Diagnosis of natural rubber latex allergy: Multicenter latex skin testing efficacy study

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Background: No characterized diagnostic natural rubber latex skin testing material is licensed for use in the United States.
Objective: We have conducted a multicenter clinical skin testing study to document the safety and diagnostic sensitivity and specificity of a candidate *Hevea brasiliensis* nonammoniated latex (NAL) extract. These data are intended to support the licensing of this reagent for the diagnosis of latex allergy in high-risk populations.
Methods: Three hundred twenty-four subjects (304 adults and 20 children) were classified by their clinical history as having latex allergy (LA group, 124 adults and 10 children) or having no latex allergy (NLA group, 180 adults and 10 children). All subjects provided blood samples and then received sequential puncture skin tests (PSTs) at 1, 100, or 1000 μg/mL protein with a bifurcated needle and NAL (Greer Laboratories) from Malaysian *Hevea brasiliensis* (clone 600) sap. A 2-stage glove provocation test was used to clarify latex allergy status of individuals with positive history/negative PST result and negative history/positive PST result mismatches.
Results: Twenty-four subjects (15%) originally designated as having LA on the basis of their initial clinical history were reclassified to the NLA group on the basis of a negative glove provocation test result. Of the 134 subjects with LA, 54 (40%) were highly sensitive to latex, with a positive PST result at 1 μg/mL NAL. The Greer NAL reagent produced a positive PST rate (sensitivity) of 95% and 99% in subjects with LA at 100 μg/mL and 1 mg/mL, respectively. The negative PST rate (specificity) in 190 subjects with a negative history with the NAL extract at 100 μg/mL and 1 mg/mL was 100% and 96%, respectively. Immediately after the PST, mild systemic reactions (mainly pruritus) were recorded in 16.1% of the adults in the LA group and 4.4% of the adults in the NLA group. No reactions required treatment with epinephrine. Only mild delayed reactions were observed in 9.6% (LA group) and 2.8% (NLA group) of subjects 24 to 48 hours after PST. Mean wheal and erythema diameters measured in the 10 children in the LA group with spina bifida at 100 μg/mL and 1 mg/mL were similar to those observed in the adults in the LA group, suggesting that children are not at increased risk for systemic reactions compared with adults.
Conclusions: A suggestive clinical history is necessary but not sufficient for a definitive diagnosis of IgE-dependent latex allergy. These data support the safety and diagnostic efficacy of the Greer NAL skin test reagent at 100 μg/mL and 1 mg/mL for confirmatory PSTs. (J Allergy Clin Immunol 1998;102:482-90.)

Key words: *Natural rubber latex, diagnosis, skin testing, serologic testing, glove provocation*

Effective management of an individual with a Type 1 hypersensitivity to natural rubber latex derived from *Hevea brasiliensis* trees begins with a definitive diagnosis.[1-7] The primary diagnostic test used by allergists and other physicians is the skin test in which allergen is introduced by puncture or intradermal injection into the skin. Presently, there are no Food and Drug Administration (FDA)–approved skin testing reagents for latex available in the US. Some clinicians have prepared their own extracts of unknown potencies from gloves or performed skin tests by puncturing directly through an uncharacterized powdered latex glove. Other allergists have resorted to a latex glove use or provocation test. There are many challenge protocols and methods of rating skin and respiratory symptoms, and there is no common source of powdered latex gloves with a known allergen content.[8-10] For these reasons, latex glove provocation procedures are generally considered unsafe and of variable diagnostic sensitivity. Sera are also being sent to laboratories for the detection of latex-specific IgE by using 1 of 3 FDA-approved serologic tests. The diagnostic sensitivity and specificity of these assays vary appreciably, but in general they have lower sensitivity than puncture skin tests (PSTs), depending on the sera used and the laboratory performing the evaluation.[11-12] A standardized and safe skin testing reagent is needed.

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Supported by National Institutes of Health grant AI-31867 and Allegiance Healthcare Corporation. Drs Hamilton and Adkinson and the Johns Hopkins University are entitled to royalties derived from sale of latex skin testing reagents prepared by Green Laboratories, Inc, as described in this study. The terms of this arrangement have been reviewed and approved by the Johns Hopkins University in accordance with its conflict of interest policy.

Received for publication Apr 30, 1998; revised June 22, 1998; accepted for publication June 23, 1998.

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0091-6749/98 $5.00 + 0 **1/1/92696**

Abbreviations used
CD: Contact dermatitis
FDA: Food and Drug Administration
HCW: Health care worker
LA: Latex allergy
NAL: Nonammoniated latex allergen preparation
NLA: No latex allergy
PEFR: Peak expiratory flow rate
PST: Puncture skin test

After the first International Symposium on Latex Allergy in 1992, we began the development of a characterized skin testing reagent. We prepared ammoniated latex, nonammoniated latex (NAL), and an extract of gloves with latex from clone 600 of *Hevea brasiliensis* trees. In initial safety and efficacy studies[13] the nonammoniated form of latex was identified over its companion ammoniated latex and glove extracts as the optimal candidate for development. That preliminary study suggested that the 100 μg/mL NAL concentration was safe (no moderate or severe systemic reactions) with good diagnostic sensitivity (96%) and specificity (100%) when used in puncture skin tests (PSTs) with a bifurcated needle. The purpose of the current multicenter study was to document the safety and diagnostic sensitivity and specificity of a candidate *Hevea brasiliensis* NAL extract. A cross-section of adults and children with LA were studied from 12 centers across the US by using a skin test protocol that was designed to minimize bias and obtain maximally objective performance data. These data support the conclusion that together with the clinical history, the NAL PST reagent permits a safe and reliable diagnosis of IgE-dependent allergy to natural rubber latex.

