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Immunological Responses of Mice following Administration of Natural Rubber Latex Proteins by Different Routes of Exposure

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Although the prevalence of IgE-mediated latex allergy has increased over the past decade, the circumstances which culminate in sensitization remain uncertain. The objective of these studies was to evaluate the role which sensitization route plays in the development of latex allergy using murine models representative of potential exposure routes by which health care workers (topical and respiratory) and spina bifida patients (subcutaneous) may be sensitized. BALB/c mice administered latex proteins by the subcutaneous, topical, intranasal, or intratracheal routes exhibited dose-responsive elevations in total IgE. *In vitro* splenocyte stimulation initially demonstrated specificity of the murine immune response to latex proteins. Subsequently, immunoblot analysis was used to compare latex-specific IgE production amongst sensitization routes. Immunoblots of IgE from subcutaneously sensitized mice demonstrated recognition of latex proteins with molecular weights near 14 kDa and 27 kDa. These protein sizes are consistent with the molecular weights of major latex allergens (*Hev b* 1 and *Hev b* 3), to which high percentages of spina bifida patients develop antibodies. Mice sensitized by intratracheal or topical administration exhibited combined IgE recognition of latex proteins near 14 kDa, 35 kDa, and 92 kDa. These molecular weights are similar to other latex allergens (*Hev b* 6, *Hev b* 2, and *Hev b* 4) commonly recognized by IgE of health care workers. Mice sensitized to latex proteins by topical, intranasal, or intratracheal exposures exhibited bronchoconstriction as evaluated by whole body plethysmography following respiratory challenge with latex proteins. Subcutaneously sensitized mice were unresponsive. These differences in latex-specific IgE immunoblot profiles and altered pulmonary function amongst the four different sensitization routes suggest that exposure routes leading to sensitization may play a role in determining the primary allergen(s), and the clinical manifestation of the allergic responses.

Key Words: **mouse; latex; allergy; IgE; topical; respiratory; plethysmography; NRL; *Hev B*; PENH; bronchoconstriction.**

Natural rubber latex (NRL) allergy has become recognized internationally as a major health concern over the past decade. Allergic responses to NRL products include contact dermatitis, asthmatic bronchospasms, and life-threatening anaphylactic shock (Slater, 1994; Landwehr *et al.*, 1996). Greater than 80% of the allergic responses to NRL products are due to delayed type hypersensitivity (DTH) directed toward chemical processing aids used during product manufacture (Conde-Salazar *et al.*, 1993; Cohen *et al.*, 1998). IgE-mediated reactions to latex are potentially more severe and have been shown to be directed towards residual NRL proteins, which remain on products following the manufacturing process. To date, eight NRL proteins (*Hev b* 1–10) have been classified as major latex allergens by the International Union of Immunological Societies. Both the biology of NRL and the manufacture of NRL products have been described in numerous publications (Levy *et al.*, 1992; Landwehr *et al.*, 1996; Warshaw, 1998).

While the majority of the literature supports a latex allergy prevalence of less than 2% in the general public (Turjanmaa, 1987; Moneret-Vautrin *et al.*, 1993; Bernardini *et al.*, 1997; Cremer *et al.*, 1998; Liss *et al.*, 1999), increased risk has been associated with several occupations and medical conditions. Numerous reports have suggested that between 8–17% of healthcare workers (HCW, *e.g.*, physicians, nurses, dentists) may be allergic to NRL (Hamilton *et al.*, 1994; Yassin *et al.*, 1994; Sussman *et al.*, 1995; Sussman *et al.*, 1997; Tarlo *et al.*, 1997). Persons employed in the manufacture of latex products have also been linked with a heightened prevalence (11%) of occupational allergies toward NRL (Tarlo *et al.*, 1990). Furthermore, young patients with disorders which require repeated surgical procedures have been associated with an increased risk of latex allergy. While HCW are primarily exposed to latex proteins dermally and by inhalation, surgical patients are additionally exposed subcutaneously. For example, up to 70% of spina bifida (SB) patients have been diagnosed with IgE-mediated latex allergy (Kelly *et al.*, 1994; Nieto *et al.*, 1996; Cremer *et al.*, 1998), and a positive correlation between the number of surgical procedures and latex allergy prevalence has been demonstrated within these patient populations (Chen *et al.*, 1997; Porri *et al.*, 1997).

Despite the increased prevalence of latex allergy over the past decade, isolated cases of IgE-mediated reactions to NRL

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products were reported as early as 1927 (Stern, 1927). Implementation of "Universal Precautions" in the late 1980's correlate with the sudden rise in latex allergy prevalence. Although the prevalence of latex allergy has increased, the primary route(s) of sensitization, and the complete spectrum of latex proteins which result in allergic responses remains unclear. Immunological assays have provided evidence that HCW demonstrate higher recognition for water soluble latex proteins while SB patients exhibit increased recognition of rubber-associated proteins (Alenius *et al.*, 1993; Hamilton *et al.*, 1996; Reunala *et al.*, 1996; Yeang *et al.*, 1996; Posch *et al.*, 1998). Latex proteins can aerosolize (Swanson *et al.*, 1994) or penetrate the skin (Hayes *et al.*, 1999) and potentially sensitize HCW, while physiological fluids from surgical patients who come in contact with NRL products may elute additional latex proteins allowing for exposure to a unique set of proteins. The objective of these studies was to evaluate the role which sensitization route plays in the development of latex allergy using murine models representative of potential exposure routes by which health care workers (topical and respiratory) and spina bifida patients (subcutaneous) may be sensitized.

