

Immune Response and Airway Reactivity in Wild and IL-4 Knockout Mice Exposed to Latex Allergens

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Key Words

Latex allergy · Animal model · IL-4 knockout · BALB/c mice · Airway hyperreactivity · IgE response · Eosinophils · Lung inflammation

Abstract

Background: Natural rubber latex has been reported as a major cause of allergy and asthma in a number of individuals. One of the occupational groups most affected by latex allergy are the health care workers who are frequently exposed to natural rubber latex products in their patient care activities. The immunopathogenesis of latex allergy is not well understood. In order to understand the immune mechanism in latex allergy, we have developed a mouse model of latex allergy. **Methods:** Both wild-type and IL-4 knockout BALB/c mice were challenged intranasally with latex proteins and their immune responses, lung pathology, and airway reactivity were evaluated. **Results:** The total serum IgE and latex specific IgE, IgG1, and peripheral blood and lung eosinophil levels in wild type BALB/c mice were enhanced by the latex exposure, while no IgE or eosinophil were detected in IL-4 knockout mice. Latex-specific IgG1 levels in the sera were lower in IL-4 knockout animals compared to wild mice. However, latex-spe-

cific IgG2a antibody was higher in all the IL-4 knockout mice compared to wild type mice. Both the wild type and IL-4 knockout animals developed increased airway resistance after antigen challenge when compared to control animals, although the airway resistance response of IL-4 knockout animals was attenuated compared to the wild-type animals. The histology of the lungs of these two groups of animals was similar. **Conclusion:** In spite of the differences in the immune responses in the two groups of mice, there were comparable lung inflammatory responses, suggesting a multifactorial pathogenetic mechanism.

Introduction

Latex-induced IgE-mediated allergy has been reported in increasing numbers of patients during the past several years, largely as a result of increased exposure to natural rubber latex proteins present in various health care devices [1–5]. The clinical spectrum of the disease ranges from local contact urticaria, allergic rhinoconjunctivitis, bronchospasm, angioedema, asthma, and life-threatening anaphylaxis [3–9]. Latex allergy has also become a serious risk among several groups of individuals who frequently use la-

tex products or are involved in latex manufacturing [3, 7, 10, 11]. Health care workers have shown a high prevalence of latex allergy attributed to frequent exposure to latex proteins from gloves and other health care products [3, 4, 12]. Among other groups of patients with substantial risk of latex allergy are those with spina bifida and those undergoing multiple surgeries, probably due to the frequent exposure to latex proteins [3, 8, 9, 13].

Although the clinical features of latex allergy are well established, our understanding of the immunopathogenesis is not clear [9]. An understanding of the immune mechanisms and airway responses to latex proteins may help to develop more reliable diagnostic criteria and to devise measures for better patient care as well as control of the disease. Hence, animal model studies may yield needed information that will enhance our understanding of the disease. Previously, we have used both rabbits and mice to develop a model of latex allergy [14, 15]. Rabbits exposed to latex proteins developed latex-specific serum IgG antibody and showed immediate skin test reactivity to latex antigen. Both lung sections and bronchial lavage fluids showed eosinophil accumulation [15]. The mouse model demonstrated elevated serum IgE and lung and peripheral blood eosinophilia and airway hyperreactivity [14].

In the present study, we have investigated the role of interleukin-4 (IL-4) in eliciting immunological and airway responses in mice exposed to latex allergens. The results indicate antigen-specific airway obstruction in both wild type (WT) and IL-4 knockout (KO) animals exposed to latex allergens, although the airway response was more pronounced in WT compared to the IL-4 KO mice.

Materials and Methods

Animals

Ten- to 12-week-old BALB/c IL-4 $\pm$ (WT) mice were purchased from Charles River Laboratories, Wilmington, Mass., USA. IL-4 gene disrupted (IL-4 KO) BALB/c mice were a gift from Dr. Robert Coffman, DNAX Research Institute of Molecular and Cellular Biology, Inc., Palo Alto, Calif., USA, as breeding pairs. These animals were bred at the Veterinary Medical Unit of the V.A. Medical Center, Milwaukee. Age- and sex-matched animals were exposed intranasally to latex allergens as described below.