METHODS

Subjects

Recruitment of subjects was conducted over a 1 year period at 12 institutions across the United States (average 34 subjects per site). The investigators are members of the American Academy of Asthma, Allergy and Immunology's Latex Committee. Of the 410 subjects enrolled, 30 were disqualified because they met one of the exclusion criteria (see Results section). The 358 adult subjects (≥18 years of age) who qualified for the study were classified into 3 groups on the basis of their clinical history: those not allergic to latex (NLA group, $n = 180$), those allergic to latex (LA group, $n = 124$), or those with contact dermatitis (CD group, $n = 54$). Skin test results from the CD group, in which the primary clinical feature was a skin reaction confined to the area of glove contact, were not used in the data analyses for determining the diagnostic performance of the latex skin testing reagent.

Because of requirements placed on the study by the FDA review panel to delay the enrollment of children for safety considerations, only 22 children (<18 years of age) were enrolled in the study. These included 10 children with spina bifida who had a history consistent with latex allergy; 10 control subjects in the NLA group with no known reactions to powdered latex rubber gloves, balloons, and catheters; and 2 subjects with CD (Type IV) reactions (rash and itching) confined to the area of glove/skin contact.

Latex skin testing reagent

Three lots of NAL were prepared by Greer Laboratories (Lenoir, NC) in a licensed good manufacturing practice facility by using a protocol that has been previously described.[13] In brief, crude Malaysian *Hevea brasiliensis* (clone 600) tree latex was collected in sterile plastic bottles containing a nonhazardous (patented) Goodyear preservative (0.1 mol/L $NaHCO_3$, 50% wt/vol glycerol, and 3 mmol/L cysteine with no azide). After 1 week of shipping on ice packs, the milky latex was ultracentrifuged for 1 hour at 4° C, and the yellowish latex "C-serum" was isolated. The NAL reagent was filtered through a 0.22-μm Millipak 40 (Millipore), adjusted to 50% glycerin and 1 mg/mL total protein by the ninhydrin method, and stored at 2° to 8° C. FDA-required safety, sterility, glycerin, and protein tests were performed as previously described.[13] General composition analyses were performed with a 12% nonreducing SDS-PAGE followed by a Western blot,[14] and a potency assessment in an ELISA inhibition assay analogous to the RAST inhibition assay was done,[15] with the E8 NAL as a reference standard (CBER, FDA, Bureau of Biologics, Bethesda, Md).

Study design

This project was conducted under Investigational New Drug application 6365 with a protocol approved by the Allergenic Products Committee of the FDA and by the institutional review board of each participating institution. The protocol was designed to blind the tester to the history taken by the investigator and to blind the subject as to the identity of the skin testing extracts. Initially, only individuals who had received no previous diagnostic skin or blood tests were included in the study. However, this severely limited access to otherwise qualified subjects. This requirement was relaxed so that subjects with previous diagnostic tests could be enrolled but with the requirement that 2 investigators independently read the skin test results to minimize bias.

After informed consent was obtained, blood was collected for serologic tests. Each subject completed a detailed questionnaire that examined their general atopic and LA history status, the type and severity of their allergy symptoms after natural rubber latex examination and surgical glove use, and other known risk factors such as food allergies. It further examined the number of surgeries, frequency of glove use, and extent of exposure to common household rubber products, such as balloons and condoms. A detailed description of the 2 most recent reactions to latex products was collected to identify the extent of exposure, rapidity of onset, and duration and severity of symptoms. The primary investigator assigned each subject to 1 of 3 groups (LA, NLA, or CD) on the basis of each subject's clinical history. Baseline peak expiratory flow rate (PEFR) measurements were performed before skin testing.

All skin testers were required to pass a validation test before participating in the study, which involved performing PSTs on 10 nonatopic subjects with saline and histamine (1.8 mg/mL; Allermed, San Diego, Calif) in duplicate. In the validation process mean diameters of the wheal and erythema observed with histamine at 15 minutes were compared with population norms for the bifurcated needle.

In the study PSTs were performed by applying glycerin-saline (negative control) and 1.8 mg/mL histamine (positive control) and NAL serially at 1, 100, and 1000 μg/mL to the volar aspect of the forearm, in duplicate, every 15 minutes. All vials were coded "A" to "E" to blind the subject. A puncture was made through each drop by rocking the needle at 45° angles through 4 complete cycles, and the needle was wiped with alcohol between each skin test. A positive PST response was defined as one producing a greater than 2-mm wheal and a greater than 5-mm erythema above that caused by the

