

Purified and Recombinant Latex Proteins Stimulate Peripheral Blood Lymphocytes of Latex Allergic Patients

Bryon D. Johnson^a Viswanath P. Kurup^b Gordon L. Sussman^c
Siti A.M. Arif^d Kevin J. Kelly^b Donald H. Beezhold^e Jordan N. Fink^b

^aDepartment of Pediatrics and ^bDepartment of Medicine, Allergy-Immunology Division, Medical College of Wisconsin; and Research Service, Veterans Affairs Medical Center, Milwaukee, Wisc., USA, ^cUniversity of Toronto, Toronto, Ontario, Canada, ^dBiotechnology and Strategic Research Unit, Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia, and ^eLaboratory of Macrophage Biology, Guthrie Research Institute, Sayre, Pa., USA

Key Words

Latex allergen · Peripheral blood mononuclear cells stimulation · IgE · Health care workers · Recombinant allergen

Abstract

Background: Natural rubber latex proteins have been implicated in severe allergy in individuals exposed to latex products, particularly health care workers. Until recently, only crude antigens were available to study the immune response in these patients. In recent years a number of relevant allergens have been purified, but few have been used in lymphocyte studies. Hence, to better understand the immunological mechanisms involved in latex allergy, we investigated the response of peripheral blood mononuclear cells (PBMCs) to various purified natural rubber latex allergens. **Methods:** Using conventional protein purification methods and gene cloning, we have obtained 6 natural rubber latex proteins. We studied allergen-specific IgE levels and PBMC responses to these allergens along with 3 crude latex antigen preparations. **Results:** Of the 28 latex-allergic health care workers studied, 16 reacted to one or more

of the allergens studied, but PBMCs from controls failed to respond to these antigens. Serum IgE to the antigens was detected in 11–90% of the patients. **Conclusion:** Fifty-seven percent of the latex-allergic patients demonstrated PBMC responses to at least one of the latex allergens tested, but there was no direct correlation between serum IgE levels and PBMC responses. However, since none of the control subjects showed any PBMC stimulation, this may prove useful in determining sensitization to latex. Among the allergens studied, the predominant mononuclear cell responses were directed against Hev b 2, while serum IgE against rHev b 6 was demonstrable in the greatest number of patients. The crude latex allergens were toxic to PBMCs and hence, the purified allergens may be of greater value in demonstrating sensitization of patients to latex allergens.

Copyright © 1999 S. Karger AG, Basel

Introduction

Natural rubber latex allergy is a recognized occupational allergy among health care workers (HCW) [1–7]. The patients are exposed to latex allergens present in various

health care products made of natural rubber latex [8–12]. Of particular concern are gloves, tubing, stoppers, and many frequently used devices in health care maintenance. These devices are made from latex of the rubber tree *Hevea brasiliensis*, which contains about 1–2% of proteins [13]. Over 240 polypeptides have been demonstrated in natural rubber latex by two-dimensional polyacrylamide gel electrophoresis (PAGE) with at least 40–80 showing binding with specific IgE or IgG antibodies [14–15]. A number of these peptides may be cleavage products from several of the major latex proteins, as demonstrated by the N-terminal amino acid sequence analysis of a number of polypeptides resolved in two-dimensional electrophoresis [16].

In recent years, considerable attention has been directed towards the identification and purification of relevant latex allergens associated with latex allergy [17]. Molecular biological methods have also been employed and a number of genes encoding latex allergens have been cloned and expressed. Currently, 8 major allergens have International Union of Immunological Sciences designation and several more allergens have been identified. Most of them have been purified to homogeneity by conventional purification methods or by molecular cloning techniques [17]. The sequences of some of these allergens have been compared for homology with other known protein allergens. A few of these latex allergens like β 1,3-glucanase, microhelix components, rubber elongation factor, patatin-like protein, chitinase, enolase, manganese superoxide dismutase, and profilins have sequence homology with other allergens while others are unique and do not share any similarity [16, 18–27]. Epitopes, particularly B-cell epitopes, have been studied for several of the major allergens, including Hev b 1, rHev b 5, and rHev b 6 [28–30]. However, very few studies have been conducted to characterize the T-cell response in latex allergy [31–33]. The major problem in earlier studies was the toxicity of crude latex proteins, either from natural rubber latex or from finished latex products, to human peripheral blood mononuclear cells (PBMCs) [34]. However, a number of relevant purified natural and recombinant latex allergens have recently become available and may not be as toxic as the crude extracts. In order to gain a better understanding of the immunological mechanisms involved in latex allergy, we have undertaken the present study to determine the response of PBMCs from latex allergic patients utilizing purified latex antigens. We also studied the IgE binding of the sera with the allergens to compare their cellular and humoral immune responses. We evaluated the PBMC responses of a total of 36 subjects, including HCW, with latex allergy and non-latex-allergic controls using six purified latex allergens. The results of the present study

demonstrate significant stimulation induction of PBMC responses with several purified antigens while other antigens elicited variable mononuclear cell responses. The IgE binding of the various purified antigens varied from 11 to 89% among the latex-allergic patients.

Materials and Methods

Patients and Controls

PBMCs from 28 latex-allergic HCW, 6 patients with rhinitis but no clinical latex allergy, and 2 normal healthy controls were studied. Informed consent was obtained from all of the patients. The study was approved by the Institutional Human Research Review Committee.

