

Analysis of T-cell reactive regions and HLA-DR4 binding motifs on the latex allergen Hev b 1 (rubber elongation factor)

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Summary

Background The rubber elongation factor in *Hevea* rubber (Hev b 1) is one of the important latex-allergenic proteins inducing the production of specific IgE antibodies in latex-exposed subjects. In addition, Hev b 1 induces lymphocyte proliferation responses. A study about detailed T-cell epitope analysis of major latex allergens has not been published yet.

Objective The aim of this study was to define the T-cell reactive regions on the latex allergen Hev b 1.

Methods Nine overlapping peptides with 19 or 17 amino acid lengths representing the complete sequence of Hev b 1 were used for T-cell epitope mapping. Peripheral blood mononuclear cells (PBMCs) of latex-sensitized patients and healthy subjects were isolated and stimulated with the synthetic peptides to determine the specific proliferation response. The examined patients were sensitized to latex by occupational exposure ($n=14$) and suffered from rhinitis, conjunctivitis, contact urticaria and/or asthma. Two control groups of non-sensitized subjects were studied, one occupationally exposed to latex ($n=4$), the other one not exposed to latex ($n=4$).

Results Positive proliferation response induced by one or more peptides was detected in 10 of the latex-sensitized patients and in two of the latex-exposed non-sensitized subjects. Each patient exhibited an individual epitope reactivity pattern. The whole protein was found to be immunogenic, but the intensity of lymphocyte responses induced by the peptides was different. The majority of the patients' PBMCs (more than 66%) responded to the peptides 31–49 and 91–109. Using the peptide selection of Stille's algorithms, three epitopes were predicted covering region 38–74 and two others covering region 82–104; the two peptides 31–49 and 91–109 are part of these regions. A strong HLA-DR4Dw4 (DRB1*0401)-binding motif according to published DR4w4 binding motifs was predicted in the region of 102–110 including peptide 91–109 to which the majority of tested HLA-DR4-positive patients responded.

Conclusions Our data indicate that the whole 14 kDa protein Hev b 1 is immunogenic having regions that demonstrate individual and varying PBMC stimulation. Experimental data of T-cell reactive regions based on PBMC-stimulation complement the information on T-cell epitope prediction. In addition, the Hev b 1 molecule contains a HLA-DR4Dw4 (DRB1*0401)-binding motif.

Keywords: latex allergy, Hev b 1, T-cell reactive regions, epitope mapping, epitope predictions, rubber elongation factor, lymphocyte stimulation, HLA-binding motif

Clinical and Experimental Allergy, Vol. 28, pp. 339–348. Submitted 17 April 1997; revised 23 June 1997; accepted 2 October 1997.

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Introduction

During the last decade, latex allergy has developed into a factor of great importance worldwide for the diagnosis of

Table 1. Latex-sensitized patients

Patient	total IgE (kU/l)	Latex-specific IgE		Hev b 1-specific IgE		Symptoms
		(kU/l)	RAST class	(kU/l)	RAST class	
Pa1	63	3.03	2	0.08	0	U + R + A
Pa2	28	5.47	3	0.32	0	U + R + C + A
Pa3	93	13.00	3	0.27	0	U + R + C + A
Pa4	25	3.24	2	0.39	1	U + R + C + A
Pa5	> 2000	58.30	5	0.79	2	U + R + C + A
Pa6	78	11.29	3	1.31	2	U + R + C + A
Pa7	41	9.08	3	0.23	0	U + R + C + A
Pa8	450	39.10	4	1.76	2	A
Pa9	35	4.27	3	0.09	0	R + A
Pa10	47	5.76	3	0.11	0	U + R + C + A
Pa11	708	6.78	3	0.39	1	U + R + A
Pa12	748	100.10	6	3.40	2	U + R + C + A
Pa13	> 2000	96.90	5	0.41	1	U + R + C + A
Pa14	410	8.50	3	0.67	1	U + R + C

U = urticaria, R = rhinitis, C = conjunctivitis, A = asthmatic complaints and/or asthma

intolerance reactions to gloves, occupational asthma and anaphylactic conditions. Prolonged exposure to latex allergen can result in sensitization involving specific IgE antibodies and subsequently the development of clinically manifested latex allergy [1]. The responsible allergens are latex proteins found in raw latex and in various latex-made products. Latex proteins with allergenic potential include hevein [2], hevein preprotein (prohevein) [3], hevamine [4] and the rubber elongation factor REF [5]. According to the allergen nomenclature designated as Hev b 1, REF is an enzyme thought to be important in the biosynthesis of rubber. Furthermore it has been shown to be one of the important latex allergens. Hev b 1 induced the production of specific IgE-antibodies in latex type I hypersensitivity [5,6] in spina bifida patients and health care workers. Based on the knowledge that allergen-specific IgE production is under the strict control of allergen-specific CD4⁺ T cells through the orchestration of regulatory cytokine production [7], we studied the T-cell mediated immune response to latex allergens, especially Hev b 1. In a previous study [8], we demonstrated that latex sap and latex glove extract as well as Hev b 1 induced lymphocyte proliferation responses in latex-sensitized patients and in a subgroup of latex-exposed subjects. Induction of CD4⁺ T cell response required molecular interactions involving the T-cell receptor (TCR), a class II major histocompatibility complex (MHC) molecule and the peptide composed of 13–25 amino acid residues with critical binding motifs. In contrast to MHC class I peptides, class II-binding peptides exhibited a significantly broader heterogeneity [9]. Accumulation of

