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Latex Sensitization by Dermal Exposure Can Lead to Airway Hyperreactivity

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Key Words
Latex allergy · IgE · Airway hyperreactivity · Murine model · Natural rubber latex · Dermal exposure · Gloves

Abstract

Background: Using non-powdered, low-protein natural rubber latex (NRL) gloves has been shown to reduce the elicitation of respiratory symptoms in latex-allergic individuals; however, the role of dermal exposure in the induction of sensitization is not completely understood. ***Objective:*** These studies were conducted to (1) determine levels of NRL protein in gloves currently in use and (2) evaluate, using a murine model, the potential for dermal exposure to induce NRL sensitization and subsequent airway hyperreactivity upon respiratory challenge. ***Methods:*** Total extractable protein and NRL allergen levels were evaluated from 38 glove samples using the Lowry and CAP inhibition assays, respectively. BALB/c mice were dermally exposed to non-ammoniated latex (NAL, 6.25–25 μg) 5 days/week for 13 weeks and monitored weekly/biweekly for IgE levels. Airway hyperreactivity was determined following respiratory challenge with methacholine (MCH) or NAL proteins on days 60 and 93, respectively. ***Results:*** Glove total protein and NRL allergen levels ranged from below the limit of detection to 946 μg/g and from 0.002 to 112 μg/g, respectively. Mice demonstrated dose-dependent increases in total serum IgE levels by day 58 with increased airway hyperreactivity observed upon respiratory challenge with MCH (day 60) or NAL proteins (day 93). ***Conclusions:*** These studies investigated the continued use of gloves with high levels of total extractable protein and NRL allergen. The potential for dermal exposure to induce NRL-specific IgE and airway hyperreactivity upon respiratory challenge suggests there should be continued concern regarding the induction of sensitization in individuals using non-powdered latex gloves.

Introduction

The induction of allergic responses resulting in asthma and upper airway disease has classically been attributed to respiratory tract exposure and therefore in the investigation of natural rubber latex (NRL) allergy, exposure assessment has focused on the airborne and dust levels of NRL allergens [1–3]. Given that approximately 5–17% of health care workers have been reported to have serum IgE specific for NRL allergens, and NRL allergic patients have suffered consequences of NRL exposure in hospital and clinical settings, intervention strategies have been initiated at many medical facilities [4–10].

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In 1997, the National Institute for Occupational Safety and Health issued an alert entitled 'Preventing Allergic Reactions to Natural Rubber Latex in the Workplace' that suggested several strategies for reducing latex exposure. The recommendations suggested the use of non-latex gloves whenever there was 'little potential for contact with infectious materials'. If NRL gloves were chosen, it was suggested that individuals use gloves that were powder-free and low in protein [11]. The Food and Drug Administration has sought to establish a limit on the NRL protein content in gloves in an effort to reduce exposure to NRL allergen [12]. Studies have demonstrated the importance of reducing the levels of glove-associated powder and the levels of NRL allergens in NRL gloves. It has been shown that glove powder may serve as a carrier for NRL allergens [13] and that personal avoidance of NRL gloves, as well as the use of powder-free or low powder content NRL gloves, results in a lower prevalence of allergic symptoms in previously sensitized individuals [14, 15].

Despite studies demonstrating the potential for penetration of NRL proteins following dermal exposure [16], the role of dermal exposure in the development of sensitization and airway hyperreactivity upon subsequent respiratory tract challenge is not well understood. Studies with the low-molecular-weight (LMW) chemicals diphenylmethane-4,4′-di-isocyanate and trimellitic anhydride have demonstrated elevated serum IgE levels and pulmonary hyperreactivity following dermal sensitization and respiratory challenge in both rat and guinea pig models [17–22]. This response is not limited to LMW chemicals, as elevations in ovalbumin (45 kD) specific IgE and IgG1 and airway hyperreactivity following dermal sensitization and subsequent respiratory challenge have also been demonstrated using murine models [23–25].

The purpose of these studies was to determine the protein and allergen levels in NRL gloves recently in use and demonstrate that repeated dermal exposure to NRL proteins has the potential to lead to sensitization and subsequent airway hyperreactivity despite the absence of previous respiratory exposure to latex proteins.