Patients were diagnosed as having latex allergy when the following criteria were present: (1) a history of skin and respiratory symptoms usually progressing from contact dermatitis through urticaria to asthma and/or anaphylaxis on latex contact, particularly with latex glove powder inhalation; (2) immediate wheal and flare skin reaction to latex glove antigens; (3) IgE antibodies to natural rubber latex antigens or latex extract antigens as detected by an enzyme-linked immunosorbent assay (ELISA) or a radioallergosorbent test (RAST), and (4) a history of allergic reaction to one or more of the cross-reactive antigens such as bananas, avocados or other food allergens. All patients were examined in detail and a questionnaire was used to record the detailed case history, onset, and progress of the disease. The first three criteria were met by all patients, while the fourth was variable. All patients showed skin test reactivity to ammoniated latex extract (Bencard, Canada) and glove extracts used in day-to-day work. On initial diagnosis, all had significant serum levels of specific IgE to latex allergens by RAST, ELISA or Western blot. The patients included nurses, technologists, physicians and medical students.

Six HCW with seasonal rhinitis, but no latex specific IgE in the serum, and negative skin test reactivity to latex allergens were studied as controls. In addition, 2 laboratory workers with no latex allergy were also studied. Clinical latex allergy, as indicated by detailed examination and case history, skin and serological tests, was ruled out in all 8 control subjects.

Antigens

In the present study, we used 3 crude and 6 purified latex antigens (table 1). Two of the crude antigen extracts were obtained from examination gloves and the third from crude latex sap. The glove extracts were made from Perry X-am and Baxter Triflex gloves as described previously [6, 12, 35]. Briefly, 10 g of glove were cut into 1-cm² pieces and extracted with 50 ml of sterile phosphate-buffered saline using a magnetic stirrer for 2 h at room temperature. The fluid was decanted, centrifuged, and the supernatant dialyzed against distilled water at 4°C. These antigens designated as gloves 1 and 2 were aliquoted and stored at –70°C for up to 6 months.

The crude latex sap antigen was prepared as described previously [36]. Briefly, Malaysian nonammoniated (MNA) latex, a gift from Ansell International, was collected in sterile containers without any additives and frozen immediately before transport to the laboratory. The samples were transported frozen to the USA. In the laboratory, the latex was allowed to thaw slowly at 4°C, and the fluid from the coagulated sap was collected and centrifuged at 3,000 rpm for 30 min. The supernatant designated as MNA was collected, dialyzed, characterized, and stored as described above [36].

Purification of Hev b 1, 2, 3, 4

Fresh latex obtained by tapping *H. brasiliensis* trees was collected into chilled containers at the Rubber Research Institute of Malaysia Experimental Station, Sungei Buloh, Selangor, and Hev b 1, 2, 3, and 4 were fractionated and purified as described [13, 37]. Briefly, the latex was centrifuged and the rubber cream (top fraction), the fraction containing lutoids (bottom fraction), and the C-serum (the middle fraction) were separated. Hev b 1 and Hev b 3 were extracted from the rubber cream and solubilized in a detergent mixture of 0.1% Triton-X 100 and 1% sodium dodecyl sulfate (SDS) and purified by preparative electrophoresis [38]. Hev b 2 and Hev b 4 were prepared from the bottom fraction of centrifuged latex as reported previously [20]. The lutoids were ruptured by repeated freezing and thawing followed by dialysis. The protein precipitated during dialysis was redissolved in 0.35 M NaCl and Hev b 2 and Hev b 4 were purified using gel filtration with Sephacryl S-200 (Pharmacia, Sweden). These two proteins were eluted with 0.35 M NaCl as the two major peaks.

Cloning and Expression of rHev b 6

The gene encoding prohevein was amplified by means of PCR from a cDNA library generously supplied by Dr. Anil Kush of the University of Singapore [39]. Primers were designed to obtain the full-length prohevein gene by PCR as described previously [28]. The amplified PCR product was purified and subcloned in the pMALc2 expression vector (New England BioLabs, Beverly, Mass., USA). *Escherichia coli* (TB1) was transformed with pMALc2 vectors with and without the prohevein gene insert. The fusion protein was expressed along with maltose binding protein. Maltose-binding protein expressed from vectors without prohevein inserts was used as a control in IgE ELISAs and in the PBMC stimulation assays.

Cloning and Expression of rHev b 7

The gene encoding Hev b 7 was cloned as described previously [40]. The GST Gene Fusion System (Pharmacia Biotech Inc., Piscataway, NJ) was used to express rHev b 7. The fusion protein Hev b 7/GST, was purified using glutathione agarose beads [40]. The purified rHev b 7 was dialyzed against water, lyophilized, and resuspended in 100 mM Tris (pH 9) and stored at -40°C .

Characterization of the Antigens

The protein content in the crude antigens, purified antigens, and recombinant proteins was determined using Lowry, Coomassie blue binding or bis-cinchoninic acid protein assays (Pierce Chemical Company, Rockford, Ill., USA) [41, 42]. The purified native proteins and recombinant antigens expressed in the fusion protein pMal 2 vector or GST fusion system were studied for their SDS-PAGE profile, Western blot analysis and for their binding with known latex allergic and nonallergic patient sera, as described before [43].

Antigen Induced PBMC Stimulation

The PBMC proliferation assays were carried out in 96-well, U-bottom microtiter plates. PBMCs were obtained from heparinized blood as described previously [32]. Two hundred microliters of medium containing 2×10^5 PBMCs were cultured in each well with or without various concentrations of latex allergens. The cells were cultured in culture medium consisting of complete RPMI-1640 medium supplemented with 2 mmol/l glutamine, penicillin (100 U/ml), streptomycin (100 $\mu\text{g/ml}$), and pooled human AB serum (C-Six Diagnostics, Germantown, Wisc., USA). The cultures were incubated at 37°C in a humidified incubator containing 5% CO_2 for a total of 6–7 days.

