Lymphocyte proliferation response to extracts from different latex materials and to the purified latex allergen Hev b 1 (rubber elongation factor)

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Background: *Type I allergy to latex is a growing problem, especially among health care workers. A detailed study of the peripheral blood cell responses to latex allergens has not been reported.*
Methods: *Peripheral blood mononuclear cells of patients and healthy subjects were isolated and stimulated with protein extracts from latex sap and latex gloves and the purified latex allergen Hev b 1 (rubber elongation factor) at different concentrations to determine the antigen-specific proliferation response. The examined patients were sensitized to latex by occupational exposure (n = 23) and had rhinitis, conjunctivitis, contact urticaria, and/or asthma. Two control groups of nonsensitized subjects were studied: one occupationally exposed to latex (n = 8), and the second, not exposed to latex (n = 8).*
Results: *In general, only latex-exposed subjects responded to the different latex antigen preparations. Lymphocyte proliferation responses to latex sap extract were found in 65% of latex-sensitized subjects and in 37.5% of the latex-exposed healthy subjects. Latex glove extract induced a significant proliferative response in 47.8% of latex-sensitized patients and in 25% of latex-exposed individuals. Hev b 1 induced lymphocyte proliferation responses in 52% of the latex-sensitized patients and in 25% of the latex-exposed subjects, indicating that Hev b 1 is relevant antigen in these latex-sensitized and latex-exposed groups. Peripheral blood mononuclear cells of 39.1% of the latex-sensitized subjects responded to all three allergen preparations (latex sap and latex glove extract, as well as Hev b 1). We could find no correlation between latex-specific IgE level and latex-induced lymphocyte proliferation response.*
Conclusion: *Our data indicate that the 14 kd protein Hev b 1 is a relevant allergen in health care workers. It can be detected by specific IgE antibodies to Hev b 1, as well as in lymphocyte proliferation assay. In addition, our study suggests that antigen-specific proliferation response to latex is associated with exposure to latex, but not with the level of specific latex IgE. This may be useful for the evaluation and prediction of latex hypersensitivity development. (J Allergy Clin Immunol 1996;98:640-51.)*

Key words: *Latex allergy, rubber elongation factor, Hev b 1, lymphocyte stimulation*

Immediate-type hypersensitivity to latex is mediated through anti-latex IgE antibodies.[1-4] Health care workers, patients requiring long-term catheterization and multiple operations, rubber workers, and atopic subjects have been shown to be at higher risk for sensitization to latex.[5, 6] Latex sensitivity was shown to have progressive phases with initial symptoms of contact urticaria, followed by

Abbreviations used

EAST:	Enzyme-linked allergosorbent test
PBMCs:	Peripheral blood mononuclear cells
SDS-PAGE:	Sodium dodecylsulfate–polyacrylamide gel electrophoresis
SI:	Stimulation index

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TABLE I. Latex-sensitized patients

Patient	Total IgE (kU/L)	Specific latex IgE (kU/L)	CAP class	Specific IgE Hev b 1 (kU/L)	EAST class	Symptoms
Sco	748	100.10	6	3.40	2	U+R+C+A
Wil	>2000	96.90	5	0.41	1	U+R+C+A
Ser	343	70.10	5	2.15	2	U+R
Eib	793	59.80	5	0.65	1	U+R+C+A
Fin	>2000	58.30	5	0.79	2	U+R+C+A
Mue	450	39.10	4	1.76	2	A
Mon	2000	23.70	4	18.08	4	R+A
Leg	183	14.20	3	0.48	1	U+R+C+A
Bra	93	13.00	3	0.27	0	U+R+C+A
Gar	78	11.29	3	1.31	2	U+R+C+A
Men	41	9.08	3	0.23	0	U+R+C+A
Woz	410	8.50	3	0.67	1	U+R+C
Sci	708	6.78	3	0.39	1	U+R+A
Bec	144	5.98	3	0.26	0	U+R+C+A
Scr	47	5.76	3	0.11	0	U+R+C+A
Bor	28	5.47	3	0.32	0	U+R+C+A
Old	435	5.26	3	0.35	1	R+C+A
Far	635	5.14	3	0.19	0	U+R+C+A
Leb	280	4.84	3	0.18	0	U+R+C+A
Roh	35	4.27	3	0.09	0	R+A
Dur	25	3.24	2	0.11	0	U+R+C+A
Bie	63	3.03	2	0.08	0	U+R+A
The	8	0.99	2	0.15	0	U+R+C+A

U, Urticaria; *R,* rhinitis; *C,* conjunctivitis; *A,* asthmatic complaints and/or asthma.

sequential development of extra-site urticaria, rhinitis, asthma, and possibly anaphylaxis.[7] The main risk factor of sensitization in the group of health care workers is prolonged and repeated use of protective gloves.[8, 9] The responsible allergens are latex proteins, which are found in raw latex and in various latex-containing products. Several of these proteins have been characterized both chemically and with regard to their specific reactivity with serum antibodies. Although more than 240 separate polypeptides can be discerned by two-dimensional electrophoresis in latex sap, less than 25% of these peptides showed reactivity with IgE antibody from patients with latex allergy.[10] Immunoblotting with human sera revealed more than 10 protein bands from natural latex and latex glove extract, which bound IgE antibodies shown in several studies. The allergenic proteins have molecular weights ranging from 10 to 100 kd.[11-13] At their primary structure level, so far, four proteins have been identified in natural rubber latex. They are prohevein (20 kd),[13-15] hevamine (29.5 kd),[13, 16] prenyltransferase (38 kd),[17] and the rubber elongation factor (14.6 kd).[18] Our group[19] isolated and identified the rubber elongation factor in latex gloves and in raw latex as a major allergen in latex. This protein (rubber elongation factor) has been designated as Hev b 1 according to the International Union of Immunological Societies' allergen nomenclature.[20] Hev b 1 is also present as an airborne allergen when donning and doffing of latex gloves release powder-bound allergens into the air (our unpublished results).

For a better understanding of the immune mechanism of latex allergy, we studied T-cell–mediated responses to latex allergens, including the purified Hev b 1 and protein extracts obtained from latex gloves and latex sap. In this study we report on the determination and comparison of lymphocyte responses to the different prepared latex antigens in latex-sensitized patients and in nonsensitized subjects with or without latex exposure.

