

Original article

Sex-related differences in the initiation of allergic rhinitis in mice

Background: Several clinical and epidemiologic studies have investigated sex differences in the prevalence of allergic rhinitis. At present, however, no reports have demonstrated such differences in experimental models with local, but not parenteral, sensitization with antigens that may reflect natural exposure to allergens. We have recently developed murine models of allergic rhinitis after repeated intranasal sensitization with antigens in the absence of adjuvants. In this study, we investigated the role of sex in the initiation of the disease *in vivo*.

Methods: Male and female CBA/J and BALB/c mice were sensitized intranasally with phospholipase A₂ (PLA₂) and *Schistosoma mansoni* egg antigen (SEA), respectively, in the absence of adjuvants. After the repeated sensitization, serum Ab titers against the sensitizing antigen and nasal eosinophilia were determined. In addition, the involvement of androgen in IgE synthesis was investigated in castrated CBA/J male mice with or without testosterone administration.

Results: Females produced significantly higher levels of PLA₂-specific IgE than males in CBA/J mice sensitized with PLA₂. On the other hand, both titers of PLA₂-specific IgG1 and nasal eosinophilia did not significantly differ between the two groups. Castrated male mice produced significantly higher amounts of PLA₂-specific IgE than sham-treated male mice. In addition, PLA₂-specific IgE production decreased in castrated mice treated with testosterone. Sexual differences in the production of Ag-specific IgE were not seen in BALB/c mice after the sensitization with SEA.

Conclusions: These results suggest that sex is responsible for the production of Ag-specific IgE, but not IgG1 or nasal eosinophilia, and that androgen appears to be involved in the *in vivo* production of specific IgE in male mice.

T. Yamamoto¹, M. Okano¹, T. Ono²,
E. Nakayama², T. Yoshino³,
A. R. Satoskar⁴, D. A. Harn Jr⁴,
K. Nishizaki¹

Departments of ¹Otolaryngology, ²Immunology and Parasitology, and ³Pathology, Okayama University Medical School, Okayama, Japan; ⁴Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, USA

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Mitsuhiro Okano, MD, PhD
Department of Otolaryngology
Okayama University Medical School
2-5-1 Shikata-cho
Okayama
700-8558 Japan

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Several clinical and epidemiologic studies have investigated sex differences in the prevalence of allergic rhinitis (1–5). Equal prevalence was seen in some reports (1, 2), whereas other reports showed sex-dominated prevalence of the disease (3–5). For example, in New Zealand adults, women showed a significantly higher prevalence of allergic rhinitis (43.5%) than men (31.9%) (5). In addition, it is well known that nasal hyperreactivity is exacerbated during pregnancy, the so-called “pregnancy rhinitis” (6).

Recent *in vitro* studies have indicated that sex hormones are involved in allergic reactions (7–12). Female sex hormones are known to increase the expression of histamine H₁ receptor mRNA on cultured human nasal epithelial cells and human mucosal microvascular cells, exacerbate eosinophilic inflammation in nasal mucosa, and enhance immunoglobulin production (7–9). On the other hand, male sex hormones affect bone marrow cells, thereby altering B-cell development, accelerate thymocyte apoptosis, and inhibit immunoglobulin production by PBMC (10–12). However, it is unclear whether these

sex hormones are involved in the *in vivo* initiation of allergic diseases such as allergic rhinitis. Astorquiza et al. reported that the IgE response, but not the IgG or IgM, is lower in male RF mice than in female mice after parenteral immunization with ovalbumin (OVA) adsorbed with alum (13). Lei et al. demonstrated that male ICR mice were more susceptible than female to non-IgE-mediated anaphylactic death after intravenous injection of bovine serum albumin (BSA) into BSA/CFA-primed mice (14).

We have recently developed two murine models of allergic rhinitis after repeated intranasal sensitization with antigen in the absence of adjuvants (15, 16). In the present study, using these models, we sought to determine sex difference and involvement of sex hormones in the initiation of allergic rhinitis. To our knowledge, this is the first report on sex difference in the initiation of allergic diseases by local, but not parenteral, sensitization of antigen without adjuvants, in models that mimic natural exposure.

