

Gastro-Intestinal Exposure to Latex Antigens Induce Allergic Responses in Mice

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Key Words

Natural rubber latex allergy • Intragastric sensitization •
Ingested allergens • Murine model

Abstract

Background: Natural rubber latex (NRL) has emerged as a major cause of respiratory allergy among specific exposed groups of individuals. Since latex allergens are dispersed in the environment it is conceivable that latex proteins are both inhaled and ingested. The mechanism of latex allergy and the immune responses following reexposure of latex allergens by the intranasal route was studied in a murine model of latex allergy developed by intragastric sensitization with NRL. **Methods:** BALB/c mice were sensitized intragastrically ('ig'), intranasally ('in') or 'ig' followed by 'in' challenge with NRL allergens. The cellular and humoral immune responses, lung function and histological changes were determined. **Results:** Peripheral blood eosinophilia was observed in the 'ig' and 'ig/in'-NRL-sensitized animals in comparison to normal controls ($p < 0.05$). The 'ig' group showed a marked increase over control mice in serum total IgE, NRL-specific IgG and IgG subclasses ($p < 0.05$). Increased levels of IL-4, IL-5, IL-10, and IL-13 were detected in 'ig'-NRL-sensitized mice. Intranasal exposure with NRL after 'ig' sensitization further enhanced the cytokine levels. A tendency towards enhanced stimulation was determined in 'ig'-sensitized mice; a signifi-

cant difference was shown in the 'ig/in'-group ($p < 0.05$). Increased airway hyperreactivity was found in 'ig'-NRL-sensitized-mice (15.1 ± 2.5 vs. 8.9 ± 1.7 cm $H_2O \cdot ml^{-1} \cdot s$, $p < 0.05$). Mucus secretion from jejunal epithelium and eosinophilic infiltration into the jejunal lamina propria were observed in the 'ig'-NRL-sensitized-mice. **Conclusions:** The results demonstrate that intragastric NRL sensitization did not induce specific tolerance, and additional intranasal exposure with latex allergens resulted in systemic allergic manifestations in the murine model.

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Introduction

Natural rubber latex (NRL) allergy is an IgE-mast cell-mediated type I respiratory hypersensitivity, recognized in recent years in individuals exposed to latex allergens [1–4]. The hypersensitivity responses induced by NRL allergens include rhinitis, bronchospasm, angioedema, urticaria, anaphylaxis and death [5, 6]. While the clinical symptoms are recognized, the immunopathogenesis of latex allergic syndrome is not well understood. Latex allergy is manifested by an immediate hypersensitivity reaction occurring in sensitized patients on subsequent exposure to NRL allergens. IgE bound to the surface of mast cells interacts with natural latex resulting in cross linking

Table 1. Sensitization protocol

	Group 1 'ig/in' PBS	Group 2 'ig/in' NRL	Group 3 'ig' NRL	Group 4 'in' NRL
Weeks 1–4, once/week	'ig' PBS	'ig' NRL	'ig' NRL	'in' NRL
Weeks 5–11, twice/week	'in' PBS	'in' NRL	'ig' NRL	'in' NRL
Last challenge, week 12	'in' & 'ip' PBS	'in' & 'ip' NRL	'ig' & 'ip' NRL	'in' & 'ip' NRL

Group 1 was treated 'ig' and 'in' with PBS (negative control). Group 2 was sensitized 'ig' and challenged 'in' with NRL. Group 3 was sensitized and challenged 'ig' with NRL. A well-described 'in' model of latex allergy was included as positive control (group 4). No adjuvant was used. A final challenge 'ig/ip' or 'in/ip' was given 24 h before euthanasia. Dose: 'ig': 200 µg NRL in 200 µl PBS/mouse; 'in': 50 µg NRL in 30 µl PBS/mouse; 'ip': 100 µg NRL in 200 µl PBS/mouse. Each group consisted of 5–10 BALB/c mice.

of IgE that leads to mediator release causing allergic and anaphylactic reactions [7–11].

While it is well recognized that the sensitization usually occurs through skin contact with latex or inhalation of antigen from latex gloves and other devices containing allergens [12], it is presumed that the latex allergens present in the environment may possibly induce responses resulting from inhalation and ingestion [13, 14]. Although the immune responses of the gastrointestinal tract are frequently directed towards the exclusion of microbial pathogens, they also function in the containment of certain ingested allergenic proteins [15, 16]. It is not known how allergens, particularly latex proteins, are handled by the intestines of mice.

Several latex allergens, particularly those that cross-react with fruit allergens, have become an important factor in the syndrome that warrants a clear understanding. The 'latex-fruit' syndrome is a term used to denote the serum cross-reactivity observed between latex allergens and avocado, banana, chestnut, fig, bell pepper, kiwifruit, and other fruits and vegetables [17–19]. It is unclear whether latex sensitization precedes or follows the onset of food allergy. Because of its widespread cross-reactivity we selected latex allergens as a sensitizing allergen to investigate the role of the gastrointestinal tract in the induction of allergy or tolerance.

The murine model of latex allergy was developed either by intragastric ('ig') sensitization with NRL or by 'ig' followed by intranasal ('in') allergen exposure. Intragastrically sensitized mice developed peripheral blood and intestinal eosinophilia, increased total serum IgE, NRL-IgG and subclasses, specific proliferation of lymphocytes, cytokine production by spleen cells and airway hyperresponsiveness. Additional 'in' exposure to 'ig' NRL-sensitized mice further enhanced the allergic responses.