MATERIALS AND METHODS

Animals

Female BALB/c mice were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Mice were 6–8 weeks of age upon arrival and were quarantined for at least one week prior to use. Mice were maintained under conditions specified within the *Guide for the Care and Use of Laboratory Animals* (NIH, 1996). Nitrile or vinyl gloves were worn while handing mice. Mice were weighed and assigned into homogeneous weight groups (n = 5). All mice were provided tap water and Agway Prolab Animal Diet (5% fat) *ad libitum*. Animal rooms were maintained between 18–26°C and 40–70% relative humidity with light/dark cycles at 12-h intervals.

Latex Test Materials

Latex proteins were prepared from raw non-ammoniated latex (NAL), kindly provided by Dr. Che Hasma Hashim of the Rubber Research Institute of Malaysia (RRIM). Raw latex was tapped from a rubber tree and immediately diluted 1:2 with a Goodyear Preservative (50% glycerol/67 mM $NaHCO_3$/2 mM L-cysteine buffer). Upon receipt, the latex/glycerol solution was centrifuged for 40 min @ 14,400×g to separate the rubber fraction from the aqueous protein phase. Collection of the aqueous layer underneath the rubber fraction was accomplished using an 18 gauge needle and a 10-cc syringe. The aqueous fraction was then centrifuged twice more at 40,000×g for 1 h per spin. The final protein extract was filtered through a 0.45 micron bottle top filter and stored at −80°C. Total protein concentration of the NAL extract was determined to be approximately 7 mg/ml using a modified Lowry Assay (ASTM, 1998; Standard D5712–95). Figure 1 shows the protein profile of the NAL extract following centrifugation and filtration. This protein extract was utilized for all sensitization and challenge exposures, with the exception of respiratory challenges performed for plethysmography evaluations.

NAL Protein Exposures

Subcutaneous exposures. BALB/c mice were injected once per week subcutaneously (s.c.) in the dorsal thorax region between the shoulders with 100 μl NAL protein diluted in Goodyear preservative (glycerol buffer). One-cc tuberculin syringes with latex-free plungers fitted with 25 gauge needles were used for all injections. Vehicle-treated mice received glycerol buffer alone. Doses ranged from 0 μg–700 μg NAL protein per injection. Mice were exposed weekly for approximately 3 months in order to characterize the dose-response and time-course of IgE production. Subsequent studies, conducted by other routes of exposure, were concluded once serum total IgE levels appeared to plateau.

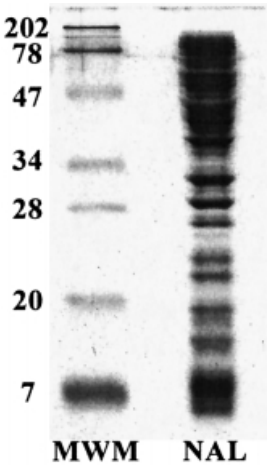

FIG. 1. 10% SDS–PAGE separation of NAL proteins (Coomassie stain). MWM = molecular weight marker; molecular weights are reported in kDa.

Topical exposures. NAL dosing solutions were prepared in Goodyear preservative and then diluted 1:1 in acetone for all topical studies. A 15–20% gradient SDS–PAGE gel suggested that the NAL protein profile was unaffected by acetone (data not shown). Each mouse was anesthetized (methoxyflurane; Schering-Plough Animal Health Corporation, Omaha, NE) and dorsal thorax or lumbar regions were clipped once per week (Mondays). The clipped surface was treated with Nair® hair removal lotion for up to 1 min and the back was washed with warm water and dried. The exposure site was abraded via tape stripping with 10 stripping discs (D-Squanme® Stripping Discs, 14 mm or 22 mm, Cuderm Corp., Dallas, TX). As demonstrated by histology, the stratum corneum of a mouse is largely removed following tape stripping with 10 discs (data not shown). Mice were dosed 5 days/week with 50 μl containing 0–150 μg NAL; all topical exposures were non-occluded.

As non-occluded, topically exposed mice were group housed (5/cage), the possibility of animals receiving an oral exposure following topical application of NAL existed. An experiment was therefore conducted to address the potential for IgE production due to oral exposure following topical applications. Mice were administered 10 μl of NAL solution 5 days per week by placing doses on the tongue using a 20 μl Gilson pipetman. Mice fed three different concentrations (1.2 ng, 78 ng, and 50 μg) of NAL did not demonstrate elevations in total IgE by day 79 (data not shown).

Intranasal exposures. Unanesthetized, BALB/c mice were instilled intranasally (i.n.) with NAL proteins (0–50 μg) diluted in glycerol buffer and PBS. Mice were administered 5 μl/nostril for 5 days per week over 72 days. Vehicle-treated mice received glycerol buffer diluted in PBS.

Intratracheal exposures. Intratracheal (i.t.) aspirations were performed similar to that described by Keane-Myers *et al.* (1998). Mice were anesthetized via methoxyflurane inhalation and restrained in a vertical position on a plexiglass stand by the upper and lower teeth. The tongue was withdrawn using padded forceps, and 50 μl of NAL/glycerol buffer/PBS dosing solution was pipetted into the back of the oral cavity. The tongue was held, to prevent swallowing, until the dose was aspirated from the oro-pharyngeal cavity. Vehicle groups received glycerol buffer diluted in PBS. Mice were dosed every 5th day for 4 weeks with NAL doses ranging from 0–50 μg.

I.t. and i.n. aspirations of 0.5% Evans blue were used to demonstrate the distribution of test article in the respiratory tract. Within 10 min following i.t.

aspiration of 50 μl, blue dye was visible in the trachea and the right and left lungs, including the cranial, medial, and caudal lobes of the right lung. Evans blue was also observed in the bronchi of the accessory lobe of the right lung. Conversely, i.n. instillation with 10 μl of Evans blue dye demonstrated no signs of lower respiratory tract exposure. Blue dye, however. was visible in the stomach of animals within 10 min of i.n. instillation.