analytic data on class II-binding peptides as well as the crystallography of HLA molecules [10,11] have provided a clue for predicting probable epitope peptides from the primary sequences of antigen molecules [12–14]. Investigations of our group concerning the relationship between HLA class II alleles and the IgE-specific immune response demonstrated only an elevated phenotype frequency of HLA-DRB1*0701 (DR7) in spina bifida patients with Hev b 1-specific IgE antibodies (40%) in comparison with unexposed controls (17%) [15]. In Hev b 1-positive health care workers, the phenotype frequencies of DR1 and DR4 were slightly elevated in comparison with unexposed controls (DR1: 28% vs. 17%; DR4: 41% vs 24%) [16].

As a part of our efforts to study the possible pathomechanisms of latex type I allergy, we investigated the T-cell reactive regions on Hev b 1 and compared the experimental data of the peptide-induced proliferation with T-cell epitope prediction based on different algorithms and determined HLA-DR4Dw4-peptide binding motifs. Based on the primary sequence of Hev b 1 (137 aa residues), nine overlapping peptides with 19 aa length representing the complete aa-sequence were used for the determination of T-cell reactive regions.

Materials and methods

Subjects

Three groups of subjects were investigated: Group 1 (Table 1) consisted of 14 latex-sensitized hospital employees

(Pa1-Pa14), especially health care workers and physicians, aged between 20 and 50 years (eight women and six men). All of them were diagnosed as having latex-related allergic symptoms like urticaria, rhinitis, conjunctivitis and/or asthma. None of the patients was on immunotherapy and/or corticosteroid therapy. All 14 subjects had positive latex skin-prick test results and latex-related specific IgE antibodies (≥ 0.35 kU/L; measured by CAP-system). In addition, eight subjects (57%) also had positive specific IgE values (≥ 0.35 kU/L) when tested with the isolated latex allergen Hev b 1 by EAST (Table 1). Eight of the 14 latex-sensitized patients (Pa1, Pa5, Pa7, Pa9, Pa11, Pa12, Pa13, Pa14) were regarded as atopics due to their case history and positive skin-prick test reaction to at least two common allergens. In sera of seven patients (Pa1, Pa2, Pa4, Pa5, Pa9, Pa10, Pa12) IgE antibodies to hevein, another important latex allergen, were detectable. Moreover, in four of these sera (Pa1, Pa2, Pa9, Pa10) no Hev b 1-specific IgE was measurable. Total serum IgE levels of patients ranged from 25 to above 2000 kU/L.

Group 2 consisted of four healthy subjects (E1-E4) aged between 28 and 42 years (four women). They were occupationally exposed to latex (especially surgical gloves), due to their professions as health care workers and technicians in research laboratories. They had no latex-related respiratory or cutaneous symptoms. Latex-specific IgE CAP results as well as Hev b 1-specific IgE EAST results were negative (< 0.35 kU/L). Total serum IgE levels ranged from 21 to 368 kU/L.

Group 3 was composed of four healthy subjects not occupationally exposed to latex (C1-C4), aged between 41 and 61 years (three men and one woman). They had no symptoms of hypersensitivity and negative latex-CAP results. Total serum IgE levels varied from below 12–442 kU/L.

Detection of total and latex-specific IgE antibodies

All sera of the subjects were tested for their concentration of specific IgE to latex allergens by the CAP system (Pharmacia, Uppsala, Sweden). The results were expressed as kU/L according to the standard curve by Pharmacia. Specific IgE values to Hev b 1 were measured using an enzyme-linked allergosorbent test (EAST) with Hev b 1 coupled to CNBr-activated paper disks in our laboratory.

Total IgE concentration in sera of the 22 subjects was determined by a Phadebas IgE PRIST (Pharmacia) with a polyclonal rabbit anti-IgE antibody.

Peptides

Nine overlapping peptides covering the whole Hev b 1 sequence (eight 19-mer and one 17-mer and designated as 1–19, 16–34, 31–49, 46–64, 61–79, 76–94, 91–109, 106–124, 121–137) were synthesized stepwise on an Applied Biosystem 432 A peptide synthesizer using Fmoc strategy as

previously described [6]. Peptides were analysed for their purity by reversed-phase HPLC (C18 column: 4×250 mm) and identified by IonSpray mass spectroscopy on an API III triple-quadrupole mass spectrometer with an IonSpray interface (Sciex, Toronto, Canada). When necessary, the peptides were purified by reversed-phase HPLC on a silica bead (18 column: 20×250 mm) to ensure that their purity was over 75% as determined by analytical RP-HPLC. Preparation and purity of Hev b 1 were described in detail in a previous publication [8].