METHODS

Subjects

Three groups of subjects were investigated. Group 1 (Table I) consisted of 23 latex-sensitized hospital employees, especially health care workers and physicians, aged 19 to 52 years (17 female and 6 male subjects). All

of them were diagnosed as having latex-related allergic symptoms such as urticaria, rhinitis, conjunctivitis, and/or asthma. All 23 subjects had positive latex skin prick test results and latex-related specific IgE antibodies (≥0.35 kU/L, measured by CAP-system [Pharmacia, Uppsala, Sweden]). In addition, 12 subjects (52%) also had positive specific IgE values when tested with the isolated latex allergen Hev b 1 by enzyme-linked allergosorbent test (EAST). Total serum IgE levels of the 23 patients ranged from 8 kU/L to above 2000 kU/L.

Group 2 consisted of eight subjects aged 26 to 44 years (6 female and 2 male subjects). They were occupationally exposed to latex (especially surgical gloves) because of their professions as health care workers, physicians, and technicians in research laboratories. They had no latex-related respiratory or cutaneous symptoms. The latex-specific IgE CAP results, as well as Hev b 1–specific IgE EAST results, were found to be negative (<0.35 kU/L). Total serum IgE levels ranged from 19 to 368 kU/L.

Group 3 was composed of eight nonoccupationally latex-exposed healthy subjects aged 31 to 61 years (6 male and 2 female subjects). They had no symptoms of hypersensitivity and negative latex CAP results. Total serum IgE levels varied from below 2 kU/L to 442 kU/L.

Detection of total and latex-specific IgE antibodies

All sera of the subjects had been tested for concentration of specific IgE to latex allergens by the CAP system. The results were expressed as kilounits per liter according to the standard curve by Pharmacia. Specific IgE values to Hev b 1 were measured by using an EAST with Hev b 1 coupled to CNBr-activated paper disks in our laboratory.[11]

Total IgE concentration in sera of the 39 subjects was determined by a Phadebas IgE PRIST (Pharmacia) with a polyclonal rabbit anti-IgE antibody.

Antigen-specific lymphocyte proliferation

For antigen-driven proliferation, peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque (Pharmacia) gradient centrifugation and adjusted to 1×10^6/ml in RPMI-1640 conditioned medium (Gibco, Eggenstein, Germany) supplemented with glutamine, penicillin/streptomycin, and 10% heat-inactivated pooled human AB sera (Bavarian Red Cross, Munich, Germany). PBMCs were incubated in 96-well plates in medium alone or with different final concentrations of the latex allergen preparation for 5 days in a humidified atmosphere at 37° C in 5% CO_2. For the final 12 hours, 37 kBq of tritium-labeled thymidine-methyl (Du Pont [Germany], NEN-Division, Dreieich, Germany) was added to each well, and incorporated radioactivity was assessed by liquid scintillation spectrometry.

Results were shown as stimulation index (SI), calculated as the ratio of the mean counts per minute obtained in the six similar cultures with allergens and that obtained in the allergen-free culture (RPMI-control). We set cutoff lines determining positive and negative responses at SI = 2.5.

Allergen preparation

Latex sap extract. Ammoniated (0.7%) *Hevea* latex sap (Kautschuk GmbH, Frankfurt, Germany) was diluted 1:1 with 50 mmol/L Tris, pH 8.0, mixed for 30 minutes, and centrifuged at 41.000 *g* for 45 minutes. The protein in the aqueous layer (C-serum) was carefully separated from the creamed rubber particles and then filtered through a 0.45 μm membrane filter. The resulting extract was further dialyzed in 50 mmol/L Tris, pH 8.0, across a 6 kd cutoff cellulose membrane tube.

Latex glove extract. Latex gloves (Sempermed, Semperit, Austria) were cut into pieces, which were then extracted by vigorously stirring for 24 hours in 0.1 mol/L acetate buffer, pH 8.0. Subsequently, the rubber pieces were removed, and the supernatant was dialyzed in distilled water across a 6 kd cutoff cellulose membrane tube for 36 hours at 4° C and then lyophilized. The dry material was then redissolved in 0.1 mol/L acetate buffer, pH 8.0, and the insoluble substance was removed by centrifugation at 4000 *g*. The supernatant was filtered through a 0.45 μm membrane, freeze-dried, and stored at −20° C.

Hev b 1. One hundred milliliters of ammoniated (0.7%) latex sap from the rubber tree *Hevea brasiliensis* was diluted 1:1 with 50 mmol/L Tris, pH 8.0, and 0.01% Triton X-100 and centrifuged at 41,000 *g* for 45 minutes. The yellow aqueous layer at the bottom of the centrifuge tube was discarded, and the serum-free rubbery supernatant was resuspended in 50 mmol/L Tris containing 0.01% Triton-X 100 and centrifuged once more. The creamed rubber particles were carefully separated from the serum layer and immediately resuspended in 160 ml of 2% sodium dodecylsulfate. The sample was stirred for 30 minutes at room temperature, and the visible rubber particles were removed by centrifugation (41,000 *g* for 45 minutes); if necessary, the sample was centrifuged once more. The supernatant was then filtered through a 0.45 μm filter (Satorius AG, Göttingen, Germany) and dialyzed in distilled water by using a cellulose dialysis tube with a molecular mass cutoff of 6 kd for 36 hours at 4° C. The dialysate was lyophilized and redissolved in 10 ml of 0.1 mol/L ammonium acetate buffer containing 0.01% NaN_3, pH 6.8. The protein-containing solution was loaded on a Sephadex 75 Superfine column (2.6 × 90 cm, Pharmacia), which was equilibrated and eluted with the same ammonium acetate buffer. Fractions of 2.5 ml were collected and examined by sodium dodecysulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1). The fractions containing the 14.6 kd protein were pooled, desalted by centrifugation through an Amicon membrane (Amicon Inc., Beverly, Mass.), lyophilized, and stored at −40° C.

The protein concentration of all three allergen prep-

arations was determined by the Bradford method with a Bio-Rad protein assay kit (Bio-Rad Laboratorium GmbH, Munich, Germany).

Statistics

Results are expressed as arithmetic mean ± SD. Differences between two groups were analyzed by using Student's *t* test for unpaired data. The Mann-Whitney U test was used to compare the SI and the specific IgE levels between the different groups. Differences with p values less than 0.05 were considered statistically significant.