Total IgE ELISA

Prior to and following NAL protein exposures, mice were tail-bled weekly or biweekly and total IgE serum levels were measured as described by Manetz and Meade (1999). Serum samples were serially diluted and then added to 96-well flat bottom microtiter plates (Immulon-2), which had been coated overnight with the rat anti-mouse IgE monoclonal antibody. Bound mouse IgE was quantified using a two step addition of a biotin conjugated rat anti-mouse IgE, followed by strepavidin-alkaline phosphatase (Sigma Chemical). P-nitrophenyl phosphate tablets diluted in substrate buffer were added and plate absorbency was determined within 30 min at 405 nm. IgE concentrations for each serum sample were interpolated from a standard curve using a multipoint analysis. Monoclonal antibodies used during the ELISA were previously characterized by Keegan *et al*. (1991) and Liu *et al*. (1980), and were purified from hybridomas kindly provided by Dr. Daniel Conrad (Virginia Commonwealth University, Richmond, VA).

In vitro Splenocyte Proliferation Assay

In light of the fact that the total IgE response is not indicative of a specific immune response, splenocyte proliferation was evaluated following *in vitro* challenge with NAL to demonstrate antigen specificity. Spleens from latex-exposed mice were collected aseptically and placed in sterile Hank's Balanced Salt Solution (pH 7.2; 15 mM HEPES). Single cell suspensions were prepared using frosted microscope slides and splenocyte counts were determined using a Z2 Coulter® Counter. Splenocytes (2×10^5) from latex-treated and control mice were incubated with either RPMI media or increasing concentrations of latex proteins (0.1 – 20 μg/ml) in RPMI at 37°C and 6% CO_2 for 72 h. RPMI media contained 15 mM HEPES, 0.225% Sodium Bicarbonate, 2 mM Glutamine, 100 U/ml Pen G, and 100 μg/ml Streptomycin Sulfate. 10% FBS and 50 μM 2-Mercaptoethanol were added just prior to incubation. Concanavalin A (1 μg/ml) and LPS (10 μg/ml) were added to control wells as positive control mitogens to assure splenocyte responsiveness. Twenty μl of ^{3}H-thymidine (5 μCi/mM) diluted 1:20 in RPMI media were added 18 h prior to cell harvesting. Cells were harvested onto filter pads. ^{3}H-thymidine uptake by splenocytes was determined via beta liquid scintillation counting and served as an indicator of splenocyte proliferation and a measure of specific allergen stimulation.

Immunoblot Analysis (AlaBLOT™)

Pooled sera from vehicle- or NAL (50 μg)-exposed mice were evaluated for latex-specific IgE using AlaBLOT™ latex-specific allergen strips (DPC®, Los Angeles, CA). Sera from NAL exposed mice were diluted between 1:2–1:5 with AlaBLOT™ sample diluent to normalize total IgE content (~1,500 ng), then incubated with latex allergen strips (nitrocellulose) for two h at room temperature. Sera from vehicle-treated mice were diluted 1:2 with sample diluent prior to incubation with allergen strips. Bound murine IgE was subsequently identified by the step-wise addition of rat anti-mouse IgE-HRP (Southern Biotech, Birmingham, AL), diluted 1:500 for one h, followed by 500 μl of BCIP/NBT substrate solution (AlaBLOT™ Universal Kit substrate) for up to 15 min. To further demonstrate specificity of the IgE produced by mice exposed to latex proteins, sera inhibition with NAL and ovalbumin was performed prior to immunoblot analysis. Test sera (250 μl) were incubated with an equal volume of NAL (1 mg), ovalbumin (1 mg), or AlaBLOT™ sample diluent for 2 h at 37°C prior to addition to latex allergen strips, as described above. Normalized band intensities were determined on scanned images of immunoblot strips using Gel Expert 97 (version 2.0). Latex protein or ovalbumin-inhibited allergen strips were compared to sample diluent treated strips to determine percent reduction in band intensities.

Whole Body Plethysmography

To determine pulmonary reactivity, enhanced pause (PENH), an indicator of bronchoconstriction (Drazen *et al*. 1999), was evaluated in naïve, vehicle, and latex- sensitized mice using whole body plethysmography following respiratory challenge with NAL protein (300 μg). Glycerol-free, lyophilized NAL protein was kindly provided by Dr. Donald Beezhold of the Guthrie Research Institute (Sayre, PA) and was reconstituted in PBS (pH 7.2) for use as the respiratory challenge solution. Prior to i.t. challenge, sensitized mice were placed into plethysmograph chambers (Buxco Electronics, Sharon, CT) and monitored for five min to determine baseline PENH values. Mice were then anesthetized (methoxyflurane) and challenged by i.t. aspiration, as described previously. Immediately following aspiration of NAL into the trachea, mice were placed into plethysmograph chambers and monitored for 25 min. The mean enhanced pause was reported over a period of 20 breaths and was calculated for each breath as follows:

$$\text{PENH} = \left(\frac{\text{expiratory time}}{\text{relaxation time}} - 1\right)\left(\frac{\text{peak expiratory flow}}{\text{peak inspiratory flow}}\right)$$

PENH values for individual mice were averaged for each minute over 25 min and recorded. The percent increase over baseline was subsequently calculated and plotted versus time for each mouse. The area under the curve for percent increase was determined for individual mice using Graph Pad Prism, version 2.01 (San Diego, CA). Group means and standard errors were calculated.