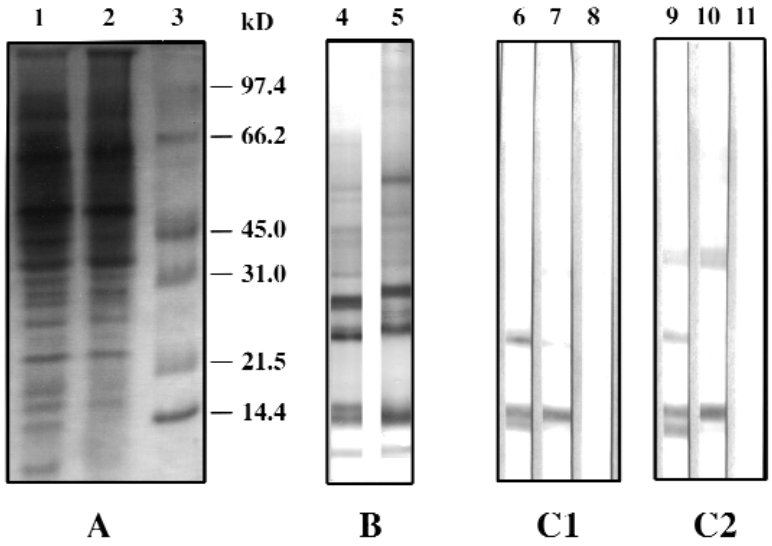

FIG 1. Distribution of allergenic proteins in Greer NAL as evaluated by SDS-PAGE and Western blot analyses. **A,** SDS-PAGE profile of Greer NAL extract (Lot GH78-9BC) *(lane 1)* compared with CBER FDA E8 NAL reference preparation *(lane 2).* Molecular weight standards are displayed in *lane 3.* **B,** Western blot analysis of Greer NAL extract *(lane 4)* and CBER FDA-E8 NAL *(lane 5)* developed with FDA human IgE anti-latex S2 serum pool. *Hevea* proteins at 4 to 5, 14 to 16, 23 to 28, 30, and 45 kd correspond to the following known allergens: Hev b 6.02 or hevein = 4.7 to 5 kd; Hev b 1 or rubber elongation factor = 14.6 kd; Hev b 6.03 or C-domain of prohevein = 14.4 kd; Hev b 3 or prenyltransferase = 23 to 27 kd; Hev b 5 or acidic protein = 16 to 24 kd; Hev b 2 or β 1/3 glucanase = 30 to 36 kd; and Hev b 7 or patatin homologue = 46 kd. Gel was not designed to identify Hev b 4 (microhelix), which is 110 kd in nonreduced gel. Western blot analysis of Greer NAL extract **(C1)** and CBER FDA-E8 NAL **(C2)** developed with two bleeds of rabbit anti-Hev b 1 (rubber elongation factor = 14.6 kd, tetramer 58 kd) or nonimmune rabbit serum: *lanes 6* and *9* = rabbit #47 bleed 101596; *lanes 7* and *10* = rabbit 48 bleed 040996; and *lanes 8* and *11* = normal rabbit serum. Hev b 1 is detected in both Greer NAL and FDA E8 NAL. Additional bands most likely reflect Hev b 1 aggregated or adsorbed onto other *Hevea* proteins. These "non 14.6 kd" Hev b 1 protein bands appear to become more prominent on immunoblot as *Hevea* latex ages (data not shown).

saline control at 15 minutes after application. The mean diameters of the wheal and erythema were measured and, their perimeters were outlined with a fine tip rolling writer pen and transferred onto transparent tape (Transpore 3 inch, 3M Company) for a permanent record on the data forms. A repeat PEFR measurement was performed at any time during the study when it was deemed necessary and at the end of skin testing. A greater than 20% decrease in PEFR observed at any time during the study stopped all testing.

Only in cases where the history was discordant with the PST results (either a positive history with a negative PST result at 100 μg/mL or a negative history with a positive PST result), a 2-stage unblinded glove provocation test was performed as described previously to screen only for immediate upper and lower respiratory responses.[9] In brief, the subject was equipped with plastic goggles and a silicone-based respirator mask equipped with 2 activated charcoal cartridges (mask model 72813, cartridge model 7251; 3M Company) to prevent ocular exposure or inhalation of latex allergen attached to glove cornstarch donning powder. The powdered latex examination glove used in the provocation studies at all 12 study sites was shown to contain a high level of extractable latex allergen on the basis of RAST inhibition analysis[15] (mean latex allergen content ± SEM: 15,072 ± 1448 AU per glove; vinyl glove contains <1 AU/glove). Latex allergen was known to be attached to cornstarch donning powder by direct binding of latex-specific human IgE antibody to cornstarch particles[16] collected from the glove used at all 12 study sites (data not shown).

In stage 1, 3 PSTs with saline were performed on the hand immediately before donning a high-allergen powdered latex examination glove on 1 hand and a synthetic (vinyl) glove on the opposite hand. Subjects were observed for skin symptoms over a 30-minute period. At stage 2, a provocational test was performed if no pruritus and no visually detectable erythema or swelling were observed by the investigator. The mask and goggles were removed and a new high-allergen powdered latex glove was blown up 3 times by the subject like a balloon and expelled gently each time into his own face. Each subject was then observed carefully over an hour period for objective evidence of any allergic symptoms. PEFR measurements were performed between the stage 1 and stage 2 provocation tests and at the end of the study to assess changes in lung function. Before leaving the site, each subject was queried for symptoms, given a diary card, and asked to contact the investigator at any time up to 48 hours to report any late asthmatic or delayed allergic reactions. A change in PEFR of greater than 20% from the subject's pretesting personal best was considered positive in this study, as well as in other studies.[17]

Serologic analyses

Total serum IgE level was measured by enzyme immunoassay (IMx; Abbott Laboratories, Abbott Park, Ill) and reported in nanograms per milliliter and as a percentile of the age-adjusted nonatopic mean.[18] IgE antibodies to common aeroallergens were measured in a single Phadiatop multiallergen screen (CAP System, Pharmacia-UpJohn, Kalamazoo, Mich) as a general marker for atopy. Natural rubber latex–specific IgE was measured by the 3 FDA-approved assays, and these results will be reported elsewhere.

