

The Latex Allergen Hev b 5 Is an Antigen with Repetitive Murine B-Cell Epitopes

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Key Words

Hev b 5 · Latex allergy · IgE antibodies · Recombinant allergens · Site-directed mutagenesis · Multivalent protein

Abstract

Background: Hev b 5 is a potent latex allergen. In this study, we characterize the linear B-cell epitopes for three monoclonal antibodies (mAbs) to Hev b 5. **Methods:** The mAbs included 2 IgG1 (6A10, 3G3) and 1 IgG2b (6F6) isotypes. We used SPOTscan analysis with overlapping octapeptides to identify the binding regions for the antibodies and then methionine substitution analysis to further define the critical amino acids (aa) in each epitope. Site-directed mutagenesis was used to selectively eliminate the IgG binding for each epitope and single and multiple mutations were expressed as recombinant GST fusion proteins. Antibody recognition of the mutant proteins was determined by inhibition ELISA. **Results:** All three mAbs recognized the same aa sequence by SPOTs analysis with slight variations, and this epitope was repeated 3 times in the Hev b 5 sequence; APETEK (63–68), PAEGEK (120–125), and PAEEEK (126–131). Sequential methionine substitution by SPOTs analogue identified K⁶⁸, E¹²², and K¹³¹ as critical aa in each epitope to change by site-directed mutagenesis. Inhibition ELISA with the mutant proteins indicated that epitope 126–131 was the dominant epitope, but mutation of epitope 120–125 was

also required to eliminate mAb reactivity to Hev b 5. The antibodies did not appear to recognize the epitope 63–68 in the recombinant fusion protein. **Conclusions:** We identified an immunodominant B-cell epitope in Hev b 5 that is repeated 3 times within the sequence, making Hev b 5 multivalent. Well-characterized monoclonals recognizing repeated epitopes would be a good choice for immunodetection of Hev b 5 in glove extracts where individual epitopes could get altered by the manufacturing process.

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Introduction

Hev b 5 is a major allergen for latex-allergic healthcare workers. This allergen was first isolated and characterized by recombinant DNA technology [1, 2], but little information exists regarding the native protein or its function in *Hevea brasiliensis*. Homologues for Hev b 5 have been identified in kiwi [1, 2], sugar beet [3], and recently in manioc (cassava) where the protein may function in secondary growth and maturation of parenchymal cells [de Souza, unpubl. obs.].

Hev b 5 has an acidic pI (pI 3.5) with an unusually high glutamic acid content (30%) [1]. The protein has a predicted molecular mass of 16 kD, but migrates at approximately 36 kD in SDS-PAGE gels, a property typical of highly acidic proteins. In Western blots, Hev b 5-specific

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antibodies recognize a broad range of protein in the 36- to 100-kD range [4, 5].

Studies have shown that Hev b 5 is one of the most common allergens in the complex mixture of proteins found in latex with 62–65% of latex-allergic patients demonstrating skin prick test (SPT) reactivity to the recombinant protein [6, 7]. Sensitized patients show SPT reactivity to as little as 10 ng/ml of recombinant Hev b 5 [6].

Despite the lack of information about the structure or function of Hev b 5, much has been learned about its antigenicity and allergenicity. The T- and B-cell epitopes for mice immunized with rHev b 5 have been identified [8, 9]. Two immunodominant human T-cell epitopes at positions 46–65 and 109–128 [9] and multiple IgE epitopes were described for human subjects allergic to latex [4]. Site-directed mutagenesis studies have demonstrated that because of the multiple IgE epitopes, at least 8 B-cell epitopes required mutation in order to produce a hypoallergenic Hev b 5 [10].

Recently, monoclonal antibodies (mAbs) were developed and used to characterize Hev b 5 and determine relative amounts of the allergen in latex products [5]. In this paper, we report the characterization of the B-cell epitopes for these mAbs. We have used site-directed mutagenesis to dissect the functional activity for these epitopes.