Statistics

Statistics were conducted using Graph Pad Prism, version 2.01. Total serum IgE levels, *in vitro* splenocyte proliferation, and PENH data from vehicle and NAL protein exposed mice were analyzed by one-way ANOVA. When significant differences were detected ($p \leq 0.05$), test groups were compared to the controls using a Dunnett's test. PENH data were also evaluated using the Prism software's Linear Trend Test. Correlation between total IgE and PENH responses were evaluated using a two-tailed, Pearson's correlation calculation. Unpaired t-tests were used to perform pair-wise comparisons of PENH data between NAL-challenged and PBS-challenged mice, between NAL-sensitized and vehicle control groups, and between naive mice and vehicle control groups.

RESULTS

Total IgE Response

Female BALB/c mice injected s.c. with 12.5 μg-700 μg of NAL protein exhibited statistically significant elevations in total IgE by day 16 (Fig. 2A). IgE increases were dose responsive and peaked near 30,000ng/ml by day 86 (Fig. 2B). IgE serum levels following s.c. administrations of 49 ng NAL were similar to those of vehicle-exposed mice, while s.c. injections with only 0.19 μg resulted in a 10-fold increase in total IgE production, a level which was not statistically significant (Fig. 2B).

Topical applications of 150 μg of NAL to tape stripped mice for two weeks resulted in significant increases in total IgE by day 16. While 50 μg topical exposures elicited IgE production in abraded mice after week 3, non-tape stripped mice showed no increase in total IgE through day 23 (Fig. 3). By day 53, IgE levels peaked near 10,000 ng/ml, and elevations in total IgE

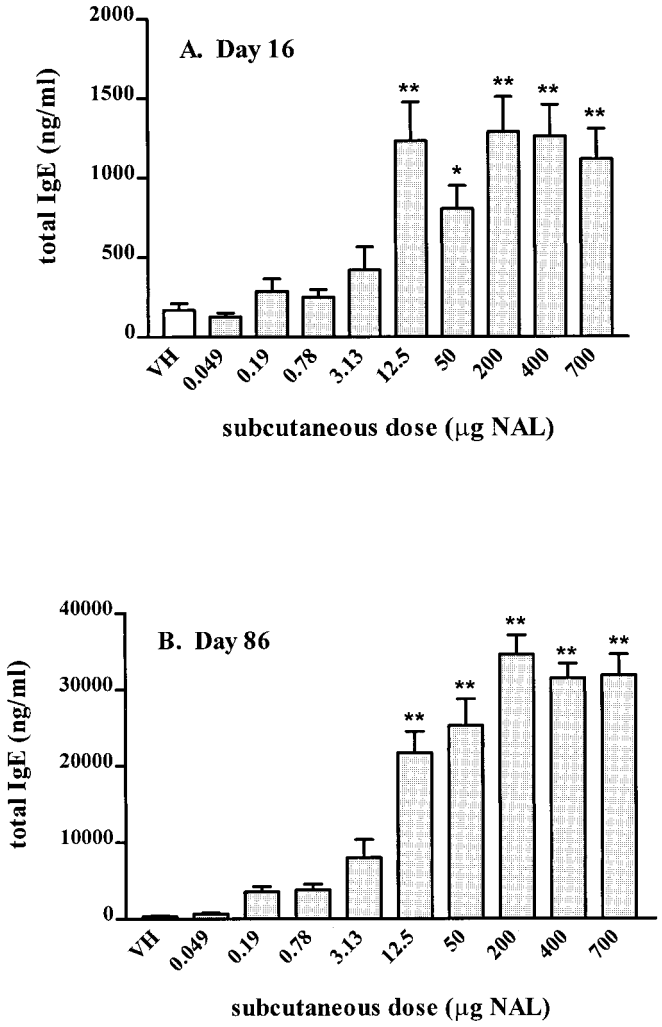

FIG. 2. Total IgE response following s.c. exposure to NAL proteins. BALB/c mice (n = 5) were injected weekly with latex protein; sera were prepared and IgE concentrations determined via ELISA. Bars represent group means ± standard error on days 16 (A) and 86 (B). Asterisks indicate statistical differences from vehicle-exposed mice at $p \leq 0.05$ (*) or $p \leq 0.01$ (**) using a Dunnett's test.

were observed in mice administered only 12.5 μg NAL topically. Any difference in total IgE concentrations between abraded and non-tape stripped mice was negligible by day 53; both groups exhibited equivalent IgE levels (5986 ± 1517 ng/ml vs. 5856 ± 3243 ng/ml).

I.n.-exposed mice did not demonstrate a statistical increase in total IgE until day 40, following 50 μg instillation (Fig. 4A). No appreciable elevations in total IgE were noted in any other dose group through day 72.

Following i.t. aspirations of NAL protein, total IgE levels were increased in the 12.5 μg and 50 μg dose groups by day 23, although only 50 μg exposures resulted in statistical significant elevations in IgE (Fig. 4B). No other group of mice demonstrated a rise in serum IgE comparable to vehicle-exposed mice following i.t. administration of NAL.

In vitro Splenocyte Proliferation

Splenocytes from mice sensitized with NAL demonstrated dose-responsive proliferation following *in vitro* challenge with latex proteins. Figure 5 shows a representative dose response following challenge of splenocytes from animals sensitized s.c. with 6.25 μg NAL. While *in vitro* stimulation of murine splenocytes with vehicle resulted in approximately 1,000 c.p.m./2 × 10^5 spleen cells, splenocytes challenged with 10 or 20 μg/ml NAL exhibited greater than 6,000 c.p.m./2 × 10^5 splenocytes. Splenocytes from vehicle-exposed mice challenged *in vitro* with 20 μg/ml NAL exhibited proliferative responses that were not different than RPMI challenged controls. Splenocytes challenged *in vitro* with Concanavalin A (1 μg/ml) or LPS (10 μg/ml) demonstrated splenocyte proliferation within historical positive values for this assay.

