

Evaluation of Percutaneous Penetration of Natural Rubber Latex Proteins

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Latex allergy is recognized worldwide as a serious health risk. To date, exposure assessment and intervention strategies have focused primarily on respiratory protection; this work evaluates the potential role of dermal protein penetration in the development of latex allergy. *In vitro* penetration models using flow-through diffusion cells and both human surgical specimens and hairless guinea pig skin (CrL: IAF/HA) demonstrated iodinated latex proteins (ammoniated and non-ammoniated) penetrating into and through both intact and abraded skin. Although less than 1% penetration was observed with intact skin, up to 23% of latex proteins applied to abraded skin were recovered from receptor fluid within 24 h of exposure. Phosphoimaging of the concentrated effluent revealed proteins ranging in size from 3 to 26 kDa. Using a ³H₂O penetration assay to evaluate barrier integrity, the amount of latex protein penetration was found to positively correlate with the degree of dermabrasion. Immunohistochemistry of the skin localized latex proteins in the Langerhans cell-rich epidermis and in the dermis. Both *in vitro* penetration studies and immunohistochemistry supported the use of hairless guinea pig skin as a surrogate for human skin in evaluating latex protein penetration. In studies performed *in vivo*, 35% of hairless guinea pigs topically exposed to latex proteins (100 μg) 5 days per week for 3 months demonstrated elevations in latex-specific IgG1. The implication for these data is that the skin is not only a plausible route for latex sensitization but can be a major exposure route when the integument has been compromised.

Key Words: latex allergy; dermal protein penetration; flow through diffusion cells; latex specific IgG1; dermal latex sensitization; immunohistochemistry; hairless guinea pig dermal penetration model.

Over the last decade, reports of IgE-mediated allergic reactions to natural rubber latex (NRL) products have increased dramatically worldwide. Several theories have been proposed for the sudden increase in prevalence. The most prominent is associated with increased latex glove usage following the universal precaution recommendations issued by the Center for Disease Control and Prevention (CDC, 1987, 1988), calling for

barrier protection to prevent the spread of HIV, hepatitis, and other bloodborne pathogens. In 1987, the United States imported one billion pairs, but in 1988 this number soared to more than 8 billion (Kwittken and Sweinberg, 1992). In 1992, the Occupational Safety and Health Administration's Blood-Borne Pathogens Rule required employers to provide gloves and other protective measures for their employees (CFR, 1992), and by 1996, latex glove importation reached 21 billion pairs (Centers for Devices and Radiological Health, 1997). Well-demarcated risk groups have been defined and include health care workers (Turnjanmaa, 1987), rubber industry workers (Tarlo *et al.*, 1990), and children undergoing multiple surgical procedures (Alenius *et al.*, 1994). Using skin-prick tests, the prevalence of allergic reactions to NRL products in the general population was estimated to be approximately 1% (Liss and Sussman, 1999), but for health-care workers, the prevalence increases to 5–12% (Liss *et al.*, 1997; Tarlo *et al.*, 1997) and can be as high as 65% among children with neural tube defects (Meeropol *et al.*, 1993; Yassin *et al.*, 1992). The antigens responsible for IgE-mediated latex allergy have been identified as the residual proteins remaining in finished NRL products (Carrillo *et al.*, 1986; Tomazic *et al.*, 1995). The *Hevea brasiliensis* tree constitutively produces many of the proteins, while others are induced when the tree is wounded during the tapping procedures (Broekaert *et al.*, 1990; Yagami *et al.*, 1998).

Although large amounts of data have been generated on the elicitation of allergic reactions, less is known on how individuals become sensitized to NRL proteins. For children with spina bifida, studies in Germany have positively correlated increasing numbers of operations with the development of latex allergy (Chen *et al.*, 1997). These studies support the possibility of mucosal or subcutaneous sensitization. For health care workers, studies have associated powdered-glove usage with increased allergic responses (Brehler *et al.*, 1997) suggestive of inhalation sensitization. Also, it has been demonstrated that many latex-allergic health care workers currently have or have previously had skin lesions due to irritant or contact dermatitis (Boxer, 1996); this raises the question of skin induction. Consequently, there has been an increasing effort to

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prevent new NRL allergic reactions through education and intervention strategies. The Food and Drug Administration banned one product that caused 15 deaths from 1988 to 1992 due to highly antigenic latex catheter tips (Slater, 1994), and the National Institute for Occupational Safety and Health issued an alert recommending the use of non-powdered, low-protein gloves when latex gloves are chosen (NIOSH, 1997). These preventative directions have sought to reduce exposure to NRL proteins mainly due to inhalation and mucosal routes. As large molecular weight proteins are generally considered unable to cross the intact skin barrier, little research has focused on the role of dermal penetration in the development of latex sensitization. These studies were undertaken to evaluate the percutaneous penetration of latex proteins into and through intact and abraded skin and to demonstrate the potential for the induction of an immunological response following dermal exposure. A better understanding of the mechanisms underlying the development of latex sensitization, including the skin's role in latex-protein allergies, is needed to provide a scientific basis for future intervention strategies.

MATERIALS AND METHODS

Animals. Hairless guinea pigs (HGP), CrL: IAF/HA (hr/hr), were either purchased from Charles River Laboratories, Inc., Wilmington, MA or taken from a breeding colony maintained at the West Virginia University animal facility. Original breeding pairs from the colony were obtained from Charles River Laboratories. Animals were maintained under conditions specified within the *Guide for the Care and Use of Laboratory Animals* (NIH, 1996). Guinea pigs were housed in polycarbonate cages in an environment regulated at approximately 72°F and 60% relative humidity. Feed consisted of TekLab Guinea Pig Chow (Harlan, Indianapolis, IN) and distilled water, both given *ad libitum*. Skin from male animals 8–13 months of age was used in the percutaneous penetration studies. Male and female animals, approximately 3 months of age at the start of the study, were used in the *in vivo* exposure studies.

