

Human T-cell epitopes of the latex allergen Hev b 5 in health care workers

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Background: Latex allergy affects health care workers as a high-risk cohort. Hev b 5 is a major latex allergen reacting with serum IgE from 92% of latex-allergic health care workers. Because CD4⁺ T-cell recognition is central to the specific immune response to allergens, identification of dominant T-cell epitopes is important for the development of specific immunotherapy for latex allergy.

Objective: Our purpose was to map T-cell epitopes of Hev b 5 in health care workers.

Methods: Six latex-allergic health care workers (grade 3 to 4 enzyme allergosorbent test score) were studied. Peripheral blood latex specific 3-week T-cell lines were generated and screened for proliferative response to overlapping 20-mer peptides of Hev b 5. Supernatants collected at 48 hours were analyzed by ELISA for IL-5 and IFN- γ .

Results: Dot immunoblotting with use of recombinant Hev b 5/maltose-binding protein indicated serum-specific IgE in 5 of 6 patients. T-cell reactivity to one or more Hev b 5 peptides was identified in these 5 donors, but not in the sixth. Hev b 5 (46-65) induced T-cell proliferation in all 5 donors. Hev b 5 (109-128) stimulated T cells from 3 of these patients. Proliferative responses were accompanied by substantial IL-5 secretion and minimal IFN- γ , indicating a T_H2-predominant cytokine profile.

Conclusions: Five of 6 latex-allergic patients demonstrated T-cell responsiveness to Hev b 5 consistent with a major T-cell reactive latex allergen. Two T-cell immunodominant regions of Hev b 5 were identified, and reactivity to these sites was associated with strong IL-5 but minimal IFN- γ production. (*J Allergy Clin Immunol* 2000;105:1017-24.)

Key words: T cells, latex, Hev b 5, peptides, T-cell epitopes, allergen, allergy

IgE-mediated hypersensitivity to latex has emerged as a serious occupational health problem since the introduction

Abbreviations used

AEU:	Allercoat enzyme allergosorbent units
APC:	Antigen-presenting cells
cpm:	Counts per minute
EAST:	Enzyme allergosorbent test
HCW:	Health care worker
³ HTdR:	Tritiated thymidine
LAL:	Low ammoniated latex
MBP:	Maltose-binding protein
rHev b 5/MBP:	Fusion protein of recombinant Hev b 5 and maltose-binding protein
T _H 2:	T helper cell type 2

of universal precautions in the mid-1980s.^{1,2} The use of latex gloves for barrier protection may lead to sensitization of health care workers (HCW) to protein allergens present in the natural rubber latex. The prevalence of latex sensitization among HCWs has been reported from 8.2% to 22%³⁻⁷ with a prevalence of up to 49% for children with spina bifida.⁸ Allergic reactions to latex range from urticaria, rhinoconjunctivitis, asthma,⁹ and angioedema to severe generalized anaphylaxis in some cases.¹⁰ Because the only form of treatment currently available is allergen avoidance and symptomatic relief, there is an urgent need for the development of specific immunotherapy for this condition. Many hospitals are developing a latex powder-free glove policy and replacing latex products with latex-free alternatives wherever possible to develop a "latex-safe" environment.¹¹ However, for some highly sensitized patients these measures remain insufficient to allow continued occupational exposure to latex products, even in the presence of a powder-free glove policy. The immunologic characterization of latex allergens is an important initial step in the development of specific immunotherapy regimens for latex allergy that are both safe and efficacious. Several allergens from the rubber plant *Hevea brasiliensis* have been identified.¹²⁻²⁰ On the basis of IgE binding studies, certain latex allergens seem to be preferentially recognized by particular risk groups (Hev b 1 and 3 by children with spina bifida and Hev b 5 and 6.02 by HCWs²¹). Hev b 5, a highly acidic and proline-rich protein with a predicted predominantly random secondary structure, has been shown to react with IgE from 92% of HCWs and 56% of spina bifida patients with latex allergy.¹⁸ Thus Hev b 5 has been identified as a major allergen in natural rubber latex and is a rational first target for specific immunotherapy.