Immunoblot Analysis

Sera collected from mice sensitized with 50 μg NAL by each of the four exposure routes demonstrated IgE specific for latex proteins on AlaBLOT™ latex-specific allergen strips (Fig. 6). Conversely, sera from vehicle-treated mice demonstrated faint recognition of latex proteins between 35–40 kDa. Simultaneous comparison of immunoblots from each exposure route demonstrated some differences in specific IgE profiles. Sera from mice sensitized s.c. with NAL demonstrated IgE recognition of latex proteins with molecular weights near 14 kDa (consistent with *Hev b* 1, *Hev b* 6.03, and *Hev b* 8), 24–27 kDa (similar to molecular weight of *Hev b* 3), as well as proteins with molecular weights ranging from 35–70 kDa, including one near 48 kDa (*Hev b* 7 = 46 kDa). Mice sensi-

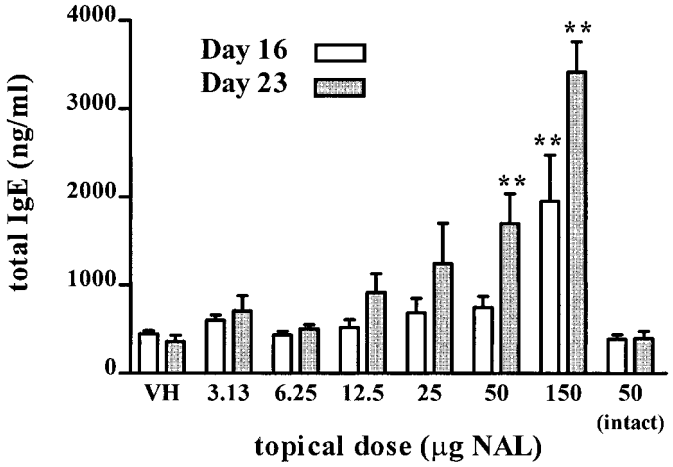

FIG. 3. Murine total IgE response following latex protein application to abraded and non-tape stripped skin. BALB/c mice (n = 5) had the hair removed from the back and were tape stripped weekly. NAL diluted in acetone was applied to the back 5 days/week. Sera were prepared and total IgE concentrations were determined via ELISA. Bars represent group means ± standard error on days 16 and 23. Double asterisks (**) indicate statistical differences from vehicle-exposed mice at $p \leq 0.01$, using a Dunnett's test.

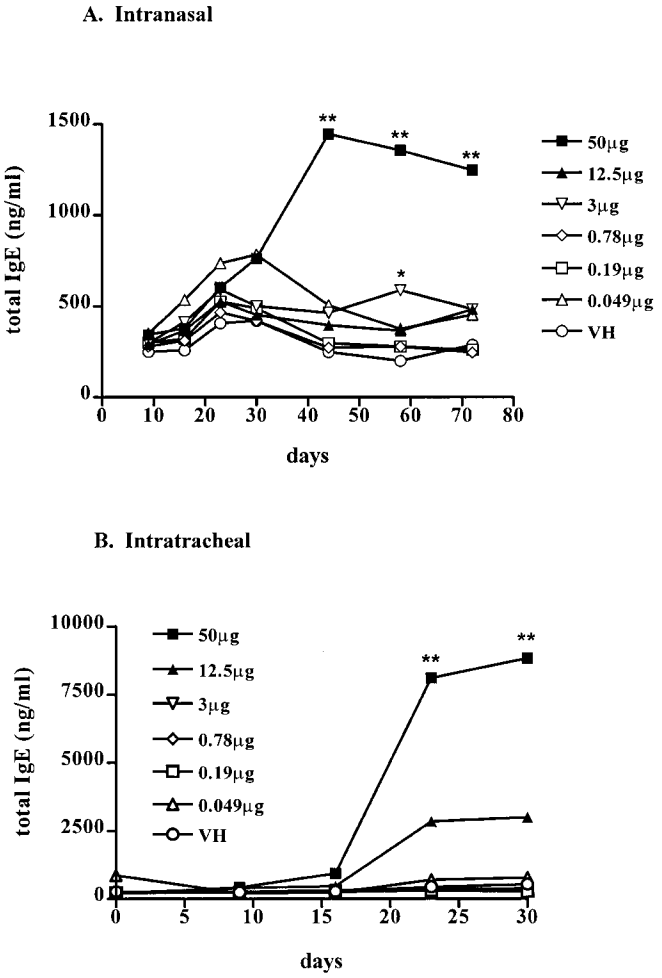

FIG. 4. Time course of total IgE response in BALB/c mice (n = 5) following respiratory tract exposure to latex protein. A. I.n. exposure: animals were administered NAL 5 days/week for 10 weeks. B. I.t. exposure: mice were anesthetized and dosed via i.t. aspirations with NAL every 5th day for 4 weeks. Sera were prepared and total IgE concentrations were determined via ELISA. Data represent group means. Asterisks indicate statistical differences from vehicle-exposed mice at $p \leq 0.05$ (*) or $p \leq 0.01$ (**), using a Dunnett's test.

tized i.t. to NAL exhibited a number of IgE bands ranging from 35–70 kDa, as well as recognition of a latex protein near 92 kDa. This protein was not detected by sera from other routes of sensitization. Topically exposed, derm-abraded mice demonstrated fewer IgE bands than s.c. or i.t. sensitized mice but showed a number of proteins between 36–60 kDa and more intense binding of a latex protein near 14 kDa. Despite the low IgE concentrations following i.n. instillation of NAL proteins, some IgE specific protein bands were detected using AlaBLOT™ allergen strips. Sera from i.n. sensitized BALB/c mice demonstrated IgE for latex proteins near the 32–40 kDa and the 60 kDa ranges.