Human skin. Human skin was obtained from two sources. First, under a protocol approved by the Institutional Review Board for the Protection of Human Subjects at West Virginia University, fresh, surgical skin samples were obtained from Ruby Memorial Hospital, Morgantown, WV. The donated skin came from females undergoing partial or full mastectomies and was received in saline immediately following surgery. Second, human skin samples were obtained from the Center for Organ Recovery and Education (CORE, Pittsburgh, PA). The fresh surgical samples were utilized in the penetration assays and for immunohistochemistry sections, and the skin received from CORE was used during immunohistochemistry procedures to adsorb cross-reacting antibodies found in the rabbit sera.

Harvesting skin for *in vitro* penetration studies. For all animal studies, HGP were euthanized by carbon dioxide asphyxiation and full thickness skin removed from the dorsal thorax and lumbar areas. The excised skin was washed with a 10% (v/v) soap solution, rinsed with water, and patted dry with paper towels. For abraded specimens, the skin was tape stripped ten times with D Squame™ discs (14-mm diameter, Cuderm Co, Dallas, TX) to remove the *stratum corneum*. A 250- μ m thickness, including the entire epidermis and extending into the upper papillary dermis, was excised with an electric dermatome (Padgett Instruments, Kansas City, MO) and 12.7 mm (0.5 in) circular punches were taken of intact and abraded skin using a steel punch (McMaster-Carr, Inc., New Brunswick, NJ). In each experiment, the entire procedure was accomplished within 2 h of the time that animals were euthanized. Processing of human surgical samples was

accomplished within 2 h of receipt from the hospital and was conducted as described for animal skin, with one exception. Human skin samples were dermatomed to a thickness of 380 μ m. Attempts were made to prepare thinner sections of the surgical samples, but the resulting tissue had visible holes and failed a barrier test prior to tape-stripping.

Test articles. Both non-ammoniated latex (NAL) and ammoniated latex (AL) proteins were evaluated in these studies. The former is a more standardized test article (Hamilton and Adkinson, 1996) containing the proteins from the *H. brasiliensis* lactiferous cells, while the latter is the starting material often used by industry to make dipped products such as gloves and condoms and more closely represents extractable proteins from NRL products.

Protein iodination. The latex protein solutions used for the penetration studies were radiolabeled with iodine(125) using the method described by Markwell (1982) with minor modifications. Carrier-free Na¹²⁵I (NEN, Boston, MA) and Iodo-Beads™ (Pierce, Inc., Rockford, IL) were added to 250 μ l of 100 mM PBS (pH 7.0) followed by addition of the latex-protein solutions. After 25 min of incubation, the radioactive solution was dialyzed for 48 h (4°C) against 8L 50 mM KH₂PO₄ (pH 7) using tubing with a molecular weight cut off of 1000 Da.

***In vitro* penetration studies.** The *in vitro* penetration assay was modeled after the method of Bronaugh and Stewart (1985). Studies were conducted with In-line, Teflon diffusion cells (PermeGear, Inc., Riegsville, PA) with an exposed skin surface area of 0.64 cm² in each cell. Skin temperature was maintained at 32°C by aluminum cell warmers. An environmental chamber covered the diffusion cells to control the ambient temperature and humidity at 26.7°C and 60%, respectively. A HEPES-balanced Hank's salt solution (Gibco) was pumped through the cells at a flow rate of 1.8 ml/h and the effluent was collected every 6 h. The entire epidermal surface area of the dermatomed skin samples was covered with 100 μ l of the radiolabeled latex mixtures (100 μ g protein). Two skin samples (one intact and one abraded) in each study were exposed with the same concentration of non-radioactive latex proteins, under identical conditions, and were used for the immunohistochemistry experiments. Following 24 h of exposure, the skin samples were washed for contribution to mass balance, and the radioactive skin samples and 500 μ l of receptor effluent for each time point were quantified. The non-radioactive skin samples were fixed in 10% buffered formalin overnight, processed, and embedded in paraffin for immunohistochemistry.

Barrier test. The barrier integrity of all skin samples receiving radioactive test compounds was verified using a 20-min ³H₂O penetration assay (Bronaugh and Collier, 1991). Briefly, 200 μ l of ³H₂O was applied to the skin sample, covering the entire exposed surface area. The solution was left on for 20 min and then blotted dry with cotton-tipped applicators (Fisher Scientific). After washing once with 300 μ l distilled H₂O, and drying the surface of residual water, the effluent was collected for an additional 60 min. Finally, 10 ml of Scintisafe Plus™ 50% scintillation cocktail (Fisher Scientific) was added to each tube and the radioactivity quantified via a Packard Tri-Carb 2500TR beta liquid scintillation counter. A 200- μ l sample of the dosing solution was counted in duplicate and averaged. The percent ³H₂O penetration for each cell was calculated by the following formula:

$$\text{effluent dpm (80 min)} \div \text{dosing solution (avg dpm)} \times 100 = \% \text{ penetrated.}$$

Only skin samples that passed the test (<0.35% ³H₂O penetration, (Bronaugh and Collier, 1991) were used for intact skin analysis and only cells failing the test were used as abraded samples.