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TABLE I. Clinical characteristics of latex-allergic patients

Patient No.	Age(y)	Sex	Clinical symptoms on contact with latex	Clinical food allergy	Clinical kiwi fruit allergy	Latex EAST score out of 4 (AEU/mL)	Kiwi EAST score out of 4 (AEU/mL)	Total IgE (IU/mL)
1	37	F	Urticaria, asthma, angioedema, anaphylaxis	Yes	No	4 (16.4)	0 (<0.18)	98
2	55	F	Urticaria, angioedema	Yes	Yes	3 (7.1)	0 (<0.18)	22
3	41	F	Urticaria, rhinitis, asthma, angioedema, anaphylaxis	Yes	Yes	3 (9.5)	2 (1.65)	291
4	65	F	Urticaria, rhinitis, asthma, angioedema, anaphylaxis	Yes	Yes	4 (>17.5)	0 (<0.18)	645
5	30	F	Urticaria, angioedema	Yes	Unknown	4 (>17.5)	0 (<0.18)	244
6	41	F	Urticaria, rhinitis, asthma, angioedema, anaphylaxis	Yes	Unknown	3 (9.5)	0/1 (0.23)	>1000

F, Female.

Interestingly, the amino acid sequence of Hev b 5 shows 46% identity to another acidic protein identified in kiwi fruit (*Actinidia deliciosa*)¹⁸ and this may provide a molecular explanation for the high frequency of fruit hypersensitivity seen in latex-allergic patients.²²

The production of allergen-specific IgE by B cells and release of inflammatory mediators by mast cells and eosinophils results in the effector response of allergic disease. However, it is well established that these events are orchestrated by allergen-specific CD4⁺ T cells with a T helper cell type 2 (T_H2) cytokine profile. T-cell reactive determinants have been reported for another major latex allergen Hev b 1²³ but not for Hev b 5. Thus characterization of the T-cell response to Hev b 5 is important as an initial step in the development of specific immunotherapy.

In this study we examined T-cell responses to overlapping peptides that span the entire length of the Hev b 5 molecule with use of latex-specific T-cell lines generated from the peripheral blood of latex-allergic HCWs.

METHODS

Subjects

Latex-allergic HCWs were recruited from the Alfred Hospital Allergy Clinic (with informed consent after approval by the Alfred Hospital Ethics Committee). All subjects had severe clinical symptoms of IgE-mediated latex hypersensitivity with confirmation of sensitization by a grade 3-4/4 score of latex-specific serum IgE (Table I) measured with the Kallestad Allercoat enzyme allergosorbent test (EAST) system (Sanofi-Pasteur Diagnostics). The latex allergen source used in the manufacture of the solid phase of the allergen is a pulverized nonpowdered commercial glove extract. The latex EAST score in nonallergic individuals is 0/4 and <0.18 Allercoat EAST units (AEU) per milliliter.

Antigens

Low-ammoniated latex. Low ammoniated latex (LAL) (Ansell) was centrifuged at 20,000 revolutions/min for 20 minutes at room temperature. The middle clear layer was collected, dialyzed against PBS overnight at 4°C, and filter sterilized, and the protein concentration was determined with use of the BCA protein assay kit with BSA as the standard (Pierce).

Hev b 5. A construct composed of the Hev b 5-encoding complementary DNA in the pMAL/c-2 vector was generated as part of a maltose binding protein (MBP) fusion protein (rHev b 5/MBP) and purified as described previously.¹⁸ As a control protein, MBP fused to the LacZ α protein was produced by expressing the pMAL/c-2 vector alone as described previously.¹⁸

Tetanus toxoid and PHA. These control antigens were purchased from Sigma.

Peptides. Synthetic peptides (20-mers overlapping by 11 or, for the 2 N-terminal peptides, 15 residues) spanning the entire length of the Hev b 5 molecule¹⁸ were purchased from Chiron Technologies (Clayton, Australia).

Immunoblotting

Patient serum IgE reactivity to latex allergens LAL (25 μ g), rHev b 5/MBP (7 μ g), and MBP (4.5 μ g) was analyzed by dot immunoblots according to our established protocols.²⁴ The amount of MBP was estimated to be equivalent to that in the rHev b 5/MBP aliquot.