RESULTS

Lymphocyte proliferation response to latex sap extract

PBMCs from 23 sensitized patients (group 1), from eight nonsensitized but latex-exposed subjects (group 2), and from eight healthy subjects (group 3) without occupational exposure to latex were stimulated with protein extract from latex sap at different concentrations (0.5 to 20 μg/ml), and the lymphocyte proliferation was investigated (Fig. 2). PBMCs of 18 subjects showed a significant proliferation response (SI ≥ 2.5) to the latex sap extract (Fig. 2, *A*). In 15 cases the responding lymphocytes were from latex-sensitized patients (15 of 23, 65%), and in three cases the cells were from latex-exposed but nonsensitized subjects (3 of 8, 37.5%). Although the PBMCs of each tested individual demonstrated their own dose-response curve to latex sap extract (typical ones are presented for 6 subjects in Fig. 2, *B*), a concentration range between 10 and 20 μg/ml was optimal. The mean SI value in group 1 was 5.72 ± 4.72 (maximal SI of each individual: $n = 23$, mean ± SD), and the mean SI value in group 2 was 3.35 ± 3.42 ($n = 8$, mean ± SD). The differences in the mean SI values between the responses observed in the latex-sensitized patients (group 1) and latex-exposed but nonsensitized subjects (group 2) were not significant ($p \geq 0.05$ by the Mann-Whitney U test). It should be noted, however, that in group 2 only one subject of three showed pronounced SIs with all five tested concentrations of latex sap extract (Fig. 2, *B*). Lymphocytes of healthy subjects without occupational latex exposure (group 3) did not show proliferation responses induced by latex sap extract (Fig. 2, *A*) (mean SI value = 1.46 ± 0.78, $n = 8$; significantly different when compared with group 1 [$p < 0.002$]).

Lymphocyte proliferation response to latex glove extract

Next, PBMCs from the test subjects were stimulated with latex glove extract in the protein concentration range between 0.5 and 20 μg/ml (Fig. 3). Antigen-specific proliferation was detected in 13 cases: in 11 patients (11 of 23, 47.8%) with a mean SI value of 3.97 ± 3.3 and in two exposed subjects (2 of 8, 25%) with a mean SI value of 1.9 ± 1.37 ($n = 8$). SI values calculated for stimulation with latex glove extract were compared between groups 1 and 2; latex-sensitized patients (group 1) had significantly higher values ($p < 0.02$). Pronounced stimulation was detected with the concentrations of 0.5, 1, and/or 5 μg/ml (Fig. 3,

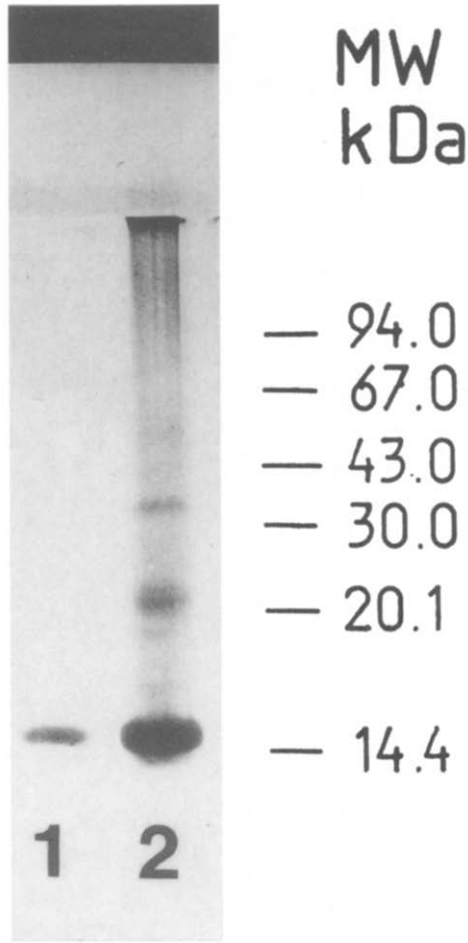
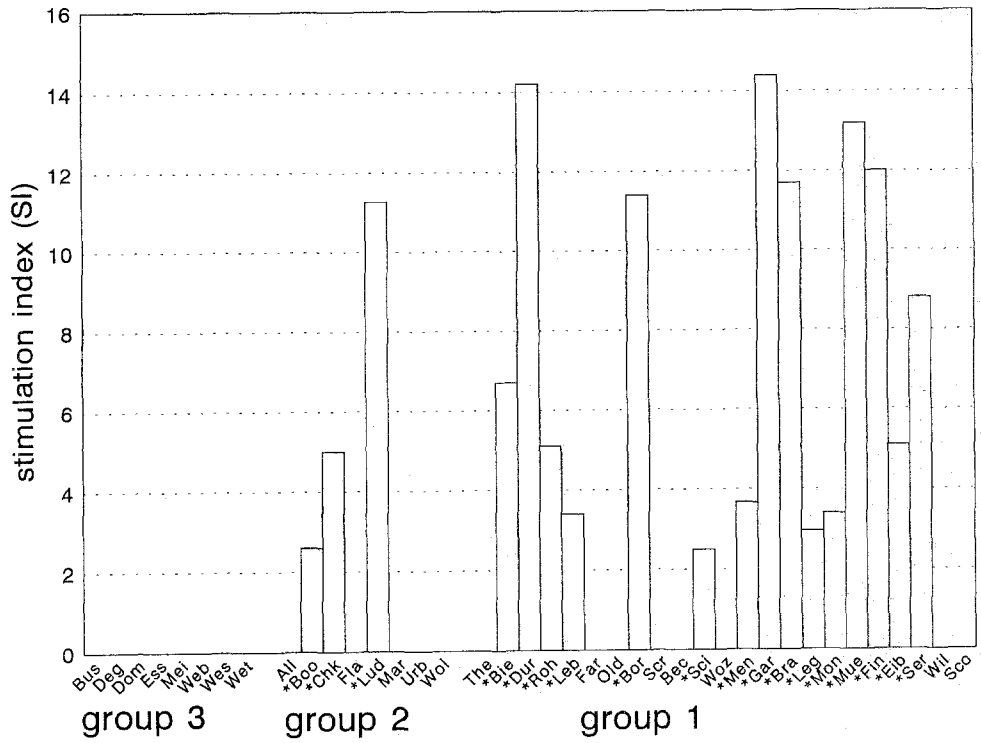


FIG. 1. SDS-PAGE of proteins in ammoniated (0.7%) *Hevea* latex sap. *Lane 1,* Hev b 1 protein released from rubber particles in 2% SDS and purified by gel chromatography by using a Sephadex-75 column. *Lane 2,* Protein extract obtained by centrifugation to remove rubber particles. Molecular masses of marker proteins *(kDa)* are indicated at right. SDS-PAGE was carried out in a Phast System (Pharmacia) with precast 8% to 25% gradient gels. Protein bands are visualized by silver staining. *MW,* Molecular weight.