Materials and Methods

Glove Studies

Gloves

Total protein and allergen content were analyzed from 16 brands of gloves obtained from glove stations in laboratories and clinical areas of a university medical center between April and June of 1999. Thirty-eight total glove samples were obtained, which consisted of 18 NRL (11 powdered; 7 non-powdered) and 20 non-latex gloves (3 powdered; 17 non-powdered) with no 2 gloves from the same lot number. The non-latex gloves obtained were manufactured from a variety of materials, including, nitrile, vinyl, neoprene, polyvinyl chloride, and chloroprene. Protein extractions were conducted by mincing 1-gram sections of glove fingers into 2- to 3-mm pieces. The resulting product was extracted in 10 ml PBS at room temperature over 4 h with continuous rocking. Following extraction, supernatants were poured off of glove pieces, centrifuged at 300 *g* for 30 min, and passed through a 0.45-μm syringe filter to remove powder and other particulate material. Supernatants were subsequently concentrated by centrifugal ultrafiltration against a 3-kD MW cut-off filter (Centriplus YM-3, Millipore Corp., Bedford, Mass., USA) to a volume of 1 ml. Concentrated supernatants were stored at –70°C until assayed for total protein and NRL allergen.

Measurement of Total Protein

Measurement of total protein in glove extracts was performed by a micro-Lowry assay according to the manufacturer's instructions using bovine serum albumin to standardize the assay (Sigma Chemical Co., St. Louis, Mo., USA). The limit of detection (LOD) of the assay was determined as the mean plus 3 standard deviations of 10 blank assays performed without added protein. The assay LOD was 22.4 μg/ml, which yielded an LOD for determination of extractable glove protein of 22.4 μg/g of glove material.

Measurement of NRL Allergen

NRL allergens were measured by assessing the ability of samples to inhibit anti-NRL IgE binding in a commercially available fluorescence enzyme immunoassay for anti-NRL IgE (CAP, Pharmacia-Upjohn Diagnostics, Uppsala, Sweden) in a manner similar to that described by Baur et al. [2]. Briefly, plasma samples high in anti-NRL IgE activity were identified by screening plasma units from a blood bank with the NRL-CAP assay. A plasma pool (9.96 kU/l) was prepared by mixing equal parts of plasma from 5 high-titer individuals. NRL allergen levels in glove extracts were assessed by pre-incubation of 4 parts plasma pool with 1 part appropriately diluted glove extract (18 h, 4°C), followed by a CAP assay for anti-NRL IgE. CAP assays were performed in duplicate. Inhibition of the CAP assay was measured as a reduction in fluorescence units and related to a standard curve generated by pre-incubation of 4 parts plasma pool with 1 part known concentrations (varying from 0.0 to 28.0 μg/ml) of NRL (Greer Laboratories, Lenore, N.C., USA) dissolved in PBS. The LOD of the assay was determined as the mean plus 3 standard deviations of 7 blank assays run in the absence of latex allergen. The assay LOD was 1.26 ng/ml, which yielded an LOD for determination of extractable glove allergen of 1.26 ng/g glove material.

Animal Studies

Animals

Female BALB/c mice were purchased from Taconic Laboratories (Germantown, N.Y., USA). Upon arrival, the mice were quarantined for 1 week prior to use. The mice were weighed, tail marked for identification, and assigned into homogeneous weight groups (n = 5). All animals were 7–9 weeks of age at the start of the study and were maintained on Agway Prolab 3500 diet and tap water ad libitum. Mice were maintained under conditions specified within NIH guide-

lines. Animal rooms were maintained between 18 and 26°C with 40–70% relative humidity and 12-hour light/dark cycles.

Natural Rubber Latex Proteins

Non-ammoniated latex (NAL) proteins were graciously provided by the Rubber Research Institute of Malaysia in Goodyear preservative (50% glycerol, *L*-cysteine, and $NaHCO_3$) at a concentration of 7 mg/ml. SDS-PAGE analysis of the NAL revealed proteins with MW ranging from 4 to 202 kD (data not shown). NAL dosing solutions (6.25, 12.5 and 25 μg NAL) were prepared in Goodyear preservative and then diluted 1:1 in acetone for all dermal studies. Separation of latex dosing solutions by a 10% gradient SDS-PAGE gel suggested that the NAL protein profile was unaffected by acetone (data not shown).

