

In vivo sensitization to purified *Hevea brasiliensis* proteins in health care workers sensitized to natural rubber latex

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Background: Thirteen proteins of natural rubber latex (*Hevea brasiliensis*) known to bind human IgE have been isolated and characterized as Hev b allergens. However, the in vivo importance of native Hev b allergens has not been defined in health care workers (HCWs) with natural rubber latex (NRL) allergy. **Objectives:** The principal aim of this study was to identify the major in vivo Hev b allergens in HCWs with NRL allergy confirmed by percutaneous sensitivity to nonammoniated latex (NAL).

Methods: Skin prick testing was performed with 7 (native) proteins purified from NAL (Hev b 1, 2, 3, 4, 6.01, 7.01, and a newly described Hev b 13) and recombinant Hev b 5 in 62 HCWs with histories of NRL allergy (group 1) confirmed by percutaneous reactivity to NAL and in 49 atopic HCWs without NRL allergy (group 2). Serial 10-fold concentrations of Hev b proteins (5×10^{-5} µg/mL to 50 µg/mL) were tested; serum samples of subjects were assayed for serum specific IgE by immunoassays.

Results: Hev b 2, Hev b 5, Hev b 6.01, and Hev b 13 produced skin reactions in more than 60% of group 1 subjects, with Hev b 1, 3, 4, and 7.01 eliciting reactions in less than 50%. Only 1 of 49 group 2 workers reacted to a single Hev b antigen (Hev b 13). Specificity of 7 Hev b allergens was 100% and 98% for Hev b 13 in identifying workers with confirmed NRL allergy. Specific IgE by AlaSTAT and CAP immunoassays was elevated in 40 of 60 (67%) and 33 of 62 (53%) of NAL-reactive workers and produced false-positive test results in 4 of 49 (8%) and 3 of 48 (6%) group 2 subjects, respectively.

Conclusion: Hev b 2, 5, and 6.01 are major in vivo allergens and Hev b 13 is a new major in vivo allergen among HCWs with allergy to NRL. (J Allergy Clin Immunol 2003;111:610-6.)

Key words: Latex, natural rubber latex, Hev b, Hev b 13, skin prick test, latex allergy, health care workers

A reported 5% to 15% of health care workers (HCWs) regularly exposed to powdered natural rubber latex (NRL) gloves have NRL allergy confirmed by skin prick test reactivity to NRL skin testing reagents.¹ Objective confirmation of IgE mediated sensitization to NRL is essential in the accurate diagnosis of work-related occupational urticaria, rhinitis, or asthma in HCWs. Although NRL skin test extracts have been commercially available in Europe and in Canada, serologic NRL serum specific IgE immunoassays (eg, AlaSTAT, IMMULITE, CAP, HY-TECH) are the only Food and Drug Administration (FDA)-cleared tests currently available in the United States for confirming NRL allergy. In vitro assays possess suboptimal diagnostic sensitivity as compared to skin prick testing in identifying HCWs with NRL allergy.² Therefore, allergists practicing in the United States urgently need commercial NRL skin test reagents.

Of approximately 50 NRL proteins that bind IgE, 13 have been purified from crude NRL extracted from the rubber tree, *Hevea brasiliensis*, and these have been designated as Hev b allergens.³ Because of the well-documented, low diagnostic sensitivity of commercial serologic IgE antibody assays and occasional false-negative skin test results with various reagents, the usefulness of recombinant Hev b allergens as potential diagnostic antigens have been assessed in HCWs.⁴⁻⁶ By using 4 purified Hev b (Hev b 1, 2, 3, and 4) and 2 recombinant proteins (Hev b 6 and 7) as antigens in an ELISA specific IgE assay, Kurup et al⁷ reported that the combination of positive test results for Hev b 2 and Hev b 7 detected 81% of HCWs with NRL allergy. However, because elevated ELISA binding was detected in 12% and 6% of normal control subjects to Hev b 2 and 7, respectively, these in vitro tests possessed some degree of nonspecificity. Yeang et al⁶ performed similar in vitro specific IgE studies with purified Hev b 1, 2, 3, and 4 and two Hev b 7 proteins (one of which has now been renamed Hev b 13). They reported that IgE antibodies were detected to 2 or more antigens in 74% of patients with NRL allergy. Yip et al⁵ performed