Prediction of T-cell epitopes and HLA-DR binding motifs

The computer program EPIPLOT designed by Menéndez-Arias & Rodríguez [17] was used for T-cell epitope prediction based on different algorithms. Potential HLA-DR4Dw4 restricted T-cell epitopes were predicted according to published DR4w4-binding motifs (reviewed in [18]), using the computer program PROTSHELL [19] (the program PROTSHELL is available from Professor Dr Claude Muller; Laboratoire National de Santé, Boite Postale 1102, L-1011 Luxembourg).

Allergen- and peptide-specific lymphocyte proliferation

For peptide-driven proliferation, PBMCs were separated by Ficoll-Hypaque gradient centrifugation and adjusted to 1×10^6 /mL in RPMI-1640 (Gibco, Eggenstein, Germany) conditioned medium supplemented with glutamine, penicillin/streptomycin and 10% heat-inactivated pooled human AB-sera (Bavarian Red Cross, Munich, Germany). The cultures of PBMCs were set up in six replicates in 96-well round-bottom trays in a 200 μ L volume generally at a concentration of 5×10^5 cells/mL. The allergen Hev b 1 or the Hev b 1-peptide was added to the wells at the beginning of the cultures that were performed in a humidified atmosphere at 37°C containing 5% CO₂ for 5 days. The optimal antigen concentrations were determined by dose-related proliferation experiments, and the final protein concentrations were 10 μ g/mL for Hev b 1 (according to [8]) and 7.5 μ g/mL for Hev b 1-peptides (data not shown). Negative controls of cells alone in the presence of medium (RPMI-1640 medium) and positive controls of cells incubated with phytohemagglutinin (PHA; 7.5 μ g/mL) were included in each assay. For the final 12 h, 37 kBq of ³[H]-labelled thymidine-methyl were added to each well, and incorporated radioactivity was assessed by liquid scintillation spectrometry.

Because background proliferation (proliferation only in the presence of RPMI) varied significantly among individuals, responses to the complete allergen Hev b 1 were expressed as stimulation index (SI) calculated as the ratio of the mean cpm (counts per minute) obtained in the six similar cultures with allergens and that one obtained in the allergen-free culture (RPMI-control). We tentatively set cut-off lines determining

Table 2. HLA-D-specificities and DR4-subtypes

Patient	HLA-D-specificities	DR4-subtypes
Pa1	DR4, DR6, DR52a, DR53, DQ6, DQ8	DRB1*0401
Pa2	DR4, DR6, DR52c, DR53, DQ6, DQ8	DRB1*0404
Pa3	n.d.	—
Pa4	DR4, DR6, DR52b, DR53, DQ6, DQ8	DRB1*0401
Pa5	DR4, DR6, DR52b, DR53, DQ6, DQ8	DRB1*0405
Pa6	DR11, DR52b, DQ7	—
Pa7	DR4, DR6, DR52a, DR53, DQ6, DQ8	DRB1*0401
Pa8	DR1, DR7, DR53, DQ2, DQ5	—
Pa9	DR3, DR4, DR52a, DR53, DQ2, DQ8	DRB1*0401
Pa10	DR1, DR4, DR53, DQ5, DQ8	DRB1*0413
Pa11	DR6, DR52a, DR52b, DQ6, DQ7	—
Pa12	DR4, DR53, DQ7, DQ8	DRB1*0401
Pa13	DR3, DR8, DR52a, DQ2	—
Pa14	DR1, DR15, DR51a, DQ5, DQ6	—

positive and negative responses at SI = 2.5 for Hev b 1-driven responses [8]. Results of the Hev b 1 peptide-induced responses were presented as mean of the six replicates in absolute counts per minute (mean $\pm$ SEM, $n = 6$).

HLA-class II typing

In 13 of the latex-sensitized patients (group 1), HLA-class II-typing was performed (Table 2). Genomic DNA was extracted from frozen white blood cells using the QIAamp blood kit (Qiagen; Germany) or the DNA Easy-Prep kit (Lifecodes, Belgium). DNA typing was performed by PCR amplification and the use of sequence-specific oligonucleotide probes (SSOPs) end-labelled with DIG-11-ddUTP according to the methods previously described [20, 21]. These methods enable the identification of the broad DRB and DQB alleles equivalent to the serological specification and in some cases a further subdivision. DR4 subtyping was carried out with the Dynal DRB1*04 PCR-SSP subtyping kit allowing the determination of alleles DRB1*0401-DRB1*0423.

Statistics

Quantitative data were expressed as the mean values $\pm$ standard error of mean (SEM). Wherever relevant, significant differences between peptide-induced proliferation responses and the medium control for each individual were calculated with the unpaired Student's *t*-test, and *p*-values of <0.05 were considered significant.

Results

Lymphocyte proliferation response to Hev b 1

Peripheral blood mononuclear cells (PBMCs) from 14

sensitized patients (group 1), from four non-sensitized but latex-exposed subjects (group 2) and from four non-sensitized subjects without occupational exposure to latex (group 3) were stimulated with the purified latex-allergen Hev b 1 (Fig. 1). Nine of the 14 latex-sensitized patients (64%) showed pronounced proliferation responses (SI ≥ 2.5 ; as described in a previous publication [8]), only four of these patients had Hev b 1-specific IgE antibodies in sera (Table 1). In addition, PBMCs of two out of four latex-exposed subjects (E1- E4; group 2) proliferated in the presence of Hev b 1. Lymphocytes of healthy subjects without occupational latex exposure (C1-C4; group 3) did not show proliferation responses induced by Hev b 1.