Material and methods

Animals

Young adult (6–10-week-old) CBA/J and BALB/c strain female and male mice were purchased from Charles River Japan (Yokohama, Japan). The mice were maintained at Okayama University Medical School according to the guidelines of the Okayama University Medical Area Research Committee.

Reagents

Purified rat anti-mouse IgE mAb was purchased from Biosource (Camarillo, CA, USA), biotinylated rat anti-mouse IgE mAb from Pharmingen (San Diego, CA, USA), PLA₂ and extravidin-peroxidase conjugate from Sigma Chemical Co. (St Louis, MO, USA), goat, anti-mouse IgG1 mAb-peroxidase conjugate from Boehringer-Mannheim (Indianapolis, IN, USA), Tween 20 from Bio-Rad (Hercules, CA, USA), tetramethylbenzidine substrate for peroxidase from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD, USA), fetal calf serum (FCS) from Gibco BRL (Grand Island, NY, USA), and testosterone from Nacalai Tesque Inc. (Kyoto, Japan). Protein assay was performed by bicinchoninic acid (BCA) assay according to the manufacturer's instructions (Pierce, Rockford, IL, USA).

Sensitization of mice

Sensitization of mice ($n=6$) to allergen was performed as previously described with slight modification (15, 16). In brief, mice were lightly anesthetized by diethyl ether (Ishizu Keiyaku Ltd, Osaka, Japan). A volume of 20 μ l of either 10 μ g PLA₂ or 5 μ g SEA concentrated in Dulbecco's PBS (Gibco) was applied three times at 1-week intervals through both nostrils with a microsyringe (Hamilton Co., Reno, NV, USA). One week after the third sensitization, the mice were inoculated with either 5 μ g PLA₂ or 2 μ g SEA for 7 consecutive days. Blood samples were taken from tail veins 12 h after the final sensitization.

Castration and androgen treatment

Castration was performed under ether anesthesia at 6 weeks of age. Sham-operated littermates were used as controls. Two weeks after the castration surgery, intranasal sensitization was begun. In some experiments, androgen replacement was administered to castrated mice 1 week after the surgery. Testosterone (50 mg) was dissolved in 400 μ l of 100% ethanol, and then concentrated to 5 mg/ml by adding 9.6 ml of sesame oil (Sigma). Subcutaneous injections of testosterone (0.75 mg) were given every 2 days. Control castrates were injected with an equal volume of the ethanol/sesame oil vehicle alone. Intranasal sensitization was started 1 week after the testosterone administration.

Serum Ab titers

Antigen-specific IgE was determined by sandwich ELISA using biotinylated antigen as the detection reagent, as described previously (15, 16). Titers are expressed as mean OD at 450 nm of six individual sera diluted at 1:4. Ag-specific IgG1 Ab was detected by indirect ELISA, as described previously (15, 16). Results are expressed as endpoint titers where the endpoint equals the final serum dilution yielding an absorbance twice background.

Histologic examination

Twelve hours after the final nasal challenge, the mice were killed by cervical dislocation. The heads were removed, fixed in 10% formalin, and decalcified with 2.5% EDTA-2 Na solution. Coronal nasal sections were stained with hematoxylin and eosin, and the number of eosinophils in nasal mucosa was counted microscopically under a high-power field (10 $\times$ 40).

Statistical analysis

Data are expressed as the mean $\pm$ standard error of mean (SEM) for each subject group. Statistical analysis was performed with Student's unpaired-*t* test and the Mann-Whitney U-test to compare the titers of Ag-specific IgE and IgG1/IgG2a, respectively, in different groups of mice. A value of $P < 0.05$ was considered significant.