Materials and Methods

Antibodies

Production and characterization of mAbs to Hev b 5 were previously described [5]. The mAbs used for this study were 6A10 (1 mg/ml), 3G3 (0.8 mg/ml) and 6F6 (0.3 mg/ml). 6A10 and 3G3 antibodies were of the IgG1 isotype, while 6F6 was an IgG 2b isotype. mAbs to irrelevant antigens (5H5 anti-LTP and GE1 anti-IgE) were used as negative controls.

Latex Proteins

Latex proteins were from ammoniated latex (AL) and non-ammoniated latex (NAL) reference reagents IRM 913 (Akron Rubber Development Laboratories, Akron, Ohio, USA) that are used in the ASTM D6499 standard method. In brief, latex from 3 different suppliers was pooled and centrifuged to remove the rubber particles and particulates. The middle aqueous layer (C serum) was recovered, dialyzed against carbonate buffer, and the concentration determined using the ASTM D5712-95 modified Lowry protein assay. The protein was lyophilized in small vials at 3 mg/vial. The protein was solubilized in carbonate buffer or H₂O to a concentration of 1 mg/ml.

SPOTscan and SPOTsalogue Membranes

Peptides spanning the entire Hev b 5 sequence (SPOTscan) were synthesized according to the manufacturer's instructions as a series of octapeptides overlapping by 2 amino acids (aa) on derivatized cellulose membranes (SPOTs, Sigma-Genosys, The Woodlands, Tex.,

USA) as previously described [4]. On a second membrane (SPOTsalogue), a series of 12 SPOTs, each consisting of an 11-aa peptide spanning the region of each of the 3 epitopes identified in the SPOTscan were synthesized. The sequences synthesized included PAPE-TEKAEV (62–72), APAAPAEGEKP (116–126) and EKPAEEEPIT (124–134). For the first SPOT of each row, the 11-aa peptide of the native sequence for each epitope was synthesized. For each successive spot, a single aa was substituted with a methionine residue in place of the native residue.

Immunoblotting

The SPOTs membranes were wetted in methanol, rinsed with Tris buffered saline (TBS), and blocked for 6 h with 5% BSA protein in 0.05% Tween 20 in TBS (T-TBS). The blocking buffer was removed and the membrane incubated overnight with the mAbs. Since antibody recognition of a linear peptide can be several orders of magnitude weaker than that of the peptide in an intact protein [11], the anti-Hev b 5 mAbs and the negative control mAbs were used at 1/300 dilution for the immunoblots. The membranes were washed 3 × with 20 ml T-TBS and reacted with alkaline phosphatase (AP)-conjugated goat anti-mouse IgG (Sigma, St. Louis, Mo., USA) diluted to 1/1,000 for 1 h. After washing 5 ×, the membrane was placed in development solution (ECF substrate, Amersham Pharmacia Biotech, Inc., Piscataway, N.J., USA). Chemifluorescence was recorded using a STORM 840 (Molecular Dynamics, Sunnyvale, Calif., USA) and after densitometry scanning, quantified using ImageQuant software (Molecular Dynamics). Epitopes were defined by determining the common or overlapping sequence of positive spots (SPOTscan) or by inhibition of binding (SPOTsalogue) as described by the manufacturer.

Site-Directed Mutagenesis

Specific point mutations were introduced into Hev b 5 in pGEX4T-1 using the QuickChange Site-directed Mutagenesis kit from Stratagene (La Jolla, Calif., USA) according to the manufacturer's instructions. Based on methionine scanning, we mutated K⁶⁸M, E¹²²M, K¹³¹M and all combinations of the three mutations. Following mutagenesis, each clone was transformed into competent BL21 cells. To verify each mutation, the sequences for each mutant clone were determined by Cycle Sequencing (Applied Biosystems) with BigDye terminators using overlapping forward and reverse primers.

rHev b 5 Fusion Proteins

The pGEX4T-1/Hev b 5 or mutant Hev b 5 plasmids were inserted into competent BL21 *Escherichia coli* and cultures of *E. coli* expressing the GST/Hev b 5 fusion proteins were grown. Fusion protein expression was induced for 4 h using isopropylthiogalactoside; the culture was centrifuged and the resulting bacterial pellet frozen, thawed, and disrupted by sonication. The GST/Hev b 5 fusion proteins were isolated over a glutathione column (Amersham Pharmacia Biotech) according to the manufacturer's instructions. The GST construct (pGEX-2T) with no insert served as a negative control. The protein concentrations were determined by a Bradford assay, and the quality of the protein was checked by SDS-PAGE. The proteins were diluted to 1 mg/ml and stored in aliquots at –80 °C.