To evaluate the specificity of lymphocyte activation we determined the expression of CD25 (IL-2 receptor) and HLA-DR both on T cells measured by flow cytometry. Incubation of PBMCs of four patients for 5 days with Hev b 1 (10 μ g/mL) increased the expression of CD25 compared with PBMCs incubated only in the presence of RPMI (Hev b 1: $6.84 \pm 0.33\%$; RPMI: $3.25 \pm 0.18\%$; $n = 4$, mean $\pm$ SEM; $P < 0.01$). In addition, the expression of HLA-DR on CD3-positive lymphocytes was elevated, too (Hev b 1: $18.0 \pm 0.75\%$; RPMI: $12.04 \pm 0.67\%$; $n = 4$; mean $\pm$ SEM; $P < 0.05$). We compared these results with data obtained with Hev b 1-stimulated PBMCs of two non-latex exposed normal donors. PBMCs of these control subjects did not show increased CD25 as well as HLA-DR expression after stimulation with Hev b 1 (data not shown).

Lymphocyte proliferation to Hev b 1 peptides

PBMCs from 14 sensitized patients (group 1) and eight from the control groups (four of group 2 and four of group 3) were incubated with the nine overlapping peptides covering the complete sequence of the Hev b 1 molecule. Lymphocytes of healthy subjects without occupational latex exposure (group 3) did not show any significant proliferation response to the individual peptide (no significant differences between medium values and culturing in the presence of peptides). Positive proliferation response induced by one peptide or several ones (significant difference between the mean of the cpm-values of the medium control and cultures in the presence of peptides) was detected in 10 of the latex-sensitized patients and in two of the latex-exposed non-sensitized subjects (E2, E4) (Fig. 2). With the exception of one patient (Pa7), all of the PBMCs with positive proliferation to the peptides were also stimulated with Hev b 1. The results (Fig. 2A + B) demonstrate each patient to have an individual peptide reactivity pattern.

To prove the peptide-specific T-cell responses we incubated PBMCs of two patients (Pa4 and Pa6) and a control subject (C3) with the peptides 1–19 and 91–109 and RPMI for 5 days and measured the expression of CD25 by flow