Generation of latex-specific T-cell lines

Latex-specific T-cell lines were isolated with use of our well-established methods for the generation of allergen-specific oligoclonal T-cell populations.^{25,26} Briefly, PBMCs (2.5 $\times$ 10⁶/well) were separated from heparinized venous blood by density centrifugation and initially stimulated for 1 week with LAL at 20 μ g/mL in 24-well tissue culture plates (Costar) in complete medium (RPMI 1640, Gibco Life Technologies, supplemented with 2 mmol/L L-glutamine, 100 IU/mL penicillin-streptomycin, and 5% screened heat-inactivated human AB serum [Sigma]). After 7 days lymphoblasts (1 $\times$ 10⁶/well) were restimulated for 1 week with LAL at a concentration of 20 μ g/mL in the presence of an equal number of irradiated (3000 rad) autologous PBMCs as antigen-presenting cells (APC). On days 2 and 4 cultures were supplemented with Lymphocult-T (5% vol/vol, Biotest Folex) and fresh medium. At the end of the second week lymphoblasts were restimulated for 1 week with rHev b 5/MBP at a concentration of 20 μ g/mL as described above. Oligoclonal T cells were recovered, washed, and tested in proliferation and cytokine assays (as described below). We have previously shown that CD4⁺ T cells are preferentially expanded in these cultures.

Oligoclonal T-cell proliferation assays

Oligoclonal T-cell blasts (5 $\times$ 10⁴/well) from the 3-week cultures were incubated in 96-well round-bottom plates (Linbro, ICN Bio-

medicals) in triplicate with equal numbers of irradiated APCs in the presence of LAL at concentrations ranging from 0.03 to 100 $\mu\text{g/mL}$, rHev b 5/MBP (10 and 20 $\mu\text{g/mL}$), MBP (10 and 20 $\mu\text{g/mL}$), overlapping peptides of the Hev b 5 sequence (10 and 30 $\mu\text{g/mL}$), Lymphocult-T (10 IU/ml), or PHA (3 $\mu\text{g/mL}$). Cultures of T cells and APCs in the absence of antigen, T cells alone, and APCs alone in the absence of antigen were included as controls. After 72 hours cultures were pulsed with 1 μCi of tritiated thymidine ($^3\text{HTdR}$) (DuPont, NEN) and harvested 12 to 16 hours later. Proliferation as correlated with $^3\text{HTdR}$ incorporation was measured by liquid scintillation spectroscopy. Results are expressed as mean counts per minute (cpm) for triplicate cultures with SD ($\leq 20\%$ for all experiments).

Production of IL-5 and IFN- γ by latex-specific oligoclonal T cells

The secretion of IL-5 and IFN- γ by oligoclonal latex-specific T cells was determined by ELISA on culture supernatants. Representative stimulatory and nonstimulatory peptides were selected and T cells were cultured in triplicate with these peptides. At 48 hours 50 μL of supernatant was harvested from the cultures, and IL-5 and IFN- γ were assayed by ELISA with use of paired cytokine antibodies (from Pharmingen, Becton Dickinson and Endogen, respectively). For this, opaque flat-bottom ELISA plates (Nalgene, Nunc International) were coated with 30 μL of capture antibody at 2 $\mu\text{g/mL}$ in 0.1 mol/L sodium bicarbonate, pH 8.2, coating buffer overnight at 4°C . After being washed 3 times with PBS/0.5% Tween the wells were blocked with 3% BSA/PBS at room temperature for 2 hours. The plates were washed and 30 μL of pooled triplicate culture supernatants was added in duplicate. After incubation overnight at 4°C and washing, bound cytokine was revealed by incubation with 30 μL of biotinylated detecting antibody (1 $\mu\text{g/mL}$ for IL-5, 0.5 $\mu\text{g/mL}$ for IFN- γ) for 45 minutes at room temperature followed by streptavidin-biotinylated horseradish peroxidase (Amersham Pharmacia Biotech) at a 1:2000 dilution for 30 minutes and enhanced chemiluminescence substrate (NEN Life Science). Light emission was read immediately on a Lumicount microplate Glow Luminometer (Packard Instrument Company). A standard curve was run each time with known concentrations of standard cytokine samples, and the concentration of IL-5 and IFN- γ in the culture supernatants was calculated accordingly. The lower limit of detection of the IL-5 assay was 20 pg/mL and the lower limit of detection of the IFN- γ assay was 2 pg/mL.