ELISA

Microtiter plates (Nunc-Immuno Plate, Maxisorp surface) were coated with 1 µg/well of GST/Hev b 5 (native or mutant) in carbonate buffer overnight at 4 °C, washed 2 × with 0.05% T-PBS, and

blocked with 3% dry milk in 0.05% T-PBS before use. An indirect ELISA was performed to examine mAb reactivity with the mutant proteins as the solid-phase antigen. The antigen-coated plates were incubated with the mAbs for 3 h, washed 3× and then incubated with a 1/8,000 dilution of HRP-labeled anti-mouse IgG (Amersham NA931) for 1 h at 37°C. A colored reaction product was produced by adding 100 µl o-phenylenediamine (0.4 mg/ml in phosphate citrate buffer with 0.04% hydrogen peroxide), incubated for 30 min, and stopped with H₂SO₄. The plate was read at 490 nm.

For the inhibition ELISA, a dilution plate (Corning) was blocked with 3% non-fat dry milk in T-PBS overnight at 4°C and washed 2× with T-PBS. One hundred microlitres of mAb was mixed with 100 µl of an appropriate inhibitor in the dilution plate. Following a 2-hour incubation at 37°C, 100 µl of the mixture was transferred from the dilution plate to the antigen-coated plate and incubated for an additional 3 h at 37°C. The plate was washed 3× with T-PBS and reacted with a 1/8,000 dilution of HRP-labeled anti-mouse IgG (Amersham) for 1 h at 37°C. The reaction product was produced using o-phenylenediamine and the plate was read at 490 nm as before. After background subtraction, the percent inhibition was calculated as $[1 - (\text{experimental OD}/\text{uninhibited rHev b 5 OD})] \cdot 100$. mAbs were used at a dilution that gave approximately 1 OD in the ELISA using native rHev b 5-coated wells under the described conditions.

Results

Epitopes for Monoclonal Anti-Hev b 5

Three mAbs were screened by SPOTscan using overlapping octapeptides to scan the entire Hev b 5 sequence. Figure 1 shows the scan for monoclonal 6F6, an IgG2b isotype. We expected 1 spot or small group of spots for a mAb, but the antibody reacted to 3 groups consisting of spots 54–55, 82–83 and 85–86. Each spot is an octapeptide overlapping by 2 aa, therefore reactivity to 2 spots results in 6 aa in common between the 2 spots. The aa sequence for each group was APETEK (position 63–68), PAEGEK (position 120–125) and PAEEEK (position 126–131), respectively. The aa sequence for the second (120–125) and third (126–131) epitopes appeared contiguous, but since a distinct gap occurred (fig. 1, spot 84) on the membrane, it appears that there are 2 epitopes. These data suggested that the mAb reacts to a single epitope that was repeated 3 times in the Hev b 5 sequence. Interestingly, monoclonals 6A10 and 3G3, both IgG1 isotypes, also reacted to the same 3 regions (data not shown). The data indicate that the 3 mAbs react to a single immunodominant B-cell epitope that is repeated 3 times in the Hev b 5 sequence. Control mAbs to irrelevant antigens (5H5 and GE1) did not react with any spots.

Methionine Substitution Analysis

Each of the hexapeptide epitopes identified by SPOTscan and their bordering regions were synthesized as a