TABLE I. Results of the LA and NLA groups

Parameter	NLA group adults	LA group adults	NLA group children	LA group children
No. of subjects	180*	124†	10	10
Age range (y)	21-69	20-64	1.8-17	6-18
Age (yrs [mean ± SEM])	38.0 ± 0.7	37.4 ± 0.8	10.4 ± 1.5	13.4 ± 1.1
Gender (M/F)	89/91	27/97	5/5	4/6
Previous latex diagnostic test, *n* (%)	8 (4.4)	44 (35.5)	3 (30)	5 (50)
Occupation (% of total group)				
HCWs‖	97.7	80.6	0	0
Students	0.6	1.6	100	100
Office worker, florist, police, librarian	1.1	13.8	0	0
Engineer, farmer, housekeeper	0.6	4.0	0	0
Spina bifida, *n* (%)	0 (0)	3 (2.4)	5 (50)	8 (80)
Atopic history				
Rhinitis, *n* (%)	70 (38.9)	74/122 (60.7)	3 (30)	4 (40)
Asthma, *n* (%)	22 (12.2)	19/121 (15.7)	2 (20)	2 (20)
Eczema, *n* (%)	21(11.7)	21/122 (17/2)	2 (20)	0
Food allergy, *n* (%)	15 (8.3)	44 (35.5)	3 (30)	2 (20)
Hx Allergy-kiwi, banana, avocado; *n* (%)	5 (2.8)	20/121 (24.8)	0 (0)	2 (20)
Latex-associated symptoms (% cases)				
Skin (hives, rash, swelling, itch)	28 (15.6)	116 (93.5)	5 (50)	9 (90)
Eyes (itchy, red, tear/watery)	22 (12.2)	110 (89)	3 (30)	9 (90)
Upper airway (mouth, nose, throat)	25 (13.9)	97 (78)	3 (30)	5 (50)
Lower airway (asthma, wheezing)	12 (6.7)	76 (61)	2 (20)	2 (20)
Gastrointestinal	1 (0.6)	9 (7.3)	2 (20)	0 (0)
Cardiovascular	4 (2.2)	23 (18.5)	1 (10)	1 (10)
Serologic results				
Total IgE (ng/mL, mean ± SEM)	206 ± 32	679 ± 156	615 ± 416	248 ± 79
Phadiatop (% positive)	86 (48)	79/117 (67.5)	3/7 (43)	7/8 (88)
Skin test results				
PST at 100 μg/mL (% positive)	0 (0)‡	119 (96.0)¶	0 (0)‡	8 (80)¶
PST at 1000 μg/mL (% positive)	5 (2.8)‡	123 (99.2)¶	2 (20)‡	10 (100)¶
Glove provocation test results				
Glove provocation (stage 1) positive	0/50	6/7 (85.7%)	ND	ND
Glove provocation (stage 2) positive	0/47	0/1 (0%)	ND	ND

ND, Not done.
*Twenty-four of the original subjects in the LA group (16.2%) with an initial positive history for latex allergy were reassigned to the NLA group on the basis of negative glove provocation challenge test results.
†Only 1 subject with an initial negative history of latex allergy and a positive skin test result was reassigned to the LA group on the basis of a positive glove provocation challenge test result.
‡1-specificity.
‖HCW group includes MD, RN, Medical Laboratory Technologist, LPN, respiratory therapist, cast technician, pharmacist, dentist, and dental hygienist.
¶Sensitivity.

Statistical analyses

All statistical analyses were performed by using SPSS^{x} (SPSS Inc, Chicago, Ill). For all analyses, the NLA control group was used as a reference for comparison. Unpaired *t* tests were used to compare continuous variables, and a chi-squared test was used to compare categorical and binomial variables. All *P* values were 2-tailed, and those below .05 were considered significant. Sample size was chosen to allow an estimated sensitivity of greater than 95% to be established with a 95% confidence interval of ±3.5%.

RESULTS

Latex reagent quality control

The NAL used in this study passed extensive lot-release criteria before being provided to investigators. Sterility and safety studies as required by FDA regulations were successfully passed. The glycerin content was shown to be 52.6% vol/vol (target range: 52.5% to 60% vol/vol). Total protein of the most concentrated NAL stock was 1.02 mg/mL (target range: 1 ± 0.1 mg/mL [mean ± SEM]). The relative potency by ELISA inhibition analysis of the NAL extract in comparison with the CBER-FDA reference (E8) NAL was 0.85 ± 0.23 (mean ± 1 SD, target = 1.0). Stability was shown by no significant loss in latex allergen potency by ELISA inhibition when the NAL reagent was stored at 4° C for 12 months (the duration of the study).