Characterization of effluent. Receptor effluents pools were generated for both intact and abraded samples. The solutions were freeze-dried, resuspended in 10% formic acid, and dialyzed against 4 L dH₂O (48 h, 4°C) to remove the salts and formic acid. The dialyzed solution was lyophilized again and resuspended in sample buffer for running on gels. A sample of this solution for both the intact and abraded skin samples was resolved on 16.5% tris-tricine ready gels (BioRad), dried, and subjected to phosphoimaging.

Immunohistochemistry. Paraffin-embedded skin samples were cut at 5 μm and placed on microscope slides. To block endogenous peroxidase, the slides were placed in a 1:1 solution of 3% H_2O_2 and methanol for 20 min and then incubated with 0.1% avidin and 0.01% biotin for 30 min (DAKO Co.) to block endogenous biotin in the skin. The slides were next coated with a 10% BSA block buffer for 1 h, followed by incubation with a rabbit polyclonal anti-latex antibody overnight at 4°C (αNAL , 1:200; αAL , 1:1000). The slides were then incubated with a biotinylated swine, anti-rabbit F(ab')₂ fragment (1:300, DAKO) absorbed with human skin, for 30 min at RT, followed by incubation with streptavidin-HRP (1:100). The slides were developed with liquid 3-amino-9-ethylcarbazole, washed with dH_2O , and counter stained with Mayer's hematoxylin (5 min).

In vivo exposure. Twenty-five hairless guinea pigs were weighed and assigned to homogeneous weight groups ($n = 5$). Due to the limited availability of HGP, it was necessary to use both males (15) and females (10), and they were assigned randomly into each group. Animals were divided into 5 exposure groups: vehicle tape-stripped, NAL intact, NAL tape-stripped, AL intact, and AL tape-stripped. Only one vehicle group was designated because of the limited availability of HGP. Prior to the first latex exposure, animals were pre-bled to establish a baseline latex specific IgG1 level. To ensure that compound application and tape-stripping occurred at the same area throughout the exposure period, a circle was outlined with a Sharpie pen on each animal's dorsal thorax area. To accomplish the tape-stripping, animals were manually restrained and a 22-mm D-Squame™ disc was applied to the skin inside the marked circle and swiftly peeled away. This was repeated 10 times for each animal in the tape-stripped groups. For the first 6 weeks of exposure, the tape-stripping occurred once every 2 weeks, during the remainder of the study, this procedure was increased to once per week. The animals designated as intact received no skin modifications during the study. NAL and AL proteins were diluted in vehicle (3 parts 50 mM KH_2PO_4 and 1 part acetone solution (pH 7–8)) to 1 mg/ml to administer the same volume (100 μl) and protein amount (100 μg) as was used in the *in vitro* penetration studies. The addition of 1 part acetone to the vehicle was necessary to break the surface tension of the vehicle when applied to the skin and to prevent the solution from rolling off. Initial attempts to apply the protein in vehicle without the addition of acetone, using Finn chambers, failed because the hairless guinea pigs would not tolerate the bandage materials used to secure the chambers, and excoriated their skin. The solutions were diluted fresh each day and applied topically with a pipette tip inside the marked circle, 5 days per week for 3 months. Post-exposure blood samples were obtained at the termination of the study. Prior to blood collection for both pre- and post-exposure samples, the animals were lightly anesthetized with ketamine (intramuscular injection, 25 mg/kg). Peripheral blood was taken and allowed to clot for 1 h at room temperature. The clotted blood was centrifuged at approximately $1500 \times g$ for 20 min and the sera fractions were stored at -20°C until use.

IgG1 ELISA. Immulon-II microtiter plates (Dynatech Labs) were coated overnight (4°C) with 100 μl of either NAL or AL proteins (5 $\mu\text{g}/\text{ml}$) in carbonate buffer, followed by a block with 5% nonfat dry milk in block buffer (PBS-Tween, Sigma Chemical Co.). The sera (1:5 initial dilution) were serially diluted (1:2) down the plate in block buffer and incubated for 2 h at 37°C followed by incubation with a horseradish peroxidase-conjugated goat anti-guinea pig IgG1 (1:2000, Bethyl Laboratories, Inc.) for 1 h at 37°C. Between each step, the plates were washed 6 times with phosphate-buffered saline containing 0.5% Tween-20. The plates were developed with substrate (p-nitrophenyl phosphate, Sigma Chemical Co.) and the optical density (OD) read at 405 nm after 1 h.

Statistical analysis. All data analysis was accomplished using Prism 2.0 software for the PC. For the *in vitro* penetration experiments, unpaired *t*-tests were performed to compare intact and abraded skin results. Correlations between the barrier tests and percutaneous penetration into the receptor fluid were assessed with either non-linear or linear regression analysis.