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Abbreviations used

ETC: Endpoint percutaneous threshold concentration
FDA: Food and Drug Administration
HCW: Health care worker
NAL: Nonammoniated latex
NRL: Natural rubber latex

skin prick testing with six Hev b recombinant proteins (Hev b 2, 3, 5, 6, 7, and 8) and found reactivity to Hev b 5, 6, or 7 among 93% of 31 HCWs tested without detecting false-positive responses in control subjects.

In this article we report on a novel skin prick test titration study with 7 purified Hev b native proteins including a newly described allergen, Hev b 13, and 1 recombinant protein (Hev b 5) in 62 HCWs with confirmed NRL allergy. The aims of this study were (1) to use in vivo techniques for identifying principal Hev b allergens that elicit specific IgE antibody responses in HCWs with NRL allergy and (2) to compare the test characteristics of skin prick test Hev b responses with FDA-cleared commercial immunoassays that detect NRL serum specific IgE.

METHODS

Study population

HCWs were recruited for participation by advertisement. Before initiating study-related activities, prospective subjects gave written informed consent that had been approved by Centers for Disease Control/National Institute of Occupational Safety and Health Human Subjects Review Board.

Two groups of HCWs underwent skin test procedures. Inclusion criteria for the NRL allergic study group, group 1, included (1) prior or current history of immediate onset cutaneous, respiratory, or anaphylactic symptoms associated with occupational exposure to NRL gloves and (2) a positive skin prick test result to nonammoniated latex (NAL) extract at a concentration of less than or equal to 1000 µg/mL of protein at the screening visit. The vast majority (88%) of group 1 workers were atopic (ie, demonstrated skin prick test reactivity to at least 1 of 12 common aeroallergens).

A group of 49 HCWs (group 2) who were atopic and without prior NRL glove related symptoms served as a control group. Criteria for inclusion in group 2 included (1) prior seasonal allergic rhinitis symptoms during tree, grass, and ragweed pollen seasons combined with a positive skin prick test result to at least 1 of 12 regional aeroallergens; (2) a history of work-related dermal exposure to NRL gloves; (3) no symptoms associated with direct or indirect exposure to NRL gloves; and (4) a negative skin prick test result to all test concentrations of 1000 µg/mL or less of NAL extract.

Because of concerns over heightened risks of NRL skin testing in asthmatic subjects, prospective subjects with active or prior histories of asthma underwent screening spirometry and were not permitted to participate unless (1) FEV₁ was greater than 70% predicted; (2) asthma symptoms occurred at a frequency of 3 or fewer times/week within 2 weeks before skin testing; (3) no nocturnal asthma episodes occurred within the previous 2 weeks; and (4) rescue inhaled β-agonists were being used fewer than 3 times per week. Two excluded volunteers included an HCW with an FEV₁ that was 50% predicted and another with FEV₁ of 85% predicted, who also reported daily asthma symptoms.

Subjects were excluded if they were (1) women who were pregnant, nursing, or not using acceptable methods of birth control; (2) unable to discontinue use of antihistamines or drugs known to inhibit histamine; (3) identified with a skin condition (eg, der-

matographism) that interfered with accurate interpretation of skin tests; (4) reported to have anaphylaxis or acute asthma after prior skin testing with NRL; (5) receiving β-blockers; (6) suffering with a concurrent respiratory disease; or (7) diagnosed with clinically significant medical conditions or diseases that could place the subjects at additional risk.

Study design

The study was conducted during 3 consecutive clinic visits separated by 1- to 4-week intervals that included a screening visit (visit 1) followed by 2 subsequent visits (visits 2 and 3). On each of these visits, skin prick test titration testing was performed with 4 different Hev b proteins and their respective diluent solutions.