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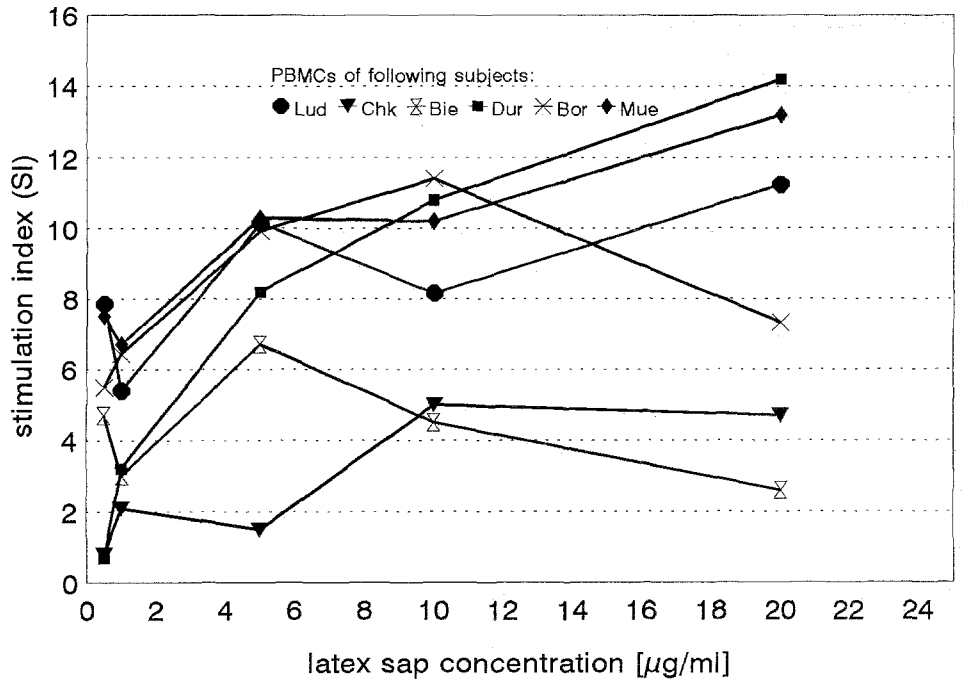
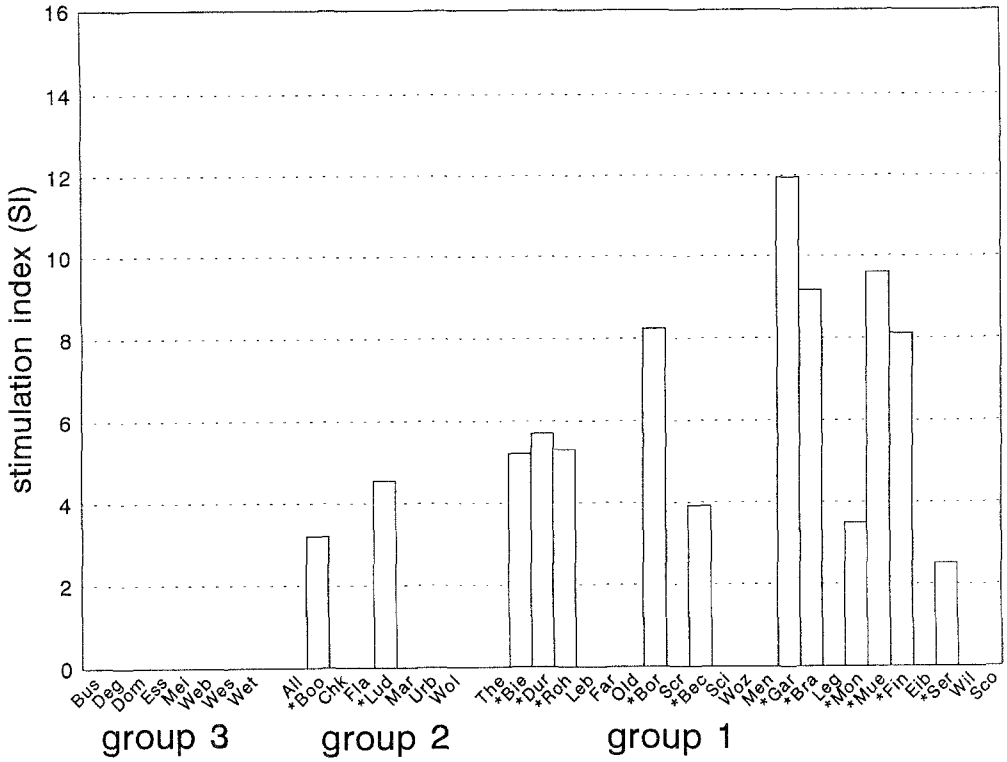


B

FIG. 2. PBMC proliferation responses to latex sap extract. Group 1: latex-sensitized patients; group 2: occupationally latex-exposed but nonsensitized subjects; group 3: healthy control individuals without occupational latex exposure. **A,** Maximal SI values for each subject are presented, and only SI values of 2.5 or greater are shown. Each value is expressed as the mean of at least six parallel determinations. *Asterisk* indicates positive proliferation response. **B,** Dose-dependent proliferation response to latex sap extract. Dose-related stimulation responses of six subjects are taken as examples (two of them belonged to group 2 [Lud and Chk] and the others belonged to group 1 [Bie, Dur, Bor, and Mue]).