Table 1. Latex allergens used in this study

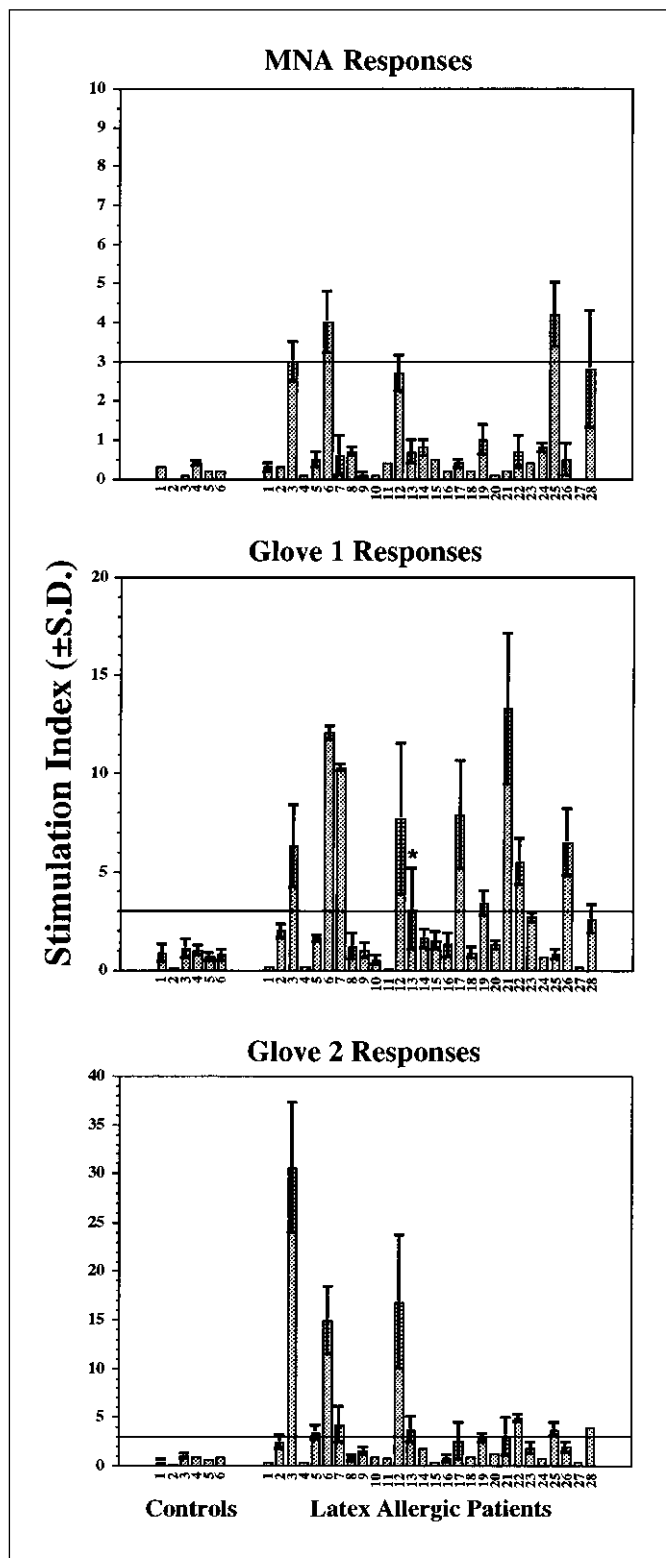
Hev b 1 – rubber elongation factor (14 kD), rubber cream
Hev b 2 – β 1,3-glucanase; latex B-serum
Hev b 3 – small rubber particle protein (24 kD); rubber cream
Hev b 4 – microhelix protein (50–57 kD); latex B-serum
Hev b 6 – prohevein (20 kD); cloned and expressed
Hev b 7 – patatin-like protein (43 kD); cloned and expressed
MNA – Malaysian nonammoniated latex; crude latex sap
Glove 1 – Perry X-am; protein extract
Glove 2 – Baxter Triflex; protein extract

The antigens were titrated at various concentrations to determine the optimal concentration for stimulating PBMC responses in vitro. The cultures were pulsed with 0.5 μCi per well of [^3H]-thymidine (specific activity 6.5 Ci/mmol; Amersham, Arlington Heights, Ill., USA) for the final 18-hour of culture. The cells were harvested on glass fiber filters and the radioactivity measured on a beta scintillation counter (Micromedex Systems, Inc.). The stimulation index (SI) was calculated as the mean counts per minute of triplicate experimental wells divided by the mean counts per minute of triplicate control wells (no antigen). The proliferation responses were considered positive if the SI was >3 and the standard deviations between the experimental mean counts per minute and control mean counts per minute were nonoverlapping. All PBMCs were also tested for mitogen (concanavalin A)-induced proliferation as described previously [32].