RESULTS

Serum IgE response to Hev b 5

Sera from the latex-allergic HCWs were tested for IgE reactivity to latex allergens by dot immunoblotting with use of LAL and rHev b 5/MBP as antigens. MBP was included as a control to exclude the possibility that binding of patient sera to the fusion protein was the result of anti-MBP antibodies. All patients except patient 2 demonstrated IgE reactivity to LAL (Fig 1). Sera from patients 1, 2, 3, 4, and 5 showed IgE reactivity to rHev b 5/MBP, which was markedly stronger than that to MBP alone, indicating the presence of a B-cell response to Hev b 5 in these patients. Patient 6 showed IgE reactivity to LAL but not to the rHev b 5/MBP fusion protein, indicating that Hev b 5 was unlikely to be a significant allergen in this case.

T cell responses to latex allergens

In preliminary primary cultures of PBMCs, the optimal response to LAL was observed at 10 to 30 $\mu\text{g/mL}$ in all

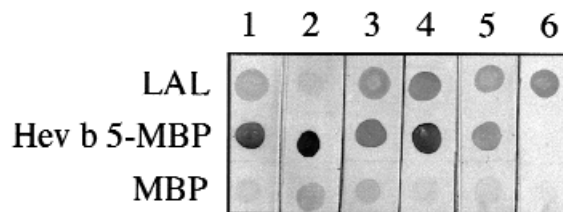


FIG 1. Dot immunoblot analysis of serum IgE reactivity with latex allergens for the latex-allergic patients 1 through 6.

cases (data not shown). The polyclonal responses to rHev b 5/MBP were considerably less than those to LAL. A similar response was observed to MBP alone, indicating the presence of MBP-specific T cells in the peripheral blood. Therefore, to enrich for latex-specific T cells while preventing the selective expansion of MBP-specific T cells, LAL at 20 $\mu\text{g/mL}$ was used for primary and secondary stimulations. Finally, Hev b 5-specific T cells were enriched by incubating the LAL-specific 2-week T-cell line with rHev b 5/MBP at 20 $\mu\text{g/mL}$ in the third antigen stimulation. The 3-week latex-specific T-cell lines from all 6 latex-allergic donors responded to LAL (Table II). The T-cell lines from patients 1 and 2 were also tested for their proliferative responses to rHev b 5/MBP and MBP alone. In contrast to the polyclonal responses, the oligoclonal responses to rHev b 5/MBP were significantly higher than those to MBP alone, indicating a selective expansion of Hev b 5-specific T cells. In fact, MBP responses were only at background levels. Mitogenicity and toxicity of latex antigens were excluded (data not shown).

Hev b 5 T-cell epitope mapping

The latex-specific 3-week T-cell lines were tested for proliferative responses to the Hev b 5 peptides. T-cell reactivity to one or more Hev b 5 peptides was identified in all 5 donors who demonstrated IgE binding to rHev b 5/MBP (Table II). Patient 6 failed to respond to any peptide (Table II). Hev b 5(46-65) induced T-cell proliferation in all 5 donors with IgE reactivity to rHev b 5/MBP (Table II). The stimulation index values observed for these responses were 5.9, 10.8, 5.0, 5.9, and 5.6. Additionally, peptide Hev b 5 (109-128) stimulated T cells from 3 of the 5 donors.

Production of IL-5 and IFN- γ by oligoclonal latex-specific T cells

IL-5 was the predominant cytokine produced by the latex-specific T-cell lines in response to stimulation with selected Hev b 5 peptides that caused T-cell proliferation (Table III). Peptides that failed to stimulate T-cell proliferation induced minimal or no cytokine production. Illustrative data from patient 4 is shown (Fig 2). Peptides Hev b 5 (37-56), (46-65), (55-74), (64-83), and (109-128) induced T-cell proliferation (stimulation index values greater than or equal to 2.5) and also secretion of IL-5, whereas peptides Hev b 5 (100-119) and (118-137) failed to stimulate either T-cell proliferation or cytokine secre-

TABLE II. Oligoclonal proliferative responses to Hev b 5 peptides of latex-allergic donors