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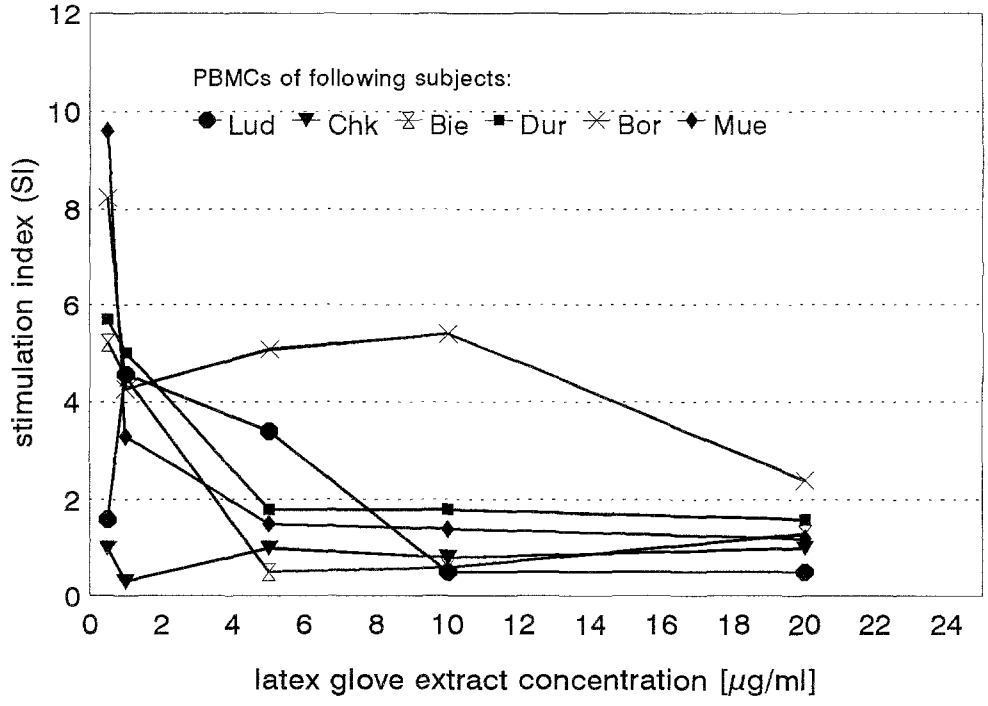
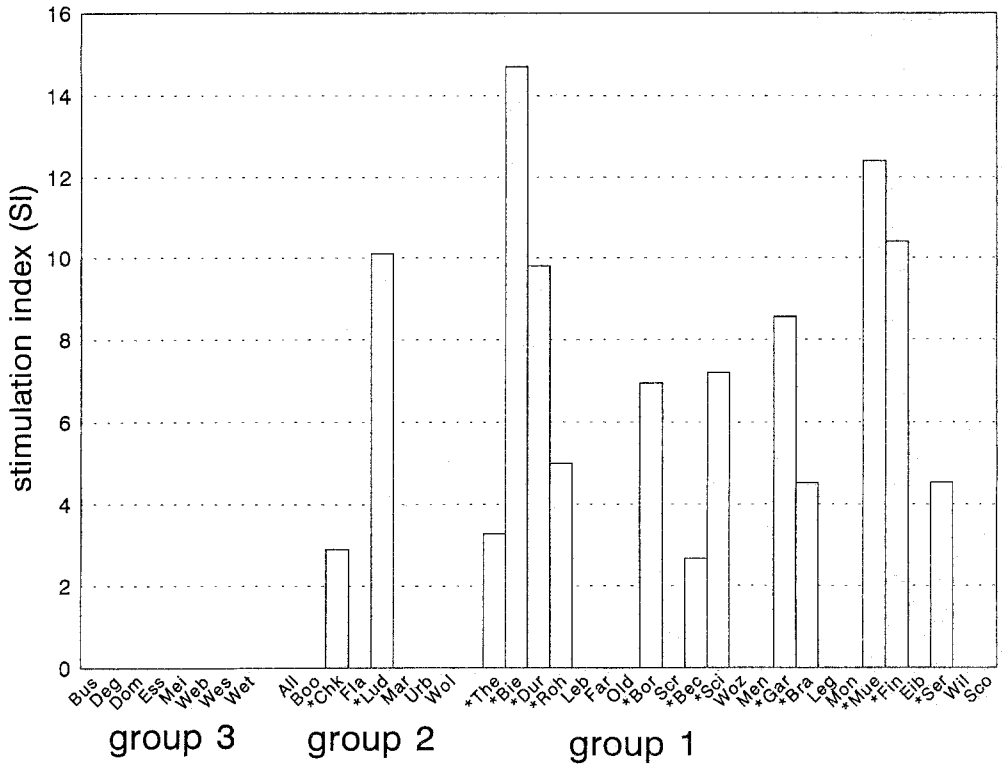


B

FIG. 3. PBMC proliferation responses to latex glove extract (for group definitions, see Fig. 2). **A,** Maximal SI values for each subject are presented, and only SI values of 2.5 or greater are shown. Each value is expressed as the mean of at least six parallel determinations. *Asterisk* indicates positive proliferation response. **B,** Dose-dependent proliferation response to latex glove extract. Dose-related stimulation responses of six subjects only are taken as examples (two of them belonged to group 2 [Lud and Chk] and the others belonged to group 1 [Bie, Dur, Bor, and Mue]).