Latex Antigen

Antigen used in the present study was obtained from the milky latex fluid of the rubber tree *Hevea brasiliensis*. The latex fluid was collected from Malaysian rubber trees by tapping them directly into sterile containers, frozen immediately, and then stored at -70°C . It was a gift from Ansell International, Melbourne, Australia. The frozen samples were transported to the laboratory on dry ice. The soluble proteins ('C' serum) were collected by thawing the frozen latex at 4°C ,

followed by centrifugation at 3,000 rpm for 30 min. The liquid phase, which contains the majority of the proteins, was collected, dialyzed, and filtered through a $0.2\text{-}\mu\text{m}$ nitrocellulose filter [14, 16, 17]. This latex extract was then aliquoted and stored at -70°C . The antigen was analyzed for protein content by the Biscinchoninic acid method according to the protocol recommended by the manufacturer (Pierce Chemicals, Rockford, Ill., USA). The protein profile was studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the allergen profile by Western blot analysis using sera from latex-allergic patients [18, 19]. The stained gel and Western blot patterns were compared with in house standard antigen and antibody [18, 19].

Sensitization of Mice with Latex Antigen

Five to 15 BALB/c WT and IL-4 KO female mice were used for each experiment. Animals were lightly anesthetized with metofane (methoxyflurane, Pitman-Moore, Mundelein, Ill., USA) aerosol as described previously [14, 20]. About $50\text{ }\mu\text{l}$ of latex extract containing $30\text{ }\mu\text{g}$ of latex proteins was introduced into the nostrils using a sterile pipettor tip. The animals were restrained in an upright position for a minute for smooth inhalation of the antigen. The treatment was carried out twice a week for 4 weeks. All animals were bled before the experiment and again bled one week after the last intranasal exposure of latex. The antibody to latex was measured by ELISA [13, 14] when the antibody levels showed 5- to 10-fold increases compared to preimmunization levels; the animals were challenged with a final dose of latex. These mice were sacrificed on the 5th day after the last challenge. The serum IgE latex-specific IgE, IgG1, IgG2a, IgG2b, IgG3, and lung histology were studied. The airway response was studied using randomly selected mice from the different groups of control and latex-challenged animals.

Total Serum IgE

The total serum IgE was measured using a rat anti-mouse IgE monoclonal antibody (Pharmingen, San Diego, Calif., USA) as described previously [14, 20]. The IgE concentration in the serum was expressed as nanograms/milliliter after comparing the optical density (OD) of mouse IgE standards.

Latex-Specific IgE and IgG Subclasses in the Sera of Mice

The latex specific IgE, IgG1, IgG2a, IgG2b and IgG3 were detected according to the method previously described with slight modifications [14, 20, 21]. Briefly, the method is as follows: the latex extract was diluted to $5\text{ }\mu\text{g}$ protein/ml in PBS (pH 7.4) and used to coat microtiter plates (Immulon II, Fisher Scientific, Itasca, Ill., USA). Serum dilutions from mice were made in PBS and added to the wells and incubated at room temperature for 3 h. After washing the wells, biotinylated anti-mouse IgE (Southern Biotechnology Associates, Inc., Birmingham, Ala., USA) IgG1, IgG2a, IgG2b and IgG3 (Zymed Laboratories, San Francisco, Calif., USA) were added and the plates incubated for 1 h. Again, the wells were washed and peroxidase-coupled streptavidin (Sigma-Aldrich, Inc., St. Louis, Mo., USA) was added. After incubation and washing, the color was developed by adding the substrate. The OD was measured using an ELISA reader using a 490-nm filter. The OD values of preimmunization and sacrifice sera were compared for latex-specific antibodies.

Eosinophils in the Peripheral Blood

Animals exposed to latex antigen were bled from the tail vein and the eosinophils were enumerated in a hemocytometer after staining with Discombe's stain [20, 22]. The mean values from at least 5 animals were collected and compared.

Lung Histology

Lungs from all the experimental animals were inflated with 1–2 ml of 10% (v/v) buffered formalin. The inflated lungs were then fixed in the same solution and processed. All sections were stained with hematoxylin and eosin and were examined microscopically. The histological changes were recorded with regard to the degree of inflammation and types of cells in the lesions as described before [14, 20, 21].

