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Role of Sensitization Routes in the Development of Type I Hypersensitivity to Natural Rubber Latex in Mice

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INTRODUCTION

Latex allergy has become recognized internationally as a serious health hazard. Allergic responses to natural rubber latex (NRL) products include contact dermatitis, urticaria, asthmatic bronchospasms and life threatening anaphylactic shock [Slater, 1994; Landwehr et al., 1996]. While the prevalence of latex allergy in the general population has been estimated between 2.5% and 6.5% [Ownby et al., 1994; 1996], increased risk has been associated with several occupations and medical conditions. It has been suggested that up to 17% of the 5.5 million U.S. health care workers (HCW) may be allergic to NRL [Kelly et al., 1994]. As might be expected, increased occurrences of allergies involving NRL in workers also have been associated with employment in latex product manufacturing [Landwehr et al., 1996]. Furthermore, 20–70% of spina bifida patients are latex sensitive or have latex specific IgE [Kelly et al., 1994; Nieto et al., 1996; Cremer et al., 1998]. Avoidance of latex exposure can be difficult for these allergic patients as it is present in an estimated 40,000 products [Murali et al., 1994; Slater, 1994]. Latex specific IgE reportedly cross-reacts with proteins found in natural foods such as bananas, avocados or kiwi fruit providing another avenue for adverse allergic reactions [M'Raihi et al., 1991; Blanco et al., 1994; Beezhold et al., 1996].

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There is evidence that HCW and spina bifida patients become sensitized to different specific latex proteins [Hamilton et al., 1996; Posch et al., 1998]. These differing patterns of sensitization may reflect the routes of exposure in these two groups as HCW are hypothesized to be primarily exposed to latex allergens dermally and by inhalation while spina bifida children are additionally exposed subcutaneously to latex via numerous surgical procedures. In these studies, we have begun to examine the hypothesis that the route of exposure influences patterns of sensitization to NRL.

METHODS

Total IgE ELISA

Following exposure to non-ammoniated latex (NAL) proteins, total IgE serum levels were measured using an antibody capture ELISA as described by Keegan et al. [1991]. The IgE standard and rat anti-mouse antibodies were purified from hybridoma cell lines kindly provided by Dr. Daniel Conrad (Virginia Commonwealth University, Richmond, VA).

AlaBLOT™ Allergen Immunoblotting

Sera from mice were incubated with AlaBLOT™ Latex Specific Allergen Strips (DPC®, Los Angeles, CA) to identify allergen specific IgE.

In Vitro Splenocyte Proliferation Assay

Splenocytes from latex exposed mice were incubated with varying concentrations of latex proteins. [^{3}H]-thymidine was added 18 h prior to cell harvesting; uptake by

splenocytes was determined via beta liquid scintillation counting and served as a measure of specific latex allergen stimulation.

Flow Cytometry

Spleen cells as well as local draining lymph node cells were evaluated using Becton Dickinson FACScan Analysis [Manetz et al., 1998]. B220+ and IgE+ cells were identified using FITC or PE conjugated rat IgG anti-mouse antibodies (Pharmigen, San Diego, CA).

Test Materials

NAL extract was purchased from Greer Laboratories, Inc. (Lenoir, NC). In addition, raw NAL diluted 1:2 in a 50% glycerol/67 mM $NaHCO_3$/2 mM L-cysteine buffer was kindly provided by the Rubber Research Institute of Malaysia (RRIM). Upon receipt, the aqueous protein extract was centrifuged and separated as recommended by RRIM.

RESULTS

Intranasal (I.N.) Instillations

Female B6C3F1 mice instilled with 10 µl of NAL (1 mg/ml protein) every fifth day over $6\frac{1}{2}$ weeks (9 exposures) demonstrated total IgE levels which were almost 4-fold higher than those of control mice (3,500 ng/ml vs. 900 ng/ml; Fig 1). Murine modified AlaBLOTs demonstrated latex specific IgE (Fig. 2). In addition, 73% of the B220+ lymph node cells from latex treated mice stained positive for surface IgE while only 13.5% did so for vehicle exposed mice. Likewise, 43% of B220+ splenocytes stained positive for IgE compared to 9% from vehicle mice.

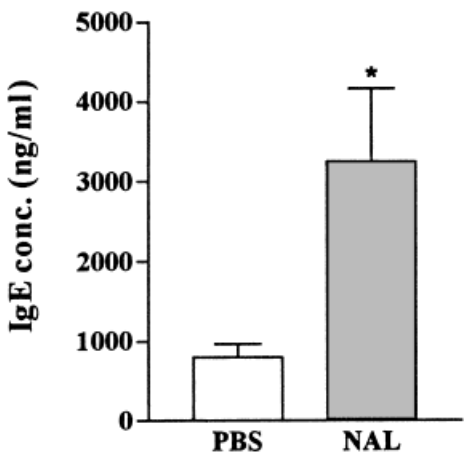

FIGURE 1. Murine total IgE levels following intranasal instillation of latex protein.

