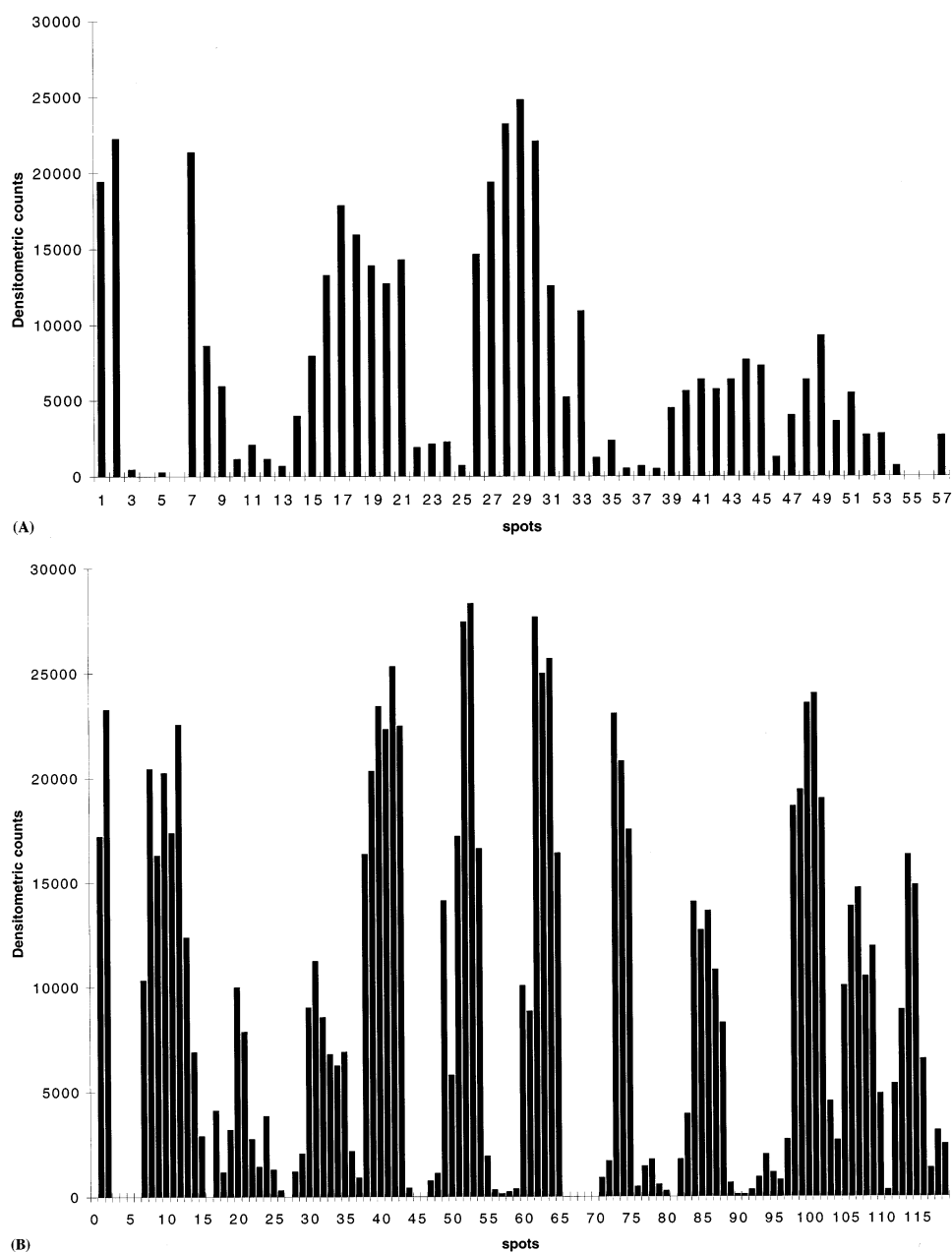


Fig. 3. (A) Densitometric scanning of the interaction between Hev b 1 derived peptides and IgE antibodies on cellulose membrane. Based on the results of first epitope analysis, peptides were synthesized at an offset of one amino acid within the identified epitope regions of Hev b 1. Eight epitope regions were selected for Hev b 1 in the amino acid residues 1–55, 60–75, and 85–110. (B) Densitometric scanning of interaction between Hev b 3 derived peptides and IgE antibodies on cellulose membrane. Based on the results of first epitope analysis, peptides were synthesized at an offset of one amino acid within the identified epitope regions of Hev b 3. Eleven epitope regions were selected for Hev b 3 throughout the molecule.