Antigen	P1	P2	P3	P4	P5	P6
T + APC alone	3.6	2.1	5.2	0.9	1.0	1.0
LAL						
100	9.5	12.6	55.9	2.2	4.1	29.9
30	7.8	9.8	65.9	2.9	4.6	27.9
10	8.8	9.4	63.4	3.1	3.3	14.6
3	7.1	7.7	37.1	2.0	1.7	9.9
Hev b 5/MBP	9.0	5.7	NT	NT	NT	NT
MBP	2.5	1.5	NT	NT	NT	NT
(1-20)						
30	2.5	2.1	8.2	2.0	2.6	2.2
10	3.0	1.6	7.6	1.6	0.9	1.0
(10-29)						
30	3.3	1.7	5.2	1.2	0.8	1.7
10	3.2	1.4	7.7	1.6	1.5	1.1
(19-38)						
30	2.4	2.4	3.0	1.6	4.0	2.0
10	2.4	1.1	6.4	1.2	0.6	0.9
(28-47)						
30	5.0	41.7	6.9	1.4	1.6	1.1
10	7.2	25.8	6.4	1.2	0.8	0.9
(37-56)						
30	8.5	38.8	2.7	3.2	1.1	0.9
10	7.1	18.3	8.8	3.0	1.4	1.4
(46-65)						
30	19.6	22.6	19.2	5.3	5.6	1.2
10	21.3	13.4	25.8	4.4	4.9	1.5
(55-74)						
30	7.7	5.6	8.2	2.8	0.7	0.9
10	5.0	2.8	9.1	2.0	1.1	0.7
(64-83)						
30	5.6	2.1	4.3	2.6	2.4	1.0
10	3.5	1.8	7.0	1.9	0.5	0.8
(73-92)						
30	2.9	14.6	3.1	1.2	0.5	1.3
10	2.5	9.8	5.3	1.2	0.8	1.0
(82-101)						
30	5.2	2.1	6.5	2.6	1.6	1.2
10	4.1	3.2	7.3	2.4	1.3	0.8
(91-110)						
30	2.6	3.2	8.1	2.2	3.2	1.2
10	2.4	2.4	7.4	1.6	1.1	0.7
(100-119)						
30	2.0	2.2	8.0	1.1	0.8	1.0
10	2.4	1.3	6.8	1.2	1.0	0.7
(109-128)						
30	5.6	42.7	17.3	3.0	0.9	0.6
10	8.2	36.9	13.7	2.3	1.3	0.9
(118-137)						
30	2.5	20.8	8.5	1.2	1.3	0.8
10	3.9	19.0	6.8	0.9	0.5	0.9
(127-146)						
30	4.4	6.6	8.6	1.6	2.5	0.8
10	3.3	3.8	7.1	2.2	2.7	1.0
(132-151)						
30	6.5	3.3	7.0	1.7	1.0	0.8
10	4.1	2.7	6.7	1.1	1.4	1.2

Latex-specific 3-week T-cell lines from latex-allergic patients (P1-P6) were stimulated with the Hev b 5 peptides at 10 and 30 µg/mL, LAL at 3, 10, 30 and 100 µg/mL, and rHev b 5/MBP at 10 µg/mL in 4-day cultures. The responses for each peptide and protein antigen are given as cpm $\times 10^{-3}$ and compared with the control response where T cells and APCs were incubated alone in the absence of antigen. Stimulation index values of ≥ 2.5 are shown in bold. NT, Not tested.