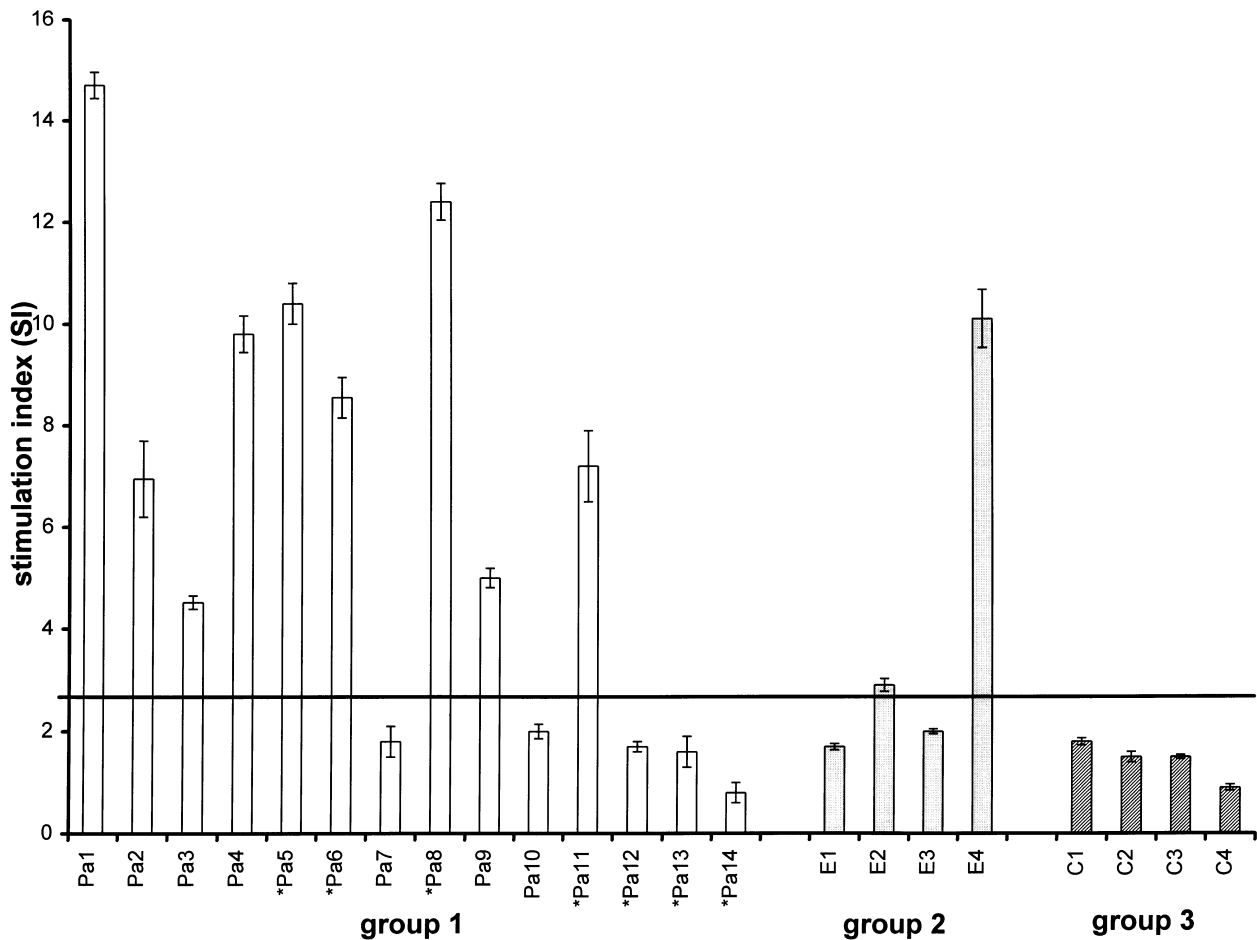


Fig. 1. PBMC proliferation responses induced by *Hev b 1*. Stimulation index values (SI) of each subject are presented. Each value is expressed as the mean of at least six parallel determinations. The cut-off line for positive responses induced by the whole molecule *Hev b 1* was SI = 2.5. Asterisks indicate positive specific IgE values to *Hev b 1*. Group 1: latex-sensitized patients; group 2: occupationally latex-exposed but nonsensitized subjects; group 3: healthy control individuals without occupational latex exposure.

cytometry. In both patients only the PBMCs incubated with the peptide 91–109 showed an increased expression of CD25 on CD3-positive lymphocytes compared with the peptide 1–19 or RPMI (Pa4: 5.8% vs 3.7% or 3.9%, respectively; Pa6: 6.23% vs 4.5% or 