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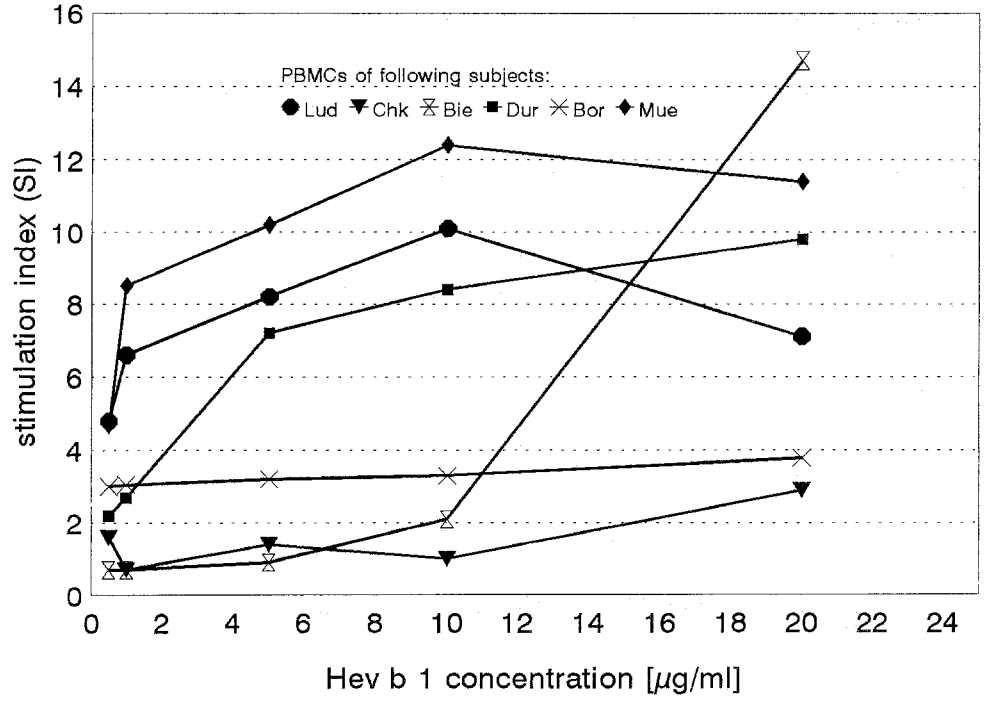

B

FIG. 4. PBMC proliferation responses to Hev b 1 (for group definitions, see Fig. 2). **A,** Maximal SI values for each subject are presented, and only SI values of 2.5 or greater are shown. Each value is expressed as the mean of at least six parallel determinations. *Asterisk* indicates positive proliferation response. **B,** Dose-dependent proliferation response to Hev b 1. Dose-related stimulation responses of six subjects only are taken as examples (two of them [Lud and Chk] belonged to group 2, and the others belonged to group 1 [Bie, Dur, Bor, Mue]).

B). PBMCs of 12 of 13 subjects showed a latex sap–specific stimulation response, too. The cells of one subject (Bec) responded to latex glove extract but not to the latex sap extract. In addition, PBMCs of six subjects (Chk, Leb, Sci, Men, Leg, and Eib) were stimulated by latex sap extract (Fig. 2, *A*) but did not show a positive stimulation response to the latex glove extract (Fig. 3, *A*). No significant proliferation was measured in the eight nonexposed healthy control subjects (group 3) with a mean SI value of 1.36 ± 0.74 (significantly different when compared with the mean SI value of group 1 [$p < 0.001$] but not with that of group 2).

Lymphocyte proliferation response to Hev b 1, the purified 14.6 kd protein from latex sap

When PBMCs of the test subjects were incubated with the purified latex allergen Hev b 1 (Fig. 4), 12 of 23 (52%) patients showed pronounced proliferation responses (mean SI value = 4.73 ± 3.98; $n = 23$). Only five of these patients had Hev b 1–specific IgE antibodies in sera (Sci, Gar, Mue, Fin, and Ser; Table I; EAST class ≥ 1). Although one patient (Mon) had an enhanced level of Hev b 1–specific IgE (EAST class 4), this patient's PBMCs did not proliferate in the presence of Hev b 1 (Table II; described in detail in the next paragraph). The highest proliferation response in each individual was induced by Hev b 1 concentrations of 10 and/or 20 μg/ml; typical dose-response curves from PBMCs of six individuals are shown in Fig. 3, *B*. In addition, PBMCs of two of eight (25%) latex-exposed subjects proliferated in the presence of Hev b 1, but only one of these two subjects (Lud) showed a significant response induced at all five Hev b 1 concentrations (Fig. 4, *B*). The mean SI value in group 2 was 3.01 ± 2.92, and no significant difference existed in comparison with the mean SI value of group 1. In the group of nonexposed subjects (group 3), no PBMCs demonstrated a proliferation response: the mean SI value was 1.61 ± 0.50, being significantly different in comparison with the value obtained with PBMCs of group 1 patients ($p < 0.001$), but without significant difference when compared with the mean SI value calculated in group 2.

Comparison of lymphocyte proliferation to all three latex antigen preparations

PBMCs of 17 of the 23 sensitized patients (part of group 1) and three of eight subjects not sensitized but occupationally exposed to latex (part of group 2) responded to one or more of the three latex antigen preparations (Fig. 5). Ten of these 20 subjects with an SI of 2.5 or greater demonstrated a positive stimulation response to all three antigen preparations. Nine of them belonged to group 1 (9 of 23, 39.1%), and another one was from group 2 (Lud; 1 of 8, 12.5%). Proliferative response to latex sap extract alone was found in four patients (Leb, Men, Leg, Eib). Proliferation response to Hev b 1 without positive stimulation induced by latex sap extract or latex glove extract occurred in only one patient (The). PBMCs of one patient (Bec) proliferated in the presence of latex glove extract and Hev b 1 but did not show significant stimulation in the presence of different concentrations of latex sap extract. In most of the PBMCs that failed to respond to Hev b 1 (from patients Boo, Leb, Men, Leg, Mon), the responses to latex sap extract and latex glove extract were not remarkably enhanced. In only two patients (Bie and Sci) was the SI calculated for Hev b 1–induced stimulation higher than the SI value of latex sap extract stimulation. With the exception of three subjects (Chk, The, and Sci), all of the PBMCs with positive proliferation response to Hev b 1 were also stimulated with latex glove extract.

TABLE II. Correlation of CAP latex values of the latex-sensitized individuals ($n = 23$) with mean SIs to latex sap extract and latex glove extract

CAP values	No. of subjects	Mean SI to latex sap extract	Mean SI to latex glove extract
1+	0	—	—
2+	3	7.46 ± 6.40*	4.23 ± 2.12*
3+	13	4.88 ± 4.52	3.92 ± 3.63
4+	2	8.3 ± 6.92	6.55 ± 4.31
5+	4	6.96 ± 4.40	3.27 ± 3.27
6+	1	1.35	1.59

*Mean SI of the group ± SD.

Relation between specific latex IgE and cellular proliferative response

To determine whether there was an association between specific IgE antibody levels to latex and lymphocyte proliferation to the three antigen preparations, sera of latex-sensitized patients (group 1) were categorized into six CAP class groups according to their specific latex IgE concentrations. The mean SI was then ascertained for both latex sap and latex glove extract for each CAP class group. These data are presented in Table II and do not reveal an association between lymphocyte proliferation and latex-specific IgE level.