Airway Response Studies

After assessing the antibody and eosinophil responses, the animals were challenged intranasally with the final dose of latex, and the measurements of pulmonary compliance and airway resistance were determined. The mice were anesthetized, tracheotomized using an 18-gauge thin-walled metal tracheostomy tube, and placed in a Plexiglas body plethysmograph chamber. A saline-filled PE 50 tube with side holes was placed in the esophagus and connected to a pressure transducer for measurement of pleural pressure. The tracheostomy cannula was connected to a 1-cm length of the 0.96 mm ID tubing through the wall of the plethysmograph, allowing the animal to spontaneously breathe room air. The esophageal catheter was connected to a pressure transducer by means of another port through the plethysmograph wall. A screen pneumotachometer connected through the wall of the plethysmograph and a Validyne (Validyne Engineering, Northridge, Calif., USA) differential pressure transducer were used to measure flow in and out of the plethysmograph. The output signal from the pneumotachometer responded in linear fashion over the range of flows tested (0–7 ml/s). The frequency response of the plethysmograph-pneumotachometer system was such that amplitude decreased by less than 10% up to a frequency of 12 Hz. The breathing frequency in the mice studied did not exceed 3.5 breaths per second.

The signals from the pressure transducer and pneumotachometer were processed using a Grass polygraph (Model 7) (Astro-Med Inc., West Warwick, RI, 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 computer using an analog-to-digital data acquisition system (CODAS, Dataq Instruments, Inc., Akron, Ohio, USA).

The digitized data were analyzed for dynamic pulmonary compliance (C_{dyn}), pulmonary resistance (R_L), tidal volume (V_T), respiratory frequency (f), and minute ventilation (V_E) from about 6–10 consecutive breaths at each recording event. C_{dyn} and R_L were calculated from pleural pressure, airflow, and volume data using the method of Amdur and Mead [23].

Prechallenge (baseline) measurements were made at 30-second intervals for 4 min. Animals were then challenged with normal saline (0.2 ml) injected into the cavernous sinus by direct puncture. Measurements were again taken every 30 s for another 4 min, and then 250 μ g of latex antigen in 0.2 ml saline was injected as before. After 30 s, measurements were made at 30-second intervals for 4 more min.

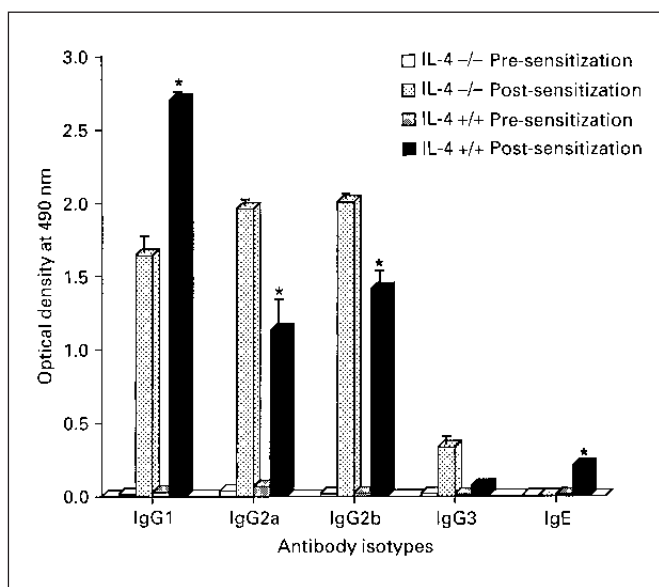


Fig. 1. Latex specific IgG1, IgG2a, IgG2b, IgG3, and IgE in the pre- and post-sera of latex challenged mice determined by ELISA. Mean optical density values (mean $\pm$ SE) from five mice are presented. Asterisk (*) indicates $p < 0.01$.

Statistical Analysis

The mean serum antibody levels, total serum IgE, and eosinophil numbers from different groups of mice were compared using unpaired two-tailed nonparametric analysis. For the airway hyperreactivity comparison in latex-sensitized mice, the changes in R_L and C_{dyn} after latex antigen challenge were analyzed by the repeated-measures analysis of variance with Tukey Kramer Multiple Comparison (SigmaStat, SPSS Science, Chicago, Ill., USA). For the comparisons, a $p < 0.05$ was considered statistically significant.

Results

Serum Antibodies

Total serum IgE in WT BALB/c mice was markedly enhanced following intranasal administration of latex antigens (592 ± 168 preimmunization to $6,620 \pm 1,410$ ng/ml of serum at the termination of the experiment). Total IgE and latex-specific IgE were not detectable in the sera of IL-4 KO mice before or after exposure to the antigen. In contrast, all latex-primed WT mice showed detectable amounts of latex-specific IgE in their sera (fig. 1).