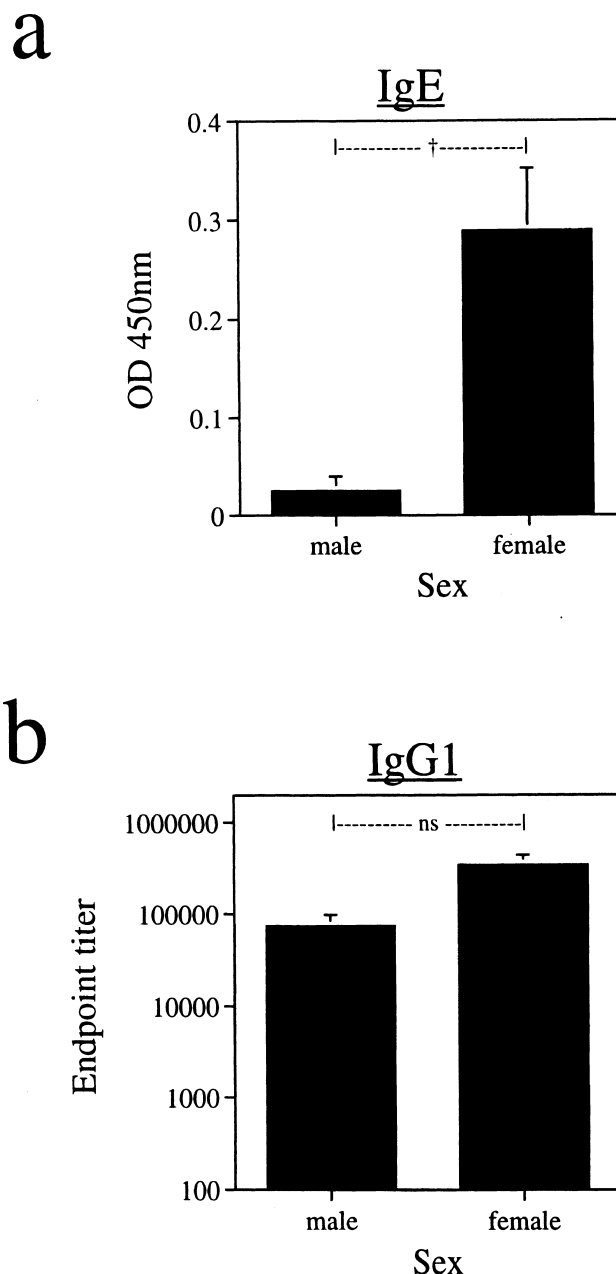


Figure 1. Serum levels of PLA₂-specific IgE (a) and IgG1 (b). CBA male and female mice were sensitized intranasally with PLA₂, as described in Material and methods. Titers of PLA₂-specific IgE and IgG1 are shown as mean OD at 450 nm of 1:4 diluted sera (a) and endpoint titer (b) $\pm$ SEM of six individual serum samples, respectively. Data are representative of three separate experiments. †: $P < 0.005$; NS: not significant.

