

Murine models for natural rubber latex allergy assessment

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Abstract

Murine models provide a powerful tool in the investigation of latex allergy and the development of intervention strategies. The immune responses to protein allergens of mice and humans are similar but differences related to the roles of IgE and IgG must be recognized. Mice have been shown to mount a dose and time-dependent IgE response to latex proteins following topical, respiratory, and subcutaneous exposures. Methods are available to evaluate cutaneous and respiratory responses to latex challenge in sensitized animals. These models have been used to investigate the role of route of exposure on the development of latex allergy and to provide a means for investigating the contribution of individual proteins to adverse respiratory and dermal responses. These models provide a mechanism for the evaluation of new technologies aimed at reducing the allergenicity of latex products, and for testing for the potential for cross-reactivity to new allergens in previously sensitized individuals. Murine models may also provide a method for testing immunotherapy strategies prior to initiating human trials. © 2002 Elsevier Science (USA). All rights reserved.

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1. Introduction

Although several animal species including the rabbit [1] and guinea pig [2] have been used to study latex allergy, most animal research in this field has been conducted using murine models. Selection of the mouse is based on the similarities in IgE-mediated responses of mouse and human; the availability of immunological reagents for the mouse; the availability of genetic variability in the mouse both in terms of conventionally bred strains and the potential for the development of transgenic animals; and the comparatively low cost of husbandry for the animals. A comparison of the immune systems of human and mouse with respect to IgE-mediated responses has been reviewed by Galli and Lantz [3]. Mice recognize and mount an IgE response to a similar repertoire of antigens as do humans. The induction of IgE is mediated by Th2 cells and the cytokines they produce, with the acute phase of the effector response being mediated primarily by tissue mast cells and the late

phase response including Th2 effector cells and other leukocytes entering the area through chemotaxis.

There are also differences in immune function between human and mouse that are important considerations when investigating type I allergy using mouse models. Although *in vitro* degranulation of human cultured mast cells via IgG has been reported [4], IgE is still considered to be the antibody class that sensitizes mast cells in humans. In the mouse, there appears to be more redundancy in function between IgE and IgG as IgG₁ also binds mast cells in mice and can mediate anaphylactic reactions [5]. In humans, the high-affinity IgE receptor (FcεRI) is found on mast cells, basophils, eosinophils, macrophages, dendritic cells, and Langerhans' cells. In mice, FcεRI is limited to mast cells and basophils, and other cells participating in the response are mediated through IgG₁ bound to FcγR. The importance of these differences becomes apparent when evaluating data generated using interleukin (IL)-4 knockout mice [6]. These animals are deficient in IgE production following latex exposure while still demonstrating latex-specific IgG₁ and an increase in airway resistance upon latex challenge.

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The majority of the published work using murine models to investigate latex allergy has been conducted using the BALB/c mouse. The decision to use the BALB/c strain is based on its Th2-dominant phenotype and high IgE production in response to protein allergens [7–12]. Other mouse strains including A/J mice [13] have demonstrated eosinophilic airway inflammation, goblet cell hyperplasia, and increased airway hyperresponsiveness following protein allergen sensitization and challenge. Although Kurup et al. [8] demonstrated an increased IgE and eosinophil response in BALB/c mice as opposed to C57BL/6 mice following intranasal exposure to latex proteins, C57BL/6 mice have been shown to demonstrate a predominant Th2 response in the lung [14] and an increase in airway hyperresponsiveness following allergen challenge [15]. These strains may prove useful in studies of latex-induced asthma.

2. Method

2.1. Routes of exposure

Studies in mice have demonstrated the ability to induce sensitization via respiratory, subcutaneous, intraperitoneal, and dermal exposures. Exposure of the entire respiratory tract can be accomplished via inhalation, either whole-body or nose only. With whole-body exposures, data interpretation can be complicated by the potential for dermal, mucosal (conjunctival), and oral (following grooming) exposures. Nose-only exposures are more stressful to the animals but eliminate these alternative routes, and acclimation to exposure chambers prior to the initiation of studies may help to reduce stress. Exposure to the upper respiratory tract can be accomplished via intranasal instillation. While the amount of material that reaches the lower airways is directly proportional to instillation volume, there is the potential for significant amounts of the dosing solution to enter the gastrointestinal (GI) tract [10]. Intranasal administration can be accomplished with or without the use of anesthesia. With the animal restrained in dorsal recumbency and the head tilted downward, 5 μ l of dosing solution is pipetted over the opening of each naris for a total of 10 μ l. The animal is held in this position until the material is inspired into the nasal cavity. Animals will tolerate a dose of up to 50 μ l, but with this amount of solution more of the material enters the lungs.