Table 1
Identified IgE binding epitopes of Hev b 1 and Hev b 3

Selected epitope regions of Hev b 1 and Hev b 3	Conserved amino acid residues of selected epitopes
Hev b1 2 AEDEDNQQGQ 11	1 AEDEDNQQ 8 : : : .
Hev b 3 2 AEEVEEERLK 11	1 AEEVEEER 8
Hev b 1 16 KYLGFVQDQQ 25	1 KYLGFVQ 7 .
Hev b 3 8 ERLKYLDFVR 17	4 KYLDFVR 10
Hev b 1 36 YLFAKDKSGP 45	1 YLFAKDKS 8 :
Hev b 3 29 TLYLYAKDIS 38	3 YLYAKDIS 10
Hev b 1 46 LQPGVDIIEG 55	1 LQPGVDIIE 9 .
Hev b 3 41 LKPGVDTIEN 50	1 LKPGVDTIE 9
Hev b 1 61 AVPLYNRFSY 70	2 VPL 4 :
Hev b 3 60 YYIPLEAVKFV 69	3 IPL 5
Hev b 1 65 YNRFSYIPNG 74	7 IP 8 :
Hev b 3 85 VPPVIKQVSA 94	1 VP 2
Hev b1 90 IDRSLPPIVK 99	6 PPIV 9
Hev b 3 103 APRIVLDVAS 112	2 PRIV 5
Hev b 1 99 KDASIQVVSA 108	5 IQ 6 :
Hev b 3 118 GVQEGAKALY 127	2 VQ 3
Hev b 3 138 AVITWRALNK 147	
Hev b 3 159 VPTAVYFSEK 168	
Hev b 3 179 QGYRVSSYLP 188	

antibody interaction are shown in Fig. 5A. The two Hev b 1 peptides, KYLGFVQDAA (b1 E2) and LQPGVDIIEG (b1 E4) demonstrated strong IgE binding ($OD_{490} \geq 0.5$). Similarly, Hev b 3 peptide (b3 E5) with amino acid residues YYIPLEAVKF (60–69) exhibited strong IgE antibody binding with SB sera ($OD_{490} > 0.8$).

The competitive ELISA using peptides from the two major latex allergens is shown in Fig. 5B. Peptides b1 E2 and b1 E4 together inhibited 58% IgE binding of Hev b 1, on the other hand, Hev b 3 peptides b3 E5 exhibited 22% inhibition in IgE binding of the complete protein.

Hence, based on immunological evaluation of linear peptides on derivatized cellulose membrane and the synthetic peptides in ELISA we have been able to identify distinct IgE binding regions of the major latex allergens Hev b 1 and Hev b 3.

4. Discussion

The development of clinical latex allergy in individuals involves a series of interactions between T and

B-lymphocytes. The mediators released from activated T lymphocytes and cognate interaction between B cells bearing appropriate antigen specific surface immunoglobulins and allergen specific T-cells lead to isotype switching and generation of allergen specific IgE (Abbas et al., 1997). The specific IgE then binds to the high affinity Fc ϵ R1 on mast cells and basophils and finally the cross-linking of surface IgE molecules with the incoming allergens triggers a life threatening immediate hypersensitivity reaction in patients with latex allergy. Because of the involvement of allergen specific IgE in the pathophysiology of allergic disease, determination of IgE binding regions of the respective allergens is certainly an important step towards the understanding of the immune mechanism in the disease.

In the present study, B-cell epitopes of two major allergens, namely Hev b 1 and Hev b 3, associated with immediate hypersensitivity responses in latex sensitized patients, have been evaluated. Both allergens demonstrated significant sequence homology and showed similar IgE binding with sera from SB patients (Alenius et al., 1993, 1996; Chen et al., 1996; Yeang et al., 1996). The current study using sera from a group of SB and HCW patients demonstrated more than 80% inhibition in IgE binding to Hev b 1 on preincubation of sera with Hev b 3. This high degree of cross-reactivity encour-

aged us to analyze in detail the presence of unique and common B-cell epitopes in Hev b 1 and Hev b 3. The result indicates that the IgE antibody binding regions are distributed all over the sequences in Hev b 1 and Hev b 3. Several previous reports have also shown continuous IgE binding epitopes for bovine β -lactoglobulin, major milk protein allergen α _{s1}-casein, pollen allergen Par j 1, peanut allergen Ara h 1, and the latex allergens Hev b 1, Hev b 5, and Hev b 6 (Ball et al., 1994; Ong et al., 1995; Chen et al., 1996; Banerjee et al., 1997; Burks et al., 1997; Colombo et al., 1998; Nakajima-Adachi et al., 1998; Slater et al., 1999).