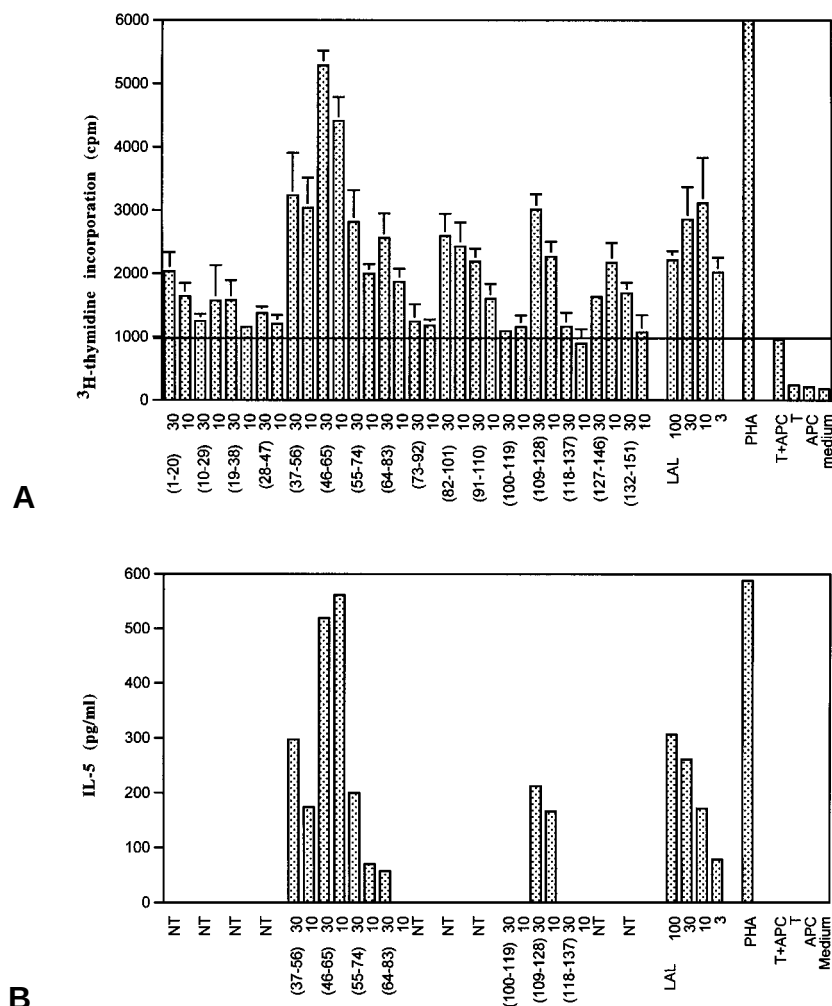


FIG 2. A, Proliferation and **(B)** IL-5 production by latex-specific T-cell line of latex-allergic patient 4. **A,** Proliferation of T cells in response to Hev b 5 peptides (30, 10 μ g/mL), LAL (100, 30, 10, 3 μ g/mL), and PHA (3 μ g/mL) assessed ^{3} HTdR incorporation (values shown are averages of triplicate samples with SD indicated). Background level of cell proliferation (T + APC) is indicated by the horizontal line at 1000 cpm. **B,** Production of IL-5 in the culture supernatants determined by ELISA. Only the indicated peptides were tested for their ability to stimulate T-cell production of IL-5. NT, Peptides that were not tested in the IL-5 assay. Values indicate averages of duplicate samples.

tion. In contrast, secretion of IFN- γ by T cells that proliferated in response to Hev b 5 peptides was decreased in comparison with IL-5 secretion in all cases.

DISCUSSION

Latex allergy affects certain high-risk groups including HCWs and patients with spina bifida or a history of multiple surgical procedures. Currently latex avoidance and symptomatic treatment are the only available forms of treatment. In severely affected patients this may be insufficient to allow continued employment in a latex-containing environment. Effective latex-specific immunotherapy is a highly desirable objective.

Hev b 5 is a major latex allergen reacting with serum IgE from 92% of latex-allergic HCWs and 56% of

patients with spina bifida. Although the predicted molecular weight of Hev b 5 is 17 kd, this protein migrates at around 24 kd on SDS-PAGE.^{17,18} This aberrant migration has been observed for other proteins that are highly acidic and proline rich.¹⁸ Although mAbs to rHev b 5/MBP have been generated,¹⁸ it has been difficult to specifically identify natural Hev b 5 on Western blots of latex extracts.^{17,18,27} Therefore we carried out dot-immunoblots with use of LAL and rHev b 5/MBP as antigen. Dot immunoblot analysis showed LAL-specific and rHev b 5/MBP-specific IgE in sera from patients 1, 3, 4, and 5, indicating the presence of a B-cell response to Hev b 5 in these patients. On the other hand, patient 6 showed IgE reactivity to LAL but not to the rHev b 5/MBP fusion protein, indicating that Hev b 5 was not a significant allergen in this case. Patient 2 had IgE reactivity to rHev b