On the screening visit (visit 1), HCWs were assessed for eligibility. If qualified, subjects underwent complete occupational histories and physical examinations. Skin prick testing was performed with NAL ranging from 1 × 10⁻³ to 1000 µg/mL or until a concentration that elicited a wheal and flare response. Screened subjects with prick test reactivity to NAL underwent skin prick tests with a panel of 12 common aeroallergens. Blood samples were obtained for NRL serum specific IgE (CAP; Pharmacia, Kalamazoo, Mich; and AlaSTAT; Diagnostic Products Corporation, Los Angeles, Calif).

Qualified subjects returned 1 to 4 weeks later (visit 2) for titration skin prick testing with native Hev b 1, 2, 3, and 4 and respective diluents. One to 4 weeks later, subjects returned on the final visit for titration skin prick testing with recombinant Hev b 5 and native Hev b 6.01, 7.01, and 13, as well as respective diluent solutions. Cutaneous wheal and flare responses were circumscribed and transferred with Transpore tape (3M Healthcare, St Paul, Minn) to a data form. Wheal areas were determined after digitization with image analysis software (Sigma Scan Pro; SPSS, Inc, Chicago, Ill).

Questionnaire evaluations

A questionnaire, modified from that used by Liss et al,⁸ was administered to each subject; this captured information pertaining to direct or indirect exposure to NRL gloves as well as symptoms related to use of NRL gloves. On the basis of responses obtained from questionnaires, diagnoses were assigned according to predefined case definitions for NRL glove-related disorders (Table I). All subjects in group 1 were categorized into 1 or more of the following occupational diagnoses: work-related contact urticaria, work-related rhinitis, work-related asthma, and work-related anaphylaxis.

Skin testing reagents

Commercial aeroallergens for atopy testing included fescue grass, American elm, maple, white oak, house dust mite, ragweed, *Alternaria*, *Cladosporium*, *Helminthosporium*, cat and dog epidermal extracts (ALK America, Wallingford, Conn). NAL was produced by Greer Laboratories (Lenoir, NC) and provided by Robert Hamilton, PhD, Johns Hopkins University. This reagent was identical to the Malaysian *H brasiliensis* (clone RRIM 600) latex preparation described by Hamilton and Adkinson.⁴ Skin testing was performed with 7 Hev b proteins purified from nonammoniated NRL, 1 recombinant Hev b (Hev b 5), as well as appropriate diluent solutions used in the preparation of each Hev b test skin prick test reagent.

Characteristics of the 8 Hev b proteins are described in Table II. Seven Hev b proteins were purified from fresh NAL obtained from the *H brasiliensis* trees. The latex that was collected in chilled containers at the Rubber Research Institute of Malaysia⁶ was centrifuged and at 4°C to 7°C separated into 3 fractions: the rubber cream, the bottom fraction, and the C-serum. The rubber particle proteins, Hev b 1 and Hev b 3, were isolated by previously described methods. Hev b 2, Hev b 4, and Hev b 6.01 proteins were purified from B-serum. Hev b 2 and 4 were recovered in 2 major eluate peaks.¹⁰

TABLE I. Case definitions for latex allergy based on questionnaire responses

Latex allergy terminology	Case definition
Work-aggravated contact urticaria	Hives with itching occurring during the skin contact with NRL gloves and Positive skin puncture test to NAL skin test antigen
Work-aggravated rhinitis	Sneezing, nasal congestion, and eye itching associated with work exposure to powdered NRL gloves and Positive skin puncture test to NAL skin test antigen
Work-aggravated asthma	Immediate onset wheezing, shortness of breath, and chest tightness associated with work exposure to powdered NRL gloves, and Positive skin puncture test to NAL skin test antigen
Work-aggravated anaphylaxis	Immediate onset dyspnea, diffuse urticaria, or hypotension requiring emergency treatment with epinephrine Positive skin puncture test to NAL skin test antigen