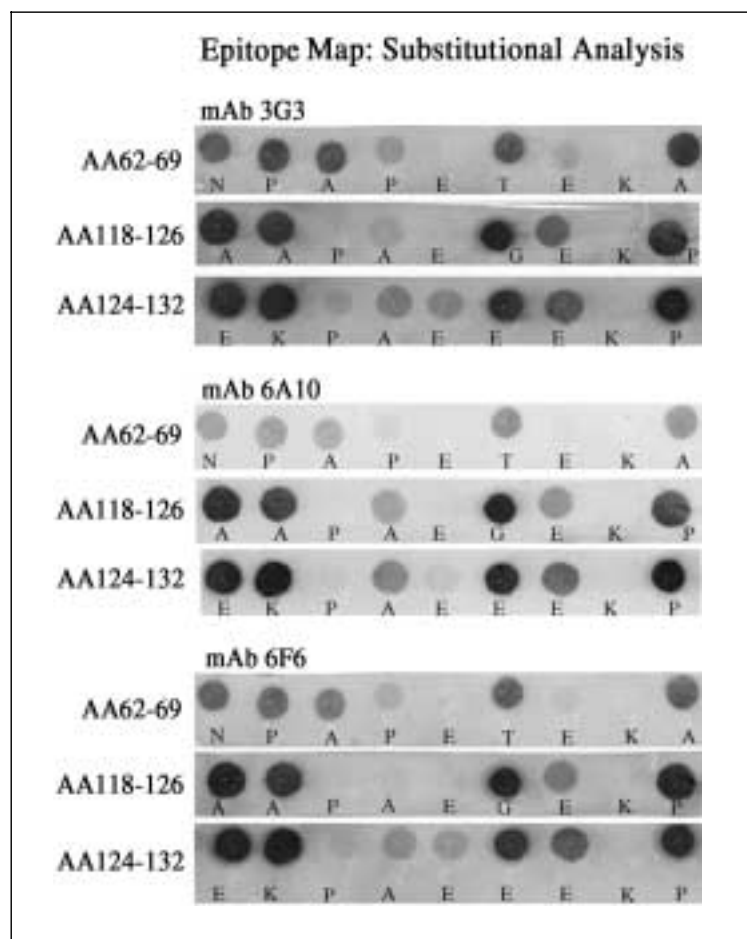
Fig. 1. SPOTs membrane stained with mAb 6F6. Each spot represents an 8-aa synthetic peptide of the Hev b 5 sequence. Peptides were sequential octapeptides overlapping by 2 aa and spanning the entire Hev b 5 sequences. Membrane was blocked and incubated overnight with the mAb at 1/300 dilution. The membrane was washed, reacted with alkaline phosphatase-conjugated goat anti-mouse IgG diluted to 1/1,000 for 1 h and reactive spots identified by chemifluorescence.

series of 11 aa peptides with sequential substitutions of methionine for each aa. Methionine was used rather than the traditional alanine because there was an alanine in the epitope sequence. Figure 2 shows the reactivity of the mAbs to the methionine-substituted peptides of each epitope. The epitopes were located to the same aa identified by SPOTscan analysis. The data show the consensus epitope to be PAExEK or APExEK where x could be T, G, E or M. Subtle differences in binding intensity were noted at the second position of the epitope (A/P) between the 2 IgG1 mAbs, 3G3 and 6A10. Also the mAb 6A10 had a weaker affinity for epitope 63–68, suggesting that the 3 antibodies are different. Based on the nearly complete elimination of reactivity (80–94% reduction by densitometry scanning) due to changes at the 3rd position (E) and the 6th position (K), we chose to make recombinant proteins with methionine substitutions K⁶⁸M, E¹²²M, K¹³¹M and all combinations of each mutation. Reactivity of the negative control mAbs 5H5 and GE1 were 1.8 and 1.4% compared with the specific mAbs when analyzed by densitometry scanning of the membranes with ImageQuant software.

Western Blot Analysis

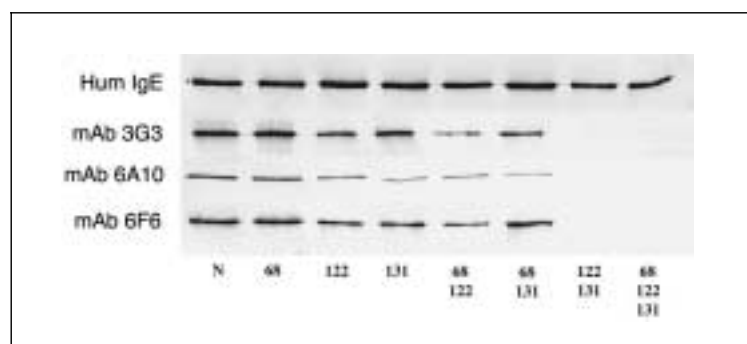
Reactivity of the mAbs to the mutant recombinant proteins was tested by Western blot. As shown in figure 3, a single mutation in any one epitope had little effect on