Intratracheal instillation or aspiration requires the use of an anesthetic agent and allows for the delivery of approximately 50 μ l of dosing solution to the lower airways and lung. In these procedures only the lower airways are exposed. Intratracheal instillation requires cannulation of the trachea with a blunted needle (approximately 22 gauge) or gavage tube. Although this

technique ensures all dosing solution is delivered to the lower respiratory tract, it is technically more difficult, requires a slightly longer anesthesia time, and has a greater potential for trauma to the animal. Intratracheal aspiration is accomplished by restraining the animal in a vertical position, extending the tongue using padded forceps, and pipetting 50 μ l of dosing solution into the oropharynx. The tongue is withheld to prevent swallowing until all of the visible material is aspirated. Once one becomes proficient in this exposure technique, inhalant agents such as isoflurane can be used, limiting anesthesia time to less than 1 min.

Subcutaneous and intraperitoneal exposures can be accomplished via injection of latex proteins or by implantation of latex-containing medical devices such as Penrose drains. Implantation requires the use of aseptic surgical technique and surgical anesthesia. For example, a combination of ketamine (84.7 mg/kg) and xylazine (8.4 mg/kg) injected intraperitoneally provides 30–45 min of anesthesia in the mouse. Alternatively the animals can be maintained on 2–3% isoflurane for an extended period.

Dermal exposures can be accomplished to clipped intact skin, or varying degrees of abrasion can be accomplished prior to exposure via tape stripping following the removal of hair using a depilatory agent such as Nair. Dermabrading the clipped, depilated skin of a BALB/c mouse with 10 D Squame disks (Cuderm, Dallas, TX) has been shown, using histology for confirmation, to remove the stratum corneum from the dosing site. The amount of tissue removed varies dependent on the pressure applied with each strip, but gross examination of the strips for the absence of cornified skin and examination of the site for the glistening appearance of the viable epidermis can be used as indicators of complete removal of the stratum corneum.

2.2. Blood collection

Indwelling jugular cannulas can be placed surgically in mice to collect sequential blood samples; however, less invasive methods are available. Sufficient quantities of blood can be obtained from mice for hematology or antibody evaluation by bleeding via the retroorbital plexus or the lateral tail vein. Retroorbital bleeding requires anesthesia and has the potential for ocular trauma, whereas bleeding via the lateral tail vein can be accomplished without chemical restraint and with minimal stress to the animal. Animals are placed for approximately 10 min in empty cages on heating pads set to moderate heat to induce vasodilation. With the animals restrained and after disinfecting the site with alcohol, one of the lateral tail veins is nicked distally with a sterile scalpel blade and drops of blood are collected into the appropriate tubes. When blood is collected to recover serum, the use of micro-serum separator tubes

will enhance the volume of recovery. Once the required amount of blood is collected, hemostasis is accomplished by applying pressure to the site with a sterile gauze. Subsequent bleeds are accomplished by alternating between the two lateral veins and making each incision proximal to the previous one. Five to eight drops (100–200 μ l) of blood can be collected in this manner weekly to allow for sequential evaluation of antibody production. At the time of euthanasia, about 1 ml of blood can be collected from mice by cardiac puncture, allowing for the evaluation of hematological endpoints as well as collecting a sufficient amount of serum to evaluate multiple antibody classes and isotypes.

2.3. Endpoints

Murine models have been used to evaluate multiple endpoints associated with latex exposure; among these are alterations in cytokine and antibody levels and pulmonary and cutaneous responses to latex challenge in sensitized animals. Using sequential bleeds, studies have demonstrated dose- and time-dependent increases in total and latex-specific serum IgE following multiple routes of exposure. Fig. 1 shows the total IgE response following latex exposure via intratracheal, subcutaneous, topical, and intranasal exposure. The method for quantitating total IgE has been published elsewhere [16,17]. Although these data cannot be compared between routes of exposure due to differences in bioavailability and in frequency of administration (intratracheal exposures occurred every fifth day, subcutaneous exposures were once weekly and topical and intranasal exposures were daily 5 days per week), in all cases IgE