TABLE II. Characteristics of *H brasiliensis* proteins used for skin testing

Name	Trivial name	Predicted physiologic roles	Molecular weight
Hev b 1	Rubber elongation factor	Rubber biosynthesis	14.6 kd
Hev b 2	Beta-1,3-glucanase	Plant defense	34-36 kd
Hev b 3	Small rubber particle protein	Rubber biosynthesis, latex coagulation	22 kd
Hev b 4	Microhelix, cyanogenic glucosidase	Plant defense	50-57 kd
Hev b 5 fusion protein (MBP-Hev b 5)	Acidic latex protein	Undocumented	16 kd
Hev b 6.01	Prohevein	Plant defense	19 kd
Hev b 7.01	Patatin homologue	Rubber biosynthesis inhibitor	44 kd
Hev b 13	Esterase	Undocumented	43 kd
NAL	Nonammoniated latex		

Hev b proteins were freshly made from 50 µg/mL stock solutions in saline fortified PBS (PBS, pH 7.4 containing 2.0% NaCl).

By using previously reported techniques, Hev b 7.01 was prepared from the latex C serum⁶ by fractionation on a Sephadex G 150 (Pharmacia, Uppsala, Sweden) column.

To prepare Hev b 13, B-serum was dialyzed overnight against 0.3 mmol/L sodium borate and 0.016 mol/L boric acid pH 7 in the cold room. After filtering through Whatman no. 1 filter paper, 10 mL of the filtrate was loaded onto carboxymethyl cellulose CM32 (Whatman, Kent, United Kingdom) column (20 × 1.5 cm) equilibrated with 0.3 mol/L sodium borate and 0.016 mol/L boric acid, pH 8.6. Proteins were eluted with a gradient of 150 mL 3 mmol/L sodium borate and 0.016 mol/L boric acid, pH 8.6, against 150 mL of 3 mmol/L sodium borate and 0.16 mol/L boric acid, pH 8.6. The unretarded materials were loaded into a DE 52 (Whatman) column equilibrated with 0.1 mol/L Tris-HCl, pH 8. The protein was eluted with a gradient of 0 to 0.5 mol/L NaCl in the same buffer, and the collected fractions were screened for Hev b 13 by immunodetection.

The method reported by Slater et al¹¹ was adopted for preparation of recombinant Hev b 5. After SDS-PAGE, the proteins were Western blotted and separately incubated with sera of patients sensitized to the respective proteins (Fig 1). The blots were then incubated with anti-IgE conjugated to alkaline phosphatase. Detection of IgE binding was by reaction with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium. Molecular markers were stained with Coomassie blue.

Skin test procedures

Allergen skin prick testing was performed with a bifurcated needle (Allergy Labs of Ohio, Columbus, Ohio). For NAL, serial 10-fold dilutions in PBS were prepared from a starting concentration of 1000 µg/mL. For the Hev b proteins, stock solutions at 50 µg/mL were prepared by using PBS containing 2.0% NaCl, pH 7.4. Tenfold dilutions were prepared from starting concentrations of 50 µg/mL, and tests were performed over a range of 5 × 10⁻⁵ µg/mL to

50 µg/mL of protein. For both NAL and the Hev b proteins the starting skin test dilution for each antigen was the most dilute solution (1 × 10⁻³ µg/mL for NAL and 50 ng/mL for the Hev b proteins). Their respective concentrations were increased by 10-fold concentrations until a positive wheal and flare skin test reaction was observed (defined as a wheal of 3 mm greater than that produced by the diluent solution). For the Hev b proteins, diluent controls were prepared that equaled or exceeded additive concentrations present when the stock Hev b solutions were diluted to 50 µg/mL of protein. Histamine phosphate (5 mg/mL) was used as a positive reference control. To assure safety and minimize risk in patients with current asthma, peak expiratory flow rates and FEV₁ were performed before each visit. If FEV₁ declined by 15% from the screening visit (visit 1), skin testing was postponed until lung function recovered to within 5% of the initial value. If systemic allergic reactions occurred during treatment, no further skin testing was performed.