Materials and Methods

Animals

Six- to eight-week-old female BALB/c (H-2^d) mice purchased from Charles River were used in this study, as BALB/c mice have been found to be useful for latex allergy studies [10, 20]. Pathogen-free animals were housed in the Veterinary Medical Unit of the Department of Veterans Affairs Medical Center. The animal research protocol was approved by the Institutional Animal Care and Use Committee.

Latex Antigens

Natural rubber latex (NRL), the non-ammoniated latex extract from the rubber tree (*Hevea brasiliensis*), was received from Malaysia and treated as described previously [10]. Prohevein, 20 kDa (Hev b6), is a precursor protein of *Hevea brasiliensis* and a major latex allergen that cross-reacts with various fruit class I chitinases; Hev b6 has been cloned and expressed as described before [18, 19, 21, 22].

Sensitization Protocol of Mice with Latex Antigens

Sensitization of mice with NRL was carried out according to the protocol scheme shown in table 1. Mice were divided into four groups of 5–10 animals each; three sensitized groups and one sham-treated group (group 1) were investigated. The control group of mice received 'ig' and then 'in' sterile phosphate-buffered saline (PBS) (group 1). Mice in group 2 were sensitized 'ig' once a week for four weeks and subsequently challenged twice a week by intranasal instillation with 50 µg of NRL antigens in 30 µl of PBS for seven weeks ('ig/in'). Another group of animals was sensitized intragastrically once a week for four weeks and then twice a week for the following seven weeks with 200 µg of NRL in 200 µl of PBS ('ig', group 3), while mice in the group 4 were exposed to NRL via 'in' once a week for four weeks, and then 'in' twice a week for the next seven successive weeks. Twenty-four hours before euthanasia mice were challenged intraperitoneally ('ip') with 100 µl of PBS (group 1) or 100 µl of NRL (groups 2 to 4). For 'in' instillation and 'ig' intubations with latex antigen, animals were anesthetized with halothane (Halocarbon Lab, N.J., USA). Animals were bled before the antigen or PBS exposure and at the termination of the experiments. The immune response was monitored in mice at random by studying peripheral blood eosinophils and antibody levels.

Enumeration of Eosinophils in Peripheral Blood

Blood from the different groups of animals was obtained from the tail and eosinophil counts were obtained using a hemacytometer after staining with Eosin Y as previously described [10].

Determination of Total Serum IgE

Total serum IgE was determined by indirect ELISA using purified anti-mouse IgE monoclonal antibody (Pharmingen). A biotinylated rat anti-mouse IgE monoclonal antibody (Southern Biotechnology) was used as the detection antibody. The antibody concentration in the serum was quantified by converting the OD values of the serum samples to picograms using a standard curve as described before [23].

Determination of NRL-Specific IgG and Subclasses

Specific serum anti-NRL antibodies were determined by ELISA using goat anti-mouse IgG (Sigma) or rabbit anti-mouse IgG1, IgG2a, IgG2b or IgG3 subclass conjugated to biotin (Zymed) as described previously [22]. A cut-off titer was determined by testing 10–12 normal mice sera (mean $\pm$ 3 SD). The determination of NRL-specific antibodies was carried out using antibody titers calculated as the reciprocal of the last dilution that gave an OD₄₉₀ above the cut off [24].

Antigen-Specific T-Cell Proliferation Assay

The spleens were obtained from mice 24 h after the last allergen challenge and were used for antigen-induced lymphocyte stimulation. Single cell suspensions were prepared by homogenization in a glass tissue grinder with 10 ml RPMI 1640 medium. Erythrocytes were lysed using red blood cell lysis buffer (Sigma). The cells were counted and resuspended in complete RPMI medium (RPMI-1640 containing glutamine, sodium pyruvate, penicillin-streptomycin and 10% heat-inactivated fetal bovine serum (FBS); Invitrogen, Carlsbad, Calif., USA). 1×10^5 cells were added in triplicate to the wells of a sterile U-bottom microtiter plate containing 100 μ l of complete RPMI with 10, 5, and 2.5 μ g/ml of NRL or Hev b6. Mitogenic stimulation was studied concurrently by incubating with various concentrations of Con A (Sigma). The cultures were incubated for 7 days at 37°C in a humidified CO₂ incubator.

Lymphocyte proliferation was determined by [³H]thymidine incorporation during the final 8 h of culture by pulsing with 1 μ Ci of [³H]thymidine (Amersham Biosciences, Piscataway, N.J., USA). Cells were harvested and the radioactivity counted using a beta-scintillation counter. The stimulation index (SI) was calculated as [³H]thymidine uptake in the presence of antigen divided by [³H]thymidine uptake by unstimulated cells. Results are expressed as the mean $\pm$ SE [25, 26].