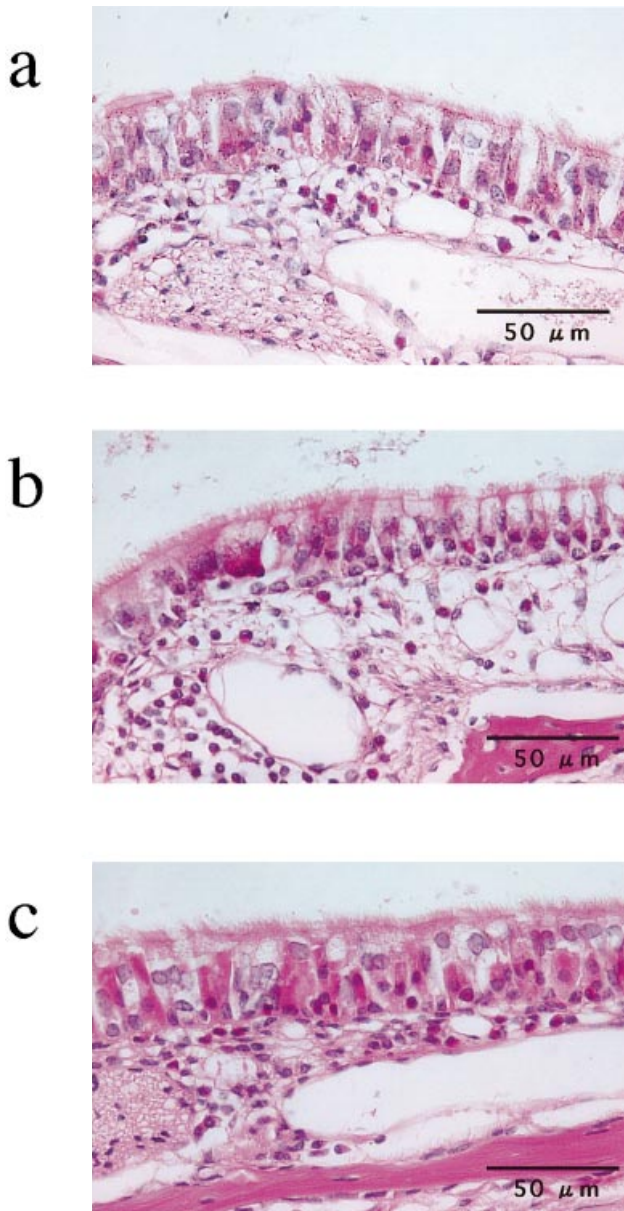


Figure 2. Nasal eosinophilia of mice sensitized intranasally with PLA₂. CBA/J mice were sensitized with PLA₂, as described in Material and methods. After nasal challenge, mice were decapitated. Nasal sections were fixed, decalcified, and stained with hematoxylin and eosin. Typical nasal sections from male (a), female (b), and castrated male (c) mice are shown.

Results

Serum Ab levels after repeated intranasal sensitization with PLA₂

CBA/J mice were sensitized with 10 μg of PLA₂ and challenged with 5 μg of the antigen. PLA₂-specific IgE was determined by ELISA after the consecutive intranasal challenge with PLA₂. Female mice produced significantly higher levels of specific IgE than male mice (Fig. 1a). The production of PLA₂-specific IgG1 was also higher in females than males; however, this difference was not statistically significant (Fig. 1b).

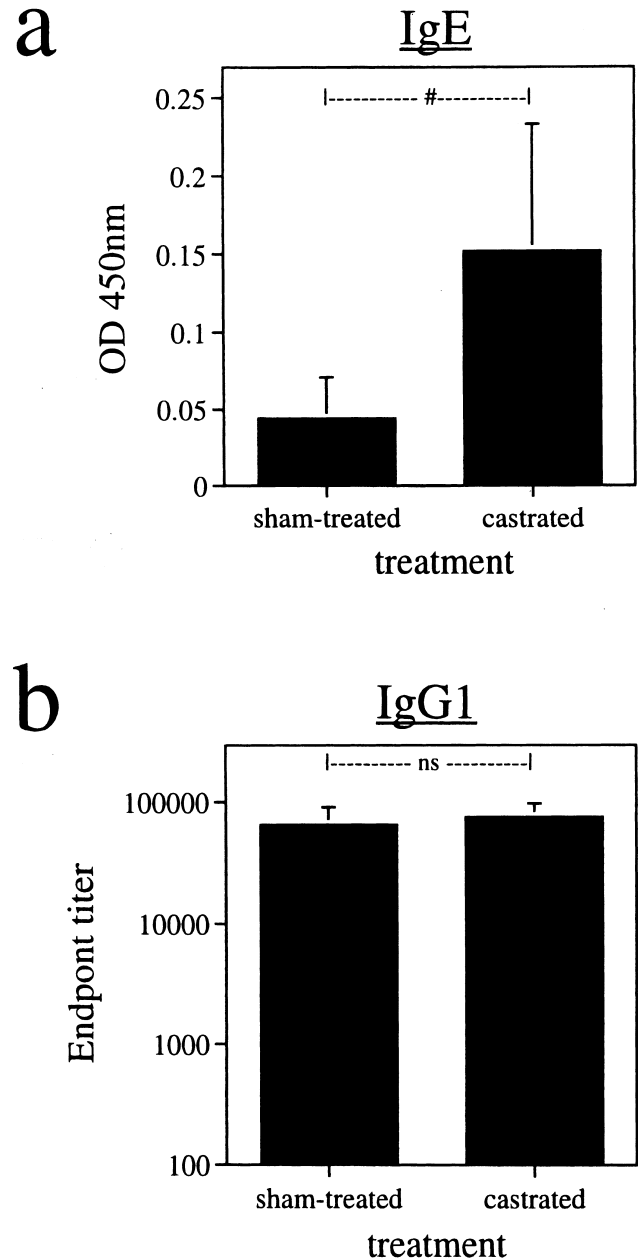


Figure 3. Serum levels of PLA₂-specific IgE (a) and IgG1 (b) from male mice treated with castration or sham operation. Titers for PLA₂-specific IgE and IgG1 are shown as mean OD at 450 nm of 1:4 diluted sera (a) and endpoint titer (b) $\pm$ SEM of six individual serum samples, respectively. Data are representative of two separate experiments. #: $P < 0.05$; NS: not significant.