The specificity of the IgE from latex-sensitized mice was further confirmed by demonstrating immunoblot inhibition with latex protein. While incubation of sera with NAL resulted in a reduction in latex-specific IgE when analyzed via immunoblot analysis, incubation with the same quantity of ovalbumin, an unrelated protein, resulted in minimal inhibition of band intensities. Latex protein inhibition resulted in 86%, 87%, and 81.5% reductions in band intensities at 40 kD, 36 kD, and 30 kD respectively, compared to buffered treated sera. Ovalbumin treatment of sera prior to immunoblot analysis resulted in only 13%, 30%, and 19% reductions in intensities for the same three mw proteins.

Enhanced Pause (PENH)

Whole body plethysmography was used to evaluate pulmonary responses of sensitized mice following respiratory challenge with NAL. To confirm the specificity of the PENH response, naive mice, mice administered vehicle i.t., and i.t. NAL (50 μg)-sensitized mice were monitored for bronchoconstriction following respiratory challenge with either PBS or 300 μg NAL. Naive mice and mice administered vehicle i.t. did not demonstrate bronchoconstriction following i.t. challenge with PBS (Fig. 7). Conversely, mice sensitized with NAL via i.t. aspiration demonstrated an almost $2\frac{1}{2}$-fold increase in bronchoconstriction following PBS challenge ($p \leq 0.05$). Naive and vehicle-treated mice challenged i.t. with NAL protein exhibited statistically significant bronchoconstriction as compared to PBS-challenged mice. Enhanced pause following NAL challenge of mice i.t. sensitized with latex protein was almost $4\frac{1}{2}$-fold higher ($p \leq 0.05$) than the PENH response in naive and vehicle mice not previously exposed to NAL.

PENH was evaluated following respiratory challenge with NAL in mice sensitized by each of the four routes of exposure. Despite an elevation in serum total IgE (up to 14,390 ng/ml),

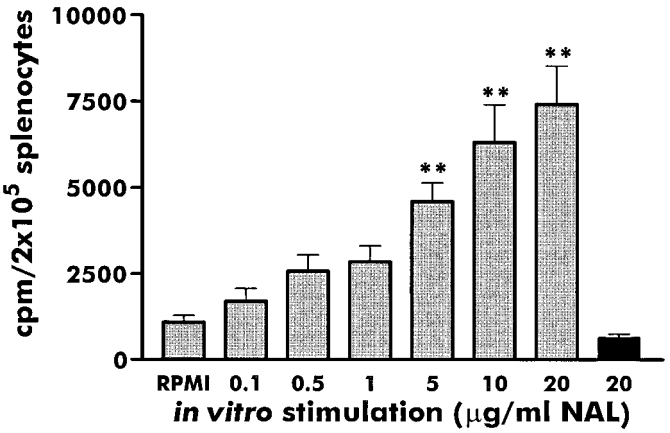

FIG. 5. *In vitro* stimulation of splenocytes with latex protein. Splenocytes from s.c. sensitized (6.25 μg) mice (dotted bars) or vehicle-treated mice (closed bar) were incubated with latex protein for 72 h; ^{3}H-thymidine was added for the final 18 h. Bars represent group (n = 5) means ± standard error. Double asterisks (**) indicate statistical differences from RPMI stimulated splenocytes at $p \leq 0.01$ using a Dunnett's test. Concanavalin A (1 μg/ml) or lipopolysaccharide (10 μg/ml) added to positive control wells resulted in 60,560 ± 1,717 c.p.m./2 × 10^5 splenocytes and 24,220 ± 551 c.p.m./2 × 10^5 splenocytes respectively.

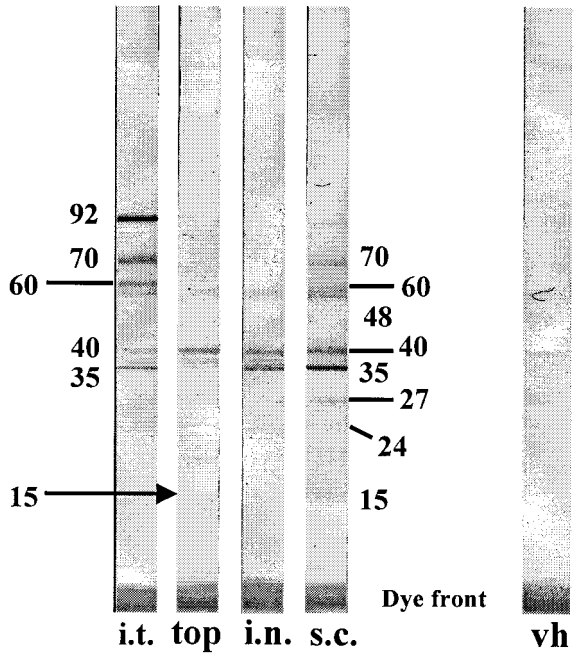

FIG. 6. Comparison of latex-specific IgE induced by different routes of sensitization. BALB/c mice (n = 5) were sensitized to NAL proteins by s.c. (day 38), topical (day 38), i.n. (day 80), or i.t. (day 30) exposures. Pooled samples were normalized for total IgE content (approximately 1,500 ng) and incubated with AlaBLOT™ latex allergen strips. Latex-specific IgE was detected using a rat anti-mouse IgE monoclonal antibody. Molecular weights are reported in kDa. Sera from glycerol buffer (vh) injected mice demonstrated faint recognition of latex proteins.