Murine Dermal Exposures

The dorsal thorax and lumbar regions of each mouse were clipped and hair was further removed using the depilatory agent Nair® (Carter Wallace, Inc., Cranbury, N.J., USA) prior to the first exposure and weekly/biweekly thereafter. The site was then tape stripped 10 times using D-Squame discs (22 mm, CuDerm Corporation, Dallas, Tex., USA) which has been shown, using histology, to effectively disrupt the stratum corneum barrier [26]. Mice were exposed 5 days a week for 93 days to 50 μl of the vehicle or increasing concentrations of NAL (6.25–25 μg). Prior to initial protein exposure and weekly/biweekly throughout the course of the study, mice were tail bled to monitor total serum IgE levels. Upon sacrifice of the animals, blood was collected via cardiac puncture and serum fractions were stored at –20°C until analysis by ELISA for total and latex specific IgE.

Total IgE ELISA

Total IgE levels were quantified using a sandwich ELISA as described by Manetz and Meade [27]. Rat anti-mouse IgE (B1E3) and biotinylated rat anti-mouse IgE (R1E4) antibodies were generated from hybridomas graciously provided by Daniel Conrad (Virginia Commonwealth University, Richmond, Va., USA).

Latex-Specific ELISA

Latex-specific serum IgE levels were measured using a modified sandwich ELISA. Immulon-2 plates were coated overnight at 4°C with 5 μg/ml (100 μl/well) of R1E4 rat anti-mouse IgE in borate-buffered saline. Plates were blocked for 1 h with freshly prepared 3% dry milk (in PBS/Tween 0.05%) prior to the addition of the serum samples. Serum samples were diluted 1:2 down each column beginning with a 1:10 dilution (100 μl/well) and then incubated for 2 h at 37°C. NAL (precipitated and stored in a 3:1 H_2PO_4:NaOH buffer) was diluted to 25 μg/ml and 100 μl was added to each well for 2 h at 37°C. This was followed by the addition of a rabbit anti-NAL antibody (100 μl/well, 1:16,000 dilution) for 2 h at 37°C. A horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG antibody (Jackson Laboratories, Bar Harbor, Me., USA) was diluted 1:2,500 and 100 μl was added to the wells for 1 h at 37°C. The plates were developed for 20 min using an *o*-phenylenediamine (OPD) substrate at 1 mg/ml (100 μl/well). After 20 min, 50 μl of 4 *N* sulfuric acid was added to the plates and the optical densities were read by a Spectramax Vmax plate reader (Molecular Devices Co., Sunnyvale, Calif., USA) at 490 nm. Sample absorbances were analyzed using Softmax Pro 3.1 (Molecular Devices). Serum with high total IgE levels (>1,500 ng/ml) obtained from mice exposed to toluene di-isocyanate was used as a negative control. High total IgE serum (>10,000 ng/ml) from mice subcutaneously injected with 25 μg of NAL proteins weekly for a period of 8 weeks was used as a positive control. Optical density (OD) levels for each mouse were back-calculated by using the reciprocal of the dilution to get the OD value for the neat sera. Average OD values were calculated for each mouse and then used to determine the overall mean OD ± standard error (SE) for each exposure group.

Evaluation of Airway Hyperreactivity

Bronchial hyperreactivity in mice was evaluated using whole-body plethysmography (Buxco Electronics, Sharon, Conn., USA). Following dermal exposures, mice were challenged via inhalation with methacholine (MCH) or intratracheal aspiration with NAL protein and airway responses recorded. Enhanced pause (PENH), an indicator of bronchoconstriction, was calculated as PENH = (expiratory time/relaxation time – 1) × (peak expiratory flow/peak inspiratory flow).