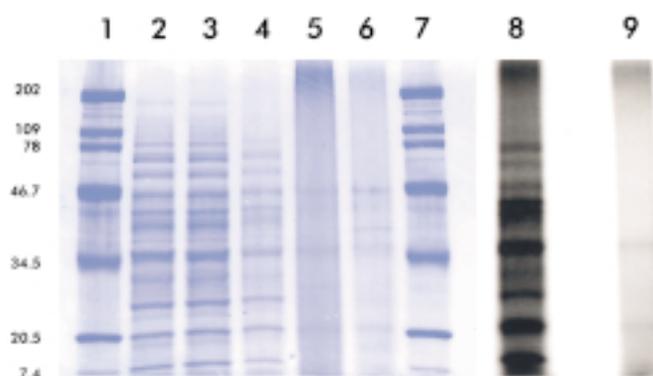


FIG. 1. Separation and radiolabeling of non-ammoniated latex (NAL) and ammoniated latex (AL) proteins. Approximately 15 μg of unlabeled and radiolabeled latex proteins were separated with 10% polyacrylamide mini-gels. The gel was visualized with Coomassie blue (Lanes 1–7), dried, exposed to a phosphoimaging screen for 48 h, and scanned for gamma radiation (Lanes 8 and 9). Lanes 1 and 7 are molecular weight standards in kilodaltons (kDa); Lane 2, NAL protein stock; Lane 3, NAL following acid precipitation; Lane 4, NAL following iodination; Lane 5, AL protein stock; and Lane 6, AL following iodination. Phosphoimaging of the dried gel revealed the radiolabeling of both the NAL (Lane 8) and AL (Lane 9) proteins.

RESULTS

Radiolabeling of Latex Proteins

Approximately 15 μg of unlabeled and radiolabeled latex proteins were separated with 10% polyacrylamide mini-gels. The gel was visualized with Coomassie blue, dried, exposed to a phosphoimaging screen for 48 h, and scanned for gamma radiation (Fig. 1). SDS-PAGE of NAL and AL proteins revealed vastly different profiles by Coomassie stain. Distinct bands were demonstrated for the NAL protein solutions while the AL proteins revealed only a few bands along with diffuse staining throughout the entire gel lane. It has been demonstrated that the manufacturing processes modify the proteins, either by altering the natural configuration or by forming polymers or complexes with other proteins (Kekwick, 1993). No visible loss in proteins was demonstrated following acid precipitation or the radiolabeling procedures. The phosphoimage revealed iodine incorporation was successful in labeling the NAL and AL protein mixtures. Of the 8 major allergens currently given nomenclature (*hev b* 1–8), all have sites available for ^{125}I -radiolabeling. Based on SDS migration comparisons, phosphoimages of the iodinated NAL and AL protein solutions produced similar bands as were visualized following Coomassie stain, demonstrating that a good representation of the proteins in each mixture were successfully radiolabeled.

In vitro Percutaneous Penetration of Latex Proteins

The data in Table 1 demonstrate latex proteins penetrated into and through both human and guinea pig skin. Following 24 h of exposure, 1.2 to 2.2% of the applied latex proteins

TABLE 1
In Vitro Percutaneous Penetration for Non-ammoniated Latex and Ammoniated Latex Proteins

	NAL proteins (% penetration)				AL proteins (% penetration)			
	Human skin		Hairless guinea pig skin		Human skin		Hairless guinea pig skin	
	Intact (n = 4)	Abraded (n = 5)	Intact (n = 23)	Abraded (n = 20)	Intact (n = 5)	Abraded (n = 5)	Intact (n = 23)	Abraded (n = 19)
Skin	0.82 ± 0.10	3.10 ± 0.36***	1.32 ± 0.12	2.57 ± 0.28***	0.73 ± 0.03	3.45 ± 1.10*	1.13 ± 0.11	2.98 ± 0.41***
Receptor fluid	0.46 ± 0.05	23.11 ± 4.57**	0.88 ± 0.09	19.14 ± 3.46***	0.47 ± 0.51	15.68 ± 6.15*	0.45 ± 0.04	7.67 ± 1.86***
Mass balance	103.40 ± 2.95	97.34 ± 2.16	93.45 ± 1.73	92.60 ± 2.21	102.03 ± 0.72	95.50 ± 4.04	101.10 ± 1.18	99.90 ± 3.11

Note. Values for skin, receptor fluid, and mass balance represent the mean percentage ± SE of the applied ¹²⁵I-labeled latex-protein dose; n, total number of skin samples combined from all penetration studies. Mass balance percentages were calculated by adding together the counts for the skin, receptor fluid, and soap and water washes. Unpaired *t*-tests performed to compare data obtained from abraded skin versus intact skin; **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

either penetrated into or through intact skin, with similar values observed for human and guinea pig skin. In all cases with human or guinea pig skin, the removal of the stratum corneum significantly increased the percutaneous penetration of both NAL and AL proteins. In studies with guinea pig skin, using ³H₂O penetration as an indication of barrier function, a correlation between the degree of abrasion and the percent NAL penetration through the skin (the percentage found in the receptor fluid) was calculated. The data fit a sigmoidal curve with an *r*² value of 0.74 (Fig. 2A). When the percent of ³H₂O penetration was less than 10%, very little NAL was recovered from the receptor fluid; however, once the skin barrier was disrupted to the extent that > 10% ³H₂O penetrated the skin, approximately 30% of the applied NAL was recovered from the receptor fluid. For AL, a linear correlation (*r*² = 0.85) was demonstrated between the barrier test and the amount of latex protein which penetrated abraded HGP skin (Fig. 2B). The differences seen in the shapes of the curves correlating the degree of protein penetration with barrier disruption for NAL and AL may relate to the sizes (Fig. 1) and conformation of the proteins in the mixtures. There may be a threshold of barrier disruption required for the larger proteins in the NAL mixture to penetrate the skin.

Due to the limited amount of human skin available, there was insufficient sample size to statistically evaluate the correlation between barrier disruption and % penetration; however, 50- and 33-fold increases in penetration through human skin following barrier disruption were observed for NAL and AL proteins, respectively (Table 1).