Five subjects in group 1 with positive PBMC

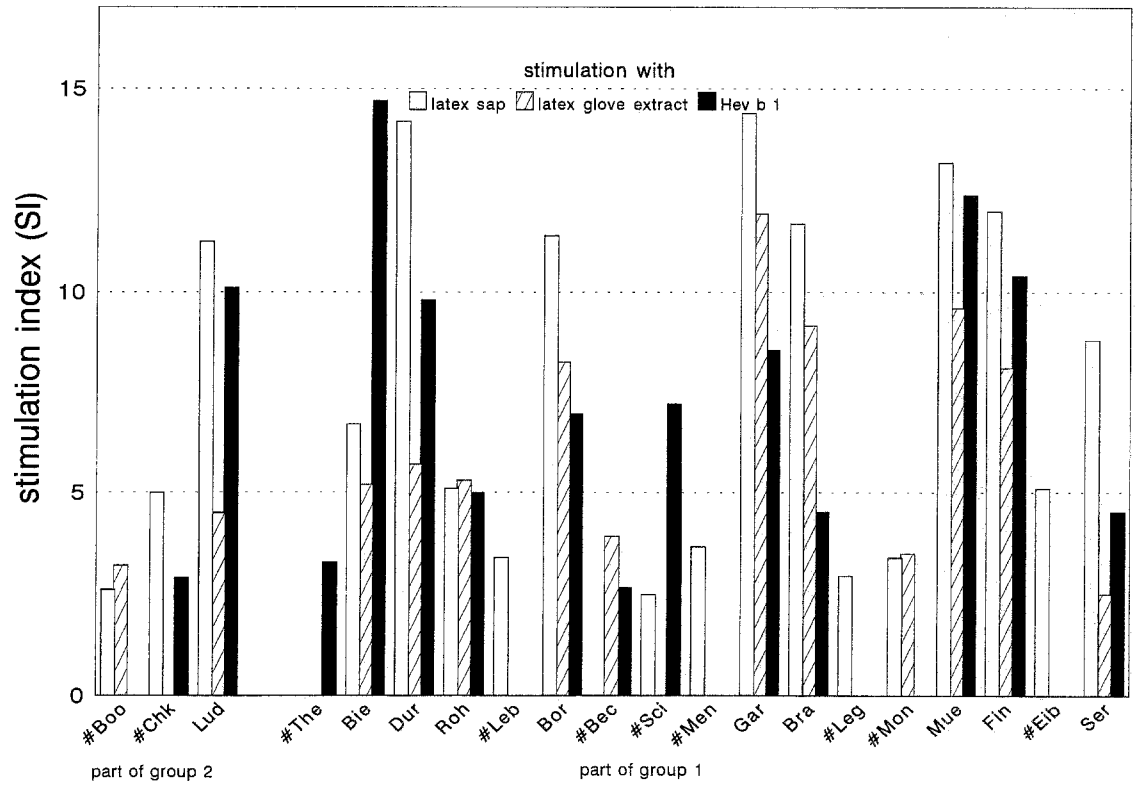

FIG. 5. Lymphocyte proliferation of subjects responding to latex sap extract, latex glove extract, and/or Hev b 1 (maximal SI values for each subject are presented). *Number sign* (#) indicates subjects (n = 10) without proliferation responses to all three antigen preparations. PBMCs of nine subjects in group 1 (9 of 23, 39.1%) responded to latex sap extract, latex glove extract, and Hev b 1. Only one subject of group 2 (Lud; 1 of 8, 12.5%) showed the same result. Mean SI values obtained from the lymphoproliferation responses of the examined subjects (n = 20) are 7.40 ± 4.28 for latex sap stimulation, 4.8 ± 3.48 for latex glove extract stimulation, and 5.21 ± 3.56 for Hev b 1 stimulation ($p \geq 0.05$).

TABLE III. Correlation of EAST Hev b 1 values of the latex-sensitized individuals (n = 23) with mean SIs to Hev b 1

EAST values	No. of subjects	Mean SI to Hev b 1
+0	11	4.83 ± 4.41*
+1	6	2.71 ± 2.20
+2	5	7.456 ± 4.46
+3	0	—
+4	1	2.20

*Mean SI of the group ± SD.

responses to Hev b 1 (5 of 12, 41.7%) had specific Hev b 1 IgE antibodies in their sera. The mean SI value in these five patients was 8.62 ± 3.0 and was not significantly different from the SI value of the seven patients responding to Hev b 1 on the cellular level without detectable sera Hev b 1–specific IgE (SI value: 6.7 ± 4.26). Like the data in Table II, data in Table III demonstrate the relationship of lymphocyte proliferation response to Hev b 1 (mean of maximal individual SI values) to the Hev b 1–specific IgE antibody levels (determined by EAST) in group 1 patients. These data clearly show that SI values in response to Hev b 1 are independent of the Hev b 1 IgE level in sera. In summary, no correlation between proliferation response (SI values) to latex sap extract, latex glove extract, and Hev b 1 and the level of specific latex IgE or Hev b 1–specific IgE could be found in our groups.

DISCUSSION

This study indicates that latex sap extract, latex glove extract, and the purified latex allergen Hev b 1 can induce a cellular immune response in vitro when incubated with PBMCs from latex-sensitized patients and occupationally latex-exposed nonsensitized subjects. When both extracts and the purified Hev b 1 were used, no significant proliferation was detectable in the healthy control group without occupational exposure to latex (group 3).

The first effort to show that T-cell–mediated immune reactions may occur in latex allergy was made by Turjanmaa et al.,[21] using latex allergen prepared from surgical gloves. They demonstrated that three of 15 patients (20%) with latex contact urticaria had positive lymphocyte proliferation test results. In addition, Murali et al.[22] showed that cells from a mixed group of patients with latex allergy responded specifically to both crude and purified latex antigens and that responses in patients with spina bifida were elevated in comparison with patients without spina bifida.