SDS-PAGE and Western blot analyses are presented in Fig 1. The SDS-PAGE (Fig 1, *A*) identified additional protein bands in the Greer NAL (lane 1) than those detected in the FDA-CBER-NAL (E8, lane 2). However, these differences are within the normal inter-lot variation that has been observed with 3 lots of *Hevea* latex col-

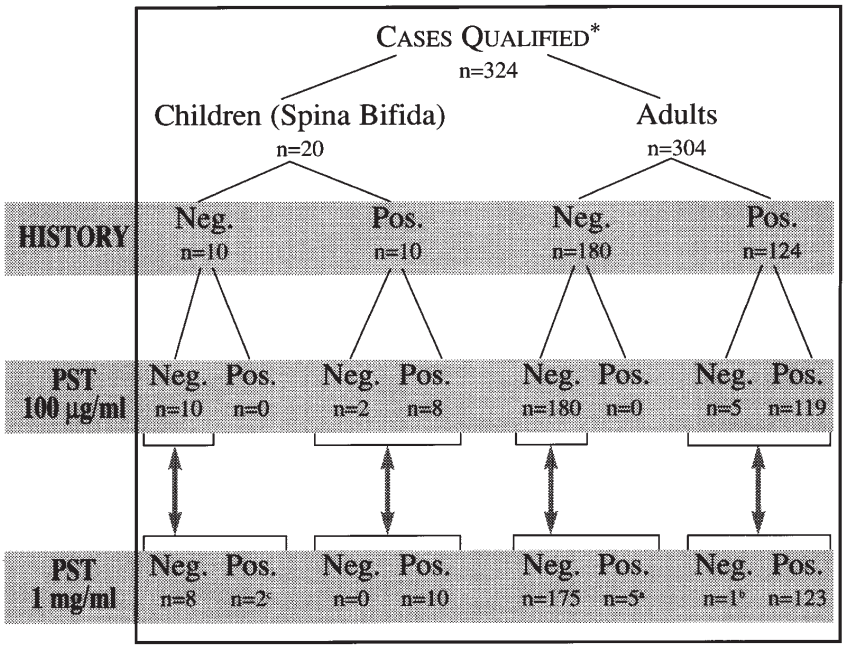

FIG 2. Positive skin test rate in children and adults in the LA and NLA groups. *Subjects were placed in the LA or NLA groups on basis of final history and results of glove provocation procedure where history and skin test results were discordant (n = 24). [a]All 5 subjects had negative provocation test responses. [b]Provocation test stage 1 negative response and stage 2 positive response. [c]Provocation tests not done.

lected from trees at different times of the year (data not shown). The Western blot analysis in Fig 1, *B* of the Greer NAL (lane 4) and CBER-E8 (lane 5) with the IgE serum pool (S2-CBER-FDA) identified Hevea proteins at 4 to 5, 14 to 16, 23 to 28, 30, and 45 kd, which correspond to known *Hevea* allergens (Fig 1). Minor shifts in the staining patterns observed between lanes 4 and 5 are within expected technical variation seen for the Western blot analyses of these extracts because the Greer NAL and FDA NAL immunoblots were each prepared from different SDS-PAGEs run on the same day.

The immunoblot (identity test) for Hev b 1 is displayed in Fig 1, *C*. Two bleeds of rabbit anti-Hev b 1 (6 vs 7 and 9 vs 10), but not the normal rabbit serum (lanes 8 and 11), identified the 14.6 kd Hev b 1 protein in both the Greer NAL (Fig 1, *C1*) and the FDA E8 NAL (Fig 1, *C2*). The other bands identified in these blots are believed to represent Hev b 1 aggregated or adsorbed onto proteins of other molecular weights.

Excluded subjects

Of the 410 total subjects recruited, 30 met predetermined exclusion criteria: pregnant (n = 2), refused skin testing after enrollment (n = 9), took medications (β-blockers, antihistamines, and tricyclic antidepressants) that precluded skin testing (n = 8), had an asthma exacerbation 24 hours before the study (n = 1), or had questionable skin test or provocation test results that could not be confirmed by repeat testing because of subject refusal (n = 10). An additional 56 subjects (54 adults and 2 children) were classified as having CD only. Of the CD group, 43% had a positive Phadiatop multi-RAST result, and 3.7% (2 of 54) had a positive skin test result with NAL at 100 μg/mL and 11.1% (6 of 54) at 1 mg/mL. The CD group with positive PST results is believed to be sensitized; however, all subjects with CD exhibited a negative glove provocation test result and could therefore have been classified as sensitized but asymptomatic. However, because their primary diagnosis was CD (skin symptoms confined to the area of glove contact), they were excluded from the Greer-NAL skin test performance data analysis. There were no significant differences in the age, gender, or total serum IgE distributions of these 86 excluded subjects and the remaining 324 subjects accepted into the analysis (data not shown).

Study group classification

The clinical history and (when appropriate) glove provocation test results were used by the investigator at the site to determine the definitive LA status of the subject. Individuals with a PST result that was concordant with the original history designation (positive history/positive PST result or negative history/negative PST result) did not receive a glove provocation test. However, 15% of the adults with probable LA by history had a negative PST result, even at the highest concentration of 1 mg/mL. All these subjects were challenged with the 2-stage glove provocation test, and all had negative results. On the basis of the negative glove provocation test result, which was considered a qualifying test, these 24 subjects originally designated in the LA group were reclassified into the NLA group. One subject in the NLA

group had a positive PST and a positive provocation test result and was reclassified to the LA group. Therefore of the 304 adults from the 12 centers who passed the entrance criteria, 124 were ultimately classified in the LA group, and 180 were classified as control subjects in the NLA group (Table I).