Methacholine Challenge. Sixty days following initial dermal exposure, animals were placed into plethysmography chambers and monitored for 5 min to establish baseline PENH values. Increasing concentrations of MCH (10, 25, and 50 mg/ml) were used for non-specific challenge and were delivered to the mice while in the plethysmography chambers using a nebulizer (DeVilbiss HealthCare Inc., Somerset, Pa., USA). Mice were exposed to each concentration of MCH for 3 min followed by exposure to dry air for 2 additional minutes. PENH measurements were calculated and recorded every 30 s for a total of 5 min for each concentration of MCH. Data were analyzed following the method described by Hamelmann et al. [28]. For each animal, the percent increase in PENH values over baseline was averaged for each concentration of MCH and these values were used to calculate the mean ± SE for each exposure group.

NAL Challenge. Antigen-specific challenges were conducted 33 days following MCH challenge and 93 days following initial dermal exposure as exposures to NAL proteins continued during the period between MCH and NAL respiratory challenges. Baseline PENH values were assessed as described above. Mice were then removed from the plethysmograph chambers and briefly anesthetized (Isoflurane, Schering Plough, N.Y., USA) to allow for intratracheal aspiration of 300 μg of NAL in 50 μl PBS. Following instillation, mice were returned to the chambers within 1 min. PENH values were collected every minute for 25 min and the percent increase in PENH values over baseline was calculated as described for the MCH challenge.

Statistical Analysis

Levels of total protein and NRL allergen in glove extracts were normalized using the following transformation: log (measured value + 1). Two-way comparisons of levels in latex and non-latex extracts were accomplished by either the Mann-Whitney rank sum test or the unpaired t test, as appropriate. Two-way comparisons of the proportions of these levels that exceeded the assay LOD were accomplished by the Fisher exact test (Sigma Stat, SPSS Inc., Chicago, Ill., USA). Differences were considered significant at a level of $p < 0.05$. Correlation between total protein and NRL allergen levels was accomplished by the Pearson product moment correlation. Data from murine studies (n = 5 for all groups) were analyzed using an analysis of variance (ANOVA) and significant differences ($p < 0.05$) between the vehicle- and NAL-exposed animals were determined using a Dunnett's t test (Graph Pad Prism 3.0, San Diego, Calif., USA). Trends were analyzed for significance using linear regression.