Our data demonstrate lymphocyte proliferation responses of latex-sensitized subjects to latex sap extract in 65% of the tested patients and in 37.5% of the latex-exposed healthy group. Latex glove extract induced a proliferative response in PBMCs of 47.8% of latex-sensitized patients and in 25% of latex-exposed individuals. According to the percentage of lymphoproliferation, there was no significant difference between latex-sensitized and latex-exposed nonsensitized subjects, and we were not able to distinguish between the cell responses of exposed subjects with or without sensitization. Only a subgroup of sensitized patients' PBMCs proliferated in the presence of latex antigen preparations. In contrast to our results obtained with latex-sensitized subjects, PBMCs of nearly all patients sensitized to the chironomid allergen Chi t 1-9[23] or to house dust mite allergens[24, 25] showed pronounced antigen-specific proliferation responses; and low to moderate responses in antigen-exposed controls were detectable. It must be considered that in the case of latex allergy, a variety of proteins are involved,[10] and the antigens used for latex-specific IgE determination (coupled to the solid phase in the CAP system) and lymphocyte stimulation (purified in our laboratory from different sources) were not identical. Furthermore, it must be mentioned that serologically negative, clinically reactive individuals might have IgE antibody levels below the detection limit of the assay. Alternatively, the latex allergen sources that were used (nonammoniated latex, ammoniated latex, gloves) may not contain the complete repertoire of clinically relevant latex allergens[26]; for example, in ammoniated latex some allergenic structures might be destroyed by ammoniation, whereas new allergenic structures might occur in latex gloves because of manufacturing processes.

In addition, individual T-cell response patterns and various T-cell epitopes are different from allergen recognition by IgE (B-cell response), and the relation between sensitization and lymphoproliferation response may be antigen-dependent.

Moreover, 12 of the 23 latex-sensitized subjects (52%) had positive specific IgE values when tested with the isolated latex allergen Hev b 1, indicating that this protein is a major allergen in the health care worker group. Hev b 1–specific lymphoproliferation was observed in 52% of the patients and 25% of the latex-exposed control subjects. With the exception of one patient, all of these subjects' lymphocytes responding to Hev b 1 had positive stimulation responses to latex sap extract and, with the exception of three subjects, also to latex glove extract.

Only five subjects with positive PBMC responses to Hev b 1 (5 of 12, 41.7%) had specific Hev b 1 IgE antibodies in their sera. On the other hand, PBMCs obtained from three patients with elevated Hev b 1–specific IgE levels (EAST class ≥ 2) were not able to proliferate in the presence of Hev b 1. The lack of correlation between antigen-specific lymphoproliferation to Hev b 1 and level of specific IgE to Hev b 1 in patients' sera also demonstrated the same trend as obtained with the two different latex extracts, suggesting that measurable antigen-specific cellular responsiveness at the T-cell level (measured by in vitro proliferation) and specific IgE values (as parameter of specific B-cell activity) are not closely associated.

The available data do not allow conclusive explanation of this lack of correlation, but we suggest that the following points be considered in interpretation of these results.

1. The correlation between cellular proliferative allergen responses and specific IgE level was allergen-dependent. In addition to our results with Hev b 1, data obtained with the purified allergen Chi t 1-9[23] confirmed our results. In contrast, investigation with the allergen Der p 1[24, 27] described a correlation between allergen-induced T-cell responses and specific Der p 1 IgE level and clinical allergic manifestation, respectively.
2. T-cell epitopes of Hev b 1 and IgE-binding sites may be complete or in part different. In the case of a purified protein such as Hev b 1, detailed analysis of T-cell, as well as B-cell epitopes[28] with peptides covering the whole sequence of Hev b 1 are necessary to determine whether T-cell epitopes or IgE-binding sites are different or similar.
3. The individual stimulation pattern of each pa-

tient's cells may be due to genetic restriction (e.g., with regard to the HLA-D locus).
4. Lymphoproliferation was only a parameter for measuring cellular responsiveness. Additional functions (parameters such as cytokine release, especially IL-4 release) of T cells seemed to be necessary to trigger IgE production. Therefore the direct correlation between high levels of specific IgE and the degree of proliferative response is not essential.
5. Other factors in addition to specific IgE level may be more closely associated with cellular proliferative responsiveness (e.g., allergen exposure, different clinical allergic manifestation [rhinitis vs asthma or eczema]) or severity of symptoms.
6. It cannot be ruled out that suppressor, feedback, or anergic effects prevented the lymphoproliferative responsiveness measured in vitro in patients with substantial total IgE or specific IgE levels.

Furthermore, the statistically significant difference in lymphoproliferative response between the patient group and the control group without occupational exposure to latex indicates that the latex extracts, as well as the purified latex allergen Hev b 1, are important indicators of relevant antigens. A higher SI in the group of latex-sensitized patients suggests a positive response and is thus a criterion of the specificity of the test antigen. These results are in agreement with previous findings for house dust mite allergens[24, 25] and the Chironomidae hemoglobin allergen Chi t 1-9.[23]

A significant correlation between latex-specific IgE level and the latex-induced lymphocyte proliferation response (SI value) could not be found; that is, PBMCs of a sensitized patient with specific latex IgE greater than 100 kU/L (CAP class 6) showed no significant antigen-specific proliferation response induced by the used protein concentrations. In contrast, in a subgroup of individuals occupationally exposed to latex without any latex sensitization or latex-specific IgE antibodies, antigen-specific proliferation was measurable. These data suggest that latex antigen–specific proliferation is more associated with the exposure to latex and not with the level of specific IgE. In contrast to our results and the results obtained with Chi t 1-9,[23] O'Brien et al.[27] demonstrated a significant association between the mite-specific IgE level and the T-cell responses to both Der p 1 and Der p 2 in house dust mite allergy. Allergen-specific influences may be important for these differences.

Finally, antigen-specific lymphocyte responsiveness should be mentioned as a predictive parameter of developing latex sensitization. Allergen recognition by T cells after their activation, including mediator release (e.g., IL-4), is a presupposition for B-cell triggering and IgE production. In the case of latex-exposed subjects without symptoms and without latex-specific IgE, some of them (especially the patient Lud) demonstrated a pronounced lymphocyte proliferation responsiveness to all tested allergen extracts and to Hev b 1. Future clinical examinations and cellular studies including the individual lymphokine pattern (IL-4 vs interferon-γ) will be necessary to clarify differences on the cellular level and steps of developing latex hypersensitivity in individual subjects.

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