Enzyme-Linked Immunosorbent Assays

All 9 antigens were studied for IgE binding activity by ELISA, as previously described [6, 43]. The concentrations of latex antigens used to coat ELISA plates (Immulon II, Fisher Scientific, Itasca, Ill., USA) were determined by checkerboard titration of each latex protein with sera from patients with latex allergy [6, 36, 43]. Polystyrene microtiter plates were coated with latex proteins diluted in PBS as described previously [6, 43]. The wells were blocked with 0.5% BSA in PBS containing Tween-20 (PBS-T) prior to the addition of a 1:25 dilution of the patient serum. After incubation at room temperature for 3 h, the wells were washed and treated with a 1:500 dilution of 100 μl biotinylated goat anti-human IgE (Vector, Burlingame, Calif., USA). The plates were incubated for 1 h followed by washing and addition

Fig. 1. PBMC responses of control subjects and latex-allergic patients to purified Hev b latex allergens. PBMCs from individual control subjects ($n = 6$) and latex allergic patients ($n = 28$) were cultured in triplicate U-bottom microtiter wells with optimal concentrations (table 2) of the designated Hev b allergens. Proliferative responses were determined following 6–7 days of culture (see Materials and Methods for details). The SIs shown for each subject were calculated as the mean counts per minutes of wells containing Hev b allergens divided by the mean counts per minute of wells containing no antigen. SI values >3 were considered as positive. The asterisk denotes a response that was considered negative because the standard deviations (SD) of the experimental and control mean counts per minute were overlapping.



of a 1:1,000 dilution of streptavidin peroxidase (Sigma, St. Louis, Mo., USA). The plates were incubated for an additional hour and peroxidase reactivity was detected by the addition of orthophenylene diamine. Color development was stopped by the addition of 2 M H_2SO_4 , and the optical density (OD) values were read in an automated ELISA plate reader using a 490-nm filter (Molecular Devices). The blanks were subtracted and the mean OD values compared.

Statistical Evaluation

SI values from the PBMC response assays and mean IgE OD values obtained from the ELISAs were compared using the t test. A p value of 0.05 was considered significant for delineating the groups.

Results

Response of PBMCs from the Latex-Allergic Patients to Different Allergens and Extracts

The results of PBMC responses from individual patients to each of the 9 antigens tested are shown in figures 1 and 2. Of the 28 latex allergic HCW patients studied, 16 patients showed a positive PBMC response to at least one of the antigens. None of the control subjects demonstrated a positive PBMC response towards any of the antigens studied (fig. 1, 2). The 16 latex-allergic patients who demonstrated a positive response showed considerable variation in their response to different antigens. Three of these patients failed to respond to any of the 3 crude latex extracts used in the study (table 2). Although none of the 16 patients studied showed PBMC responses towards all 9 antigens, the majority reacted to 4 or more of them.

The overall PBMC responses of the 28 latex-allergic patients against each individual antigen varied considerably. For each of the 9 antigens tested, the mean SIs of the latex-allergic patients were significantly greater than those of the control subjects (fig. 3). Among the purified Hevea proteins, Hev b 2 induced the greatest level of reactivity (fig. 3). Hev b 2 also induced high SIs from individual latex-allergic patients (fig. 1) with 80% of the positive responders

Fig. 2. PBMC responses of control subjects and latex-allergic patients to crude latex-allergens. PBMCs from individual control subjects and latex allergic patients were cultured in triplicate U-bottom microwells with optimal concentrations (table 2) of the designated crude latex allergens. Proliferative responses were determined following 6–7 days of culture (see Materials and Methods for details). The SIs shown for each subject were calculated as the mean counts per minute of wells containing Hev b allergens divided by the mean counts per minute of wells containing no antigen. SI values >3 were considered positive. The asterisk denotes a response that was considered as negative because the standard deviations (SD) of the experimental and control mean cpm were overlapping.

Fig. 3. Combined PBMC responses to latex allergens of latex-allergic patients and control subjects. PBMCs from individual control subjects ($n = 6$) and latex-allergic patients ($n = 28$) were cultured in triplicate U-bottom microwells with optimal concentrations (table 2) of the designated latex allergens. Proliferative responses were determined following 6–7 days of culture (see Materials and Methods for details). The data shown are the mean SIs ($\pm$ SEM) for each group of subjects for each individual latex allergen. * $p < 0.05$.