Data analysis

The primary end point in this study was the end point skin prick test threshold concentration or the lowest concentration of antigen that elicited a positive skin prick test result defined as a wheal of at least 3 mm greater than that evoked by the diluent solution. Data were analyzed by using either SAS (SAS Institute Inc, Cary, NC) or SPSS for Windows (SPSS Inc, Chicago, Ill). Nonparametric statistics were used to perform all statistical tests because Kolmogorov-Smirnov goodness of fit analyses indicated by far the majority of outcomes were not normally distributed. Differences in proportions of subjects with positive skin test results to the Hev proteins were evaluated by using the chi-square test. Spearman correlation coefficient was used to probe the association between variables. An averaging method was used to minimize bias in cases in which sera had results below the limit of detection of the assay.¹² A *P* value of .05 or less was considered statistically significant.

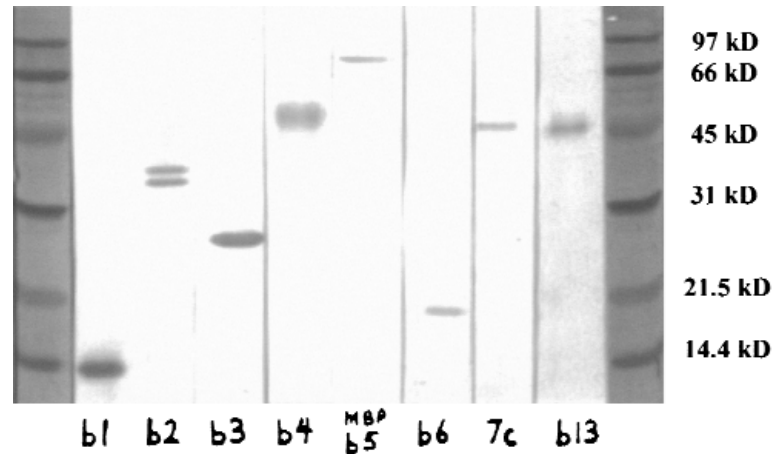


FIG 1. Western blots show IgE binding to the purified allergenic latex proteins Hev b 1, Hev b 2, Hev b 3, Hev b 4, recombinant MBP-Hev b 5, Hev b 6.01, Hev b 7.01, and Hev b 13. Molecular-weight markers are shown.

RESULTS

Subjects

Sixty-seven HCWs with NRL allergy qualified for group 1 and satisfied the aforementioned inclusion criteria. Only 2 prospective participants were excluded as a result of asthma, of which one had severe airway obstruction. Three men and 64 women participated (mean age, 36.1 years; range, 21 to 67 years) in group 1. Group 1 workers wore NRL gloves for a mean 5.2 years (range, 0 to 32 years) before the onset of work-aggravated NRL glove associated symptoms. Group 1 workers wore 16 ± 17 (mean $\pm$ SD) pairs of latex gloves per day. At the time of the study, it had been a mean of 4.0 years (range, 1 to 13 years) since group 1 subjects had last been directly exposed to latex gloves. Fifty-five of 62 (89%) group 1 workers were atopic and exhibited skin prick test reactivity to at least 1 common aeroallergen. There were 49 atopic workers (42 women, 7 men; mean age, 33 years) who participated in group 2.

Of those qualifying in group 1, 62 completed all 3 study visits and underwent skin prick testing with NAL and all 8 Hev b proteins. Five of the 67 subjects recruited in group 1 did not complete the entire protocol; 3 of these were withdrawn after experiencing systemic reactions to NAL during visit 1, and 2 subjects, who had no adverse events, withdrew their consent after completing visit 1. There were 3 reactions to NAL and 2 to a Hev b reagent. All were manifested by complaints of nasal, ocular itching and chest tightness, although wheezing was not auscultated during reactions. One required treatment with epinephrine, and the remainder required an H₁ blocker (antihistamine) only; all responded immediately to treatment.