Fig. 2. Methionine substitution analysis for each of the Hev b 5 epitopes for the mAbs. Each spot represents an 11-aa peptide overlapping the region of the epitope with successive substitutions of each aa with a methionine. The aa that was changed to methionine is indicated below each spot; N represents the native sequence. The blots were aligned in the way that similar aa sequences for each epitope were in the same position in the figure, eliminating 3 spots from each strip where there was no change in intensity. The aa position is indicated on the left and the mAb used on the top of each blot.



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Fig. 3. Western blot analysis of the mutant Hev b 5. Designated below each lane is the recombinant native Hev b 5 or mutant Hev b 5 (20 ng) separated by SDS-PAGE on a 15% gel and transferred to nitrocellulose. The membrane was blocked with 3% non-fat dry milk, and reacted with monoclonal anti-Hev b 5 (1/300) overnight. The blot was then reacted with AP-labeled secondary antibody (1/5,000) and developed with BCIP/NBT substrate. Human IgE was sera pooled from 4 latex allergic individuals with SPT reactivity to Hev b 5, and then the blot was reacted with monoclonal anti-IgE (1/1,000), and AP-labeled anti-mouse IgG (1/5,000) before developing in BCIP/NBT.



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the ability of the mAbs to recognize the mutant proteins. When mutation E¹²²M was combined with K¹³¹M, or all three mutations were present, the mAbs no longer recognized the proteins. Because the SPOTs data suggest that there are 3 separate epitopes, we assume that the antibody could bind to either epitope 120–125 or epitope 126–131, but epitope 63–68 was not recognized in the recombinant protein. Sera pooled from Hev b 5-allergic patients reacted equally to all of the mutants, because the IgE in

this serum could recognize 11 different epitopes, as shown in our previous work [10].

ELISA

Using an indirect ELISA, we examined the ability of the antibodies to react with solid-phase mutant Hev b 5 proteins (table 1). Antibodies were used at dilutions that gave approximately 1 OD in the indirect ELISA when 1 µg of native rHev b 5 was adsorbed to the plate,

1/64,000 dilution for 3G3, 1/14,000 for 6F6 and a 1/2,000 for 6A10. As observed by Western blot, mutation K⁶⁸M had no effect on antibody binding in the ELISA. Mutation of E¹²²M (Mut 122, Mut 68/122) reduced antibody binding by 20% for 3G3, 35% for 6A10, and 25% for 6F6. Mutation K¹³¹M (Mut 131, Mut 68/131) reduced antibody binding by 50% for 3G3, 87% for 6A10 and 63% for 6F6, suggesting that the antibodies have the strongest affinity for epitope 126–131.

Next, we examined the interaction of antibody with mutant Hev b 5 in solution using an inhibition ELISA. Plates were coated with native, Mut 68/122, or Mut 68/131 and then, the mAbs were inhibited with native or mutant recombinant proteins. As shown in table 2, the native

sequence is a more efficient inhibitor of antibody binding than the mutants when plates are coated with the native sequence. On a Mut 68/122-coated plate (epitope 126–131 unchanged), again the native sequence is the strongest inhibitor, but the relative inhibition by the mutant proteins increases. With the Mut 68/131-coated plate where epitope 120–125 is unchanged, the ability of the soluble mutants to inhibit increases further. Thus when a mutant with a single epitope is used as the solid phase, more antibody is available to bind to the soluble epitopes resulting in an apparent increase in inhibitory activity. The ability of the antibody to bind to the mutated antigen as solid phase is reduced (lower OD), but the antibody appears to have a higher reactivity with the soluble protein.