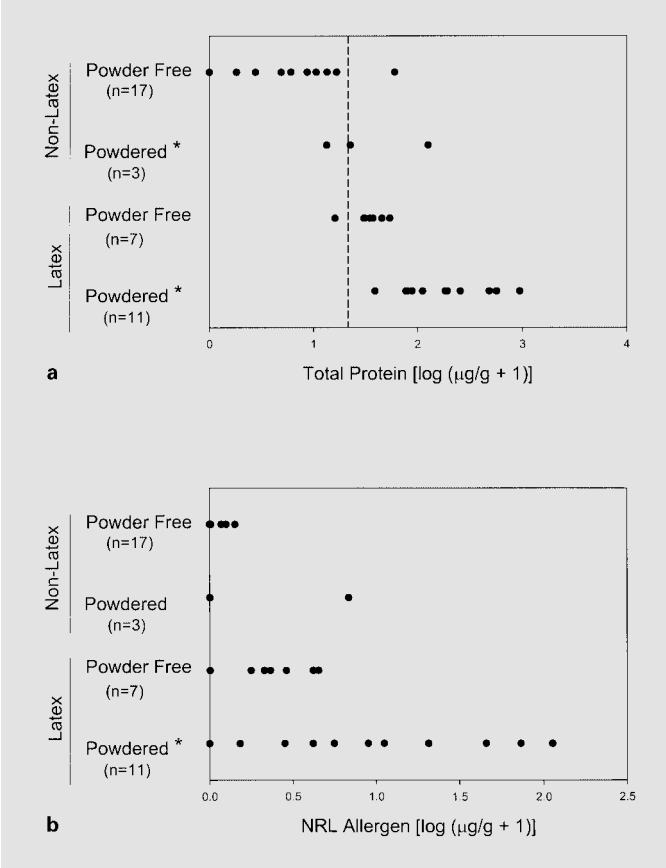

Fig. 1. Total protein (a) and NRL allergen (b) levels in glove extracts. Levels in latex gloves are greater than those in non-latex gloves, both from the standpoints of amount of protein and allergen extracted and proportion of samples exceeding the LOD (see text). Due to number of non-latex gloves with low levels of NRL allergen in b, 14 of the values for non-powdered and 2 values for powdered non-latex gloves are superimposed at the 0 value. The dashed vertical line in a denotes the LOD for glove total protein [log (LOD + 1) = 1.37]. The LOD for glove NRL allergen is not shown because of its very low value [log (LOD + 1) = 5.5×10^{-4}]. * = Statistical difference from powder free gloves, $p < 0.05$.

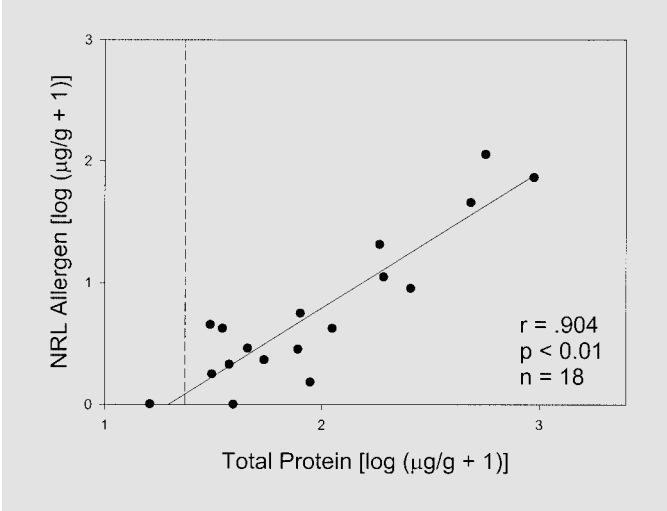

Fig. 2. Correlation between level of total protein and NRL allergen in NRL glove extracts. The dashed vertical line denotes the LOD for glove total protein [log (LOD + 1) = 1.37]. The LOD for glove NRL allergen is not shown because of its very low value [log (LOD + 1) = 5.5×10^{-4}].

Results

Total Protein and NRL Allergen in Medical Gloves

Extractable total protein levels were significantly greater for NRL than for non-latex gloves and levels were significantly higher for powdered NRL gloves in comparison to non-powdered NRL gloves (fig. 1a). However, a wide range of values was noted for both types of gloves and there was appreciable overlap. For non-latex gloves, mean levels of total protein were below the LOD for a 1-gram section of glove material with values ranging from below the LOD to 125 μg/g. For non-powdered NRL gloves, levels detected were 34.7 ± 4.5 μg/g (mean ± SD) with a range of values from below the LOD to 53.2 μg/g. Powdered NRL gloves had a mean level of 274.8 ± 84.9 μg/g with values ranging from 38.0 to 946.3 μg/g.

Extractable NRL allergen levels were significantly greater for NRL than for non-latex gloves. Levels of allergen in NRL glove extracts were significantly higher in powdered gloves when compared with non-powdered gloves (fig. 1b). With the exception of a single outlying high value, NRL allergen levels in non-latex gloves were low and tightly grouped. Of interest is that this single high outlying value was measured in a glove taken early in the study from the top of an open glove box in a clinical laboratory where powdered NRL gloves were also in use. Gloves were subsequently taken from the middle of such boxes, with no further high levels of extractable NRL allergen measured in non-latex gloves. Whereas only 9 of 20 extracts from non-latex gloves exceeded the LOD for the assay, all of the NRL glove extracts were within the detectable range of the assay ($p < 0.001$). Mean levels of NRL allergen measured in powder-free NRL gloves were 1.69 ± 0.48 μg/g (mean ± SD) with values ranging from 0.009 to 3.50 μg/g while levels of allergen in powdered NRL gloves had a mean of 25.18 ± 11.03 μg/g with a range of values from 0.002 to 112 μg/g. Mean levels of NRL allergen measured in non-latex gloves were 0.3 ± 1.3 μg/g (mean ± SD) with a range of values from 0.00 to