Work-related symptoms were classified according to criteria described in Table I. As mentioned, all 67 participants had experienced immediate skin reactions to NRL gloves. Of these, 15 (22%) HCWs reported immediate-onset rash or pruritus with donning of NRL gloves as the

sole clinical manifestation of NRL allergy, 23 (34%) also experienced work-aggravated rhinitis, 25 (37%) also had work-aggravated asthma, and 4 (6%) also satisfied criteria for work-aggravated anaphylaxis (Table I).

Skin test results

By definition, group 1 subjects exhibited skin prick test reactivity to NAL, with 97% reacting at endpoint percutaneous threshold concentrations (ETCs) ranging between 0.001 and 10 μ g/mL of NAL extract; 2 other workers had an ETC of 1000 μ g/mL. Among workers in group 1, the highest prevalences of skin prick test sensitivity (exceeding 60%) were elicited by Hev b 2, Hev b 5, Hev b 6.01, and Hev b 13 (Fig 2), which were more frequent than prevalences of 23%, 24%, 39%, and 45% of workers detected to Hev b 1, Hev b 3, Hev b 4, and Hev b 7.01, respectively. The proportions of subjects with positive skin prick test results to Hev b 1, Hev b 3, Hev b 4, or Hev b 7.01 were significantly lower than proportions of reactors ($P < .05$) to all other Hev b test reagents. The proportion of subjects with positive skin prick test results to Hev b 2, b 5, b 6.01, and b 13 were statistically indistinguishable ($P > .05$).

It is noteworthy that 5 (8%) of group 1 subjects with skin prick test sensitivity to NAL failed to exhibit skin prick test reactivity to Hev b antigens. There were no differences between in vivo allergenic potencies of Hev b proteins with the exception of Hev b 13, which had a significantly lower median ETC of 0.05 μ g/mL relative to the other Hev b proteins ($P < .05$, chi-square).

The percutaneous sensitivity of various combinations of Hev b proteins was examined. Despite the finding that 5 (8%) subjects in group 1 failed to react to any of the Hev b test proteins, the combination of Hev b 2, 3, 4, 5, and 13 identified the maximal number (92%) of subjects with NAL skin prick test positive results. Combined reactivity to Hev b 2 and Hev b 4 identified 68% of group 1 subjects, whereas Hev b 1 and Hev b 3 together identified only 32% of NAL-sensitized HCWs. One of 49 group 2

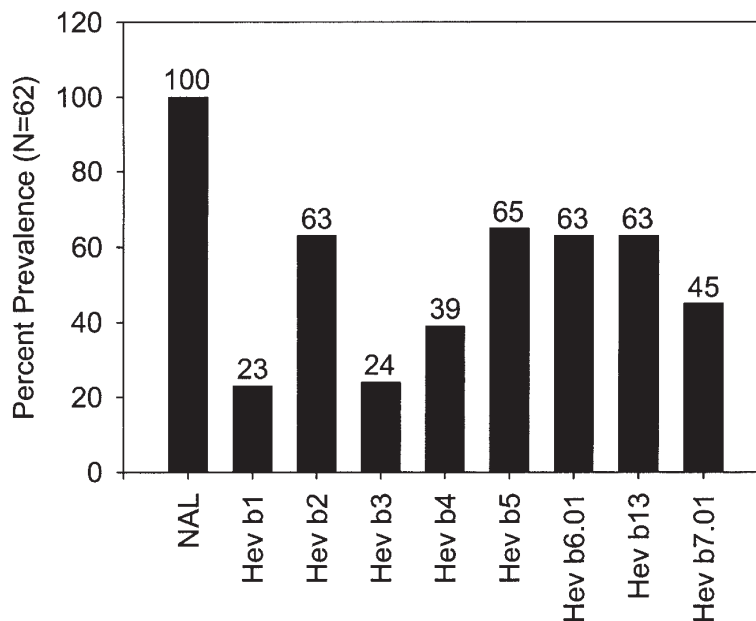


FIG 2. Percent prevalences of group 1 NAL-sensitized workers exhibiting positive skin prick test responses to 8 *Hevea brasiliensis* proteins (percentages are shown above bars).