Extensive sequence homology of the identified epitopes of Hev b 1 and Hev b 3 was detected at their N-terminal epitopes E1–E4. In a recent study, Chen et al. (1996) mapped the B-cell epitopes of Hev b 1 using sera from SB patients, monoclonal antibodies and rabbit antisera against Hev b 1 and identified common allergenic and antigenic epitopes in the amino acid residues of 31–64 and 121–137. In contrast to their findings, we identified a specific Hev b 1 epitope having amino acid residues from 16–25 reacting with IgE of SB patients. Similarly common IgE epitopes for Hev b 3 could be detected near the C-terminal region of the protein in the amino acid residues of 138–188. However, the IgE binding specificity of Hev b 3 for SB

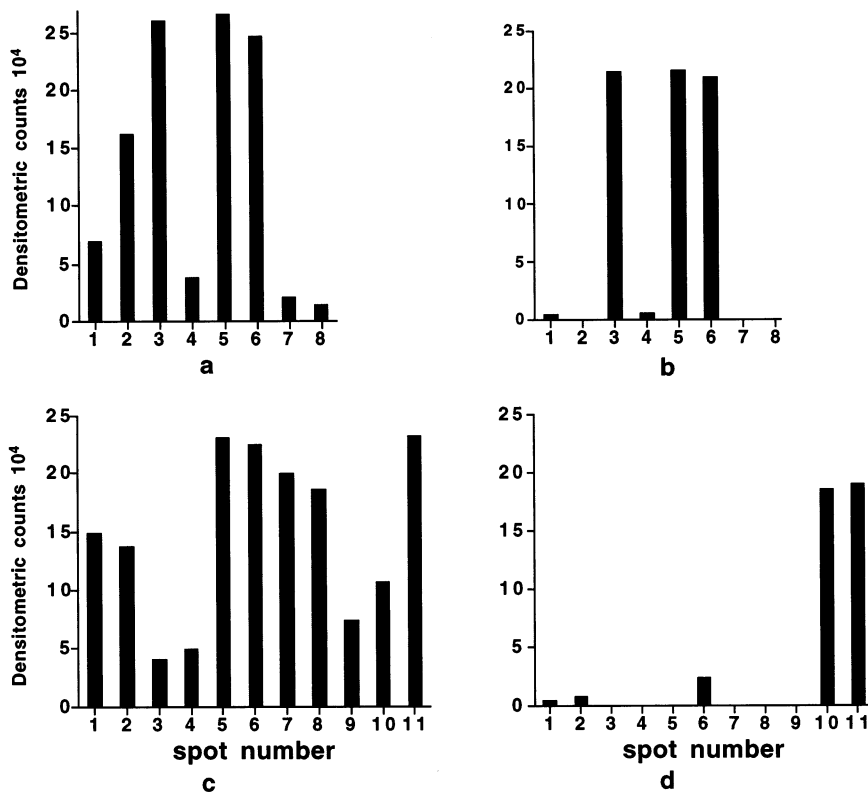


Fig. 4. Eight selected epitopes of Hev b 1 were synthesized on cellulose membrane and evaluated for IgE binding with (a) pooled sera from SB patients and (b) pooled sera from HCW. Eleven selected epitopes of Hev b 3 on cellulose membrane were evaluated for IgE binding with (c) pooled sera from SB patients and (d) pooled sera from HCW.