Histologic changes of the nasal mucosa

As shown in Fig. 2, both male and female mice showed similar migration of eosinophils into the nasal mucosa. Mean numbers of eosinophils per high-power field (10×40) $\pm$ SEM in nasal septum from four nasal sections in males and females were 10.00 ± 1.88 and 12.17 ± 2.39 , respectively.

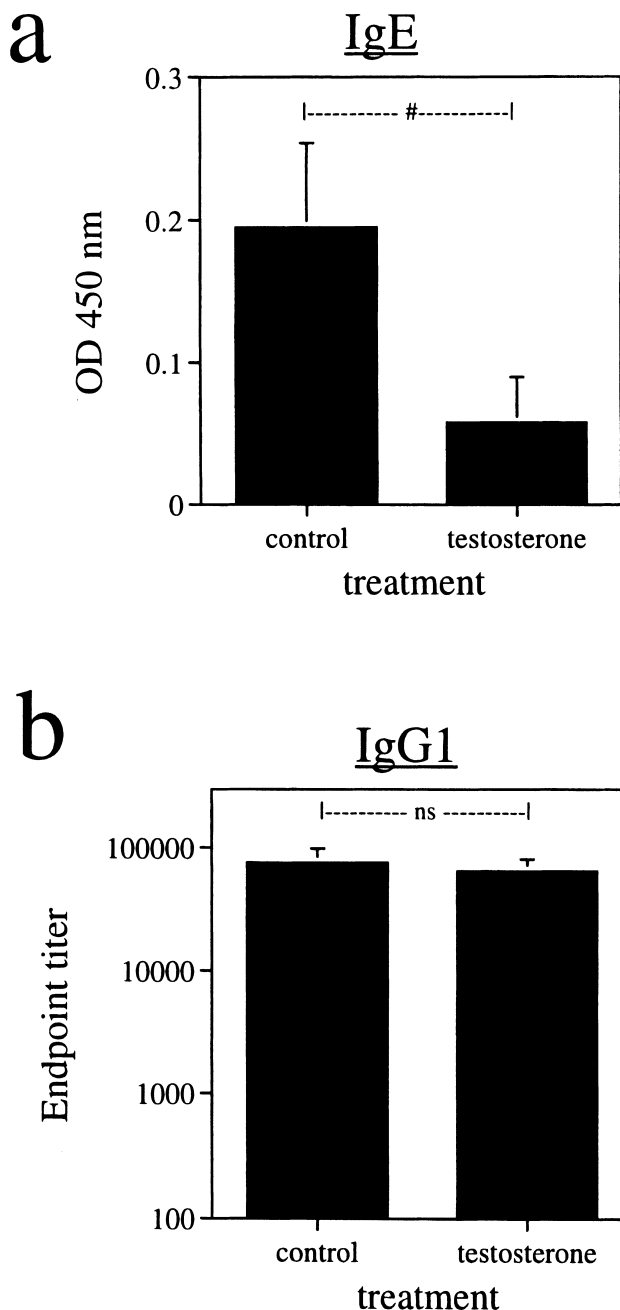


Figure 4. Serum levels of PLA₂-specific IgE (a) and IgG1 (b) from castrated male mice subcutaneously injected with testosterone or control ethanol/sesame oil vehicle. Titers for PLA₂-specific IgE and IgG1 are shown as mean OD at 450 nm of 1:4 diluted sera (a) and endpoint titer (b) $\pm$ SEM of six individual serum samples, respectively. Data are representative of two separate experiments. #: $P < 0.05$; NS: not significant.