Cytokine Production in vitro by Antigen-Stimulated Spleen Cells

Cytokine (IFN- γ , IL-4, IL-5, IL-10, and IL-13) and chemokine (Eotaxin/CCL11, RANTES/CCL5) levels were determined in the supernatants of antigen-stimulated spleen cells and controls by ELISA according to the manufacturer's (Pharmingen, R&D Systems) instructions. Briefly, spleen cells were cultured at a concentration of 10×10^6 /ml for 60 h with NRL (5 μ g/ml). Cell-free supernatants were collected and assayed for the presence of cytokines. Anti-IFN- γ mAb (R4-6A2), anti-IL-4 mAb (11B11), anti-IL-5 mAb (TRFK5), anti-IL-10 mAb (JES5-2A5), anti-IL-13 mAb (38213), anti-Eotaxin, and anti-RANTES antibodies were used to coat the

plates. Culture supernatants were added to the wells and incubated for 3 h at RT. Biotinylated rat anti-mouse IFN- γ mAb (XMG1.2), IL-4 mAb (BVD6-24G2), IL-5 mAb (TRFK4) and IL-10 mAb (SXC-1) or biotinylated goat anti-mouse IL-13, Eotaxin and RANTES were used as detection antibodies. HRP-Streptavidin conjugate at a dilution of 1:2,500 (Sigma) was then added. The color was developed with OPD (ortho-phenylenediamine, Sigma) and the reaction was stopped with 4 N H₂SO₄. The color was read at 490 nm using an automatic ELISA reader (Bio-Tek Instruments, Inc., Winooski, Vt., USA). The concentration (pg/ml) of each cytokine/chemokine in the supernatant was determined by comparison with the standard curve. Antigen-specific cytokine secretion was calculated by subtracting the cytokine content of the supernatants from spleen cells incubated with RPMI alone [27, 28].

Histology Studies

Lungs and portion of small intestine were collected and fixed in 10% buffered formalin for histological examination. The tissue was processed and sections stained with hematoxylin and eosin (HE) or periodic acid Schiff (PAS) were evaluated microscopically for morphologic changes. The pulmonary changes in antigen-exposed mice were evaluated by a veterinary pathologist (J.D.H.) and compared with normal controls as previously described [27].

Airway Response Studies

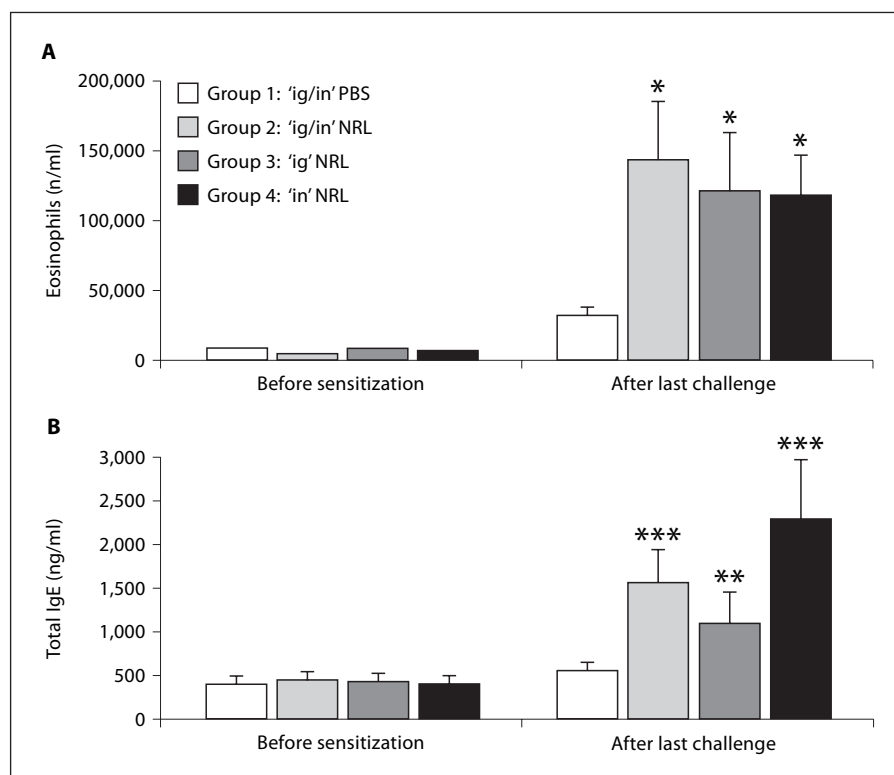
Pulmonary airway responses were studied 24 h after the last NRL challenge. Animals were anesthetized and tracheotomized and lung mechanics were evaluated using a whole-body Plexiglas plethysmograph chamber as described previously [23, 29].

Changes in pulmonary resistance (cm H₂O/ml/s) were measured in response to a nonspecific challenge with methacholine. Prechallenge (baseline) measurements were made at 30-second intervals for 3 min. Animals were then challenged intraperitoneally with cumulative concentrations of methacholine (Sigma Chemicals, St. Louis, Mo., USA) ranging from 30 to 2,000 μ g and diluted in sterile 0.9% sodium chloride (Baxter, Ill., USA). Each successive dose was injected at 3-min intervals and the cumulative doses were 30, 100, 300, 1,000 and 3,000 μ g. Data from six to ten breaths were recorded every 30 s for 3 min. The signals from the pressure transducer and pneumotachometer were processed using a Grass polygraph (Model 7, Astro-Med Inc., West Warwick, R.I., USA) recorder. The flow signal was integrated using a Grass polygraph integrator (Model 7P10) to measure corresponding changes in lung volume. Pressure, flow and volume signal outputs were digitized and stored on a computer using an analog-to-digital data acquisition system (CODAS, Dataq Instruments, Inc., Akron, Ohio, USA). The digitized data were analyzed for pulmonary resistance (RL) from six to ten breaths at each recording event. RL was calculated from pleural pressure, airflow and volume data using the method of Amdur and Mead [30].