Higher levels of latex-specific IgG1 antibody isotype in the sera of mice sensitized intranasally with latex antigens was detected in both WT and IL-4 KO mice (fig. 1). However, this increase was considerably higher in WT mice compared to KO mice, whereas all the IL-4 KO animals im-

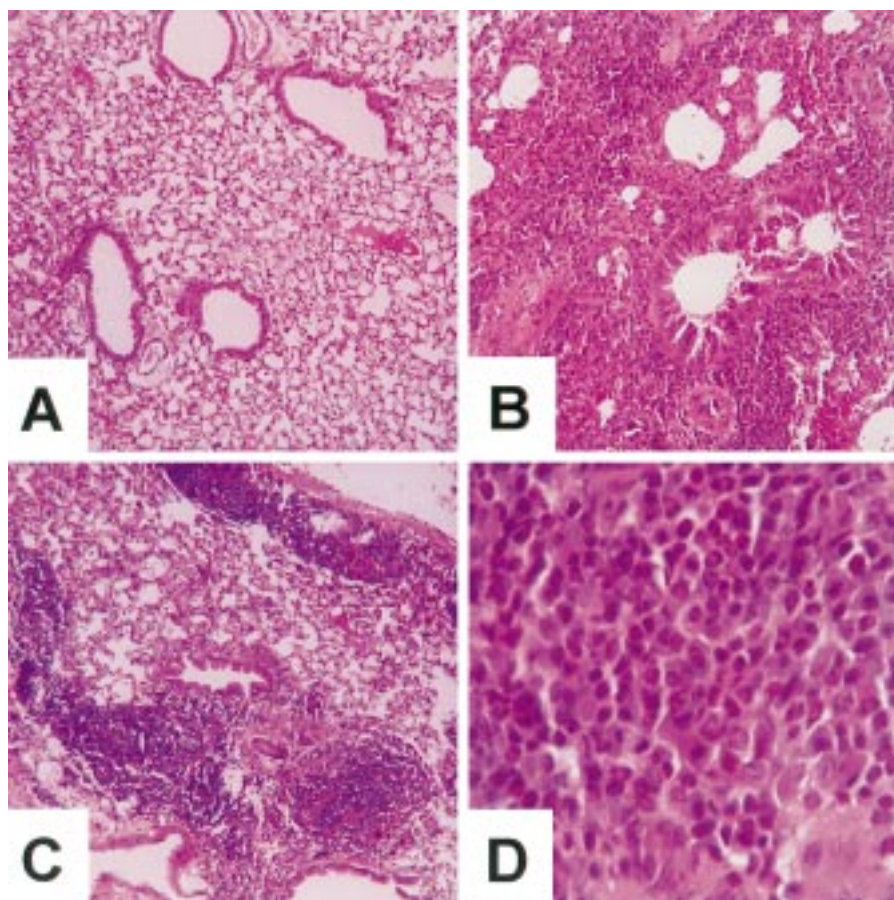


Fig. 2. Histology of the lungs of mice. **A** Lung of a mouse from the normal control group showing normal structure. HE. $\times 63$. **B** Lung of latex-sensitized wild-type (WT) mice showing moderate peribronchiolar and perivascular chronic inflammation. The bronchiolar epithelium is hyperplastic. HE. $\times 40$. **C** IL-4 $-/-$ mice (KO) sensitized with latex showing moderate chronic inflammatory infiltrates, adjacent to bronchioles and blood vessels with no eosinophils. HE. $\times 40$. **D** Higher magnification of B showing many eosinophils, but with similar peribronchiolar or perivascular inflammatory infiltrates as in KO mice. HE. $\times 160$.

munized intranasally with latex proteins showed elevated specific IgG2a antibody. The latex-specific IgG2b antibody also showed an increase compared to WT mice. IgG3 antibody showed no difference among the groups.

Eosinophils

The eosinophil infiltration in the peripheral blood was detected in both groups of mice exposed to latex antigens. IL-4 KO mice showed lesser increase compared to wild animals exposed to latex allergens ($8 \pm 2SE$ to $13 \pm 2SE \times 10^4$ /ml of blood). The WT BALB/c mice showed a 5-fold increase ($9 \pm 2SE$ to $46 \pm 6SE \times 10^4$ /ml blood) eosinophils from the base lines while the increase was under 2-fold in IL-4 KO mice.