In our previous validation study,[9] the stage 1 (contact) glove provocation test with a puncture through saline before donning a highly allergenic powdered latex rubber glove was shown to be positive in 100% of the 17 subjects in the LA group with a positive history and a positive PST response at 100 μg/mL. In this study 6 of 7 "weakly sensitive" subjects in the LA group with a positive PST response only at 1 mg/mL had a positive stage 1 provocation test response. The stage 2 (inhalation) glove provocation test was not done on these subjects for safety reasons. The seventh individual with a positive history and a positive PST response at 1 mg/mL had a negative stage 1 and stage 2 provocation test response. There were no positive glove provocation test responses among the 50 stage 1 and 47 stage 2 glove provocation tests that were performed on randomly selected control subjects from the NLA group (Table I).

Of the 324 subjects accepted into the study, 18.5% had a previous serologic test for latex-specific IgE antibody. Prior skin test results through gloves or unlicensed extracts were not seriously considered because of the difficulty in interpreting results obtained with uncharacterized reagents. Among the 124 adults in the LA group, 35.5% had a previous blood test, whereas only 4.4% of the 180 subjects in the NLA group had previous serologic testing.

Study group demographics

The demographics, atopic history, and symptoms associated with latex glove exposure of the study subjects are summarized in Table I.

Adults. Of the 304 accepted adults, the age distribution did not differ significantly between the LA (37.4 ± 0.8 [mean ± SEM]) and NLA (38 ± 0.7) groups. Seventy-eight percent of the LA adult group was female. This female preponderance has also been described in other studies[7,13] and possibly reflects the high percentage of adult health care workers (HCWs) in the study (81% of the LA group and 98% of the NLA group). The small number of subjects in the LA group who were not HCWs included students, office workers, florists, police officers, librarians, engineers, farmers, and housekeepers who all had a history of chronic exposure to rubber gloves through occupational exposure or medical and dental care or use of balloons and/or condoms. Approximately 60% of the LA and 40% of the NLA groups had a history of atopy, most commonly with symptoms of seasonal rhinitis, and a few had symptoms of asthma and eczema. An atopic history was further supported by a positive multiallergen RAST result (67.5% in the LA group and 48% in the NLA group). A significantly higher number of adults in the LA group had a history of food allergy (35.5% in the LA group and 8.3% in the NLA group, $P < .001$), with 70% of the subjects in the LA group with food allergies relating histories of gastrointestinal symptoms after consumption of kiwi, bananas, or avocados. Skin, eye, and upper airway symptoms were experienced by greater than 78% of the subjects in the LA group when working around powdered latex gloves (Table I). Interestingly, 14% to 16% of adults in the NLA group also related similar symptoms while working with powdered latex gloves.

Children. The 20 study children with spina bifida ranged in age from 1.8 to 18 years. There were equivalent numbers of men and women in both the LA and NLA pediatric groups (Table I).

TABLE II. Reactions reported in multicenter latex skin testing study*

Adverse reactions observed in 124 subjects in the LA group*	*n*
Systemic reactions immediately after skin testing	
Mild	20 (16.1%)
Pruritus only	10
Rash	1
Pruritus and rhinitis	2
Pruritus at skin test site, mild tearing of eyes, rhinitis	1
Pruritus at skin test site, mild tearing of eyes, rhinitis <1 hour, mild chest symptoms	1
Hives, eye itching, mild throat tightness	1
Rash-pruritus, eyes itching-tearing, rhinitis, mild throat tightness	1
Hives, mild chest tightness	1
Hives, rash, pruritus, throat tightness, mild chest tightness	1
Transient PEFR drop of 28% requiring no medication†	1
Itchy eyes	1
Moderate	1 (0.8%)
Itchy-tearing-red eyes, rhinitis, cough, sneezing, asthma, nausea (headache delayed)‡	1
Severe	0
Delayed systemic reactions (at 24 to 48 hours from diary)*	
Mild	12 (9.6%)
Skin itching (6 hrs), red itchy eyes	3
Red eyes	2
Numbness of tip of tongue	1
Rash, itchy eyes, runny nose, sneezing, cough, mild chest tightness	1
Rhinitis	4
Itchy throat, wheezing, and cough	1
Moderate	0
Severe	0

*In the NLA control group, 4.4% of the 180 subjects complained of pruritus, rash, rhinitis, and ocular itching tearing and redness immediately after skin testing. A similar array of delayed reactions were reported by 2.8% of the control subjects in the NLA group.
†One asthmatic subject (number 6) experienced a decrease of 22% in PEFR with scratchy itchy eyes, cough, shortness of breath, and chest tightness while wearing a mask and goggles during a stage 1 glove provocation test. These symptoms required no medications for maintenance.
‡Given 2 mg of Chlor-Trimeton by mouth after reporting eye itching, cough, and sneezing; albuterol after asthma symptoms; and aspirin next day for headache.

Skin test reagent performance

Diagnostic performance of the Greer PST reagent in the adults and children in the LA and NLA groups are

summarized in Table I and Fig 2. Only 40% of the subjects in the LA group had positive PST results at 1 μg/mL NAL. These were considered the highly sensitized group. The positive PST rate (diagnostic sensitivity) in subjects in the LA group increased from 95% at 100 μg/mL to 99% at 1 mg/mL NAL. The negative PST rate (diagnostic specificity) in subjects in the NLA group was 100% at 100 μg/mL and decreased to 96% at 1 mg/mL. These data support the use of both the 100 μg/mL and 1 mg/mL NAL in sequence to maximize safety and overall efficacy of the Greer NAL PST reagent in identifying subjects with LA.