Statistical Analysis

The antibody levels in the sera, peripheral blood eosinophils, cytokines in culture supernatants, antigen-induced lymphocyte stimulation and the methacholine challenge in the various experimental animal groups were compared by repeated-measures of unpaired Student's t test using Excel computer program. For all of the above statistical comparisons, $p < 0.05$ was considered statistically significant.

Fig. 1. Peripheral blood eosinophils and total serum IgE levels detected in NRL-treated and control mice. **A** Eosinophils in the peripheral blood before sensitization and after latex antigen challenge compared with unexposed controls before treatment (day 0), and after last challenge. * $p < 0.05$. **B** Total serum IgE responses in the sera of mice before treatment (day 0) and after last challenge (week 12). Mean and SEM values from 10 mice are presented. ** $p < 0.01$; *** $p < 0.001$.



Results

Intragastric Sensitization with Latex Allergens Induces Peripheral Blood Eosinophilia in Mice

Mice continuously treated 'ig' with NRL antigens (group 3: $12.2 \times 10^4 \pm 3.9 \times 10^4$ eosinophils/ml) showed peripheral blood eosinophilia at levels similar to animals NRL-exposed 'in' (group 4: $12 \times 10^4 \pm 2.8 \times 10^4$ eosinophils/ml). Interestingly, mice sensitized 'ig' and treated 'in' with latex allergens (group 2: $14.4 \times 10^4 \pm 4.1 \times 10^4$ eosinophils/ml) also showed peripheral blood eosinophilia. The eosinophilic infiltration in the peripheral blood detected in mice sensitized 'ig' with NRL showed significantly ($p < 0.05$) higher levels compared to the normal controls (group 1: $3 \times 10^4 \pm 0.4 \times 10^4$ eosinophils/ml) (fig. 1A).

IgE Response in Mice Sensitized with Latex Allergens

Mice receiving NRL allergens 'ig' did show statistically significant increases in total serum IgE levels (group 3: $1,118 \text{ ng/ml} \pm 340$; $p < 0.01$). Furthermore, the group of mice sensitized 'ig' and then challenged 'in' with NRL allergens demonstrated a significant enhancement in total serum IgE levels (group 2: $1,563 \text{ ng/ml} \pm 401$ vs. group

1: $559 \text{ ng/ml} \pm 169$; $p < 0.001$). The total IgE serum levels in the 'ig/in' group were comparable to those observed in intranasal NRL-exposed mice (group 4: $2,285 \text{ ng/ml} \pm 699$) (fig. 1B).

Latex-Specific IgG Subclass Antibodies in Sensitized Mice

Mice exposed to latex allergens 'ig' (group 3: $2.67 + 0.37 \log_{10}$ titer) had specific IgG antibodies at titers significantly higher than those seen in normal controls (group 4: $1.64 + 0.024 \log_{10}$ titer, $p < 0.05$); nevertheless, those values were significantly lower than those observed in mice sensitized 'ig/in' or 'in' alone ($p < 0.01$ and $p < 0.001$, respectively). High levels of latex-specific IgG antibodies were detected in the sera of mice sensitized 'ig' and challenged 'in' (group 2: $5.8 + 0.23 \log_{10}$ titer). These values were comparable to those shown in a murine latex allergic model developed by 'in' exposure of latex allergens (group 4: $6.16 + 0.2 \log_{10}$ titer) (data not shown).

Although 'ig' sensitized mice showed significantly enhanced IgG1 and IgG2b sera antibodies to latex compared to control animals ($p < 0.01$), very high titers of all IgG isotypes (IgG1, $p < 0.001$; IgG2a, $p < 0.01$; IgG2b,

Fig. 2. Specific IgG subclass antibodies detected in NRL-treated and control mice. Latex-specific IgG1, IgG2a, IgG2b and IgG3 in sensitized mice compared with control animals (asterisk alone) or compared to group 3 (joined symbols). Five to ten animals from each group were analyzed. The sera before treatment were <1.5 $\log_{10}$ titer for all groups. Data are presented as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