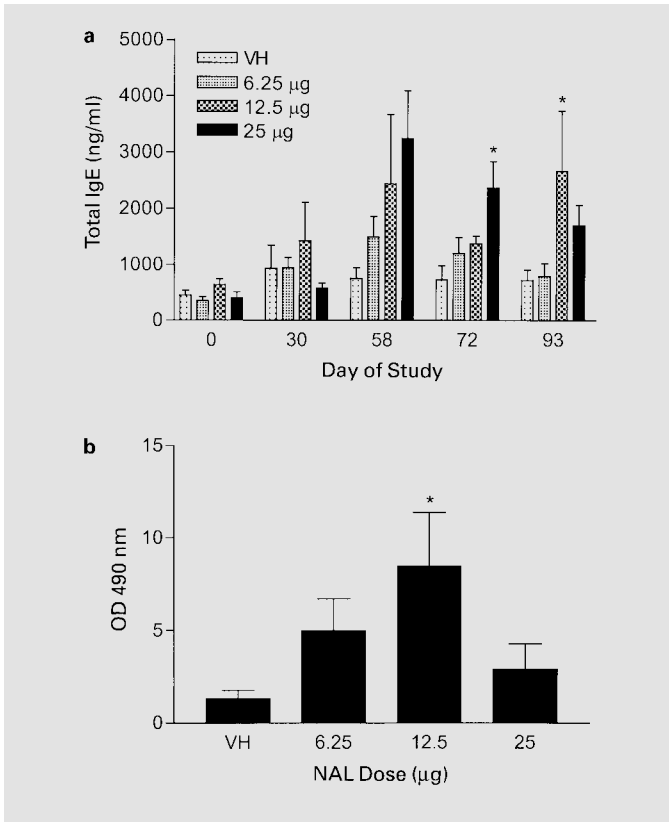

Fig. 3. a Time course and dose response of total serum IgE levels in mice exposed dermally to NAL proteins. b Latex specific serum IgE levels on day 93 in mice exposed dermally to NAL proteins. Significance of difference from vehicle (VH) was determined using a Dunnett's t test and significance is indicated by * ($p < 0.05$).

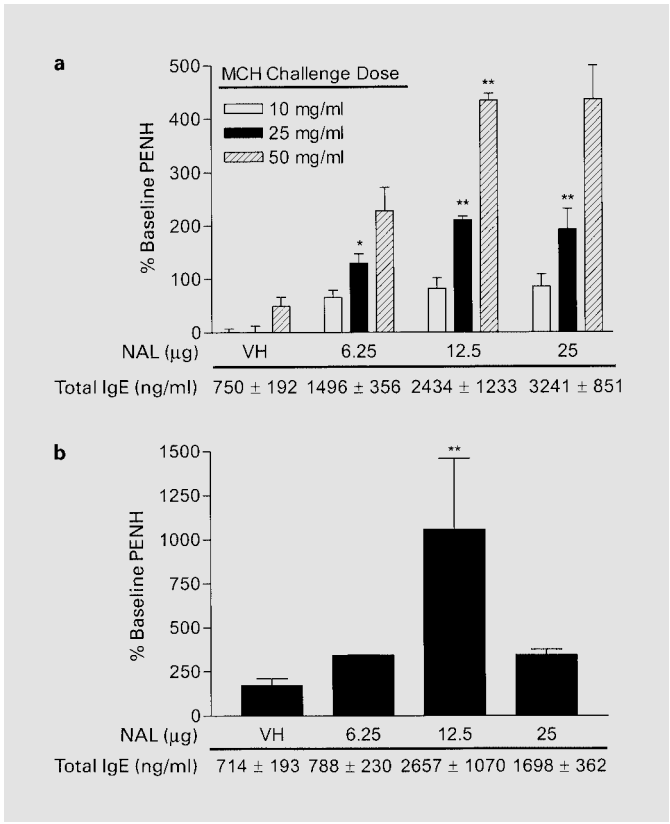

Fig. 4. Bronchoreactivity, as indicated by the percent increase in PENH values over baseline, in exposed mice following non-specific challenge with methacholine (MCH) (a) and specific challenge with NAL (b). For each challenge dose of MCH, mice dermally exposed to NAL were compared to dermally exposed vehicle animals and statistical significance was determined using a Dunnett's t test. For the NAL challenge, significance was determined by comparing mice dermally exposed to NAL to dermally exposed vehicle animals using a Dunnett's t test. Significance is indicated by * ($p < 0.05$) and ** ($p < 0.01$).