One individual (number 6) with an initial history consistent with latex allergy had negative skin test responses at all 3 NAL concentrations. This individual was classified as having a false-negative response because she exhibited a positive glove stage 1 result with a transient 22% decrease in PEFR, scratchy itchy eyes, cough, shortness of breath, and chest discomfort (while wearing a mask and goggles). In contrast, of the 180 adults in the NLA group, 5 exhibited a positive PST response at the 1 mg/mL NAL concentration. All 5 of these subjects experienced no allergic symptoms during either stage of the glove provocation test, indicating that either these are technically false-positive skin test results or the subjects are sensitized to latex but asymptomatic.

Safety

Table II displays the reactions observed in the study subjects immediately after and at 24 to 48 hours after the PSTs. Of the subjects in the LA group, 16.1% (20 of 124) reported mild systemic reactions of pruritus, rash, rhinitis, tearing and itching of the eyes, and hives immediately after skin testing. One individual had a PEFR that transiently decreased 28% after skin testing, but he experienced no chest tightness and required no medication. Another subject with LA (number 379) experienced rhinoconjunctivitis, asthma, nausea, and headache after PST and was given 2 mg of Chlor-Trimeton, albuterol, and aspirin for management. There were no systemic reactions that required epinephrine. A spectrum of mild delayed reactions were reported by 9.6% of the LA group at 24 to 48 hours after PSTs. Interestingly, the corresponding immediate and delayed reaction rates in the NLA control group were 4.4% and 2.8%.

The mean diameter of the wheal and erythema observed in the children in the LA group with spina bifida did not differ significantly from those obtained in the adults in the LA group at 100 μg/mL (children in the LA group: 6.9 ± 0.7 mm wheal, 26.1 ± 2.7 mm erythema, n = 10; adults in the LA group 7.6 ± 0.4 mm wheal, 28.0 ± 1.2 mm erythema, n = 105).

DISCUSSION

Latex allergy continues to be a problem for approximately 10% of occupationally exposed HCWs and a higher percentage of individuals, such as children with spina bifida, who experience extensive surgical exposure to natural rubber gloves. At present, the primary mode of treatment is avoidance, preferably by removal of latex (especially powdered gloves) from the environment. In cases where occupational exposure to latex allergen is difficult to control, the individual with LA may be advised to change to a new work environment. Accurate diagnosis of LA becomes critical for individuals and institutions that face these difficult issues in a health care or occupational setting.

A good clinical history is the first step in the diagnostic algorithm.[1-4,7] Results of this study confirm our previous work,[13] suggesting that a careful history is necessary but not sufficient to make a definitive diagnosis of LA. A false-positive history can occur even when the individual has established risk factors for latex allergy, such as skin symptoms associated with powdered latex glove use; an atopic history; or sensitivity to bananas, avocados, and kiwis. In this study 15% of the subjects originally classified on the basis of their history as having LA had a negative PST response. All these subjects had a subsequent negative 2-stage glove provocation test result that the investigator at the site used as a qualifying criterion to reclassify the subject into the NLA group. We employed a contact (stage 1) and inhalation (stage 2) glove provocation test as a qualifying gold standard for latex allergy status of the individual. Our stage 2 inhalation challenge was patterned after the procedure of Vandenplas et al[17] in which subjects opened a powdered glove package and shook both gloves for 3 minutes while wearing vinyl gloves to prevent direct skin contact with latex. Four of 12 asthmatic subjects had immediate asthmatic reactions, and 3 subjects had both immediate and delayed reactions after this inhalation challenge, with spirometry as a measure of change in pulmonary function. On attempting to replicate this procedure, we found that the process of simply shaking powdered gloves for 3 minutes produced highly variable levels of airborne allergen (data not shown). By blowing up a powdered latex glove and gradually dispersing cornstarch donning powder containing allergen directly into the individual's face during inhalation, we attempted to improve on the sensitivity of the procedure. Despite this modification, the procedure still may not be sufficiently sensitive to identify minimally allergic patients. Moreover, it cannot be properly blinded to eliminate bias, and patients undergoing this procedure may have symptoms that may not be associated with latex exposure associated with the challenge. This may have occurred in this study with 1 asthmatic subject with a positive history and a negative skin test who experienced a transient decrease of 22% in her PEFR with cough, shortness of breath, and chest discomfort while wearing a mask and goggles during the stage 1 contact portion of the glove provocation procedure. She was therefore classified as having a false-negative skin test response because of these symptoms. Despite the limitations of the glove challenge protocol, its use is sufficiently safe and instructive to evaluate patients with a negative PST response who have a suggestive history of latex sensitivity.

Because the skin test is a convenient and sensitive diagnostic method for evaluation of allergic disease, a characterized FDA-licensed diagnostic latex reagent

would be useful. In our earlier study[13] we identified NAL over ammoniated latex and extracts of latex gloves as the source material of choice for PST reagent development. NAL produced the highest diagnostic sensitivity and specificity and was the most reproducible on quality control tests. Because seasonal variation (moisture, temperature, and soil conditions) causes the amount of allergenic proteins produced by *Hevea* trees to vary up to 25-fold between batches of latex, the goal in latex reagent production has been to insure that the allergens are present and (theoretically) in molar excess in relation to IgE antibody levels in sensitized individuals.[19] The Greer NAL used in the current study has been shown by Western blot analyses to contain Hev b 1 and other proteins with molecular weights that correspond to most of the known *Hevea* allergenic proteins.