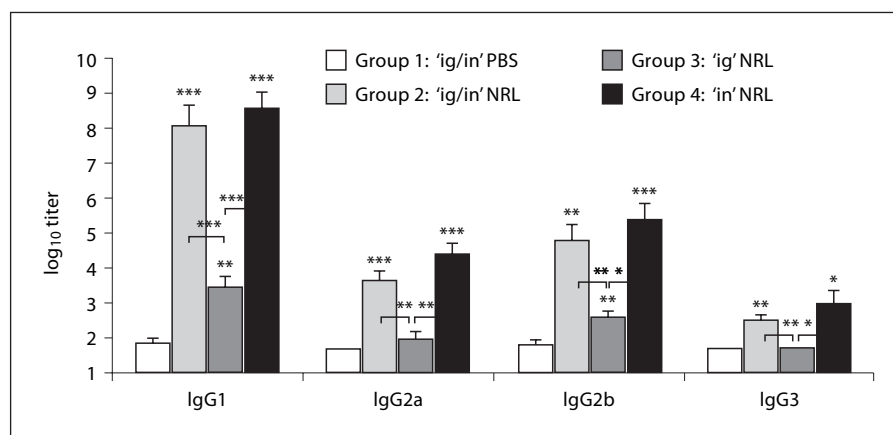
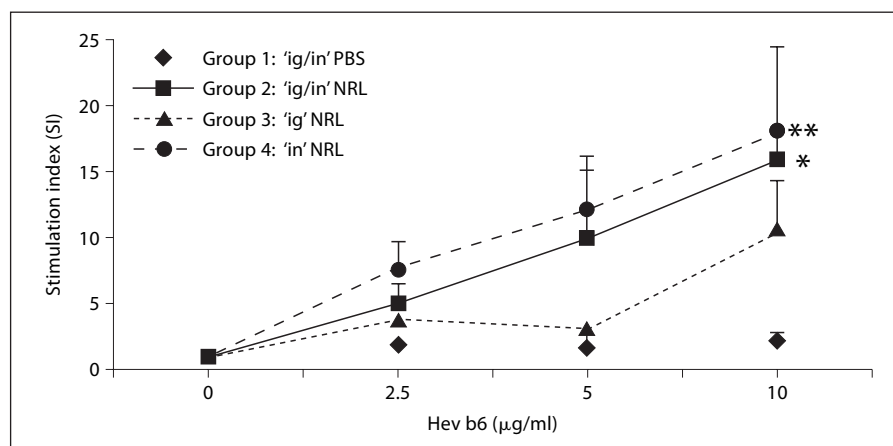


Fig. 3. Proliferative responses of spleen cells from NRL-sensitized and non-sensitized mice to the recombinant protein Hev b6. Stimulation index (SI) values were shown as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$. Five to ten animals from each group were studied.



$p < 0.01$, and IgG3, $p < 0.01$ vs. normal mice) were demonstrated in 'ig/in' NRL-sensitized group of mice (group 2). The levels observed in the latter group were similar to those determined in the 'in' exposed group (group 4) (fig. 2).

Antigen-Induced Lymphocyte Stimulation in Sensitized Mice

To characterize T-cell responses to NRL antigens, the spleen cells from sensitized mice or normal controls were cultured in vitro with NRL or Hev b6. NRL allergens were unable to induce specific proliferation, possibly because of toxic effects on the cells. Spleen cells from 'ig' sensitized mice showed a trend towards an enhancement in the stimulation index (SI) (group 3: 10.7 ± 3.79), but the values were not statistically significant compared to normal animals (group 1: 2.3 ± 0.56). The spleen cells from mice exposed via 'ig/in' (group 2: 15.98 ± 8.6) demonstrated specific-Hev b6 proliferation of lymphocytes at similar levels to those observed in mice sensitized with

latex allergens 'in' (group 4: 18.08 ± 6.41). The enhanced SI seen in both groups were significantly higher than that observed in the normal mice ($p < 0.05$ and $p < 0.01$, respectively) (fig. 3).

Cytokine Expression in Response to Latex Sensitization

The allergen-induced cytokine expression by spleen cells was evaluated in the various groups of animals. Significant levels of IFN-gamma were detected in the supernatant of in vitro NRL-stimulated spleen cells in all groups of sensitized animals, regardless of their mode of sensitization compared to normal controls. A tendency toward higher levels of other cytokines, particularly IL-4, IL-5, IL-10 and IL-13, were noted in the NRL-sensitized mice compared to normal control animals (table 2). Chemokines such as eotaxin (CCL11) and RANTES (CCL5), that are considered to play important roles in the process of the maturation, migration and activation of eosinophils [22–23], were also determined and

Table 2. Cytokine and chemokine production (pg/ml) by spleen cells from NRL-sensitized and control animals in response to NRL allergens

	Group 1 'ig/in' PBS	Group 2 'ig/in' NRL	Group 3 'ig' NRL	Group 4 'in' NRL
INF- γ	302 $\pm$ 237	7,501 $\pm$ 2,193*	4,792 $\pm$ 254*	3,657 $\pm$ 1,339**
IL-4	0 $\pm$ 0	17 $\pm$ 15	14 $\pm$ 3**	31 $\pm$ 16**
IL-13	29 $\pm$ 29	138 $\pm$ 36*	63 $\pm$ 12	179 $\pm$ 37**
IL-5	22 $\pm$ 22	142 $\pm$ 87	68 $\pm$ 52	139 $\pm$ 34*
IL-10	43 $\pm$ 43	286 $\pm$ 28**	244 $\pm$ 62*	453 $\pm$ 80**
RANTES	1,120 $\pm$ 1,048	14,316 $\pm$ 6,226	3,115 $\pm$ 1,690	19,092 $\pm$ 4,213**

IFN- γ , IL-4, IL-13, IL-5, IL-10 and the chemokine RANTES were quantified by ELISA. Antigen-induced cytokine/chemokine expression was quantified after comparison with reference standards of mouse cytokines/chemokines. The values presented were subtracted from the spontaneous release of unstimulated spleen cells. Data represent means in pg/ml $\pm$ SEM of each group.