(atopic, NAL skin test negative result) workers reacted to a single Hev b antigen (Hev b 13). Thus, in this group, the diagnostic specificity of skin prick testing with 7 Hev b proteins was 100% and 98% for Hev b 13 in the identification of workers with NAL sensitivity and NRL allergy.

The FDA-cleared AlaSTAT and CAP assays for NRL specific IgE identified 40 of 60 (sensitivity, 67%) and 31 of 62 (sensitivity, 50%) of the group 1 workers who had skin test positive results to NAL and 4 of 49 (8%) and 3 of 48 (6%) group 2 control subjects with NAL skin test negative results, respectively. There were no significant associations ($P > .05$) between the duration since last direct exposure to NRL gloves and results of CAP and AlaSTAT assays. Of 40 group 1 workers with positive AlaSTAT results, 38 (95%) had a positive skin test result to at least 1 Hev b, whereas 78% exhibited skin prick test reactivity to Hevs b 2, 5, and 6, and 75% were skin prick test reactive with Hev b 13. A significant positive correlation was found between quantitative CAP and AlaSTAT results in group 1 workers ($r = 0.872$; $P < .05$). Because both CAP and AlaSTAT evaluate binding with nonammoniated NRL extract, the relationship of immunoassay data with the NAL skin prick test ETC was evaluated. In group 1 subjects, inverse correlations were observed between the NAL-ETC (expressed as the product of wheal area and end point percutaneous threshold concentration) with the results of both AlaSTAT ($r = -0.32$; $P < .05$) and CAP ($r = -0.286$; $P < .05$) expressed as IU/mL. Four of 47 (9%) group 2 subjects exhibited positive AlaSTAT results in comparison to 4 of 48 (8%) workers with positive CAP assay results. There were no significant differences observed in NRL serum specific IgE status between group 1 workers presenting with or without history of work-related respiratory reactions and anaphylaxis.

DISCUSSION

This is the first in vivo study of skin test reactivity to native Hev b proteins purified from nonammoniated NRL in HCWs with NRL allergy confirmed by skin prick test reactivity to an NAL extract. Skin reactions to these proteins were highly specific in that only 1 of atopic HCWs with NAL skin test negative results with known exposure to NRL gloves exhibited reactivity to a single Hev b protein (Hev b 13). The results of this study indicate that Hev b 2, 5, 6.01, and 13 are major in vivo allergens in HCWs with NRL allergy. Skin prick test reactivity to each of these proteins was detected in more than 60% of HCWs with NRL allergy tested. This is slightly different from the results reported by Yip et al,⁵ who confirmed skin prick test reactivity to an ammoniated NRL reagent in 31 HCWs with NRL allergy. In the latter study, Hev b recombinant proteins (Hev b 2, 3, 5, 6, 7, and 8) were evaluated. Skin prick test reactivity in at least 50% of subjects was achieved with recombinant Hev b 5 and 6 (62% and 66%, respectively), whereas only 7% were reactive with recombinant Hev b 2. In our study, 63% had in vitro skin prick test reactivity with purified Hev b 2. Our results are consistent with previous studies reported by Kurup et al,⁷ who found that 65% of 31 HCWs with NRL allergy reacted with Hev b 2 purified from nonammoniated NRL. Thus, in contrast to recombinant Hev b 2, native Hev b 2 appears to be a very potent and biologically relevant in vivo NRL allergen in sensitized HCWs.

In the present study 45% of subjects reacted to purified Hev b 7.01, a result that was also comparable with the experience of Yip et al,⁵ in which 42% of subjects reacted to recombinant Hev b 7. This would suggest that

the recombinant Hev b protein possesses a profile as an *in vivo* allergen that resembles our purified native protein. A novel protein, identified as early nodule specific protein of legumes (recently designated as Hev b 13), elicited positive skin test results in 63% of subjects with NAL allergy. Thus, Hev b 13 appears to be a clinically important NRL allergen in HCWs.