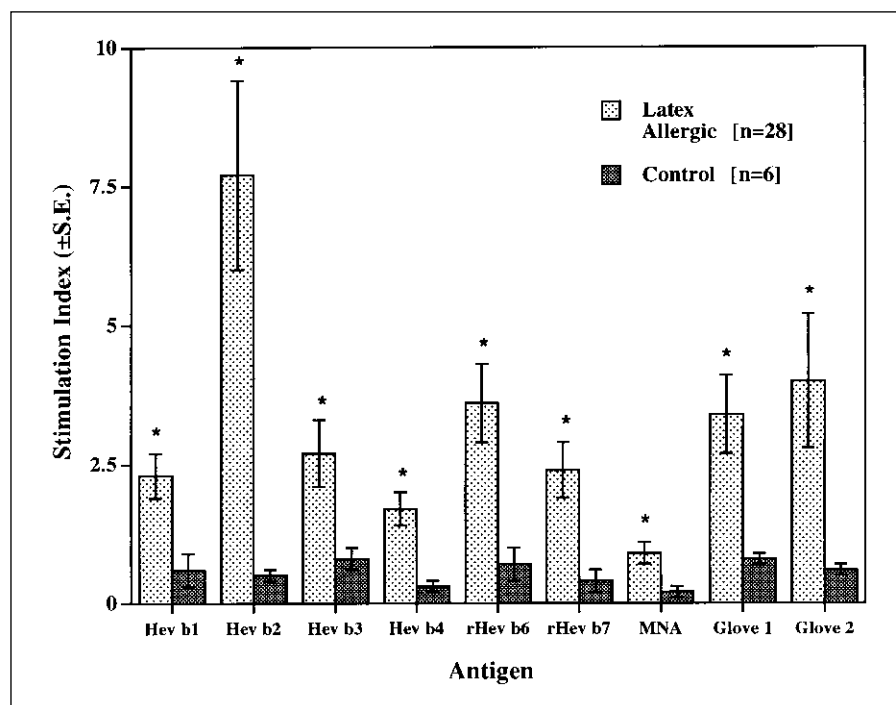
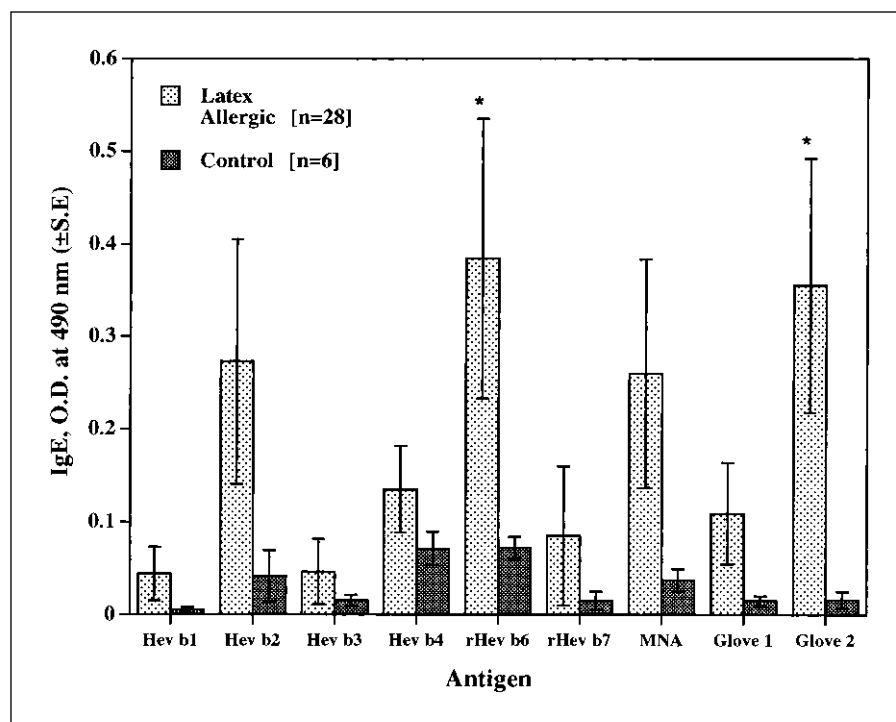


Fig. 4. Specific IgE against different purified and crude latex-antigens in the sera of latex allergic patients and controls. Antigen-coated wells of microtiter plates were allowed to react with a 1:25 dilution of the various sera. The color developed was read at 490 nm using an ELISA plate reader. The data shown are the mean $\pm$ SEM for each individual latex allergen. * $p \leq 0.05$.



exhibiting stimulation indices of 5 or greater. The least reactivity was noted against Hev b 3 and Hev b 4 (fig. 3). The natural rubber latex extract (MNA) stimulated the PBMCs of only 2 latex-allergic patients (fig. 2, table 2) and the overall mean SI was low (fig. 3). The 2 glove antigen prepara-

tions each induced responses from 9 latex-allergic patients (fig. 2), and the overall responses to these antigens were greater than the MNA response (table 2).

Table 2. Responses of PBMCs from latex-allergic patients and control subjects to latex allergens^a

	Proliferative response (SI > 3) ^b								
	Hev b1 (2.5 µg/ml)	Hev b2 (5.0 µg/ml)	Hev b3 (2.5 µg/ml)	Hev b4 (1.25 µg/ml)	rHev b6 (2.5 µg/ml)	rHev b7 (2.5 µg/ml)	MNA (1.25 µg/ml)	Glove 1 (2.5 µg/ml)	Glove 2 (2.5 µg/ml)
Controls (n = 6)	–	–	–	–	–	–	–	–	–
Latex-allergic patients No. (n = 12)	–	–	–	–	–	–	–	–	–
2	–	+	–	–	+	–	–	–	–
3	+	+	+	–	+	+	–	+	+
5	–	+	–	–	–	–	–	–	+
6	+	+	+	–	+	+	+	+	+
7	–	+	–	–	+	–	–	+	+
12	+	+	+	+	+	+	–	+	+
13	–	+	+	–	+	–	–	–	+
17	–	+	–	–	+	+	–	+	–
19	–	+	+	–	+	–	–	+	–
21	–	+	–	+	+	+	–	+	–
22	–	+	–	+	+	+	–	+	+
23	+	+	–	–	–	–	–	–	–
24	–	+	–	–	–	–	–	–	–
25	+	+	+	–	–	–	+	–	+
26	+	+	–	+	+	+	–	+	–
28	+	+	+	–	+	–	–	–	+

^a Following dose titration experiments, the antigen concentrations used were found to be optimal for inducing proliferative responses.

^b The SI was calculated as the mean cpm of triplicate experimental wells containing antigen divided by the mean cpm of triplicate control wells (no antigen). The proliferative responses were considered positive (+) if the SI was >3 and the standard deviations between the experimental mean counts per minute and control counts per minute were nonoverlapping.