Finally, we examined the reactivity of the mAbs with both AL and NAL natural latex protein using the inhibition ELISA. Incubation with either AL or NAL resulted in approximately the same amount of inhibition indicating a similar level of Hev b 5 in both NAL and AL (table 3). mAb 6A10 appeared to have a nearly equal reactivity with AL and NAL, while mAbs 3G3 and 6F6 reacted slightly stronger to NAL. For mAbs 3G3 and 6F6, the relative increase in inhibition that we observed on Mut 68/122- and Mut 68/131-coated plates in the previous experiments (table 2) was again seen with the native latex proteins as the inhibitor. This suggests that both epitopes are available on the natural protein. Interestingly, when NAL was dissolved in water (table 3), there was approximately 50% less inhibitory activity, indicating a reduced solubility for Hev b 5 in water. Thus, buffer composition is a very important consideration when extracting latex products for allergen determinations, and extraction in water significantly underestimates the amount of allergen present.

Table 1. mAb reactivity to mutant Hev b 5 proteins by indirect ELISA

Protein	mAb 3G3 1/64,000	mAb 6A10 1/2,000	mAb 6F6 1/14,000
GST	0.002	0.003	0.006
Native	1.244	0.919	0.805
Mut 68	1.237 (99)	0.902 (98)	0.806 (100)
Mut 122	0.988 (80)	0.578 (65)	0.595 (75)
Mut 68,122	0.984 (80)	0.595 (66)	0.605 (76)
Mut 131	0.649 (54)	0.078 (12)	0.289 (39)
Mut 68/131	0.597 (50)	0.079 (13)	0.269 (37)
Mut 122/131	n.d.	0.005 (0)	0.001 (0)
Mut 68/122/131	0.002 (0)	0.002 (0)	0.003 (0)

Data presented as OD-bkgd (% native rHev b 5 binding). Wells were coated with 1 µg/ml of recombinant proteins. n.d. = Not determined.

Table 2. mAb reactivity with mutant Hev b 5 proteins by inhibition ELISA

Coating ¹	OD ²	Inhibitor (100 µg/ml) ³			
		native	68/122	68/131	68/122/131
<i>mAb 3G3</i>					
Native	0.700	84.1±2.4	60.8±1.9	24.9±3.1	6.1±3.7
Mut 68/122	0.555	92.9±1.3	81.7±1.3	44.8±3.6	7.0±2.3
Mut 68/131	0.311	98.5±3.5	94.9±6.1	72.6±6.3	6.2±6.6
<i>6A10</i>					
Native	1.282	59.7±4.0	52.4±1.8	9.0±1.5	3.6±1.7
Mut68/122	0.810	63.8±3.2	53.9±0.4	10.9±0.6	2.9±0.7
Mut 68/131	0.144	97.7±4.3	99.2±6.8	53.3±19.3	3.8±8.3

¹ Wells were coated with 1 µg of protein.

² Data represent OD-bkgd.

³ Data represent mean ± SD of percent inhibition from 3 separate experiments.

Table 3. mAb reactivity with natural latex protein by inhibition ELISA

	3G3			6A10			6F6		
<i>AL, µg/ml</i>									
Coating	100	50	25	100	50	25	100	50	25
Native	63.1	16.9	9.2	59.3	28.5	11.6	61.8	14.9	7.7
68/122	70.9	32.0	14.0	58.3	31.4	13.4	71.0	33.0	7.9
68/131	74.3	50.1	23.2	50.3	35.4	17.2	63.4	45.6	33.6
<i>NAL in carbonate, µg/ml</i>									
Coating	100	50	25	100	50	25	100	50	25
Native	70.4	24.1	9.1	56.9	32.8	13.3	70.8	35.7	10.1
68/122	76.0	43.0	20.5	49.2	32.6	15.3	73.9	49.0	17.8
68/131	76.5	61.9	35.9	42.3	34.2	19.9	64.3	56.1	25.9
<i>NAL in H₂O, µg/ml</i>									
Coating	100	50	25	100	50	25	100	50	25
Native	16.0	11.5	8.6	27.2	12.8	5.8	20.2	13.0	10.1
68/122	30.5	21.3	12.5	27.7	18.8	10.4	33.1	20.0	12.6
68/131	53.7	29.2	31.5	37.9	30.1	17.7	46.9	27.8	20.0

Wells were coated with 1 µg of recombinant proteins. AL and NAL stock solutions were diluted to 1 mg/ml total protein. Data represent percent inhibition from 3 separate experiments.