TABLE III. IL-5 and IFN- γ levels produced by latex-specific T-cell lines when stimulated with Hev b 5 peptides

Patient	Hev b 5 peptides tested (30,10 μ g/ml)	IL-5 (pg/mL)		IFN- γ (pg/mL)		IL-5/IFN- γ ratio	
		30	10	30	10	30	10
P1	28-47 (-)	68	0	0	0	—	—
	37-56 (-)	0	0	0	0	—	—
	46-65 (+)	279	344	2	2	139.5	172
	55-74 (-)	0	0	0	0	—	—
	64-83 (-)	0	14	0	0	—	—
	73-92 (-)	0	0	0	0	—	—
	100-109 (-)	0	0	0	0	—	—
	109-128 (-)	69	0	0	0	—	—
P2	28-47 (+)	678	706	112	122	6.1	5.8
	37-56 (+)	767	744	77	74	10	10.1
	46-65 (+)	408	58	54	18	7.6	3.2
	55-74 (-)	0	0	4	2	—	—
	64-83 (-)	0	0	4	2	—	—
	73-92 (+)	287	141	45	18	6.4	7.8
	100-119 (-)	17	0	4	2	4.2	—
	109-128 (+)	595	293	97	73	6.1	4
P3	46-65 (+)	3186	3799	227	388	14	9.8
	64-83 (-)	205	109	129	132	1.6	0.8
	73-92 (-)	24	27	83	112	0.3	0.2
	109-128 (+)	1559	1403	292	257	5.3	5.5
P4	37-56 (+)	296	173	6	0	49.3	173
	46-65 (+)	519	561	12	25	43.2	22.4
	55-74 (+)	199	70	0	0	199	70
	64-83 (+)	56	0	4	0	14	0
	82-101 (+)	280	285	0	0	280	285
	110-119 (-)	0	0	0	0	—	—
	109-128 (+)	212	166	0	0	212	166
	118-137 (-)	0	0	0	0	—	—
P5		Not tested					

Supernatants were removed at 48 hours from T-cell cultures and tested in an ELISA for the presence of IL-5 and IFN- γ . Proliferation inducing and noninducing peptides are indicated by (+) and (-), respectively. The levels of IL-5 and IFN- γ produced in picograms per milliliter are shown at peptide concentrations of 30 and 10 μ g/mL. Where both cytokines were detectable, the IL-5/IFN- γ ratio is shown at peptide concentrations of 30 and 10 μ g/mL.

5/MBP but not to LAL. A possible explanation for this observation is that the natural Hev b 5 in LAL does not present IgE-binding epitopes for this patient's serum. This may be a result of degradation of the natural Hev b 5 protein caused by the process of ammoniation. Nevertheless, the T-cell repertoire of patient 2 included recognition of Hev b 5 (Table II). This is consistent with the fact that B-cell recognition of antigen is conformationally dependent, whereas T cells recognize processed linear peptides with no recognition of conformational determinants.

We generated latex-specific T-cell lines from 6 latex-allergic HCWs and analyzed the T-cell response to Hev b 5 with use of overlapping peptides that spanned the entire length of this molecule. The 5 patients with IgE reactivity to the rHev b 5/MBP by dot blot analysis demonstrated T-cell responses to the Hev b 5 peptides. As expected, the latex-specific T-cell line from patient 6 who lacked IgE reactivity to rHev b 5/MBP failed to proliferate to the Hev b 5 peptides. Because T-cell lines from 5 of the 6 latex-allergic patients responded to Hev b 5 peptides, we have established that Hev b 5 is a major T-cell allergen in the highly allergic HCW group. It will also be of interest to determine whether other patient groups (for example, atopic but not latex-allergic controls and spina bifida

patients) have a similar T-cell repertoire, and longitudinal studies of this nature are the subject of continuing research in our department.