Latex Specific IgE Antibody in the Sera

The combined results of the IgE ELISAs are shown in figure 4. The latex-allergic patients had significantly higher allergen-specific serum IgE levels when tested initially during the diagnosis (data not shown). Among the controls, only 2 subjects showed significant levels of IgE antibody. One showed reactivity towards 2 antigens (Hev b 2 and Glove 2), while the other one reacted to 1 antigen (rHev b 7). Of the 28 latex-allergic patients studied, 11–89% had detectable serum IgE levels against individual antigens (data not shown). Specific IgE against rHev b 6 was significantly elevated in latex-allergic patients compared to normals, while other antigens showed marked variations. One latex-allergic patient had no detectable serum IgE to any of the antigens tested.

The serum IgE levels and PBMC response data were compared to determine whether there was any correlation between the two sets of data. As shown in table 3, there was no correlation between latex-specific serum IgE titers and PBMC responses. Of the 3 latex-allergic patients with

high serum IgE levels to various allergens studied, only 1 patient (subject 19) showed significant PBMC stimulation with the corresponding allergens. Similarly, several of the subjects with low or nondetectable IgE levels showed strong PBMC responses to particular antigens and vice versa, indicating no demonstrable correlation between the antibody response and T cell response in the latex-allergic patients (table 3).

Discussion

This study is the first to report on PBMC responses of latex allergic patients towards a panel of 6 purified latex proteins. Previous studies investigating lymphocyte responses to latex antigens had reported on responses to only 1 or 2 purified latex proteins [31, 32, 44]. While the PBMC response assays used in the current study did not look directly at T cells, these assays provided a relative measure of T cell reactivity towards the antigens tested. In other studies,

Table 3. IgE and PBMC response in patients with latex allergy

IgE and PBMC response %	Antigens								
	Hev b1	Hev b2	Hev b3	Hev b4	rHev b6	rHev b7	MNA	glove 1	glove 2
IgE + PBMC +	0	10	2	3	11	3	1	7	9
	0	35	7	11	39	11	4	25	32
IgE + PBMC –	5	3	1	10	14	5	19	12	16
	18	11	4	35	50	18	68	43	57
IgE – PBMC +	7	6	5	1	1	4	1	2	0
	25	21	18	4	4	14	4	7	0
IgE – PBMC –	16	10	20	14	2	16	7	8	3
	57	36	71	50	7	57	25	29	11

+ = Significant IgE antibody and SI of PBMC compared to normals; – = no significant difference between normal controls and the patients in the IgE antibody level or PBMC stimulation.

we have found that mononuclear cell proliferative responses towards latex allergens are dominated by CD4+ T cells [unpubl. data], which is not surprising since CD4+ helper T cells have been shown to play an important role in IgE production [45].

Among the purified antigens tested in the current study, Hev b 2 induced the strongest responses, as evidenced by the greatest number of responding patients (16/28 patients) and by the intensity of the overall response (table 2, fig. 3). All patients that responded to at least 1 of the allergens tested also responded to Hev b 2. Among the purified *H. brasiliensis* proteins, rHev b 6 induced the second highest overall PBMC response (table 2, fig. 3; $n = 12$). These results suggest that the Hev b 2 and rHev b 6 antigens may be significant to the cell-mediated immune response in latex-allergy. Hev b 3, on the other hand, consistently induced PBMC stimulation in only 25% of the latex-allergic HCW patients. This may be due to the fact that this antigen, a membrane bound allergen, has been considered to be more specific for spina bifida patients with latex allergy [38, 46, 47]. Only 3 (11%) of the latex-allergic patients showed IgE reactivity to Hev b 3, while 1 out of 4 elicited a PBMC response to this antigen. As reported previously, this antigen is less reactive to IgE in the sera of HCW patients [28, 46], indicating that it is not a significant allergen in HCW patients.

Three crude antigen preparations were tested in this study. Two latex glove extracts induced responses from 9 patients each (table 2). We anticipated that the glove extracts would not be the most potent stimulators of PBMC proliferation since the extracts are composed of a mixture of proteins at varying concentrations. MNA induced poor responses (fig. 2, tables 2, 3) and was found to be toxic to

PBMCs at concentrations above 1.2 $\mu\text{g/ml}$ (data not shown). Reducing the MNA concentration increased the viability of PBMC, but failed to result in enhanced stimulation (data not shown). This was likely due to suboptimal concentrations of relevant proteins in the diluted crude extract. Similar toxicity of crude latex allergens to T-cell lines has been reported [34].

The lack of PBMC responses to any of the latex antigens by the control subjects indicates that the stimulatory response of PBMCs by the various latex allergens are specific and nonmitogenic. Although most of the latex-allergic patients studied showed elevated latex-specific IgE when tested at the time of the diagnosis of latex allergy, several of them demonstrated decreased latex-specific IgE levels when the PBMC response was studied. Most of these patients were not exposed to latex for over a year and showed markedly reduced IgE levels to latex allergens. In spite of the high IgE responses to the crude antigens (67–89%), relatively few patients (3–32%) showed PBMC responses to the same antigen preparations. On the other hand, 35 and 39% of IgE-positive patients showed PBMC responses to Hev b 2 and rHev b 6 allergens, respectively (table 3), suggesting that there may be a better correlation between IgE levels and PBMC responses when purified specific latex antigens are used.