Pulmonary Histology

Normal mice showed intact lung structure without any evidence of inflammatory infiltrates (fig. 2A). Latex-sensitized WT mice showed infiltrates with chronic inflammatory cells in the interstitial tissues of the lungs (fig. 2B). These

inflammatory cells primarily accumulated around small vessels and bronchioles and consisted of plasma cells, small number of lymphocytes, epithelioid histiocytes and occasional neutrophils. The intervening areas of lung parenchyma were minimally involved. In addition, there were many eosinophils (fig. 2D), which were numerous in some mice. The alveolar epithelium at the site of the peribronchiolar inflammation was often hyperplastic, containing many goblet cells. Alveolar spaces contained macrophages and lymphocytes. Latex-sensitized IL-4 KO mice showed similar degrees of inflammatory infiltrates as seen in WT mice, primarily adjacent to the bronchioles and small vessels (fig. 2C). Only occasional eosinophils were noted in these animals, although nodular lymphocyte infiltrates presenting as aggregates were seen along with similar chronic inflammatory cells as observed in the lungs of the WT animals.

Airway Hyperreactivity

Baseline (prechallenge) lung mechanics data are presented in table 1. The baseline values for R_L were lower in

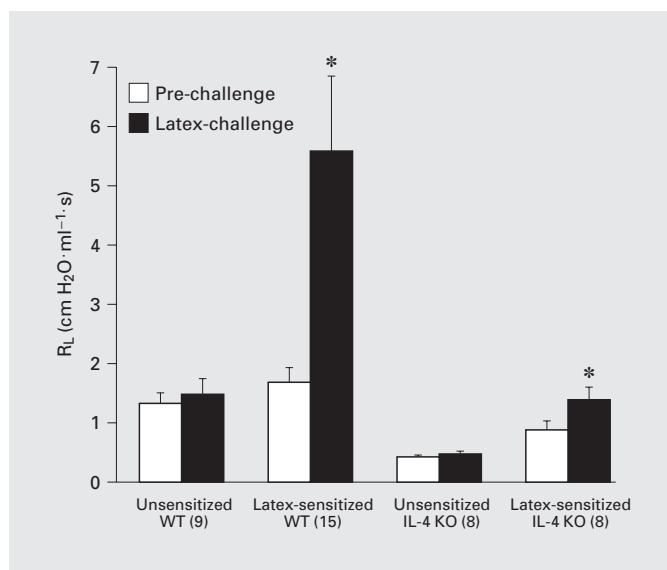


Fig. 3. Airway responses to latex antigen challenge in unsensitized and latex-sensitized WT and KO mice. Values represent means $\pm$ SE. Asterisk (*) indicates significant difference from prechallenge ($p < 0.05$).

Table 1. Baseline pulmonary mechanics, R_L and C_{dyn} for the four groups of mice

Mice	R_L cm H ₂ O/ml/s	C_{dyn} ml/cm H ₂ O
Wild type (WT)		
Unsensitized (n = 9)	1.34 $\pm$ 0.185	0.0215 $\pm$ 0.0015
Latex sensitized (n = 15)	1.704 $\pm$ 0.258	0.0237 $\pm$ 0.0016
IL-4 knockout (KO)		
Unsensitized (n = 8)	0.422 $\pm$ 0.032	0.0292 $\pm$ 0.0015
Latex sensitized (n = 8)	0.866 $\pm$ 0.155	0.0208 $\pm$ 0.0032

All values are mean $\pm$ SE. Differences between C_{dyn} for the two IL-4 knockout groups were significant ($p < 0.05$). R_L for the latex-sensitized group was significantly greater than R_L for the IL-4 knockout groups ($p < 0.05$).

IL-4 KO mice compared to WT mice, but only the difference between latex-sensitized WT mice and unsensitized IL-4 KO mice was significant. Baseline differences between unsensitized and latex-sensitized mice were not significantly different within either group. Saline challenge did not have any significant effect on preantigen challenge R_L in any of the groups. Challenge with latex antigen results in a significant increase in R_L for latex-sensitized WT and IL-

4 KO mice but not in unsensitized mice in either group (fig. 3). While the R_L increase in WT mice was nearly 300%, R_L in IL-4 KO mice increased only by about 50%.

In addition to the increase in R_L following latex challenge in latex-sensitized mice, there were small but significant decreases in C_{dyn} . Baseline values are presented in table 1. Following latex challenge, compliance fell to 0.0171 ± 0.0026 (SE) ml/cm H₂O in latex-sensitized WT mice and to 0.0164 ± 0.0026 (SE) ml/cm H₂O in latex-sensitized IL-4 KO mice, both values were statistically lower than prechallenge values ($p < 0.05$).