4.7%, respectively). T cells of the control subject did not show any increased CD25 expression after incubation with the peptides. According to the flow cytometric results we have an additional hint that the proliferation responses measured are allergen- or peptide-specific.

Summarizing the individual results and calculating the percentage of the reacting patients ($n = 12$), the whole protein was found to be immunogenic (Fig. 3). But the intensity of lymphocyte responses induced by the peptides varied. The majority of patients' lymphocytes (more than 66%) responded to the peptides 31–49 and 91–109. In contrast, the peptide 1–19 induced only in the PBMCs of three patients (25%) a positive proliferation response.

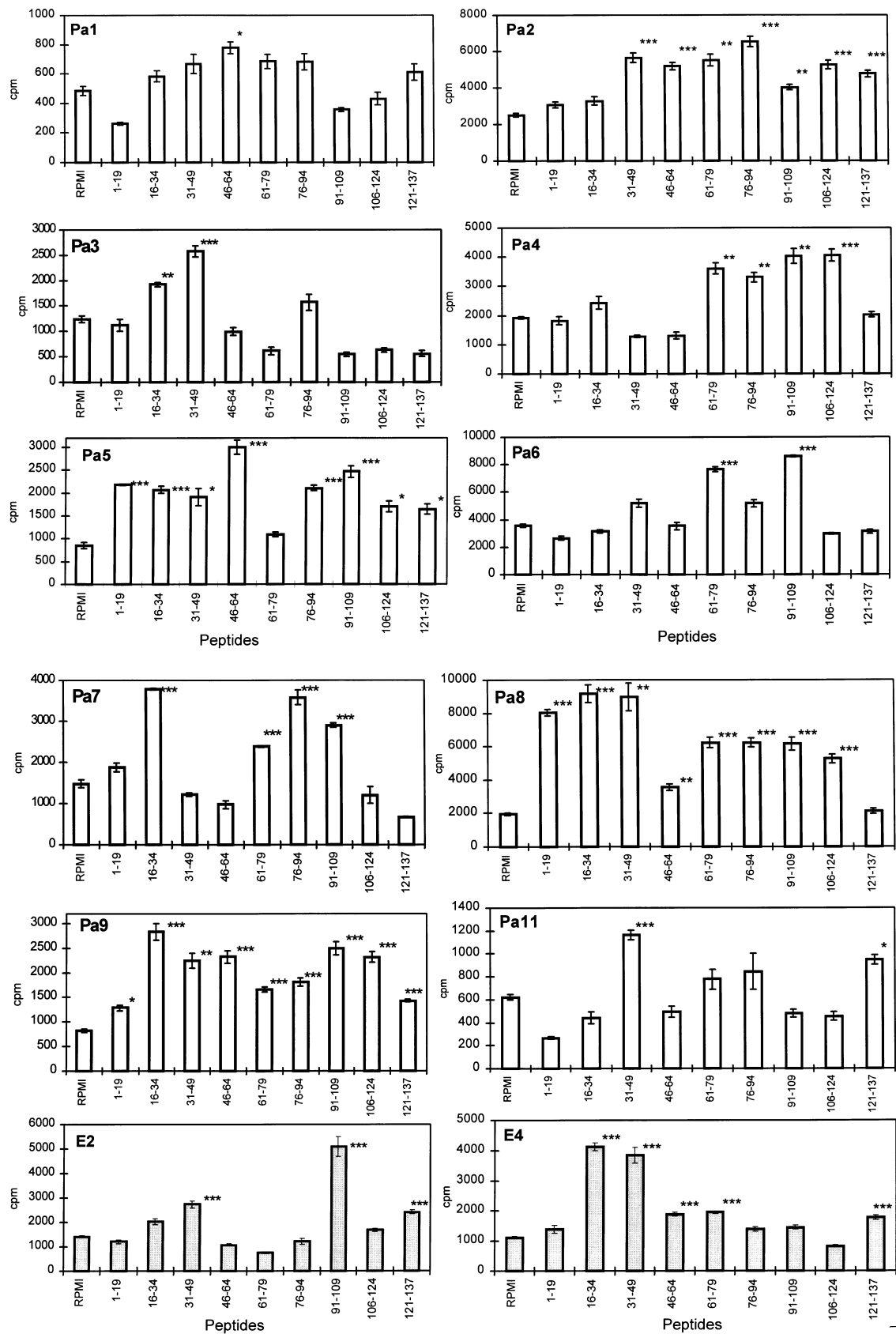
From nine of these subjects (9/12) responding to one or