* $p < 0.05$; ** $p < 0.01$ versus normal control mice.

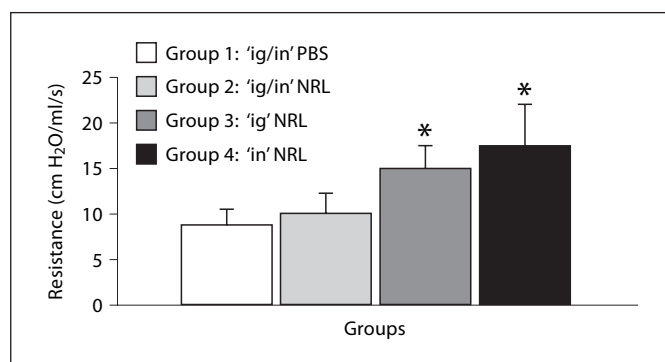


Fig. 4. Airway responses to a non-specific stimulation with cumulative doses of up to three mg of methacholine in mice (7–9 per group) sensitized with latex allergens. Data are presented as mean $\pm$ SEM of pulmonary resistance (cm H₂O $\cdot$ ml⁻¹ $\cdot$ s). Asterisks indicate significant differences (* $p < 0.05$) from non-sensitized controls.

compared between various groups. Eotaxin was under detectable levels, while a trend toward higher levels of RANTES was detected in supernatants of the stimulated spleen cells from mice sensitized with NRL allergens (table 2).

Airway Hyperreactivity

Airway response to latex allergens was studied in NRL-sensitized animals and controls. Methacholine challenge resulted in a significant increase in mice sensitized 'in' with latex (group 4: 17.46 $\pm$ 4.78 cm H₂O $\cdot$

ml⁻¹ $\cdot$ s) compared to normal mice (group 1: 8.88 $\pm$ 1.68 cm H₂O $\cdot$ ml⁻¹ $\cdot$ s, $p < 0.05$), as previously described [26]. Interestingly, a similar augmented response was observed in mice sensitized and challenged 'ig' with NRL. Airway resistance in the 'ig' sensitized group (group 3) was 15.11 $\pm$ 2.49 cm H₂O $\cdot$ ml⁻¹ $\cdot$ s, significantly increased compared to normal mice ($p < 0.05$). Mice exposed 'ig' and challenged 'in' with latex allergens did not show significant enhancement in airway resistance (group 2: 10.0 $\pm$ 2.33 cm H₂O $\cdot$ ml⁻¹ $\cdot$ s) (fig. 4).

Histological Studies of the Lung and Small Intestine

The histology of small intestine (jejunum) sections from mice sensitized 'ig' with latex allergens (group 3) showed many mononuclear, PMN and eosinophils (11–30/hpf), in the lamina propria with numerous (>30/hpf) mucus-containing goblet cells in the villi and Paneth cells in the crypt area. Abundant numbers of eosinophilic granules were located in the Paneth cells (fig. 5A, B, E, F; table 3A). Fewer morphologic changes were observed in the jejunum of 'ig/in' NRL-sensitized mice (table 3A). No significant changes were observed in either HE or PAS stains in jejunal sections from PBS control animals (group 1, fig. 5C, D, G, H). No significant changes were observed in the NRL 'in' group 4 (table 3A).

The lungs from mice sensitized 'in' with NRL (group 4) showed moderate peribronchial and perivascular chronic inflammatory responses characterized by mononuclear, PMN and eosinophilic infiltration, epithelial hyperplasia, and few (4–10/hpf) goblet cells (fig. 6D; table 3B) [10, 23]. Similar histological features were ob-

Fig. 5. Inflammatory changes in the jejunum of NRL-sensitized mice. **A** Moderate eosinophilic enteritis in jejunum from 'ig' NRL-sensitized mouse (group 3) (40 $\times$; HE). **B** Higher magnification of the jejunum section showed abundant eosinophilic granules located in Paneth cells in the mucosal crypt area. Many eosinophils were scattered throughout the lamina propria (400 $\times$; HE). Normal jejunal morphology was observed in PBS-treated control mice (group 1) at 40 $\times$ (**C**) and 400 $\times$ (**D**) magnification (HE). **E–H** Numerous PAS-positive goblet cells were observed along villi of NRL-sensitized mice (**E**: 40 $\times$; **F**: 400 $\times$), compared to PBS-negative controls (**G**: 40 $\times$; **H**: 400 $\times$) (PAS).