Effect of castration on IgE production and nasal eosinophilia

Next, we investigated the effect of castration on the initiation of allergic rhinitis in male mice. Castrated mice produced a significantly higher amount of PLA₂-specific IgE after the nasal challenge than sham-treated male mice (Fig. 3a). On the other hand, the production of PLA₂-specific IgG1 was similar for castrated and

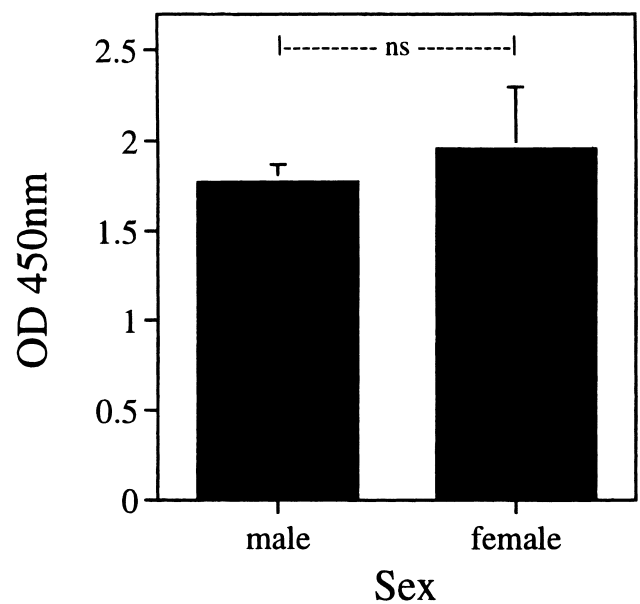


Figure 5. Serum levels of SEA-specific IgE. BALB/c male and female mice were sensitized intranasally with SEA, as described in Material and methods. Titers for SEA-specific IgE are shown as mean OD at 450 nm of 1:4 diluted sera $\pm$ SEM of six individual serum samples. Data are representative of two separate experiments. NS: not significant.

sham-treated male mice (Fig. 3b). In addition, nasal eosinophilia was also similar for the two groups (Fig. 2c). Mean numbers of eosinophils per high-power field (10×40) $\pm$ SEM in nasal septum from six nasal sections in castrated males and sham-treated males were 11.00 ± 2.03 and 9.50 ± 1.82 , respectively.

Effect of testosterone on the production of IgE and nasal eosinophilia in castrated mice

The results just described suggested that androgen hormones might be important in regulating the production of IgE; therefore, we subcutaneously administered 0.75 mg of testosterone every other day to castrated mice. As a control, castrated mice were administered the same volume of the solvent containing sesame oil and ethanol. The testosterone-treated mice produced significantly lower levels of PLA₂-specific IgE, but not IgG1, than control mice (Fig. 4a and b). Nasal eosinophilia did not differ between the two groups (data not shown).

Sex difference in mice sensitized with SEA

In contrast to the sensitization with PLA₂, both male and female mice produced similar levels of SEA-specific IgE after the nasal challenge (Fig. 5). The production of IgG1 against SEA and nasal eosinophil levels were also similar in males and females after the nasal challenge with this antigen (data not shown).

Discussion

In the present study, we demonstrated that, after intranasal sensitization with PLA₂, male CBA/J mice produced significantly lower amounts of antigen-specific IgE, but not IgG1, than female mice. In addition, castrated mice produced higher amounts of IgE than sham-treated males, and these castration-induced changes were reversed by androgen replacement. Castration is known to reduce serum levels of androgen dramatically to 1/5 in CBA male mice, which we used in this study (17). Astorquiza et al. reported that IgE response, but not IgG or IgM, is lower in male RF mice than in female mice after parenteral immunization by OVA adsorbed to alum (13). They also demonstrated that such sex-related differences were not present in castrated mice. Our current data using local *in vivo* sensitization in mice are consistent with this report, and suggest that male sex hormones are negatively involved in the *in vivo* production of antigen-specific IgE in male mice.

Although the mechanisms of the immunosuppressive roles of male sex hormones on the production of IgE *in vivo* remain unclear, recent experimental studies have demonstrated the interaction between male sex hormones and the immune system. Increased IL-2 and IFN- γ production by peripheral T cells from normal mice was seen after castration (18). In the murine model of trauma hemorrhage, male sex steroids are known to be involved in immunodepression due to loss of IL-2 and IL-3 production from splenocytes (19). The nonobese diabetic (NOD) mouse is a widely used animal model for insulin-dependent diabetes mellitus (IDDM) characterized by Th1 cell-mediated autoimmune insulinitis (20). Fitzpatrick et al. demonstrated that the kinetics of insulinitis was exacerbated in castrated NOD mice, and resembled that observed in untreated age-matched females (21). In addition, we have reported that IFN- γ transcripts were commonly detected in female DBA/2 mice, but not in males, after infection with *Leishmania mexicana*, which causes a Th1 response (22). Although we have not determined the role of cytokines in the present study, the sexual dimorphism of Th2-mediated IgE synthesis may be, at least in part, due to the effect of the male sex hormone on the production of cytokines. In fact, androgen receptors are not found on peripheral T cells, but are found on thymocytes and bone-marrow cells, and appear to be related to altered B-cell development and accelerated thymocyte apoptosis (10, 11, 23).