Discussion

The intranasal instillation of latex allergens in the present study resulted in the development of a characteristic allergic response in BALB/c mice. These features included elevated total serum IgE, latex specific IgG antibodies particularly IgG1, peripheral blood eosinophilia, and characteristic inflammatory responses in the lungs with eosinophil infiltration. These features are typical of a Th2-mediated response and are the salient features of human latex allergy [24]. Evaluation of the airway responses in these animals after exposure to latex proteins revealed increased airway resistance. This response is similar to human allergic asthma where patients challenged with the specific allergen develop smooth muscle constriction leading to airway spasm. BALB/c WT mice possess an intact immune system with a predominant IL-4-mediated response to latex allergen. Since the Th2 response, an IL-4- and IL-5-mediated response, is characteristic in allergy, we used IL-4 KO mice to elucidate the role of IL-4 in this model of latex allergy. IL-4 KO mice showed an enhanced Th1 response, probably due to the lack of IL-4-induced suppression of Th1 cytokines [21]. We anticipated a reduced Th2 response and exaggerated Th1 response in these KO animals. There was no detectable IgE in these animals, and only a slight increase in the peripheral blood eosinophils was evident following challenge with latex allergens. The lung inflammation did not significantly differ between the WT and KO animals, although more eosinophil infiltration was detected in the former group. The interstitial inflammation and perivascular and bronchial infiltration observed in this model resembled allergen-induced reactions reported in other animal models [14, 21].

The WT mice showed marked enhancement in IgG1 antibodies to latex, although this was not so with the IL-4 KO mice. It has been shown that IL-4, in conjunction with CD40-CD40L, induces the switching of IgG1 isotypes [26].

In the present study, low levels of IgG1 were detected in the IL-4 KO animals, while in contrast, it was the major antibody isotype detected in the WT animals. However, IgG2a, an isotype dependent on Th1 cytokine, IFN- γ was increased more in the KO mice compared to WT mice substantiating the presence of a predominantly polarized Th1 response in the KO animals [27].

As we have shown previously [28], the airway response in latex-sensitized WT mice is unique in that latex-sensitized mice challenged directly with latex antigen develop a significant increase in R_L . A similar result was obtained in the present study. However, differences were observed between WT and IL-4 KO mice. The KO mice had lower baseline R_L values compared to WT mice (table 1). This may be due to the predominant Th2 response in WT animals and suppressed Th2 response in the KO animals. Upon challenge with latex antigen, R_L in the latex-sensitized KO animals did increase significantly, but the increase was much less dramatic compared to WT animals (fig. 3). The results are consistent with the immunological findings. In WT mice, the Th2 cytokine response causes elevated IgE levels and increased eosinophil numbers. With no measurable IgE and only a few eosinophils in KO animals, the R_L increase was greatly attenuated. Nevertheless, the significant increase in R_L that occurred even in KO mice suggests that other factors not currently understood may also be involved.

In some mice, the large increases in R_L following latex challenge were accompanied by much less dramatic decreases in C_{dyn} . It has been suggested that contraction of airway smooth muscle could increase parenchymal stresses in a radial direction around airways resulting in the decreased C_{dyn} that often occurs when R_L is increased [29]. It is also possible that the bronchoconstriction causes closure of air

spaces, a subsequent decrease in lung volume and thus only an apparent decrease in C_{dyn} [30].

These results – immunological, histological, and physiological – demonstrate that a pronounced Th2 response is essential for airway response. However, both WT and KO animals showed almost similar histology. Hence, these results suggest that the airway resistance may be the result of binding of allergens to IgE present on the mast cells and basophils in the bronchi and the release of mediators. The activation of eosinophils and release of products such as major basic protein, eosinophil peroxidase, into the lungs may also be responsible for the lung pathology and airway response. The definite, but reduced, airway response in IL-4 KO animals also suggests that in the absence of IgE and eosinophils other mechanisms, such as Rantes, eotaxins, may be involved in latex-induced allergy and asthma.

In conclusion, we have demonstrated a latex-allergic response in WT mice comparable to latex allergy in humans. The results suggest that, in addition to IgE antibody and eosinophils, other mediators such as chemokines and other factors may play a role in latex allergy, and hence, need further study.

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