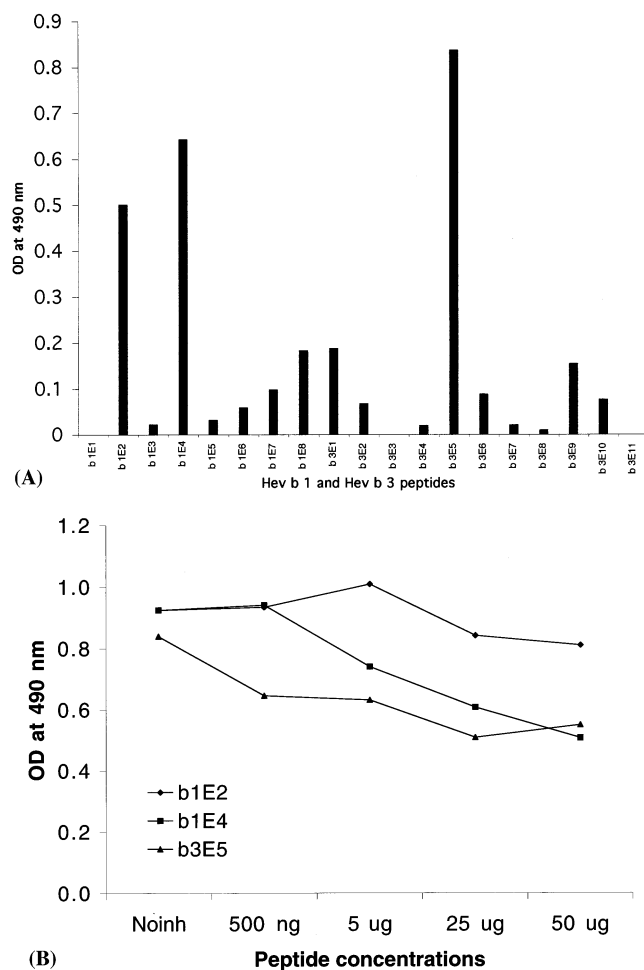


Fig. 5. (A) Hev b 1 and Hev b 3 peptide specific ELISA. Eight peptides from Hev b 1 and 11 from Hev b 3 were synthesized using F-moc amino acids. Synthetic peptides were then used for coating the wells of microtiter plates at a concentration of 12 µg/ml. The IgE antibody from SB patients binding to Hev b 1 derived peptides (b1E1–b1E8) and Hev b 3 derived peptides (b3E1–b3E11) are shown as closed bars. Average of two independent test results for each peptide is presented. (B) Competitive inhibition of IgE binding in SB patients sera by peptides b1E2 and b1E4 from Hev b 1 and b3E5 from Hev b 3. ELISA absorbance values of the uninhibited sera and peptide preincubated sera (500 ng–50 µg) were represented on the 'ordinate' axis. Average of two independent test results for each peptide is presented.

patients were found to be located near the N-terminal region of the protein. Hence the specific IgE binding of these two proteins with sera from SB patients could be attributed to the presence of unique epitopes in these molecules. In a recent study, B-cell epitopes near the C-terminal region of Hev b 5, another major latex allergen reacting with human IgE from patients with latex allergy, while multiple B-cell epitopes have been reported throughout the allergen reacting with IgG antibodies from BALB/c mice (Hsieh et al., 1997; Slater et al., 1999). Although, Hev b 5 epitopes revealed enhanced binding to KXEE or KEXE motifs where X may be empty, threonine or alanine, we could not

demonstrate any such association of amino acids in the present study. Similarly, epitopes comprising predominantly acidic (D,E) and basic amino acids (K,R) reported to have major IgE binding specificity with the peanut allergen Ara h I is also not seen in our study (Burks et al., 1997; Slater et al., 1999).

The systematic designing and evaluation of peptides representing antibody binding regions of Hev b 1 and Hev b 3 enable us to understand the similarity and molecular interactions of these two allergens. In a recent study on the epitope analysis of α_{s1} -casein, an immunodominant IgE epitope (amino acid 181–199) was identified near the C-terminal region of the protein (Nakajima-Adachi et al., 1998). Similarly, epitopes recognized by IgE for other allergens, such as various pollens, peanuts, house dust mites and cat (Fel d 1) were also found to localize in particular regions (Oshika et al., 1992; van Milligen et al., 1994; Ong et al., 1995; Zhang et al., 1996; Rabjohn et al., 1999). Studies on immunoglobulin gene rearrangements have shown that among V_H genes encoding IgE from atopic patients, the VH5 family of genes is used preferentially. These observations suggest that only a particular repertoire of B cells differentiate and produce allergen specific IgE antibody indicating the less diverse nature of IgE epitopes of different allergens (van-der Stoep et al., 1993; Snow et al., 1995; Rabjohn et al., 1999).

In conclusion, elucidation of the unique and shared IgE binding epitopes of Hev b 1 and Hev b 3 is a key step in the understanding of latex hypersensitivity because of their probable involvement in the pathogenesis of the disease. In particular, peptides representing linear IgE binding epitopes with significant inhibitory effects on IgE reactivity of Hev b 1 and Hev b 3 indicate the possibility of their use in patient care modalities including immunotherapy.

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