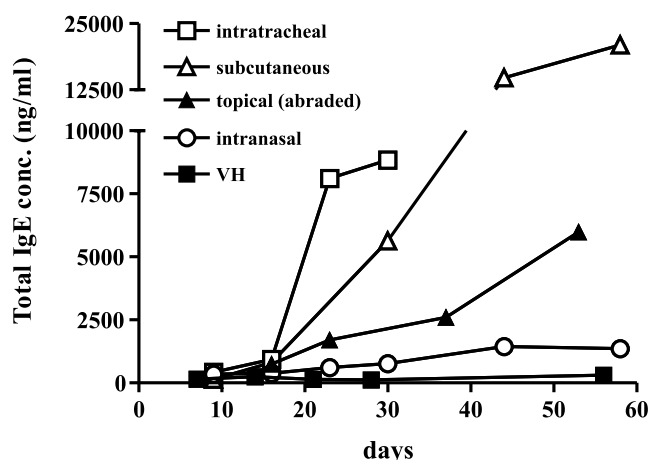


Fig. 1. BALB/c mice ($n = 5$) were administered nonammoniated latex proteins (50 μ g per dose) by intratracheal (once/5 days), subcutaneous (weekly), intranasal (5 days/week), or topical (5 days/week) exposure. Topically exposed mice were clipped and tape-stripped at the exposure site weekly. Total IgE was measured via ELISA.

levels were observed to increase in a dose- and time-dependent manner. Hayes et al. [18] demonstrated that the degree of percutaneous penetration of latex proteins was significantly correlated with the degree of dermal abrasion. By exposing groups of mice to 50 μ g of latex proteins daily 5 days per week, it was demonstrated that although dermabrasion led to an earlier rise in total IgE levels, by Day 53 there was no difference in IgE levels between animals with intact skin and those with compromised skin [19].

Enzyme immune assays can be used to demonstrate latex-specific IgE. Methods using latex protein as the capture antigen have been previously described [6,8]. A modified sandwich ELISA has been used by our laboratory to quantitate latex-specific IgE. Immulon-2 plates are coated overnight at 4°C with 5 μ g/ml (100 μ l/well) R1E4 rat anti-mouse IgE (generated from a hybridoma generously provided by Daniel Conrad, Virginia Commonwealth University) in borate-buffered saline (BBS). Plates are blocked for 1 h with 3% dry milk (in PBS/Tween 0.05%) prior to addition of the serum samples. Twofold dilutions of serum samples are added beginning with a 1:10 dilution (100 μ l/well) and incubated for 2 h at 37°C. Nonammoniated latex (NAL, precipitated and stored in a 3:1 H_2PO_4 :NaOH buffer) is diluted to 25 μ g/ml and 100 μ l is added to each well for 2 h at 37°C. This is followed by the addition of a rabbit anti-NAL antibody (100 μ l/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) is diluted 1:2500 and 100 μ l is added to the wells for 1 h at 37°C. The plates are developed for 20 min using an *o*-phenylenediamine (OPD) substrate at 1 mg/ml (100 μ l/well). After 20 min, 50 μ l of 4 N sulfuric acid is added to the plates and the optical densities are read with a Spectramax V_{max} plate reader (Molecular Devices, Sunnyvale, CA) at 490 nm. Sample absorbances are analyzed using Softmax Pro 3.1 (Molecular Devices). Serum with high total IgE levels (>1500 ng/ml) obtained from mice exposed to toluene diisocyanate is used as a negative control. High total IgE serum (>10,000 ng/ml) from mice subcutaneously injected with 25 μ g of NAL proteins weekly for a period of 8 weeks is used as a positive control. Additionally, commercially available tests for latex-specific IgE such as the CAP assay (Pharmacia) and ALAStat (Diagnostic Products) can be modified to quantitate IgE in mouse serum.

Pulmonary responses to latex exposure in mice can be investigated by evaluation of bronchial alveolar lavage fluid, by histology, and by measurement of airway hyperreactivity. Following repeated intranasal exposures with 30 μ g of latex protein in 50 μ l of sterile PBS, Kurup et al. [8] demonstrated an elevation in interstitial lymphocytes, histiocytes, plasma cells, and eosinophils by pulmonary histology. Fig. 2 shows the lungs of mice