Discussion

The latex allergen Hev b 5 is a potent allergen, inducing the production of high-affinity IgE antibodies in healthcare workers exposed to high-allergen natural rubber latex gloves [1, 2, 6, 7]. Hev b 5 is recognized by more than 60% of latex-allergic individuals, making it one of the major allergens in this mixture of proteins [1, 2, 6, 7]. We are interested in the question why certain proteins in latex such as Hev b 5 and 6 are the predominant allergens when patients are exposed to a mixture of 60 or more latex proteins. Previous studies using B-cell epitope mapping demonstrated that the human IgE response to Hev b 5 included multiple epitopes [4]. Using site-directed mutagenesis and polyclonal IgE, the data indicated that several of the allergenic epitopes with similar sequences might be cross-reactive [10].

In this paper, we have characterized three previously described mAbs that were generated by injecting mice with rHev b 5/MBP in Freund's adjuvant [5]. Linear epitope mapping using SPOTs technology demonstrated that these three mAbs all react to the same protein sequence. The mAbs recognized a consensus sequence of (PA)(AP)ExEK where x could be E, T, G or M. Interestingly, the epitope was repeated 3 times in the Hev b 5 sequence: APETEK, PAEGEK and PAEEEEK. The PAEGEK epitope was previously reported as a B-cell epi-

tope for mice and was detected using sera pooled from mice injected with rHev b 5/MBP in alum or Freund's adjuvant [9]. The PAEEEEK epitope was observed among the multiple human IgE epitopes in sera from Hev b 5 allergic healthcare workers [10]. In these earlier studies, the precise location of the epitopes could not be mapped because of the use of polyclonal sera or longer synthetic peptides. Taken together, however, the studies indicate that the B-cell epitope recognized by these mAbs comprises an immunodominant epitope of Hev b 5.

Methionine scanning was used to determine the fine epitope specificity for each binding location. Typically, B-cell epitopes consist of 6–10 aa in close proximity, either sequentially or because of protein folding, that define approximately 50–90 nm² on the antigen surface [12, 13]. Site-directed mutagenesis has been extensively used to alter epitopes in order to study antibody-antigen interactions. Mutation of single aa that are contact residues can drastically alter antibody binding, but changes in non-contact residues can also result in changes in the confirmation of the contact residues and thereby influence binding. The mutations we introduced did not appear to induce global changes in the protein since polyclonal IgE from patient sera was still able to recognize the mutated proteins (fig. 3), even though the monoclonals could no longer recognize them.

We observed that only 2 of the 3 epitopes (120–125, 126–131) were recognized in the soluble recombinant protein while the third region (epitope 63–68) was not recognized. Epitope 63–68 appears to be of lower affinity and cryptic in that the monoclonals failed to recognize mutant proteins that express only epitope 63–68 but not epitope 120–125 or epitope 126–131. While epitope 63–68 is cryptic in the recombinant protein, it might be exposed in the natural protein, as its 3D structure is not known. However, our studies using natural latex protein which contains Hev b 5 further indicate that the epitope is not a functional one. Using natural latex protein we could show that epitopes 120–125 and 126–131 are active in the native material. We did not test gloves to see which of the epitopes remain unaltered by the manufacturing process, but it has been shown by Western blotting that Hev b 5 has a smeared profile in glove extracts [4, 5].

We had previously postulated that repeated sequences in Hev b 5 may contribute to the potency of Hev b 5 as an allergen. Using the mAbs, we now show that repeated sequences can serve as epitopes in Hev b 5. Two of the repeat epitopes for these mAbs are contiguous and the third appears to be cryptic. Given the surface area occupied by an antibody, steric hindrance would be expected to prevent antibody binding to both sites on the same molecule, thus for this epitope we could not show a functional multivalency. The data indicate, for these mAbs at least, that only a single epitope would be occupied by an antibody at a time. So while the protein has repeated epitopes, at least for these mAbs there does not appear to be any potential for cross-linking of the proteins because of the multivalency.

mAbs are being developed to help establish reproducible assays for measuring specific allergen levels in latex products. Two-site sandwich ELISAs are an excellent approach for these tests, and characterizing the epitopes recognized by mAbs is extremely important for this application. Attempts to use the mAbs reported here for 2-site ELISAs were not successful [5], apparently because they recognize the same epitope. In combination with a different Hev b 5 antibody, a sensitive 2-site ELISA could be produced. The advantage of using these mAbs may be that, because of the repeated epitopes, they would be a good choice to capture Hev b 5 in glove extracts where epitopes might get altered by the manufacturing process.