mice immunized s.c. with NAL (0.049–50 μg) did not demonstrate increases in PENH values. Conversely, mice topically sensitized with NAL (0.78–50 μg) demonstrated slight bronchoconstriction, which was dose responsive ($p < 0.05$) following respiratory challenge with latex proteins. Derm-abraded mice sensitized topically with 50 μg NAL exhibited a $2\frac{1}{2}$-fold increase in PENH values, while non-tape stripped mice also showed bronchoconstriction which was approximately 2-fold greater than vehicle controls despite serum IgE levels which were approximately 6-fold lower than those from derm-abraded mice (4,627 ng/ml vs. 779 ng/ml).

Respiratory challenge of mice exposed i.n. to high-dose NAL (50 μg/ml) demonstrated almost a 4-fold increase in mean enhanced pause compared to vehicle i.n. mice ($p < 0.01$). Animals in this group were the only ones to demonstrate an elevation in serum total IgE (1,248 ng/ml) and the only ones to demonstrate bronchoconstriction following respiratory challenge. Mice sensitized i.t. with 50 μg NAL demonstrated an approximate $2\frac{1}{2}$-fold increase in enhanced pause compared to vehicle mice following respiratory challenge. The responses by the 50 μg group may have been negatively skewed; five days prior to PENH determinations, mice from this group demonstrated signs of bronchoconstriction upon dosing. Several studies investigating rat mast cell degranulation and morphology suggest that mast cells require between 14 days and 1 month to fully recover from a degranulating stimulus (Kruger *et al.*, 1981; Hammel *et al.*, 1989; Levi-Schaffer *et al.*, 1990). Additionally, one mouse from the 50 μg dose group anaphylaxed following dosing and was sacrificed prior to the termination of the study. Surprisingly, the low-dose group (0.049 μg) demonstrated signs of bronchoconstriction with almost a 2-fold increase in PENH following respiratory challenge. The remaining groups did not show any evidence of bronchoconstriction and exhibited PENH values similar to vehicle mice.

DISCUSSION

Although the prevalence in IgE-mediated latex allergy has risen since the mid-1980′s, the primary exposure route(s) which lead to latex sensitization remain undefined. The experiments described herein were designed to develop murine models of IgE-mediated latex allergy which are representative of potential human sensitization routes. Murine models have been used to assess the allergenicity of proteins by various routes of administration. Elevations in IgE production have been demonstrated in mice following injection, topical application, or respiratory exposure to protein allergens such as ovalbumin or Alcalase (Renz *et al.*, 1992; Hilton *et al.*, 1994; Saloga *et al.*, 1994; Robinson *et al.*, 1996; Wang *et al.*, 1996). Furthermore, increased immunoglobulin concentrations have been reported by other investigators in mice exposed to a single

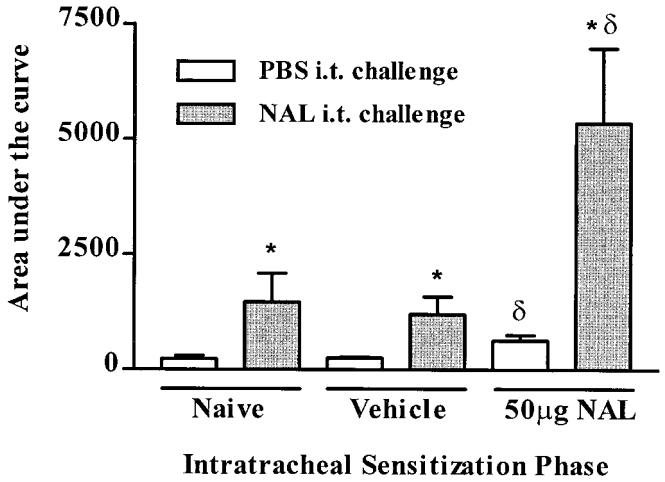

FIG. 7. PENH response by naive, vehicle, and NAL-sensitized mice. BALB/c mice (n = 5 except for 50 μg NAL exposed and challenged group where n = 4) were dosed via i.t. aspirations with glycerol buffer or NAL for 4 weeks. Naive and i.t.-exposed mice were challenged i.t. with PBS or NAL, then evaluated for bronchoconstriction. Percent over baseline PENH was calculated and plotted vs. time; area under the curve was subsequently determined. Bars represent means ± standard error. Asterisks (*) indicate statistical differences from the appropriate PBS-challenged mice, while delta symbols (δ) indicate statistical differences between NAL-sensitized and vehicle control mice. Statistical significance was determined at $p \leq 0.05$ using a one-tailed, unpaired t-test.

concentration of latex proteins via i.p. injections or i.n. instillation (Kurup *et al.*, 1994; Slater *et al.*, 1998; Thakker *et al.*, 1999). The time-course studies described here have compared dose-responsive increases in total IgE following administration of latex proteins by four different sensitization routes (subcutaneous, topical, intranasal, and intratracheal) and evaluated specific responses following latex protein challenge.