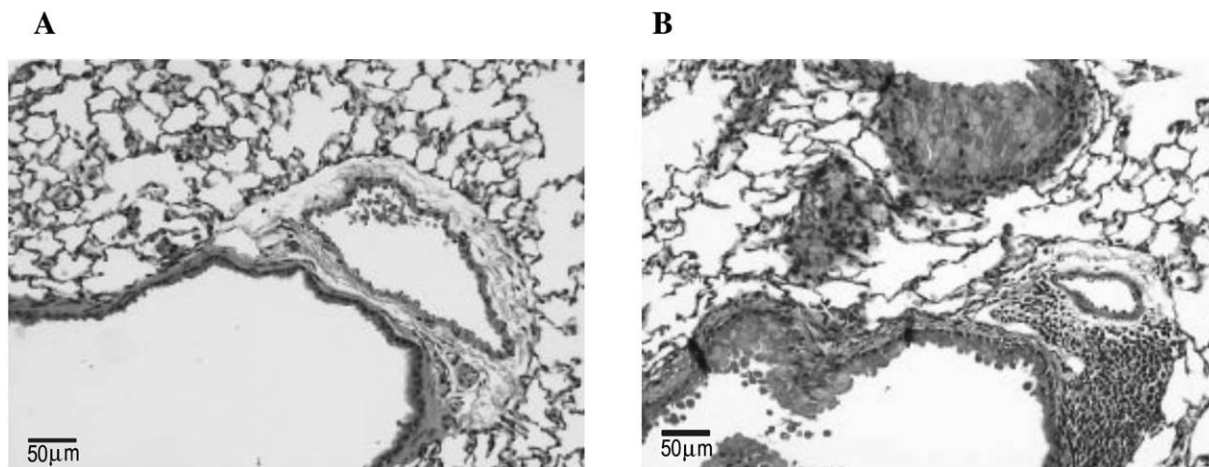


Fig. 2. BALB/c mice were exposed via intratracheal aspiration to either 50 μ l vehicle (A) or 50 μ g of nonammoniated latex proteins in 50 μ l of vehicle (B) every fifth day for a total of six exposures. Following sacrifice, the lungs of animals were removed, fixed in 10% formalin, and stained with eosin and hematoxylin for standard histopathological evaluation.

that were exposed to 50 μ g latex proteins in 50 μ l vehicle via intratracheal aspiration every fifth day for a total of six exposures. As compared with the vehicle-exposed group (Fig. 2A), the latex-exposed animals (Fig. 2B) exhibited perivascular and peribronchial mixed cell infiltrates consisting mainly of lymphocytes with fewer neutrophils and macrophages. Goblet cell metaplasia with excess mucin production was evident in the bronchi.

The mouse has been increasingly used to evaluate respiratory function following exposure to allergens [20]. Methods used to evaluate airway function have involved primarily removal of the lungs from the animal, invasive instrumentation and direct cannulation of the airway, or noninvasive, whole-body assessment. Direct cannulation of the airway results in direct measures of airway pressures but includes confounding interference due to concurrent anesthesia and physical obstruction from cannulation itself. While whole-body plethysmography allows for repeated evaluation of a response over days or months, the sensitivity is reduced as assessment of pulmonary function is indirect and also incorporates contributions by the nasal airway. Both approaches have been used in recent years to evaluate pulmonary reactivity in mice following “sensitization” with natural rubber latex proteins. Using invasive instrumentation, investigators reported altered pulmonary conductance and compliance in tracheotomized mice “sensitized” with latex protein and challenged parenterally with either methacholine or latex protein [6,12]. Whole-body plethysmography has been used in our laboratory to demonstrate increased airway hyperreactivity in mice “sensitized” topically with latex protein and challenged intratracheally with the same (Fig. 3).

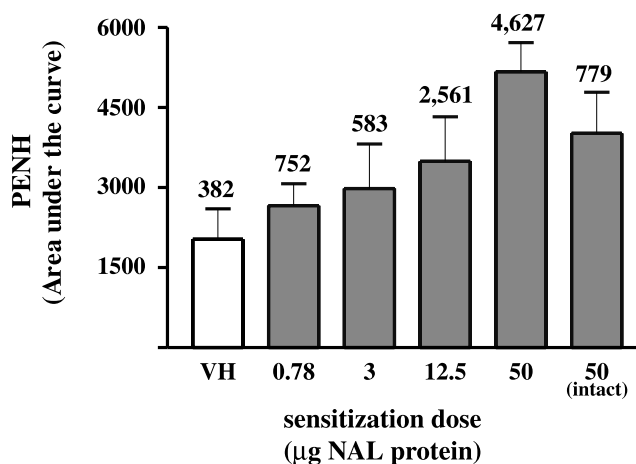


Fig. 3. Clipped BALB/c mice, with or without tape stripping, were “sensitized” via topical administration of nonammoniated latex proteins 5 days/week for 5 weeks. Mice ($n = 5$) were challenge intratracheally with 300 μ g latex protein and then monitored for bronchoconstriction via whole-body plethysmography. Percentage increase over baseline PENH was determined over 25 min and the area under each curve was calculated. Bars represent mean areas $\pm$ SE. Values above error bars represent serum total IgE concentration (ng/ml) less than 24 h after PENH measurements.