An interesting finding was the reduced solubility of Hev b 5 in water, perhaps explained by Hev b 5 acidic pI 3.5. This observation is important because some methodologies for extracting and quantifying allergen levels on latex-containing products use water as the extraction medium. Our data demonstrate that dissolving latex proteins in water reduces the Hev b 5 levels by 50%, indicating reduced solubility. Thus, PBS pH 7.4 or carbonate buffer pH 8.1 should be used because extraction with water will result in a significant underestimate of the level of allergens in the product.

In conclusion, we identified an immunodominant B-cell epitope in Hev b 5 that is repeated 3 times within the sequence, making Hev b 5 multivalent. Repeated epitopes may help to explain the potent nature of this allergen.

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References

- 1 Akasawa A, Hsieh LS, Martin BM, Liu T, Lin Y: A novel acidic allergen, Hev b 5, in latex. Purification, cloning and characterization. *J Biol Chem* 1996;271:25389–25393.
- 2 Slater JE, Vedvick T, Arthur-Smith A, Trybul DE, Kekwick RGO: Identification, cloning, and sequence of a major allergen (Hev b 5) from natural rubber latex (*Hevea brasiliensis*). *J Biol Chem* 1996;271:25394–25399.
- 3 Fowler MR, Gartland J, Norton W, Slater A, Elliott MC, Scott NW: RS2: A sugar beet gene related to the latex allergen Hev b 5 family. *J Exp Bot* 2000;51:2125–2126.
- 4 Beezhold DH, Hickey VL, Slater JE, Sussman GL: Human IgE-binding epitopes of the latex allergen Hev b 5. *J Allergy Clin Immunol* 1999; 103:1166–1172.
- 5 Sutherland MF, Drew A, Rolland JM, Slater JE, Supioglu C, O'Hehir RE: Specific monoclonal antibodies and human immunoglobulin E show that Hev b 5 is an abundant allergen in high protein powdered latex gloves. *Clin Exp Allergy* 2002;32:583–589.
- 6 Yip L, Hickey V, Wagner B, Liss G, Slater J, Breiteneder H, Sussman G, Beezhold D: Skin prick test reactivity to recombinant latex allergens. *Int Arch Allergy Immunol* 2000;121:292–299.
- 7 Bernstein DI, Biagini RE, Karnani R, Hamilton R, Murphy K, Bernstein C, Arif SA, Berendts B, Yeang HY: In vivo sensitization to purified *Hevea brasiliensis* proteins in health care workers sensitized to natural rubber latex. *J Allergy Clin Immunol* 2003;111:610–616.
- 8 Slater JE, Paupore EJ, O'Hehir RE: Murine B-cell and T-cell epitopes of the allergen Hev b 5 from natural rubber latex. *Mol Immunol* 1999; 36:135–143.
- 9 de Silva HD, Sutherland MF, Suphioglu C, McLellan SC, Slater JE, Rolland JM, O'Hehir RE: Human T-cell epitopes of the latex allergen Hev b 5 in health care workers. *J Allergy Clin Immunol* 2000;105:1017–1024.
- 10 Beezhold DH, Hickey VL, Sussman GL: Mutational analysis of the IgE epitopes in the latex allergen Hev b 5. *J Allergy Clin Immunol* 2001; 107:1069–1076.
- 11 Aalberse RC: Structural biology of allergens. *J Allergy Clin Immunol* 2000;106:228–238.
- 12 Aalberse RC, Akkerdaas JH, Van Ree R: Cross-reactivity of IgE antibodies to allergens. *Allergy* 2001;56:478–490.
- 13 Padlan EA: X-ray crystallography of antibodies. *Adv Protein Chem* 1996;49:57–133.