more of *Hev b 1* peptide, data of HLA-specificities are available (Table 2). Six of them expressed particular HLA-DR4 (Pa1, Pa2, Pa4, Pa5, Pa7, Pa9); with the exception of one patient (Pa1; PBMCs responded only in the presence of peptide 46–64), PBMCs of these patients responded to more than four peptides, predominately in the region of 61–109.

*Comparison of experimental data with T-cell epitope predictions and MHC II peptide binding motifs of *Hev b 1**

For T-cell epitope predictions, the computer program EPI-*PLOT* [17] was used.

Five amino acid sequences (38–56, 45–63, 56–74, 82–100, 104–122) were predicted as T-cell epitope in the *Hev b 1* molecule using the peptide selection of Stille's algorithm [22] which was based upon the calculation of strip-of-helix hydrophobicity (Fig. 3). These predicted epitopes included



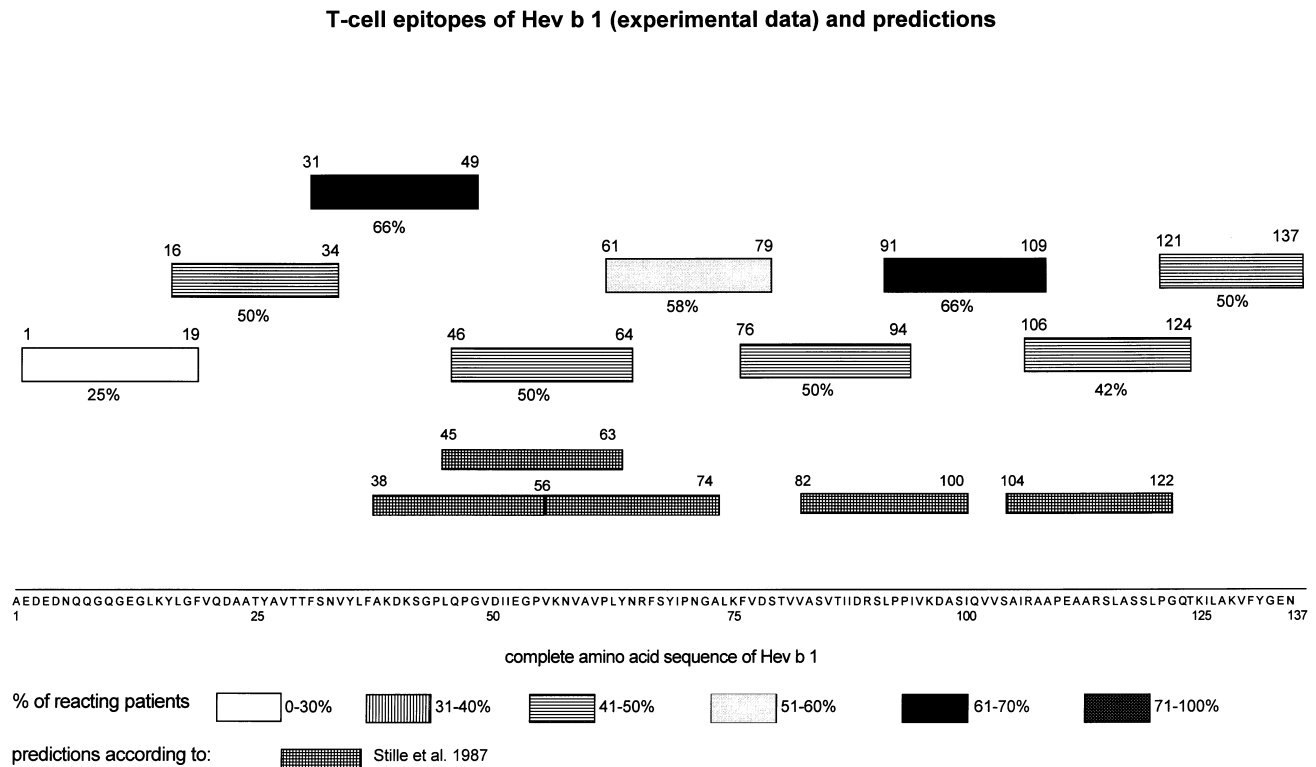


Fig. 3. T-cell epitope of Hev b 1 – summary of the experimental data and the predicted T-cell epitopes. The percentage of patients (100% = 12 subjects) reacting to one or more peptide was calculated. The different intensity of response is indicated by the bars with different patterns. T-cell epitopes of Hev b 1 were predicted according to the algorithms of Stille *et al.* [22].

regions in the Hev b 1 molecules with pronounced cellular proliferation response (more than 50%) 31–49, 61–79 and 91–109.

According to the approach of Hammer, Sinigaglia and colleagues [23,24] (summarized by Rammensee [18,25,26]), potential HLA-DR4Dw4 restricted T-cell epitopes were predicted to published DR4Dw4-binding motifs using the computer program PROTSHELL [19]. Using a stringent level of amino acid composition for the anchor positions P1, P4, P6 and P9 (Table 3), only one HLA-DR4Dw4 (HLA-DRB1*0401) binding motif was found in the region 102–110. Calculating with less stringent criteria (e.g. P2 is variable), HLA-DR4Dw4 binding motifs were also found in the regions 19–27, 77–85, 37–45 and 51–60. The best HLA-DR4Dw4 binding motive (102–110) was part of a peptide sequence inducing proliferation in 66% of

the reacting individuals, whereas the latter four binding motifs were predicted in peptides which were able to induce proliferation in 50% of the reacting donors.

Discussion

In addition to our previous study [8], we demonstrated that Hev b 1, the 14 kDa protein (REF), induced lymphocyte proliferation responses in 64% (9/14) of latex-sensitized patients and in two of four occupationally latex-exposed subjects. In most of these cases, the cellular response of the patients to Hev b 1 was independent of the presence of specific Hev b 1 IgE antibodies. Stimulation of PBMCs of latex-sensitized patients and non-latex-allergic subjects with the overlapping peptides induced a significant proliferation response in 10 out of 14 latex-sensitized patients and in two

Fig. 2. PBMC proliferation responses induced by nine peptides covering the complete sequence of Hev b 1. The individual proliferation responses of 10 latex-sensitized patients (group 1) and two latex-exposed non-sensitized subjects are shown (for group definitions, see Fig. 1). White bars: proliferation responses of group 1 patients; grey bars: proliferation responses of two subjects of group 2 (E2, E4). The mean cpm-values of six replicate wells and SEM are expressed. Asterisks indicate significant differences between the mean maximal counts of wells that contained the peptides and the wells with RPMI for each individual (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$).

Table 3. HLA-DR4Dw4 (DRB1*0401) motifs and T-cell epitopes of Hev b1

	Relative Position ¹ (P1 xx P4 x P6 xx P9)			
	P1	P2	P3	P4
1) stringent conditions	FYWILV	MAVLQ (no R, K)	TSVAG	AGSTQN (polar, aliphatic)
T-cell epitopes ² :	Hev b 1 (102–110):	IQVVSIRA		
2) less stringent conditions	FYWILY	variable	TSVAG	GASTNQA (MVLHPKPY)
T-cell epitopes:	Hev b 1 (19–27)	FVQDAATYA		
	Hev b 1 (77–85):	FVDSTVVAS		
	Hev b 1 (37–45):	FAKDKSKPL		
	Hev b 1 (51–59):	IIEGPVKNV		

¹ Anchor positions (P1, P4, P6 and P9) and main residues (marked in bold type) in HLA-DR4Dw4 (DRB1*0401) ligands are shown (according to Rammensee [18,25] and Handtmann [19]). The first anchor is set as relative position 1.

² Epitopes are aligned to the respective motifs. Aligned anchor residues are in bold type.

out of the four latex-exposed non-sensitized subjects. Peptides were classified as being stimulatory if the mean of the cpm-values in the presence of the peptide was significantly different from the mean of the cpm-values in the medium-control. The cpm-values obtained by peptide induced proliferation were low compared of those of the whole allergen molecule Hev b 1 or of other T cell epitope studies using PBMCs and, e.g. overlapping fragments of Der p 1 [27]. However, in more than 65% of our positive peptide proliferation tests, the SI-values were above 2.0. In addition, it has to be considered that we used eight 19mer and one 17mer peptides; each of them presented only a small part of the 137 amino acid sequence covering the whole Hev b 1 molecule. In contrast, O'Brien *et al.* [27] used overlapping recombinant fragments of Der p I with a length of 39–114 amino acids; it might be possible that these fragments contained more than one T-cell reactive region leading to much higher proliferation rates.