Several peptides of the Hev b 5 molecule stimulated T-cell proliferation in our patient group. Of these peptides, Hev b 5 (46-65) induced proliferation in the T-cell lines of all rHev b/MBP IgE-reactive donors, whereas Hev b 5 (109-128) stimulated T cells from 3 of these 5 donors. Both the magnitudes and the frequencies of responses to these peptides suggest they contain immunodominant T-cell epitopes of Hev b 5. The high frequency of reactivity is consistent with degenerate binding of this peptide to different MHC class II molecules in an outbred human population.²⁸ Such degeneracy is highly desirable for the development of immunotherapeutic approaches in human allergic diseases.²⁹

Several other peptides, Hev b 5 (37-56), Hev b 5 (55-74), and Hev b 5 (118-137), that were overlapping with the 2 immunodominant regions above stimulated responses in 1 or more subjects (Table II). These may represent shared epitopes or 2 separate epitopes, and further fine mapping would be required for clarification.

A database search showed Hev b 5 (46-65) to have 60% sequence identity with a peptide sequence of a par-

asite protein from *Strongyloides stercoralis*, which binds IgG and IgE from patients with strongyloidiasis.³⁰ This is of interest because both allergenic and parasitic antigens induce a T_H2 polarized cytokine response and induce IgE synthesis. None of our patients had visited a region endemic for *Strongyloides* in the previous 2 years and none had symptoms or peripheral blood eosinophilia to suggest subclinical infection.

A search for Hev b 5 (109-128) identified 62.5% sequence identity with a region of the kiwi fruit protein pKIWI501. The sequence similarity observed between the whole Hev b 5 and pKIWI501 molecules is most striking in the N- and C-terminal regions. Therefore the C-terminal region Hev b 5 (109-128) may contain a T-cell epitope that explains the dual reactivity seen clinically with latex and kiwi fruit. All 5 patients in our study with Hev b 5 IgE reactivity were also known to have food allergy. Interestingly, 3 of these patients, in addition to latex allergy, described angioedema or anaphylaxis on contact with kiwi fruit. However, kiwi fruit allergy did not precede the development of clinical latex allergy in any of these patients. Of the other 2 patients, one denied kiwi fruit allergy and the other patient had avoided kiwi fruit because of fear of possible cross-reactivity. Recognition of antigen components of related allergens (including avocado, banana, kiwi fruit, and chestnut) by cross-reactive IgE in latex-allergic subjects is well recognized³¹ and may lead to multiple allergies. Alternatively, T cells that recognize cross-reactive epitopes may provide "intermolecular" help³² and induce the production of specific antibodies to another allergen contributing to multiple clinical allergies.

Murine T-cell epitopes of Hev b 5 have been identified previously³³ in BALB/c mice with the same set of overlapping synthetic peptides. Mice were injected with rHev b 5/MBP and subsequent in vitro splenocyte proliferation assays were performed to identify T-cell determinants. The dominant murine T-cell determinants were Hev b 5 (1-20), (37-56), (73-92), (82-101), (109-128), (118-137), and (127-146). We have demonstrated that all 7 of these peptides also represent human T-cell determinants. There is precedent in the literature for shared murine and human allergenic T-cell epitopes.³⁴ Interestingly, the immunodominant human T-cell determinant Hev b 5 (46-65) recognized by all 5 subjects with IgE reactivity to rHev b 5/MBP in the current study was only a minor murine T-cell determinant.³³ This emphasizes the need to confirm T-cell epitope mapping performed in the mouse by human studies as a prelude to vaccine development for clinical use.

The cytokine data showing marked IL-5 and minimal IFN- γ production by oligoclonal latex-specific T cells after stimulation with proliferation-inducing peptides are consistent with the immunodominance of the Hev b 5 peptides identified. Peptide-specific T-cell proliferation and dominant T_H2-type cytokine profile (high IL-5/IFN- γ ratio) are compatible with the T-cell response supporting IgE synthesis to this allergen and eosinophil activation, which are necessary for the development of clinical allergy.

The full characterization of T- and B-cell epitopes of the Hev b 5 molecule might facilitate the design of specific immunotherapy regimens for latex hypersensitivity. Studies with mutant proteins of Phl p 5b from timothy grass³⁵ or T-cell epitope peptides of bee venom phospholipase A₂³⁶ have been encouraging. Earlier studies investigating peptide vaccines for the cat allergen Fel d 1 were disappointing, but these studies used large protein determinants rather than T-cell epitope peptides.³⁷ The retention of highly immunodominant T-cell epitopes in peptide epitope vaccines or mutant proteins with abrogated IgE binding would be desirable for therapeutic use.

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