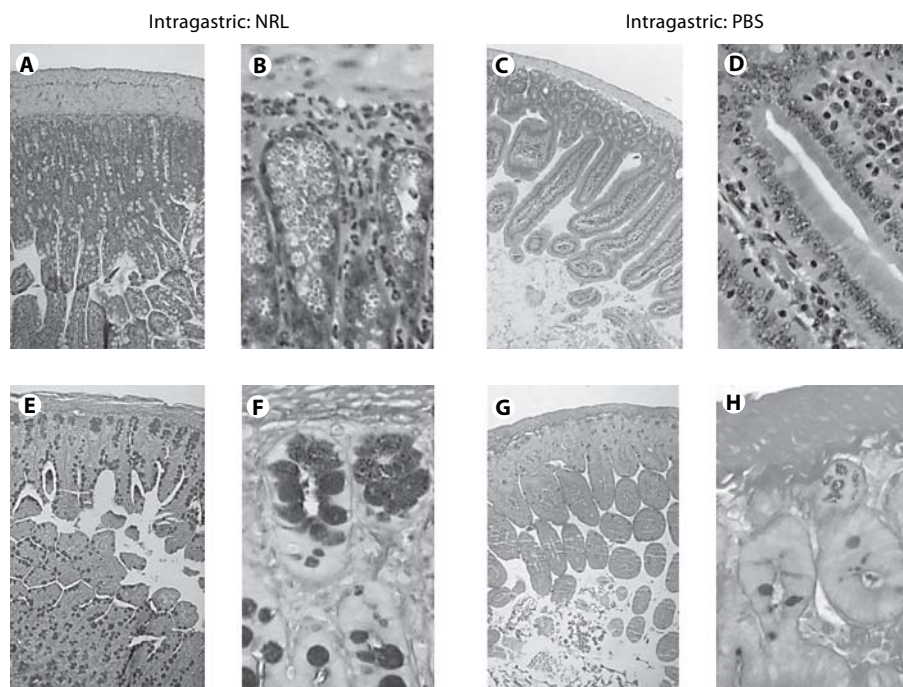
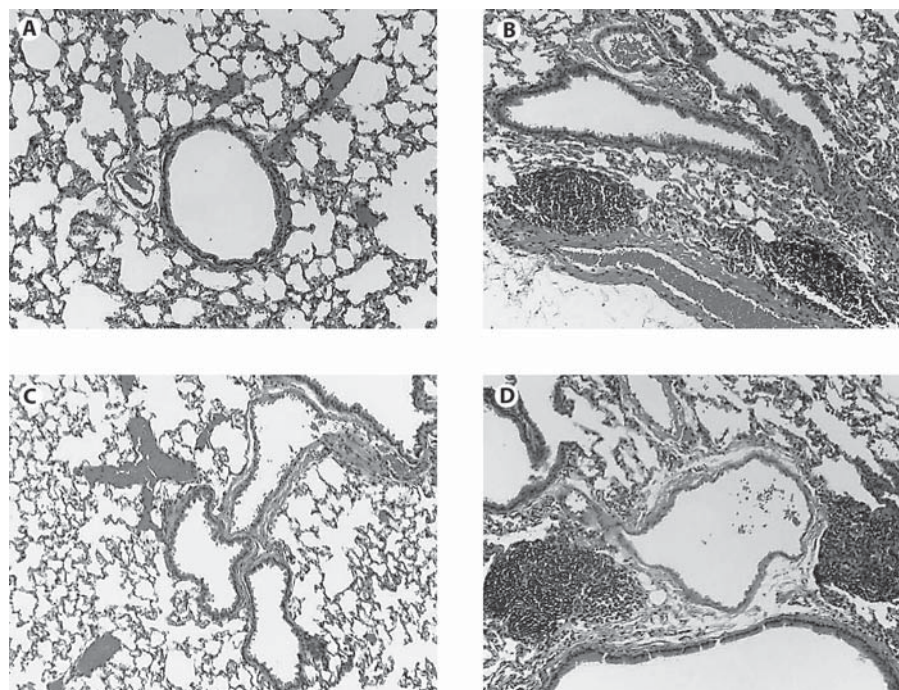


Fig. 6. Inflammatory changes in the lungs of NRL-sensitized mice. **A** Normal mouse lung architecture (40 $\times$; HE). **B** Lung tissue from an 'ig/in' NRL-sensitized mouse showing perivascular infiltration of inflammatory cells similar to those observed in 'in' NRL-treated group (**D**). **C** Lesser extension of inflammation was observed when mice were exposed to NRL via 'ig'.



served in group 2 ('ig/in') (fig. 6B; table 3B). The PBS controls (group 1) exhibited normal lung morphology (fig. 6A). Lung sections of NRL 'ig' sensitized mice (group 3) showed minor inflammatory infiltration (fig. 6C; table 3B).

Discussion

In latex allergy, natural sensitization through various routes of exposures has been reported. They include contact through the skin, mucosal surfaces and wounds, or

Table 3. Histological examinations of the intestine and lungs of NRL treated and control animals

A Jejunal sections stained with HE and PAS were examined for histological changes.

	Group 1 'ig/in' PBS	Group 2 'ig/in' NRL	Group 3 'ig' NRL	Group 4 'in' NRL
<i>Small intestine/jejunum</i>				
Lamina propria eosinophils	0	1	3	0
Lamina propria mono and PMNs	0	2	3	0
Eosinophilic granules in Paneth cells	0	1	3	0
Jejunal epithelium goblet cells	0	1	2	0
Inflammation severity	0	1	2	0

B Sections of the lung from mice exposed to NRL.