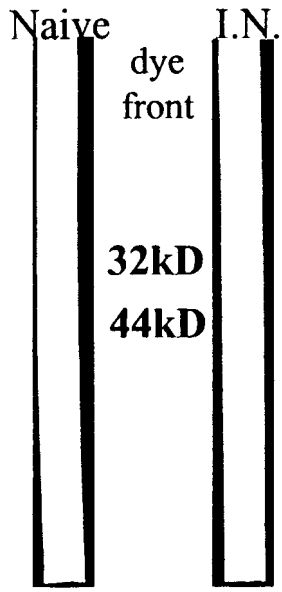

FIGURE 2. Murine anti-IgE following intranasal instillation of latex proteins.

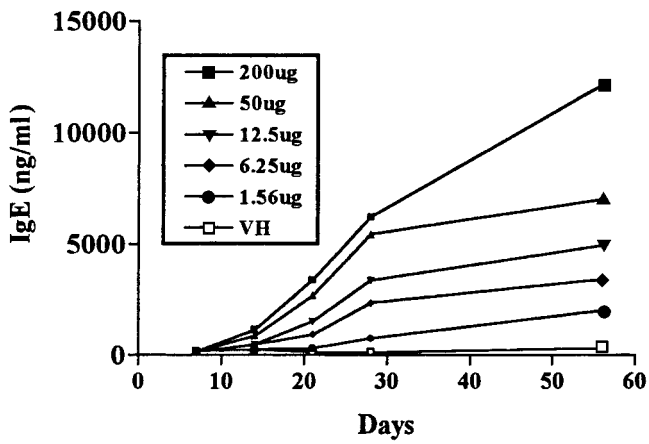

FIGURE 3. Murine total IgE time course s.c. injections of latex proteins.

Subcutaneous (S.C.) Injections

Female BALB/c mice injected s.c. in the dorsal thorax region weekly demonstrated significantly increased total IgE levels by day 14 following injections of only 50 µg of latex protein (Fig. 3). By day 56, IgE levels peaked above 12,000 ng/ml following 200 µg injections. There were a greater number of latex specific immunoblot bands following s.c. exposure than those observed following intranasal instillations (Fig. 4).

Topical Applications

Once per week the dorsal thorax region of female BALB/c mice was clipped and tape stripped using

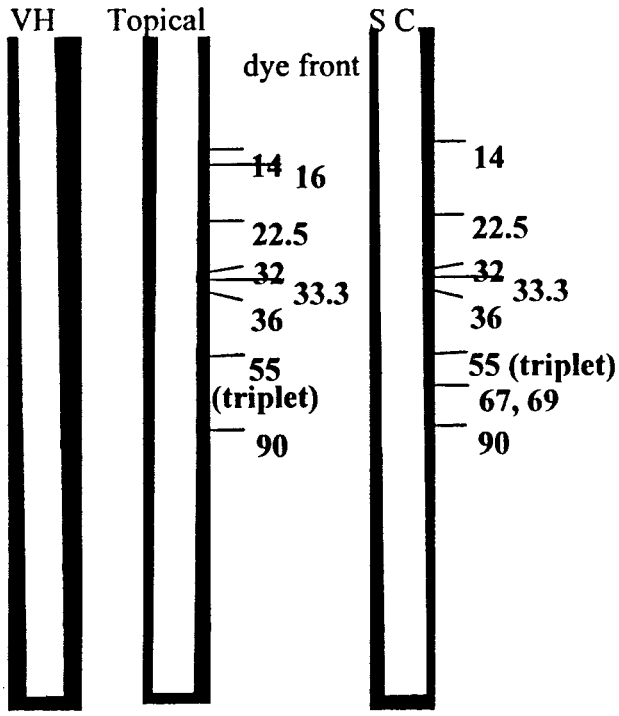

FIGURE 4. Murine anti-IgE immunoblots following latex protein exposure.

Desquame® Stripping discs (Cuderm Corp., Dallas, TX); 50 µl NAL was applied to the site five days per week over 7 weeks. Preliminary data indicate a significant increase in total IgE levels by day 16 following applications with ~110 µg of NAL proteins. Similar immunoblot profiles were demonstrated following topical NAL treatment as compared to that observed following s.c. injections (Fig. 4).

CONCLUSIONS

These experiments suggest that mouse can serve as an acceptable test system to mimic human latex exposure as mice exposed to latex proteins subcutaneously, intranasally, and topically all demonstrated specific IgE responses. The increase in latex specific IgE following topical application of latex proteins suggests that human dermal exposure to products such as latex gloves has the potential to contribute to latex sensitization. Although preliminary immunoblots from s.c. and topically exposed mice show similar profiles, a number of bands appear to be unique, thereby supporting the possibility that different routes of human exposure may lead to the varied allergen-specific IgE profiles observed in health care workers and spina bifida patients. These models will be beneficial in testing intervention strategies designed to prevent NRL hypersensitivity.

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