In summary, we have demonstrated that Hev b 2 and rHev b 6 latex proteins are capable of inducing strong cellular immune responses in a significant number of latex-allergic HCW patients. These results suggest that Hev b 2 and rHev b 6 may play dominant roles in the latex-specific T-cell responses of latex-allergic patients. In addition, the present study confirms that purified antigens can be used effectively to study and evaluate lymphocyte responses in latex

allergic patients. Purified latex allergens are less toxic than crude antigen extracts and can be used in evaluating the lymphocyte response to a specific antigen. Although there was no significant correlation between the IgE levels and PBMC stimulation when crude antigens were used, enhanced PBMC responses were observed when purified antigens were used. Hence, PBMC response assays may be of value in identifying sensitized patients with low or undetectable levels of IgE to latex. Such findings may help in avoiding serious anaphylactic reactions resulting from latex exposure in sensitized subjects with low levels of IgE or those who are in relapse.

Acknowledgment

This investigation was supported partly by USPH Service, Ansell International, Children's Hospital Foundation, Guthrie Foundation for Medical Education and Research the US Veterans Affairs and by IR-PA grant 01-04-04-0007 from the Ministry of Science Technology and Environment, Malaysia. The technical assistance of Laura Castillo and Emily Becker and the editorial assistance of Donna Schrubbe are gratefully acknowledged.

References

- 1 Fink JN (ed): Latex Allergy Philadelphia, Saunders, 1995.
- 2 Hamilton RG, Yeang HY: Differential sensitization of latex allergic children with spina bifida (SB) and adult health care workers (HCW) to rubber particle associated (RPA) allergens. *J Allergy Clin Immunol* 1997;99:S266.
- 3 Posch A, Chen Z, Raulf-Heimsoth M, Baur X: Latex allergens. *Clin Exp Allergy* 1998;28:134-140.
- 4 Turjanmaa K, Alenius H, Makinen-Kiljunen S, Reunala T, Palosuo T: Natural rubber latex allergy. *Allergy* 1996;51:593-602.
- 5 Slater JE: Rubber anaphylaxis. *N Engl J Med* 1989;320:1126-1130.
- 6 Kelly KJ, Pearson ML, Kurup VP, Havens PL, Byrd RS, Setlock MA, Butler JC, Slater JE, Grammer LC: A cluster of anaphylactic reactions in children with spina bifida during general anesthesia: Epidemiologic features, risk factors, and latex hypersensitivity. *J Allergy Clin Immunol* 1994;94:53-61.
- 7 Sussman G, Tarlo S, Dolovich J: The spectrum of IgE-mediated response to latex. *JAMA* 1991;265:2844-2847.
- 8 Turjanmaa K: Incidence of immediate allergy to latex gloves in hospital personnel. *Contact Dermatitis* 1987;17:270-275.
- 9 Swanson M, Burbank M, Hunt L, Yunginger J, Warner M, Reed C: Quantitation of occupational latex aeroallergens in a medical center. *J Allergy Clin Immunol* 1994;94:445-451.
- 10 Vanderplas O, Delwicke TP, Evrard G, Aimont P, van der Brempt X, Jamart J, Delaunois L: Prevalence of occupational asthma due to latex among hospital personnel. *Am J Resp Crit Care Med* 1995;151:54-60.
- 11 Yassin M, Lierl M, Fischer T, O'Brien K, Cross J, Steinmetz C: Latex allergy in hospital employees. *Ann Allergy* 1994;72:245-249.
- 12 Kelly KJ, Kurup VP, Zacharisen M, Resnick A, Fink JN: Skin and serologic testing in the diagnosis of latex allergy. *J Allergy Clin Immunol* 1993;91:1140-1145.
- 13 Moir GFJ: Ultrastructure and staining of *Hevea* latex. *Nature* 1959;184:1626-1628.
- 14 Alenius H, Kurup V, Kelly K, Palosuo T, Turjanmaa K, Fink JN: Latex allergy: Frequent occurrence of IgE antibodies to a cluster of 11 latex proteins in patients with spina bifida and histories and anaphylaxis. *J Lab Clin Med* 1994;123:712-720.
- 15 Kurup VP, Alenius H, Kelly KJ, Castillo L, Fink JN: A two-dimensional electrophoretic analysis of latex peptides reacting with IgE and IgG antibodies from patients with latex allergy. *Int Arch Allergy Immunol* 1996;109:58-67.
- 16 Posch A, Chen Z, Wheeler C, Dunn MJ, Raulf-Heimsoth M, Baur X: Characterization and identification of latex allergens by two-dimensional electrophoresis and protein microsequencing. *J Allergy Clin Immunol* 1997;99:385-395.
- 17 Breiteneder H: The allergens of *Hevea brasiliensis*. *Allergy Clin Immunol Int* 1998;10:101-109.
- 18 Breton F, Coupe M, Sanier C, d'Auzac JD: Demonstration of β 1,3-glucanase activities in luteoids of *Hevea brasiliensis* latex. *J Nat Rubb Res* 1995;10:37-45.
- 19 Chye ML, Cheung KY: β 1,3-glucanase is highly-expressed in laticifers of *Hevea brasiliensis*. *Plant Mol Biol* 1995;29:397-402.
- 20 Sunderasan E, Hamzah S, Hamid S, Ward MA, Yeang HY, Cardoso MJ: Latex B-serum β 1,3-glucanase (Hev b 2) and a component of the microhelix (Hev b 4) are major latex allergens. *J Nat Rubb Res* 1996;10:82-99.
- 21 Dennis MS, Henzel WJ, Bell J, Kohr W, Light DR: Amino acid sequence of rubber elongation factor protein associated with rubber particles in *Hevea* latex. *J Biol Chem* 1989;264:18618-18626.
- 22 Czuppon AB, Chen Z, Rennert S, Engelke T, Meyer HE, Heber M, Baur X: The rubber elongation factor of rubber trees (*Hevea brasiliensis*) is the major allergen in latex. *J Allergy Clin Immunol* 1993;92:690-697.
- 23 Beezhold DH, Sussman GL, Kostyal DA, Chang NS: Identification of a 26-kD latex protein allergen in health care workers. *Clin Exp Immunol* 1994;98:408-413.
- 24 Vallier P, Balland S, Harf R, Valenta R, Deviller P: Identification of profilin as an IgE-binding component in latex from *Hevea brasiliensis*: Clinical implications. *Clin Exp Allergy* 1995;25:332-339.
- 25 Graham LS, Sticklen MB: Plant chitinases. *Can J Bot* 1994;72:1057-1083.
- 26 Martin MN: The latex of *Hevea brasiliensis* contains high levels of both chitinases and chitinases/lysozymes. *Plant Physiol* 1991;95:469-476.
- 27 Miao Z, Gaynor JJ: Molecular cloning, characterization and expression of Mn-superoxide dismutase from the rubber tree (*Hevea brasiliensis*). *Plant Mol Biol* 1993;23:267-277.
- 28 Banerjee B, Wang X, Kelly KJ, Fink JN, Sussman GL, Kurup VP: IgE from latex allergic patients binds to cloned and expressed B cell epitopes of prohevein. *J Immunol* 1997;159:5724-5732.
- 29 Beezhold DH, Kostyal DA, Sussman GL: IgE epitope analysis of the hevein preprotein; a major latex allergen. *Clin Exp Immunol* 1997;108:114-121.
- 30 Beezhold DH, Hickey VL, Slater JE, Sussman GL: Human IgE binding epitopes of the latex allergen Hev b 5. *J Allergy Clin Immunol* 1999;103:S162.
- 31 Raulf-Heimsoth M, Chen Z, Rihs H-P, Kalbacher H, Liebers V, Baur X: Analysis of T-cell reactive regions and HLA-DR4 binding motifs on the latex allergen Hev b 1 (rubber elongation factor). *Clin Exp Allergy* 1998;28:339-348.
- 32 Murali PS, Kelly KJ, Fink JN, Kurup VP: Investigations into the cellular immune responses in latex allergy. *J Lab Clin Med* 1994;124:638-643.
- 33 Rihs H-P, Cremer R, Chen Z, Baur X: Molecular analysis of DRB and DQB1 alleles in German spina bifida patients with and without IgE responsiveness to the latex major allergen Hev b 1. *Clin Exp Allergy* 1998;28:175-180.