Results of this study verify in a multicenter trial the safety and diagnostic sensitivity and specificity of a candidate NAL PST reagent, which we have previously characterized.[13] Positive PST rates of 95% and 99% were observed in subjects with positive LA histories at 100 μg/mL and 1 mg/mL, respectively. Negative PST rates of 100% and 96% were observed in subjects with negative LA histories at 100 μg/mL and 1 mg/mL, respectively. In retrospect, we note a potential unintentional selection bias involving the enrollment of a higher percentage of nonatopic subjects in the NLA than in the LA group (Table I), which may have led to an enhanced diagnostic specificity assessment. Five subjects in the NLA group with a negative history, negative 100 μg/mL PST, and a negative glove provocation test had positive PST responses at the 1 mg/mL NAL concentration. Three of these 5 were serologically positive as determined by the UpJohn Pharmacia CAP System (data not shown), suggesting that these subjects are sensitized but asymptomatic individuals.

The use of the 1 mg/mL NAL concentration enhanced the diagnostic sensitivity of the PST reagent with only minimal loss in specificity. A subset of the study participants included an entire department of anesthesiology and critical care at a tertiary care facility. Of the 168 anesthesiologists who received PSTs with the multicenter protocol, 10% had a positive PST response at 1 mg/mL and a negative glove provocation test response. For purposes of this study, these subjects were placed in the NLA group. We believe, however, that these represent individuals who are sensitized but asymptomatic due to being newly sensitized and having limited allergen exposure. Some of these individuals were also allergic to avocados, kiwis, and bananas, which contain proteins cross-reactive with latex allergens[20-21] and which may have led to their weakly positive skin test responses. These are being followed to study if they develop increased sensitivity while working in a powdered latex glove environment. These observations suggest a rationale for including 1 mg/mL NAL PSTs for individuals with a positive history who have a negative PST response at 100 μg/mL or high-risk HCWs with a negative history. We believe that performance of a 100 μg/mL NAL PST followed 15 minutes later with a 1 mg/mL NAL PST should insure maximal safety.

Because safety was a primary consideration in the design of this study, we selected the puncture over the intradermal skin test procedure. Other studies have reported a 0.04% to 1.4% frequency of severe adverse reactions (asthma and anaphylaxis) after PSTs.[22-25] We are unaware of any reports of the frequency of mild allergic reactions (not requiring medication) after PSTs. Kelly et al[26] reported that PSTs with an uncharacterized latex glove extract or raw latex produced severe systemic reactions in 8.4% of the 107 individuals with a positive history tested with a multi-test prick device. The frequencies of severe adverse reactions reported in other studies after PSTs with latex glove extracts are much lower.[12,27] Differences in these reported adverse reaction rates most probably stem from the highly variable protein content of the different glove extracts used in these studies.[28]

To maximize safety in this study, we avoided the use of glove extracts and performed PSTs with a characterized NAL in duplicate from 1 μg/mL to 1 mg/mL protein. Of the 124 individuals with LA in our study, 20 (16.1%) reported mild systemic skin, eye, and upper airway symptoms immediately after skin testing. Mild delayed reactions were observed in 9.5% of the LA group (Table II). There was 1 individual who displayed a transient 28% PEFR decrease after skin testing that required no medication. Another individual who experienced rhinoconjunctivitis, asthma, nausea, and headache after undergoing a PST required Chlor-Trimeton, albuterol, and aspirin for management. Mild reactions were also reported in 4.4% of the control subjects in the NLA group. Because the original protocol required investigators to stop skin testing if the PST response was positive at 100 μg/mL, only 26 of the 124 adults with LA were actually skin tested with the highest concentration of 1 mg/mL. None of these subjects reported any reactions either immediately after skin testing or 48 hours later.

This study was performed with the bifurcated needle. Nelson et al[29] have shown that different devices used for PSTs produce wheal and erythema reactions that vary greatly in intensity of responses at both positive and negative test sites. They have also shown that the bifurcated needle is among the more traumatic and thus more sensitive devices. However, performance results obtained with the bifurcated needle should not be extrapolated to other skin testing devices. It will therefore be necessary to verify the diagnostic performance and safety of the NAL observed in this study with other PST devices.

Although differences have been reported in the specificity of IgE latex antibodies detected in the sera of children with spina bifida and adult HCWs,[30-31] there is no reason to believe that these 2 groups should be differentially sensitive to an unpurified NAL skin test material. However, there has been concern about whether children with LA are at greater risk than adult HCWs for adverse reactions to PSTs. In this study 10 children with spina bifida in both the LA and NLA groups were evaluated with the same multicenter protocol used for the adults. The wheal and erythema diameters observed in the skin of the children with LA were not different from those observed in the adults with

LA. Moreover, there was no increase in the frequency of reported reactions observed in the pediatric group. We believe these preliminary data support the safety of this diagnostic procedure in children with LA and spina bifida.

In summary, results of this multicenter study support the safety and diagnostic accuracy of the Greer NAL for use in the diagnosis of natural rubber latex allergy. The candidate NAL PST reagent consistently displayed an excellent diagnostic sensitivity and specificity at both the 100 μg/mL and 1 mg/mL dose. Safety was confirmed, with few mild reactions observed. Use of the clinical history alone may lead to misdiagnosis; however, a skin test result must also be viewed within the context of the patient's history. This is especially important in cases of asymptomatic individuals who have a positive skin test response and a confounding allergy to cross-reactive foods.

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