Immunohistochemical staining was used to localize latex proteins to distinct regions of the skin. Representative sections of human and guinea pig skin exposed to AL proteins and incubated with rabbit anti-latex sera are shown in Figures 3 and 4, respectively. In the intact skin, latex proteins were seen in the stratum corneum non-viable layers as well as in the viable epidermal layers, which are rich in antigen presenting Langerhans cells. Removal of the barrier layers resulted in more intense staining in the epidermis with visualization of latex proteins in the vascularized dermal layers, which gives rise to the possibility of systemic absorption following skin exposure. Similar patterns of staining were observed in human and guinea pig skin exposed to NAL (data not shown). Positive antibody staining of latex proteins in the skin helps confirm that the radioactive counts recovered in the penetration assays were due to latex proteins and not unincorporated radiolabel. Also, the radioactive counting of the skin samples following extensive washes and immunohistochemistry dem-

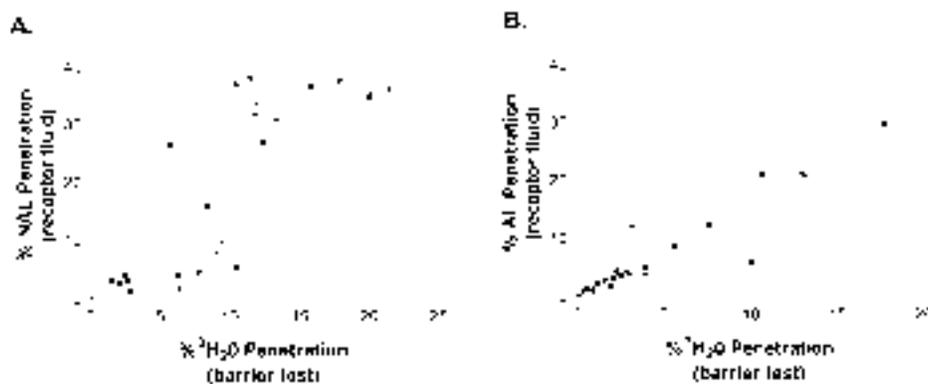


FIG. 2. Correlation between skin barrier condition and latex protein penetration through HGP skin into the receptor fluid. (A) NAL: points represent 20 values combined from 4 studies and the data were evaluated by non-linear regression analysis. (B) AL: points represent 19 samples combined from 4 studies and the data were evaluated by linear regression analysis.

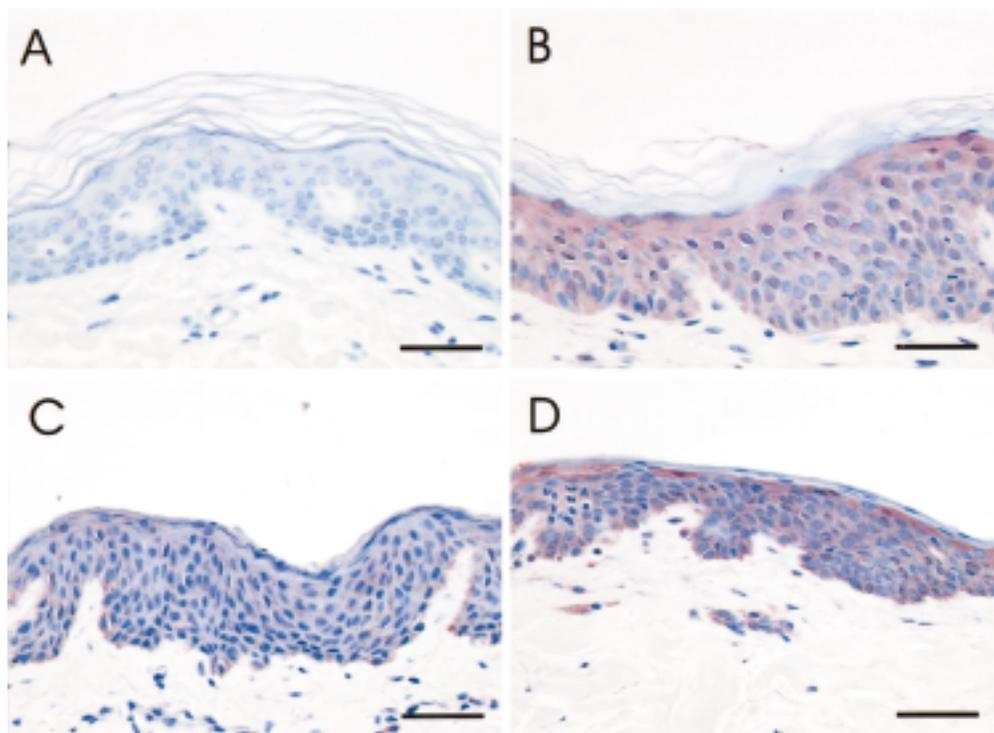


FIG. 3. Immunohistochemistry of human skin exposed to ammoniated latex protein. Intact (A) and abraded (C) skin controls not exposed to latex proteins but incubated with rabbit anti-AL sera and biotinylated secondary antibody. (B) Intact and (D) abraded skin following 24-h exposure to 100 μg AL proteins and incubated with rabbit anti-AL sera and biotinylated secondary antibody. Bars, 50 μm .

onstrate that the skin is able to retain latex proteins after a 24-h exposure period.