Protein from NAL was used as the test material due to the improved homogeneity in protein profiles as compared to ammoniated latex or NRL glove extracts; this allowed for more direct comparisons amongst sensitization routes without introducing variability due to test articles. Furthermore, NAL extracts have been shown to possess comparable diagnostic sensitivity and specificity in comparison to AL and glove extracts, and an NAL preparation is under consideration by the FDA as a standardized skin prick test reagent (Hamilton *et al.*, 1996, 1998). The concentrations of latex proteins chosen for evaluation in these studies were consistent with potential human exposure levels. A number of laboratories have described the elution of latex proteins from NRL gloves (Alenius *et al.*, 1994; Slater, 1994; Yunginger *et al.*, 1994; Tomazic *et al.*, 1995). Approximately 75% of the powdered gloves evaluated by these investigators had greater than 100 μg of latex protein per gram of glove; therefore, a latex glove weighing 8 grams could contain 800 μg of protein. Swanson *et al*. (1994) quantified between 8–978 ng/m^3 latex aeroallergens in several areas of a medical center where powdered gloves were used. Approximately 34% of the airborne particles collected were respirable ($\leq$ 7 μm). Based on human minute ventilation rates, a 40-h work week could result in respiratory tract exposure up to 10 μg of latex proteins per week.

The stratum corneum is considered to be the major barrier between topical antigens and the dermal immune system, but it is frequently compromised in HCW due to dermatitis caused by extensive glove usage and hand washing. Using *in vitro* diffusion cells, Hayes *et al*. (1999) demonstrated that, following 24 h of exposure to a single dose of 100 μg latex proteins, approximately 26% of NAL penetrated into or through tape stripped human skin, while less than 1.5% of the applied dose penetrated intact, non-abraded skin samples. Skin abrasion not only improves penetration of proteins through the skin but contributes to the ensuing immune response. Tape stripping of skin has been reported to increase Langerhans cell expression of MHC II and CD86 (Nishijima *et al.*, 1997), and induce Th2-dominant cytokine responses in the skin of BALB/c mice (Kondo *et al.*, 1998). Consistent with these factors, serum IgE became elevated more quickly in animals which had been tape stripped to remove the stratum corneum. However, by day 50, the effects of tape stripping appeared minimal, as IgE levels were comparable between tape stripped and non-tape stripped animals.

The skin and lungs are considered good anatomical locations for IgE production following antigen exposure (Wu *et al.*, 1996; Frazer *et al.*, 1999) and, while topical and i.t. administration of latex proteins resulted in marked increases in IgE, i.n. instillation elicited a much lower response. The low IgE response following i.n. administration of latex proteins may be due to the limited antigen distribution in the lower respiratory tract. Robinson *et al.* (1996) demonstrated that only an approximate 30% of an i.n. administered 60 μl dose of ^{125}I BSA was recovered from the lungs of mice, with the majority being recovered from the nasal cavity and gastro-intestinal tract.

A recent report demonstrated a significant correlation between specific IgE levels and both the incidence and severity of asthma and urticaria following latex challenges in humans (Kim *et al.*, 1999). Studies have described altered pulmonary function in mice sensitized with ovalbumin by one route (topical or i.p.), and challenged either i.t. or by nebulization (Saloga *et al.*, 1994; Keane-Myers *et al.*, 1998). Thakker *et al*. (1999) demonstrated altered pulmonary conductance and compliance in mice sensitized i.n. with latex proteins and challenged with allergen intravenously. In these studies, mean total IgE levels correlated with PENH responses ($p \leq 0.05$) following topical ($r^2 = 0.87$), i.n. ($r^2 = 0.73$), and i.t. ($r^2 = 0.66$) sensitization. Conversely, mice sensitized s.c. demonstrated the highest serum total IgE response (14,390 ng/ml), but showed no signs of bronchoconstriction as measured by enhanced pause.

Evidence that HCW and SB patients have a higher prevalence of sensitization to particular latex proteins has emerged. While the rubber associated proteins *Hev b* 1 (14 kDa) and *Hev b* 3 (22–27 kDa) are recognized more frequently by IgE from SB patients, *Hev b* 2 (35–36 kDa), *Hev b* 4 (100–110 kDa), and *Hev b* 6.01, 6.02, and 6.03 (20 kDa, 5 kDa, and 14 kDa, respectively) are freely soluble proteins to which a higher percentage of allergic HCW develop antibodies (Alenius *et al*., 1993; Hamilton *et al.*, 1996; Reunala *et al.*, 1996; Yeang *et al*., 1996; Posch *et al.*, 1998). Realizing that molecular weights alone can not be relied upon to identify specific latex proteins (*Hev b* 1, *Hev b* 6.03, and *Hev b* 8 have similar molecular weights), the different latex-specific IgE profiles observed in these studies following distinct sensitization routes is consistent with the hypothesis that the route of sensitization contributes to the varied specific antibody production among latex allergic patients (*i.e*., HCW and SB patients).

These studies suggest that NRL sensitization may occur following s.c., respiratory, and topical exposure to latex proteins. Mice sensitized to latex proteins demonstrated immunological responses which are consistent with those described for latex allergic patients. Sensitized mice demonstrated *in vitro* splenocyte proliferation consistent with that described for latex allergic patients by multiple laboratories (Murali *et al.*, 1994; Raulf-Heimsoth *et al.*, 1996; Ebo *et al.*, 1997). Peripheral blood mononuclear cells purified from allergic individuals and stimulated with latex proteins (0.5–20 μg/ml) for 5–7 days showed stimulation indexes between 2.5–14 at optimal *in vitro* challenge concentrations. Additionally, mice showed elevations in total and latex-specific IgE, as well as *in vivo* bron-

choconstriction following respiratory challenge with latex protein. Differences in latex-specific IgE profiles and pulmonary function following sensitization of mice by four different routes suggest that exposure routes leading to NRL sensitization may play a role in determining the primary allergens and the clinical manifestation of the immune response. These murine models of latex allergy appear to be representative of human latex allergy and should be useful in developing and evaluating new intervention techniques and strategies.

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