On the other hand, sex differences were not seen in CBA/J mice after intranasal sensitization with PLA₂ in terms of the production of either Ag-specific IgG1 or nasal eosinophilia. In the present study, we measured IgG1 antibodies since this isotype is the predominantly produced IgG after intranasal sensitization with both PLA₂ and SEA (15, 16). The production of both Ag-

specific IgE and IgG1 is positively regulated by the Th2 response in mice, and Ag-specific IgE and nasal eosinophilia are two of the major hallmarks of the initiation of allergic rhinitis (24, 25). However, recent investigations, especially experimental allergic models using gene-deficient animals, have demonstrated that the production of Ag-specific IgE and the production of IgG1 and/or local eosinophilia are not definitely correlated (26–28). IL-4 appears not to be essential for IgG1 class switching or nasal eosinophilia, but plays a crucial role in IgE production (28, 29). For example, we recently reported that IL-4-gene-deficient (IL-4^{–/–}) BALB/c mice sensitized with SEA displayed no titers of Ag-specific IgE, but did show significantly lower, but not abolished, titers of specific IgG1 than wild-type control mice. Ag-stimulated nasal lymphocytes from SEA-sensitized IL-4^{–/–} and IL-4^{+/+} mice produced similar amounts of IL-5 and IL-10. Furthermore, the severity of nasal eosinophilia was similar in both groups (28). IL-5 is known to induce IgG1 isotype switch recombination in mice (30). This cytokine also plays a critical role in mediating antigen-induced eosinophil infiltration (31). Thus, we may suggest that IL-5 production, which appears to be linked with IgG1 production and local eosinophilia, may not be different between male and female mice after intranasal sensitization with PLA₂, and an investigation to detect mRNA signals for IL-4, IL-5, and IFN- γ by RT-PCR is currently underway in our laboratory.

Such sex-related differences in the induction of allergic rhinitis are not seen in BALB/c mice after intranasal sensitization with SEA. We chose to use BALB/c mice because this strain is a high responder for intranasal sensitization with SEA (15). Both male and female BALB/c mice showed similar levels of Ab production and nasal eosinophilia after the sensitization with SEA. The procedure of sensitization with PLA₂ and SEA was identical in the present study. Thus, at least two possibilities may be taken into consideration. The nature of antigen/allergen appears to be important for the induction of a sex difference in the initiation of allergic rhinitis. In fact, we recently showed that the allergenicity of PLA₂ and SEA, as shown by the induction of Ag-specific IgE production, was different (16, 32). Carbohydrates on SEA were critical for induction, although those on PLA₂ were irrelevant. In an epidemiologic study, Johnson et al. reported that girls demonstrated significant increases in IgE to mite and cat, and decreases in IgE to ragweed and timothy from 2 to 4 years of age, whereas there was little change in boys except for an increase in IgE to ragweed (33).

Mouse strain may be an important factor in sex-related induction of allergic rhinitis. It is known that sex-related onset of the disease was strain-specific in several murine models (34, 35). In murine models of Sjögren's syndrome, for example, the severity of

inflammation was greater in the lacrimal glands of female MRL/lpr, MRL +/+, NZB/NZW F1, C3H/lpr, and gld mice, and male NOD mice than in their counterparts. No sex-related variations were present in bcl-2(+)/lpr and bcl-2(-)/lpr mice (35).

In conclusion, we have demonstrated a clear sex difference in Ag-specific IgE production in a murine model of allergic rhinitis, which is Ag and strain dependent. This difference is related to the presence of male sex steroids. Results presented here may provide insight into the involvement of sex hormones and endocrine disruptors in the initiation of allergic

diseases; hence, modulation of the immune system by the hormones may lead to the development of a new immunotherapy for allergic diseases.

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