This study confirmed the findings reported by other investigators that Hev b 1 and 3 are minor allergens in HCWs, even though these antigens are known to elicit specific IgE responses with high frequency in spina bifida patients with NRL allergy.^{7,9,13,14} Of the most allergenic proteins—Hev b 2, 5, 6, and 13—that were encountered in the present study, serum IgE specific to Hev b 2, 5, and 13 were found to be among the most prevalent detected in adult HCWs in a previous investigation.⁶ It should be noted that in that report, Hev b 13 was referred to as Hev b 7b and Hev b 6 was not studied.

As already stated, various combinations of the 8 Hev b proteins exhibited a maximal 92% prevalence of concordance with skin test responsivity to NAL. Therefore, it is obvious that 8% of workers with NRL allergy could not be identified with this combination of Hev b proteins. This experience resembles data reported by Yip et al,⁵ in which only 93% of HCWs with NRL allergy reacted to 1 or more of 6 recombinant Hev b proteins. Taken together, the inability to detect Hev b reactivity in a minority of workers with NRL allergy could be due to noninclusion of heretofore unrecognized minor Hev b protein allergens. Nevertheless, other explanations are possible. In evaluating the allergenicity of purified latex proteins, the subjects had been tested with single proteins, whereas the NRL reagent used for the skin test contained multiple allergenic proteins. The additive effect of subthreshold reactive levels of allergens in NRL might conceivably induce a reaction that is not elicited by each protein of a similar concentration acting separately.

Individual Hev b skin tests were significantly correlated by ETC or positive test at any concentration. This is likely explained by the fact that subjects with NRL allergy are exposed to multiple latex allergens *en bloc*, resulting in simultaneous acquisition of sensitization to various combinations of proteins.

NRL serum specific IgE was detected in less than two thirds of workers with NRL allergy and was somewhat less sensitive in detecting NRL allergy than in previous reports. Hamilton et al² compared sensitivity and specificity of 3 FDA-approved commercial specific IgE assays. In the latter study, CAP and AlaSTAT exhibited 76% and 73% sensitivity, respectively; specificity for both assays exceeded 96%. The sensitivity of the HY-TEC assay (Hycor Biomedical, Garden Grove, Calif) was 91%, but the cutoff used for a positive test resulted in a high number of false-positive tests yielding a specificity of 73%.² Thus, this study confirms that NAL skin prick testing is superior to *in vitro* specific IgE assays in identifying workers with confirmed NRL allergy without compromising specificity. We also found significant inverse correlations between the ETCs for NAL and

results of AlaSTAT and CAP assays, which indicates that more highly NAL sensitized HCWs (based on skin prick testing) are more likely to exhibit elevated NRL serum specific IgE levels and vice versa.

Exhaustive precautions were taken in this study to prevent systemic allergic reactions (including extreme allergen dilutions). However, these occurred in 8% of group 1 subjects. It should be emphasized that with our protocols none of the reactions was considered severe or life-threatening and all responded immediately to treatment. The risk of systemic reactions associated with skin prick testing lends support to the common practice of screening patients suspected of NRL allergy with FDA-approved specific IgE assays and reserving skin prick testing for those with negative test results.

In summary, this investigation has confirmed that Hev b 2, 5, and 6 are principal *in vivo* NRL allergens and has also identified a new major allergen, Hev b 13. The designation of major allergen is based on a published definition for which allergen specific IgE is demonstrable in greater than 50% of patients tested.¹⁵ The 8 Hev b proteins evaluated in this study were demonstrated to be highly specific skin prick test reagents for HCWs with confirmed NRL allergy. Because not all NAL sensitized workers exhibited specific IgE responses to this panel of Hev b proteins, further studies are needed to identify additional heretofore unrecognized NRL allergens.

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