To determine which proteins from the mixtures were penetrating through the skin samples, the effluents were concen-

trated and the concentrates resolved with electrophoresis (16.5% SDS-PAGE). Protein bands were not clearly visible from effluent collected from intact skin samples, due to the small amount of protein that penetrated (Table 1). In studies

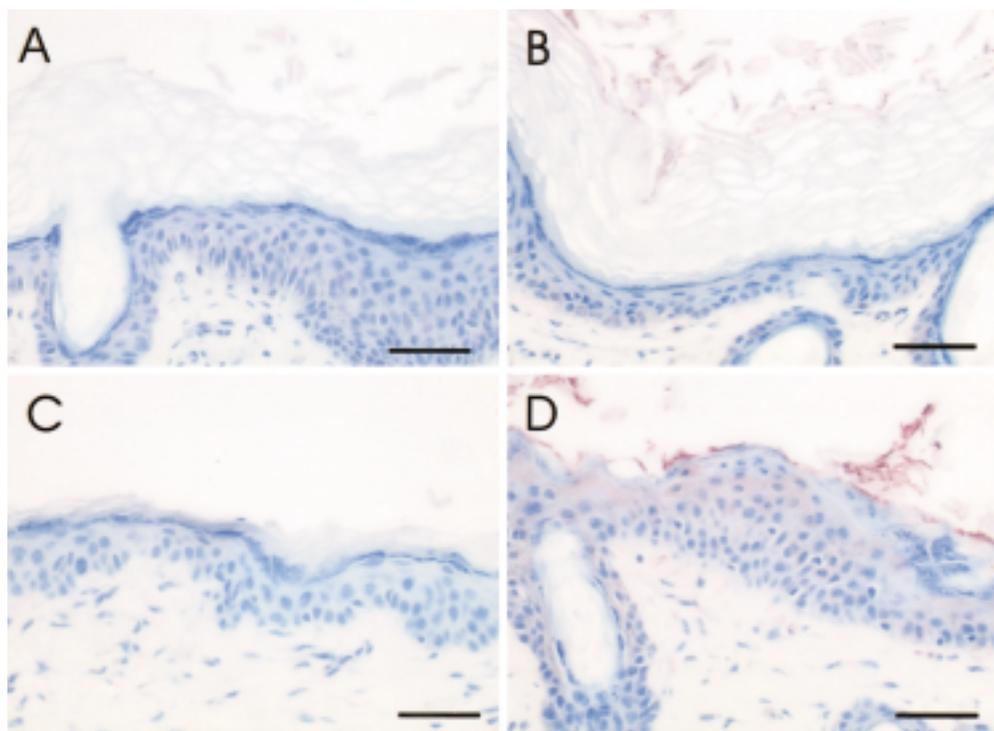


FIG. 4. Immunohistochemistry of hairless guinea pig skin exposed to ammoniated latex protein. Intact (A) and abraded (C) skin controls not exposed to latex proteins but incubated with rabbit anti-AL sera and biotinylated secondary antibody. (B) Intact and (D) abraded skin following 24-h exposure to 100 μg AL proteins and incubated with rabbit anti-AL sera and biotinylated secondary antibody. Bars, 50 μm .

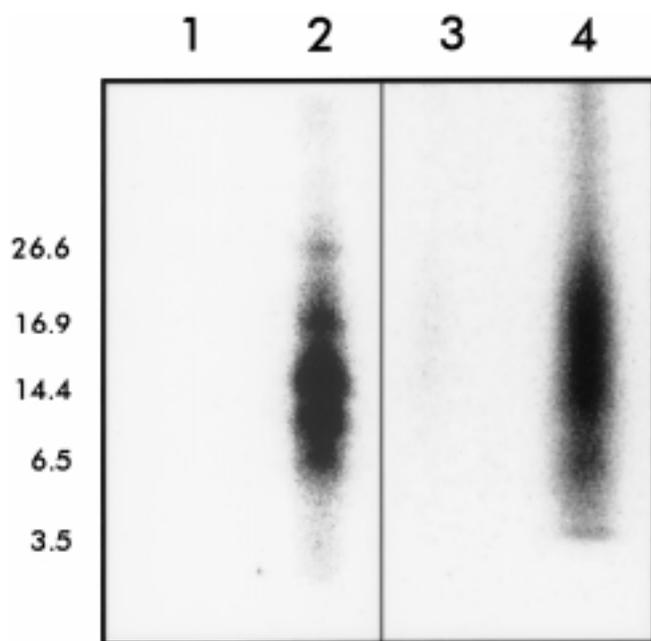


FIG. 5. Characterization of NAL and AL proteins penetrating through skin. Receptor fluid effluents were concentrated, separated by 16.5% SDS-PAGE, and subjected to phosphoimaging. Lane 1 (intact skin) and Lane 2 (abraded skin) represent the phosphoimage of effluent concentrates from skin exposed to NAL proteins while Lane 3 (intact skin) and Lane 4 (abraded skin) represent the effluent concentrates from skin exposed to AL proteins. Numbers at left represent sizes in kDa.

using abraded skin exposed to NAL and AL, however, the phosphoimage revealed that proteins ranging in size from 3 to 26 kDa had penetrated the epidermis and the upper section of the dermis (Fig. 5).

In Vivo Exposure

In vitro results demonstrated latex proteins can penetrate into and through human and hairless guinea pig skin and that HGP skin is an acceptable surrogate for human skin when evaluating latex protein penetration. A hairless guinea pig *in vivo* model then was used to further evaluate the potential for latex penetration, using the development of latex-specific IgG1 as a biomarker of exposure, following topical application of the same quantity of protein as was used in the *in vitro* penetration studies. Following 3 months of exposure, 4/10 animals (3 intact, 1 tape-stripped) topically exposed to NAL proteins and 3/10 animals (2 intact, 1 tape-stripped) exposed to AL proteins demonstrated a latex-specific IgG1 response (Fig. 6). The mean delta value (post-dosing OD - pre-dosing OD) \pm SE for the control group was 0.08 \pm 0.02. The sensitized animals had delta values greater than 0.3 with OD readings at the 1:5 dilution ranging from 0.4 to 3.3.