	Group 1 'ig/in' PBS	Group 2 'ig/in' NRL	Group 3 'ig' NRL	Group 4 'in' NRL
<i>Lungs</i>				
Perivascular/bronchial inflammation	0	3	1	3
Small bronchiole cells	0	1	0	2
Small bronchiole hyperplasia	0	2	0	1
<i>Blood vessels</i>				
Perivascular edema	0	1	0	0
Perivascular cuffing	0	3	0	3
Eosinophils	0	0	1	1
Perivascular mono and PMNs	0	4	1	4
Inflammation severity	0	2	1	2

Perivascular and peribronchial/jejunal eosinophilic infiltration grades: 0 = none, 1 = occasional cells (1–3/hpf), 2 = few cells (4–10/hpf); 3 = many cells (11–30/hpf); 4 = numerous (>30/hpf). Inflammation severity grades: 0 = no perivascular or peribronchial inflammation; 1 = 1–2 foci of inflammation; 2 = dense inflammation in central portion of lung lobe extending into the middle third of the parenchyma, 3 = extension of inflammation from the center to periphery of lobe approaching the visceral pleura. Other morphological change scores: 0 = no change, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe.

inhalation of airborne allergens released from latex products, or a combination [31–34]. While the clinical findings and exposure to latex leading to the induction of latex allergy in individuals exist, no information is available on sensitization and the events leading to the onset of the disease. The primary exposure route(s) which lead to latex sensitization still remain to be defined [28, 35].

Murine models have been established to investigate latex exposure and the immunopathogenesis of latex allergy. The experimental models of latex allergy showed a strong immunologic response when animals were exposed via intraperitoneal, subcutaneous, topical, intranasal or intratracheal routes [10, 28]. Since NRL allergens can be detected in the environments where latex devices are used or processed, there is substantial evidence that airborne latex proteins present in the environment are

also inhaled and ingested [12, 13, 36]. The present study reports a novel approach for the induction of allergy in mice through intragastric exposure without the use of any adjuvant and the evaluation of immunological, inflammatory and airway responses in the exposed mice.

Proteins and antigens ingested via the oral or intragastric route have been reported to induce tolerance in mice [37–40]. The results reported here show that exposure of NRL antigens 'ig' did not induce oral tolerance or render any protection from a later challenge with the same antigen. In fact, gastro-intestinal sensitization induced peripheral blood eosinophilia, total serum IgE, antigen-specific IgG1, airway resistance and local inflammatory response. Repa et al. have shown evidence that the route of sensitization may influence systemic and local immune responses when the major birch pollen allergen Bet

v1 is used to sensitize mice [41]. In addition, diverse responses have been observed when mice were sensitized with different allergens or allergen preparations. For example, the recombinant latex proteins rHev b1 or rHev b3 induced a strong immune response when mice were immunized intraperitoneally, but intranasal treatment prior to 'ip' sensitization led to a reduction in allergen-specific IgG1/IgE levels, significant suppression of allergen-induced basophil degranulation, lymphocyte proliferation and cytokine (IL-4, IL-5, IFN- γ) production [42]. Our findings suggest that the first encounter with the NRL allergens through the 'ig' route contributes towards specific progressive immunological, inflammatory and airway responses in mice.

Studies have reported that mice sensitized to latex protein by different routes of exposure exhibited bronchoconstriction [10, 28]. Our results showed that mice sensitized via the intragastric route with NRL developed increased airway hyperreactivity. Differences in pulmonary function response following sensitization and challenge in our study may suggest that exposure routes leading to sensitization may play a role in determining the immune, inflammatory and airway responses.

The primary exposure to food allergens is via the oral route, even though allergic manifestations can occur from direct inhalation of aerosolized particles containing allergenic foods and induce local and various generalized reactions [43, 44]. Murine models of food allergy have been very useful in understanding the immunopathogenesis and progression of the disease, in the development of methods for the evaluation of the allergenic potential of novel proteins, and in the development of new therapeutic strategies [45–47]. In the present study, after administration of the NRL allergens 'ig', mice were exposed with the same allergen by the intranasal route (group 2: 'ig/in'). These animals demonstrated systemic immunological responses including increase of peripheral blood eosinophils, elevated levels of serum total IgE, specific IgG and subclasses, specific antigen-induced stimulation of spleen cells and enhanced cytokine levels detected in the spleen cell cultures. Although the 'ig/in' NRL-treated mice showed lung inflammatory responses similar to those observed in mice exposed 'in' to NRL, they failed to demonstrate airway hyperreactivity; this disparity is currently unexplainable. The results suggest that airborne NRL allergens ingested by atopic or highly exposed individuals could lead to latex sensitization, and subsequent encounter with the allergen might evoke generalized allergic responses in sensitized individuals.

Allergic sensitizations to ingested food proteins that cross-react with airborne allergens constitute a major factor for respiratory tract allergies. Exposure to airborne food allergens reported to induce chronic asthma and other respiratory reactions includes cereal grain dust, fish particles, peanut dust, and raw green beans [44, 45]. In some cases, by clinical history, the latex allergy preceded the food allergy, whereas the converse was true for other patients [48, 49]. The homology between latex and fruit-legume proteins also makes it possible that the food-sensitized individuals might have reactions to airborne latex allergens that may trigger generalized allergic reactions [17–19, 48, 49].

In summary, intragastric administration of latex allergens without adjuvant failed to induce tolerance, and subsequent exposure via the intranasal route resulted in the development of generalized latex allergic manifestations in mice. The animal model of intragastric NRL sensitization described would be useful to gain a better understanding of the immunopathogenesis and immunoregulatory mechanisms underlying latex allergy and fruit-latex syndrome.

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