5.84 µg/g. Excluding the single high outlier, levels of NRL allergen were 0.05 ± 0.11 µg/g (mean ± SD), with values ranging from 0.00 to 0.42 µg/g. For NRL gloves, a strong correlation ($r = 0.904$) was noted between extractable total protein and NRL allergen levels (fig. 2).

Dermal Exposure Leading to Airway Hyperreactivity

Mice were dermally exposed to increasing concentrations of NAL proteins for up to 93 days. All animals appeared clinically normal throughout the course of the study. There were no statistically significant differences in body weights when comparing exposed mice to their respective control groups.

Serum IgE Levels. By day 58 after initial exposure, a dose-dependent ($p < 0.05$) increase in total serum IgE levels was observed with animals exposed to 25 µg of NAL demonstrating greater than a 4-fold increase in IgE as compared to vehicle-treated animals (fig. 3a). By day 93, levels of total serum IgE had decreased in animals exposed to 25 µg of NAL; however, total serum IgE levels remained elevated in animals exposed to 12.5 µg of NAL at this time point. On day 93 after initial exposure, latex-specific serum IgE was evaluated and as seen with total serum IgE levels, only animals exposed to 12.5 µg of NAL demonstrated a significant elevation (fig. 3b).

Airway Hyperreactivity following MCH Challenge. Sixty days following initial dermal exposure, mice were challenged non-specifically with MCH. All dose groups previously exposed dermally to NAL proteins demonstrated a significant ($p < 0.01$) dose-dependent increase in airway hyperreactivity in response to increasing concentrations of MCH. Mice previously exposed dermally to 12.5 or

25 μg of NAL demonstrated significant ($p < 0.01$) increases in airway hyperreactivity upon respiratory challenge with 25 or 50 mg/ml of MCH as compared to vehicle-treated animals (fig. 4a). Following challenge with 50 mg/ml MCH, the increase in airway hyperreactivity among mice previously exposed to NAL was positively correlated ($p < 0.05$) with the animals' increase in serum IgE levels.

Airway Hyperreactivity following NAL Challenge. Following MCH challenge, mice continued to be dermally exposed as initially described until day 93 when animals received an antigen-specific respiratory challenge with 300 μg NAL proteins. Mice previously exposed dermally to 12.5 μg of NAL proteins demonstrated a greater than 1,000-fold increase in airway hyperreactivity following NAL challenge as compared to vehicle-treated animals (fig. 4b). This increase in specific airway hyperreactivity coincided with an increase in total and NAL-specific serum IgE levels in this group. By day 93, total serum IgE levels in mice exposed to 25 μg NAL had declined to values lower than those observed for mice exposed to 12.5 μg and comparable to those observed for mice exposed to 6.25 μg. Levels of airway hyperreactivity were not significantly elevated in mice previously exposed dermally to either 6.25 or 25 μg of NAL proteins.

Discussion

Since the issuance of 'Universal Precautions' by the Center for Disease Control and Prevention in the late 1980s, the number of powdered and non-powdered latex gloves imported into the United States has risen from approximately 2 billion gloves in 1988 to over 20 billion gloves in 1996 [12]. Coincidently, the number of reported cases of latex allergy has also risen and is thought to be due in large part to increased use of NRL gloves and exposure to NRL allergens [4, 5, 10]. Several studies between 1994 and 1996 investigated the levels of total extractable protein from NRL gloves. Yunginger et al. [29] used a modified ninhydrin method to assess NRL glove protein levels and reported total extractable protein levels ranging from below the limit of detection to 9,250 μg/g of glove material. Using a modified Lowry assay (ASTM D5712-95) incorporating a protein precipitation step to assess NRL glove protein levels, Beezhold et al. [30] found a mean extractable protein level of 145 μg/g of glove material (calculated from 29 μg/ml). The Lowry assay was also used in the studies reported here to demonstrate total extractable protein levels of 181 μg/g of glove material, which are comparable to those determined by Beezhold et al. [30] in 1996. Although a precipitation step was not used in this assay, a 3-kD filter was used in the concentration step and was expected to reduce any LMW chemicals which may have otherwise interfered with the assay. Although different methods were used to evaluate total protein levels in these studies, reductions in the reported levels of extractable protein from NRL gloves since 1994 may be related to improvements in the glove manufacturing process [31].