In the 12 subjects with positive peptide proliferation, the responses were induced by one or more peptides. We could show that the peptide reactivity patterns are different from donor to donor, but all nine peptides were stimulatory in at least one of the individuals. This indicates that multiple T-cell epitopes located in each region of the entire Hev b 1 molecule are capable of stimulating T cells from both allergic and non-allergic individuals. We provided evidence that PBMCs of healthy but latex-exposed individuals responding to Hev b 1 recognized the same T-cell reactive regions as latex-sensitized patients. Comparable results were described by Ebner *et al.* [28] for the Bet v 1 (major birch pollen allergen). This study was done with allergen-specific T-cell lines and T-cell clones demonstrating that nonallergic individuals recognize the same T-cell epitopes

of the allergen as atopic patients. Differences in the cytokine profile (IL-4 vs IFN γ) could be observed. The possibility that multiple T-cell reactive regions may be present in allergenic proteins like Hev b 1 is also suggested for other allergens, e.g. Der p 1 [29,30], Chi t 1.01 [31], Bet v 1 [32] and phospholipase [33]. Furthermore, we observed that the intensity of lymphocyte responses induced by the different peptides varied, but the majority of individuals examined here (66%) responded to peptides covering the regions 31–49 and 91–109. Only minor proliferation response could be detected by incubating PBMCs with the peptide 1–19.

We predicted T-cell epitopes of Hev b 1 by the algorithm proposed by Rothbard and Taylor [14], Margalit *et al.* [13] (data not shown) and Stille *et al.* [22]. The comparison of predicted Hev b 1 T-cell epitopes with the experimental data showed a lack of conformity, especially with the prediction by Rothbard [14] and Margalit [13]; these algorithms were not better than random. In agreement with the findings of Deavin *et al.* [34] that the strip-of-hydrophobic helix algorithm of Stille *et al.* [22] was the only significant predictor against human data set, we found predicted Hev b 1-T-cell epitopes in Hev b 1 using Stille's algorithm [22] which were part of the peptides inducing proliferation response in the majority of tested individuals. Our comparison of prediction and experimental data for Hev b 1 supports the statement of Deavin *et al.* [34] that the algorithm of Stille *et al.* [22] was better than random.

Advances in the field of antigen presentation led to a general consensus that epitope predictions ought to be based on a molecular understanding of the events that determine antigen presentation, e.g. antigen processing and MHC-binding [35]. This knowledge could be useful for a precise prediction of T-cell epitopes. Both pool sequencing and the screening of phage libraries have been used for the

identification of several allele-specific class II binding motives. A typical class II ligand consists of 12–25 aa, with the groove-contacting stretch roughly in the middle [26]. X-ray crystallographic data for HLA-DR1 [10,11], and detailed studies by Hammer [12,23] and Sinigaglia [24] demonstrated that ligand residues at P1, P4, P6 and P9 act as anchors for the HLA-DR1 and HLA-DR4Dw4 motifs.

According to our finding that cells of six of our donors responding to one or more Hev b 1 peptides express particular HLA-DR4, we determined the HLA-DR4Dw4 (DRB1*0401) binding motifs in the Hev b 1 molecule. Using a stringent level of aa composition for the four anchor positions, an HLA-DR4Dw4 (DRB1*0401) binding motive could only be detected in the region 102–110. According to our experimental data that the peptide 91–109 was a good inducer of lymphocyte proliferation there seemed to be a conformity between the predicted HLA-DR4Dw4 (DR1*0401) binding motif and the detected T-cell epitope. HLA-DR4Dw4 (DRB1*0401) binding motifs calculated with less stringent criteria were found in regions covered by aa-sequences with pronounced proliferation induction activity (in cells of 50% or more of the tested individuals proliferative responses were measured). For verification, a detailed MHC-peptide binding study will be necessary.

However, HLA-DR4Dw4 (DRB1*0401) binding motives are necessary, but it has to be considered that peptides must also be recognized by T-cell receptors. This may be a possible reason for our observation that two of our latex-sensitized patients who expressed HLA-DR4 did not respond to Hev b 1 or an individual Hev b 1 peptide. In addition to MHC-binding, other factors are important for the induction of T cell proliferation, e.g. T cell receptor recognition and/or various co-stimulatory molecules. Otherwise, it has to be considered that other allergenic proteins, e.g. hevein, may also induce proliferation responses. This has to be proved. Considering these aspects, the prediction of T-cell epitopes based on their ability to bind to MHC molecules may be insufficient. More direct approaches should be used for the localization of T-cell epitopes in allergens like Hev b 1.

In conclusion, our data define regions in the Hev b 1 molecule which are capable of inducing lymphocyte responses in the majority of latex-exposed and latex-sensitized individuals, respectively. Moreover, the Hev b 1 molecule contains an HLA-DR4Dw4 (DRB1*0401) binding motive. The ability to define 'immunodominant' and 'disease-related' T-cell-reactive regions is of potential practical value in the design of immunotherapeutics.

Acknowledgements

We thank Gerda Borowitzki, Maria Düser, Claudia Krekel

and Andrea Möller for their excellent technical assistance and Helga Urban for typing the manuscript. The work was supported by the HVBG (Central federation of the statutory accident insurance institutions in industry and trade), St. Augustin, Germany.

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