IgE-mediated cutaneous responses to latex proteins can be demonstrated by active cutaneous anaphylaxis (ACA) in mice. Previously sensitized animals are challenged by injection intradermally in the right ear pinna with latex protein (10 μ g in 10 μ l PBS) and in the left ear with 10 μ l PBS as a control. Animals are immediately injected intravenously with 250 μ l of 0.5% Evan’s Blue dye and are observed for up to 30 min. Extravasation of the dye into the tissue surrounding the antigen injection site is measured as an indication of increased vascular permeability induced by mast cell degranulation (Fig. 4).

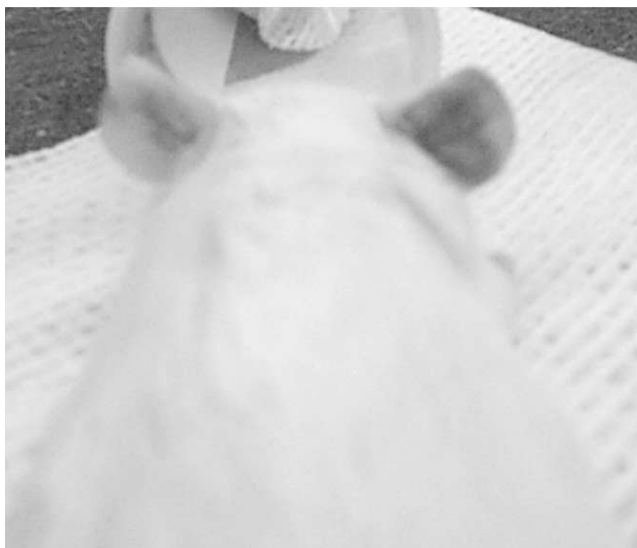


Fig. 4. BALB/c mice were “sensitized” via subcutaneous administration (weekly) of latex protein. Approximately 10 μ l PBS was injected into the left ear while an equal volume of PBS containing 10 μ g latex protein was injected into the right ear. Immediately following ear injections, 250 μ l of 0.5% Evans Blue was injected intravenously (tail vein). Dye extravasation into the ear challenged with latex protein occurred within 10 min.

2.4. Applications

Murine models have been used to investigate the sensitization potential of latex products manufactured using novel methods for reducing the allergenicity (unpublished proprietary data). These models may be used to determine the antigenicity of the products by exposing animals to product extracts and evaluating their antibody response. These models may also be useful in testing the efficacy of barrier creams. The potential for cross-reactivity between antibodies generated to latex proteins and the allergens present in products manufactured using novel techniques may be evaluated by cross-testing using Western blot analysis. Additionally, animals can be sensitized to extracts of one product and tested for ACA or increased airway hyperreactivity following exposure to the other.

Immunoblot analysis has been used to investigate the development of antibody responses to individual latex proteins. Preliminary studies by Woolhiser et al. [19] demonstrated that the route of exposure differentially influenced the latex proteins that induced antibody production. Murine models may also be used to investigate the relative importance of specific proteins in the elicitation of cutaneous, systemic, and pulmonary responses. Given the limited availability of individual proteins, animals can be sensitized to latex extracts and the potential for individual proteins to elicit responses can be determined following differing routes of administration.

Immunotherapy strategies are being developed based on the alteration of IgE-binding epitopes of individual proteins [21]. Murine models provide a mechanism for evaluating the effectiveness of these strategies prior to human trials.

Murine models have been used to investigate the role of coexposure to chemicals/agents in the health care environment on the development of latex allergy. Slater et al. [22] demonstrated an increase in Hev b 5-specific IgE when mice were coexposed to endotoxin and recombinant Hev b 5. Studies in our laboratory have demonstrated a suppression of latex-specific IgE when animals were coexposed to NAL and endotoxin [23]. The difference in these responses may be related to the complex mixture of antigens in NAL compared with the single protein Hev b 5. Numerous low-molecular-weight chemicals are used in the manufacture of latex products, and these are often irritants and/or sensitizers. Little is known of the effect of coexposure to these chemicals with latex proteins. Animal models provide a mechanism for the evaluation of these relationships with the possibility of developing intervention strategies for the reduction of latex allergy.

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