No direct comparison can be made between NRL allergen levels in these and previous studies because of the use of different allergen standards. Previous investigations have demonstrated a weak correlation between total extractable proteins from NRL gloves and the levels of NRL allergens [29, 32, 33]. However, using a modified Lowry assay, Beezhold et al. [30] demonstrated a positive correlation between total extractable protein and allergenicity by evaluating the percentage of NRL-allergic volunteers with positive skin prick tests to NRL glove extracts. The studies presented here also demonstrated a positive correlation between total extractable protein by the Lowry assay and NRL allergen levels using the CAP inhibition assay.

It has been shown that many health care workers suffer from either irritant or contact dermatitis due to occupational exposure [10, 34–39] which may lead to a compromised skin barrier and modulation of the cytokine environment in the skin [40–42]. A previous study by Hayes et al. [16] demonstrated that the degree of NRL protein penetration correlates with the degree of human skin abrasion. In order to mimic this condition in a murine model, mice in these studies were tape-stripped to disrupt the skin barrier. D-squame discs do not contain any NRL proteins [personal communication with CuDerm Corporation] and therefore do not contribute to NRL exposure. Tape stripping perturbs the skin allowing for enhanced penetration of NRL proteins as well as increasing the expression of MHC class II on Langerhans cells [43] and biasing the skin to a Th2 cytokine response upon disruption [44].

It has been shown that the use of non- or low-powdered NRL gloves by previously sensitized individuals results in a reduction in symptoms indicative of latex allergy (i.e. allergic rhinitis, contact urticaria) [14, 15] as well as a reduction of latex-specific serum IgE levels [45]. However, few studies have been conducted evaluating the incidence of sensitization among individuals using non-powdered latex gloves. Sussman et al. [46] identified a population of health care workers that was skin prick test nega-

tive to NRL proteins and re-tested them 1 year later to identify newly sensitized individuals. No significant differences were observed between the incidences of sensitization in individuals using low-protein powdered [2 of 208 (1.0%), mean total protein levels of 557 μg/g of glove] or non-powdered NRL gloves [2 of 227 (0.9%), mean total proteins less than 1% of low-protein powdered gloves]. Levy et al. [47] skin prick tested a group of dental students in England and France to identify the prevalence of sensitization to NRL proteins. Approximately 15% of the French students and 5% of the English students that were exposed to 'powdered protein-rich' NRL gloves were sensitized while none of the students exposed to 'powder-free protein-poor' tested positively by skin prick test. One limitation of these studies is their relatively short duration. A retrospective study conducted by Allmers et al. [48] reported that the first indication of latex allergy developed after an average of 5 years of exposure. Additionally, numerous case reports have suggested that sensitization to NRL proteins may require greater than 10 years [10]. Time course studies in animals have also shown that longer time is required to sensitize via dermal exposure as compared with respiratory exposure [26].

NRL proteins have been shown to elute easily from gloves [49] and healthcare workers have been shown to change their gloves as many as 20 times a day [12]. This combined with the prevalence of dermatitis suggests the potential for extensive dermal penetration of NRL proteins among healthcare workers. Because of the difficulty of investigating the role of route of exposure in the development of sensitization to NRL proteins in humans, a murine model was used to demonstrate the importance of dermal exposure to NRL proteins in the induction of an IgE-mediated response. These studies demonstrate that continued dermal exposure to NRL proteins may result in elevated total and NRL-specific serum IgE levels and subsequent airway hyperreactivity upon respiratory challenge.

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