DISCUSSION

The purpose of these studies was 2-fold: first to investigate the percutaneous penetration of latex proteins in an *in vitro*

model using human skin and HGP skin and to evaluate hairless guinea pig skin as a surrogate for human skin for studies on latex protein penetration, and secondly, to demonstrate in the hairless guinea pig the development of a latex-specific immunoglobulin response following topical exposure. Published literature on skin penetration of proteins is limited. *In vitro* studies evaluating proteins of molecular weights similar to those represented in NAL have shown minimal skin penetration in various models. Only 0.05–0.08% of the applied dose of an 1800-Da melanotropin analogue was found to penetrate the skin of haired rats and mice using 1-chambered (static) diffusion cells (Dawson *et al.*, 1988). Low or undetectable amounts of synthetic analogues of growth hormone releasing factor (3000 and 3929 Da) were found to penetrate human and hairless guinea pig skin using flow-through and static cells (Kumar *et al.*, 1992; Loden and Fajerson, 1988). Using hairless rat skin in 1-chambered (static) diffusion cells, Ogiso *et al.* (1997) found undetectable or low amounts of penetration of elcatonin (3364 Da) and insulin (5800 Da), respectively. Similar results were obtained in studies evaluating the dermal penetration of larger molecular weight proteins. Less than 1% of the applied dose of heparin (600–20,000 Da) was found to penetrate human skin in a 1-chambered (static) model (Stuttgen *et al.*, 1990). Low penetration was observed following dermal application of human serum albumin (66,300–69,000 Da) to human and rabbit skin in a 1-chamber (static) model (Tregear, 1966). Similar results were observed in these studies investigating the dermal penetration of AL and NAL proteins through intact skin. Less than 1% of the applied doses of NAL or AL were recovered from intact human skin following 24 h of exposure, with less than 0.5% penetrating through the skin and being recovered in the receptor fluid. Comparable results were seen when HGP skin was used. It is also important to consider that the data presented here were generated from samples taken from a single anatomical location from each species, breast tissue from humans and back tissue from guinea pigs, and that penetration through human skin may vary, depending on the anatomical region which is exposed (Feldmann and Maibach, 1970).

Many latex-sensitive individuals suffer from hand dermatitis either brought on idiopathically or by various factors including hand washing (Larson *et al.*, 1986), glove use (Turjanmaa, 1994), and/or atopy (Lammintausta and Kalimo, 1981) and therefore an abraded skin model may more closely represent the skin condition of individuals at risk for latex allergy. Taylor and Praditsuwan (1996) correlated a clinical diagnosis of latex protein allergy with atopy (77%) and pre-existing hand eczema (82%). In these studies, when skin samples were abraded prior to exposure to latex proteins, penetration was significantly enhanced, with the skin retaining approximately 3% of the applied dose and a mean of 23% recovered from the receptor fluid from human skin exposed to NAL. The percent penetration positively correlated with the degree of abrasion, which may explain the lower mean percent penetration seen in the