- 34 Cormio L, Turjanmaa K, Talja M, Andersson LC, Ruutu M: Toxicity and immediate allergenicity of latex gloves. *Clin Exp Allergy* 1993;23:618–623.
- 35 Yunginger JW: Variance in antigenicity of latex products; in Fink JN (ed): *Latex Allergy*. Philadelphia, Saunders, 1995, pp 61–70.
- 36 Kurup VP, Murali PS, Kelly KJ: Latex antigens; in Fink JN (ed): *Latex Allergy*. Philadelphia, Saunders, 1995, pp 45–59.
- 37 Yeang HY, Yip E, Samsidar H: Characterization of zone 1 and zone 2 rubber particles in *Hevea brasiliensis* latex. *J Nat Rubb Res* 1995; 10:108–123.
- 38 Yeang HY, Cheong KF, Sunderasan E, Hamzah S, Chew NP, Hamid S, Hamilton RG, Cardosa MJ: The 14.6 kD rubber elongation factor (Hev b 1) and 24 kd (Hev b 3) rubber particle proteins are recognized by IgE from patients with spina bifida and latex allergy. *J Allergy Clin Immunol* 1996;98:628–639.
- 39 Kush A, Goyvaerto E, Chye ML, Chua N-H: Laticifer-specific gene expression in *Hevea brasiliensis* (rubber tree). *Proc Natl Acad Sci* 1990;87:1787–1790.
- 40 Kostyal DA, Hickery VL, Noli JD, Sussman GL, Beezhold DH: Cloning and characterization of a latex allergen (Hev b 7): Homology to patatin, a plant PLA2. *Clin Exp Immunol* 1998;112:355–362.
- 41 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–275.
- 42 Bradford MM: A rapid and sensitive method for the quantitation of microorganism quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–254.
- 43 Kurup VP, Yeang HY, Sussman GL, Bansal NK, Beezhold DH, Kelly KJ, Hoffman DR, Williams B, Fink JN: Detection of IgE antibodies in the sera of patients using purified latex allergens. *Clin Exptl Allergy*, in press.
- 44 Raulf-Heimsoth M, Chen Z, Liebers V, Allmers H, Baur X: Lymphocyte proliferation response to extracts from different latex materials and to the purified latex allergen Hev b 1 (rubber elongation factor). *J Allergy Clin Immunol* 1996;98:640–651.
- 45 Mosmann TR, Coffman RL: Th1 and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145–173.
- 46 Alenius H, Palosuo T, Kelly K, Kurup V, Reunala T, Makinen-Kiljunen S, Turjanmaa K, Fink JN: IgE reactivity to 14-kD and 27-kD natural rubber proteins in latex-allergic children with spina bifida and other congenital anomalies. *Int Arch Allergy Immunol* 1993; 102:61–66.
- 47 Lu LJ, Kurup VP, Hoffman DR, Kelly KJ, Murali PS, Fink JN: Characterization of a major latex allergen associated with hypersensitivity in spina bifida patients. *J Immunol* 1995;155: 2721–2728.

Copyright: S. Karger AG, Basel 1999. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.