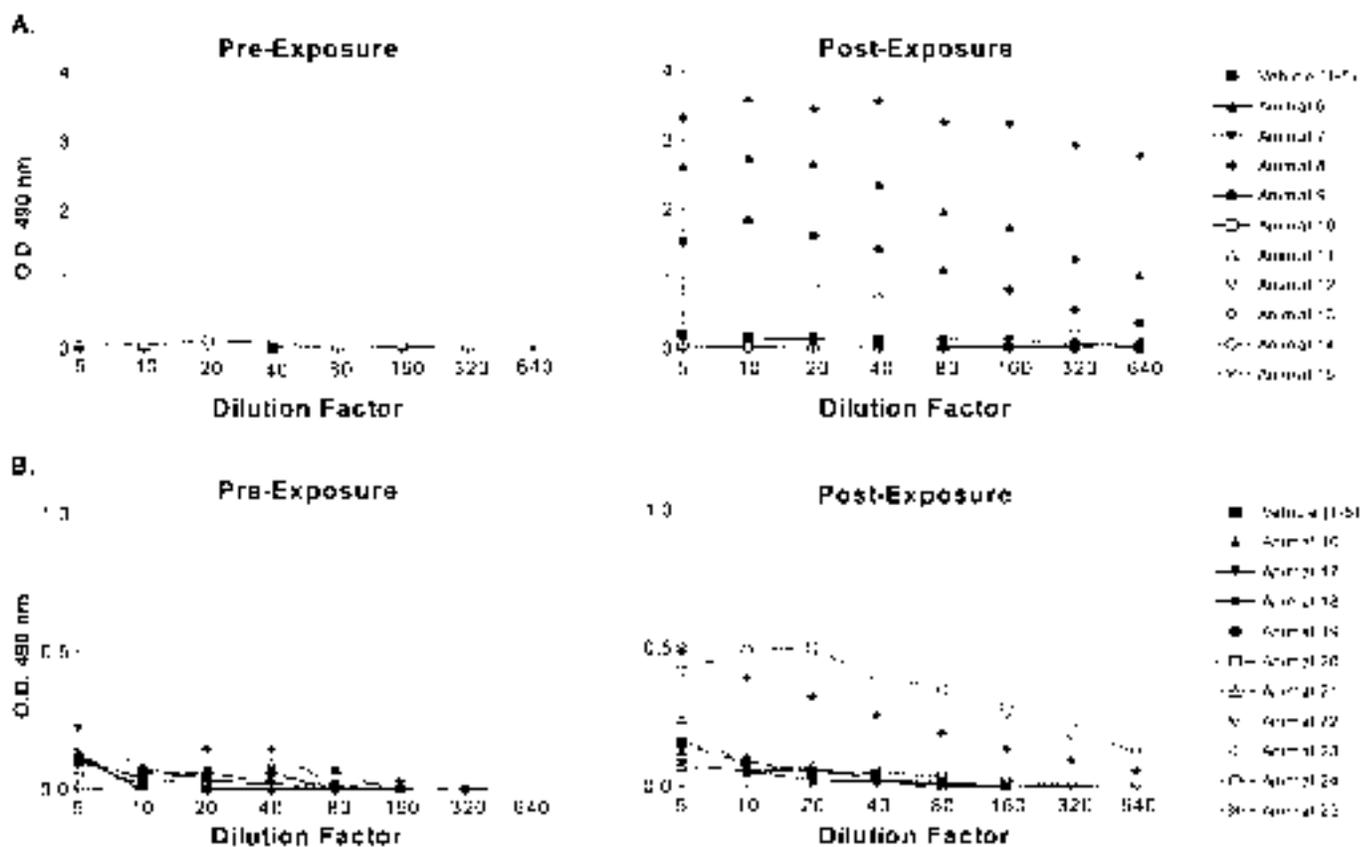


FIG. 6. Latex-specific IgG1 antibody response to NAL (A) and AL proteins (B). HGP were exposed topically to vehicle or 100 μ g NAL or AL, 5 days per week for 3 months. Groups of animals were tape-stripped to disrupt the stratum corneum or left intact. Sera were collected prior to first exposure, to establish baseline latex-specific IgG1 levels and at the termination of the study. Solid lines represent data from intact animals, data from abraded animals are represented by hashed lines. The vehicle sera ($n = 5$) were individually quantified and values shown represent the mean for the group.

HGP skin exposed to AL (7.67%) as compared to NAL (19.14%). In the $^3\text{H}_2\text{O}$ penetration test, which evaluates the degree of barrier disruption, 10 of 20 skin samples exposed to NAL permitted greater than 10% $^3\text{H}_2\text{O}$ penetration as compared to only 3 of 19 samples from the AL study. Similarly, in evaluating the penetration of vasopressin (1000 Da), using skin from haired rats in 1-chambered (static) cells, Banerjee and Ritschel (1989) demonstrated approximately 2% penetration when the hair was clipped from the animals' skin as compared to approximately 50% penetration when the skin was shaved.

In vivo studies were conducted using the development of an immunoglobulin response as a biomarker of exposure to further demonstrate that latex proteins are able to penetrate intact and abraded skin. Due to the limited number of hairless guinea pigs available, optimization studies were not conducted in this strain. Instead, the study design was based on the results of dose-response and time-course studies conducted in BALB/c mice (Woolhiser *et al.*, 2000). Only 35% of the hairless guinea pigs exposed dermally to latex proteins developed a latex-specific IgG1 response over the 3-month period. This may reflect a different time course of development of IgG1 in the guinea pig as compared to IgE in the mouse, or it may reflect

an altered immune status in the hairless guinea pig. The immune status of this species has not been well defined. It is known that in some other species, the mutations leading to the hairless phenotype also result in altered immune function (Harris *et al.*, 1993). Based on expectations due to the penetration data (i.e., a higher degree of penetration through abraded versus non-abraded skin), there may appear to be a contradiction in the number of animals that developed latex-specific immunoglobulin following exposure to abraded (2 of 10 animals) versus intact (5 of 10 animals) skin. However, Woolhiser *et al.* (2000) demonstrated that although IgE levels were elevated earlier in animals exposed to latex proteins through abraded skin, by day 53 post-initial exposure, levels were comparable in animals exposed through intact and abraded skin. Sera samples were evaluated from guinea pigs at 3 months following initial exposure.

In reviewing the relevance of skin exposure in the workplace, Fiserova-Bergerova (1993) concluded that concerns for skin exposure will increase as respiratory exposures are diminished. This may prove to be the case for latex allergy. Latex proteins have been shown to bind to glove donning powder and be aerosolized (Beezhold and Beck, 1992; Tomazic *et al.*,

1994) with latex protein levels in highly powdered glove use areas of hospitals reaching 121 ng/m³ (Swanson *et al.*, 1992). Given that the most common serious adverse effects of latex allergy are related to asthmatic responses, much attention has been focused on reducing aerosolized levels of protein and thereby reducing the elicitation of respiratory symptoms. Less attention has been given to investigating the role of the route of exposure in the development of latex sensitization. These studies demonstrated that latex proteins are able to penetrate excised human and guinea pig skin, and that exposure to 100 µg of latex proteins 5 days per week for 3 months induced an IgG1 response in 35% of exposed animals. Woolhiser *et al.* (2000) demonstrated that BALB/c mice exposed to 50 µg of latex protein through abraded skin developed elevated levels of IgE in as little as 23 days. In terms of human exposure, latex examination gloves have been shown to contain from 0.2 to 4000 µg proteins per glove (Zehr and Beezhold, 1995) and latex proteins have been demonstrated to transfer onto the skin by donning gloves (Beezhold *et al.*, 1994). The implication for these data is that the skin is not only a plausible route for NRL sensitization, but can be a major exposure route when the integument has been compromised. The data further support the recommendations that only reduced-protein latex gloves should be used to avoid skin exposure and potential sensitization to NRL proteins.

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