

Roles of Fc γ RIIB in Nasal Eosinophilia and IgE Production in Murine Allergic Rhinitis

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The low-affinity IgG Fc receptor, Fc γ RIIB, displays inhibitory potential in experimental models such as autoimmune diseases. However, whether this receptor is involved in the onset of allergic diseases remains unknown. This study examines the role of Fc γ RIIB in the initiation of allergic rhinitis in mice. Repeated intranasal sensitization with *Schistosoma mansoni* egg antigen (SEA) induced SEA-specific IgE and marked nasal eosinophilia in high-responder BALB/c mice. Fc γ RIIB gene-deficient (–/–) BALB/c mice displayed severe eosinophilia compared with that of wild-type counterparts. However, Fc γ RIIB –/– mice conversely produced less SEA-specific IgE. The production of interleukin (IL)-4 but not of IL-5 or IFN- γ by nasal mononuclear cells was also decreased in Fc γ RIIB –/– mice, suggesting that the exacerbation of nasal eosinophilia in Fc γ RIIB –/– mice is independent of the local IL-5 levels. The findings in low responder C57BL/6 mice were similar. In addition, nasal eosinophilia in Fc γ RIIB –/– mice passively sensitized with SEA was exacerbated, and conversely, specific IgE production was inhibited after a nasal challenge. These results suggest that Fc γ RIIB plays a regulatory role in the initiation of allergic rhinitis that is independent of either mouse strain or type of sensitization.

Keywords: Fc receptor; rhinitis; mouse; IgE; eosinophil

Allergic rhinitis is the most prevalent allergic condition initiated by immediate hypersensitivity. Over 10% of the population in developed countries suffers from allergic rhinitis, which creates societal burdens such as medical expenses and loss of productivity (1, 2). Antigen-specific IgE production and nasal eosinophilia are characteristic hallmarks of this condition (1, 3). In addition, atopic humans and mice often produce antigen-specific IgGs, especially IgG4 and IgG1, respectively (4–8). Atopic humans usually express higher IgG4 levels than healthy individuals (4, 5), and antigen-specific IgG1 is often produced before specific IgE in mice (7, 8). Thus, the role of antigen-specific IgG and the interaction between IgG and Fc receptors in the pathogenesis of allergic rhinitis should be understood.

Among Fc receptors in mice, Fc γ RI, Fc γ RIII, and Fc ϵ RI share a common γ chain that contains an immunoreceptor tyrosine-based activation motif in the intracytoplasmic domain (9, 10). These receptors appear to elicit cellular responses such as phagocytosis, antibody-dependent cell-mediated cytotoxicity, anaphylaxis, neutrophil chemotaxis, or autoimmune diseases via bind-

ing antibody–antigen complexes (11–15). On the other hand, Fc γ RIIB is the major Fc receptor expressed on B cells, mast cells, macrophages, neutrophils, and eosinophils in humans and mice (9, 16, 17). This receptor consists solely of an α chain containing an immunoreceptor tyrosine-based inhibition motif in the intracytoplasmic domain, and it suppresses cell activation triggered by cross-linking B-cell receptors or Fc receptors (18–20). In several models of autoimmune diseases, mice deficient in the Fc γ RIIB gene (Fc γ RIIB –/–) develop enhanced immune-complex-mediated alveolitis, collagen-induced arthritis, systemic lupus erythematosus, and Goodpasture's syndrome (21–24). In addition, Fc γ RIIB –/– mice produce more IgM, IgA, and IgG in response to both thymus-dependent and -independent antigens, and both IgG- and IgE-mediated anaphylactic responses are enhanced (25, 14). However, little is known about whether Fc γ RIIB is involved in the initiation of atopic diseases such as allergic rhinitis (26). Moreover, the role of Fc γ RIIB in antigen-specific IgE production has not been demonstrated.

We recently developed a strain-dependent murine model of allergic rhinitis based on repeated intranasal sensitization with *Schistosoma mansoni* egg antigen (SEA) in the absence of adjuvants (6). This study examines the involvement of Fc γ RIIB in the initiation of allergic rhinitis in both high- and low-responder mouse strains. To our knowledge, we are the first to describe the role of Fc γ RIIB in the initiation of allergic diseases through not only systemic but also local sensitization by antigen without adjuvants in models that mimic natural exposure. In addition, we either passively or intraperitoneally sensitized the mice with SEA to determine whether the findings from Fc γ RIIB –/– mice after intranasal sensitization with SEA depended on a particular route and/or type of sensitization.

METHODS

Animals and Antigens

All experiments were performed using young adult (6–10 weeks old) female BALB/c and C57BL/6 (Fc γ RIIB +/+) mice purchased from Charles River Japan (Yokohama, Japan). Homozygous Fc γ RIIB gene-deficient (Fc γ RIIB –/–) BALB/c and C57BL/6 founder mice were gifts from Dr. Jeffery V. Ravetch (Rockefeller University, New York, NY) (24). Fc γ RIIB –/– mice were bred and maintained under specific pathogen-free conditions at Okayama University Medical School according to the guidelines established by the Okayama University Medical Area Research Committee. All experimental protocols and procedures in this study were approved by institutional animal care and use committee. SEA was prepared as described (6). Concanavalin (Con) A was purchased from Sigma (St. Louis, MO).

Sensitization of Mice

Mice were either actively or passively sensitized with SEA. We designed both local and systemic sensitization (active sensitization) before nasal challenge with SEA. In local sensitization, mice were intranasally sensitized with SEA in the absence of an adjuvant as described (6, 27). In systemic sensitization, the mice were intraperitoneally sensitized with 5 μ g of SEA adsorbed to 1 mg of alum (Kyowa Kagaku, Kagawa, Japan)

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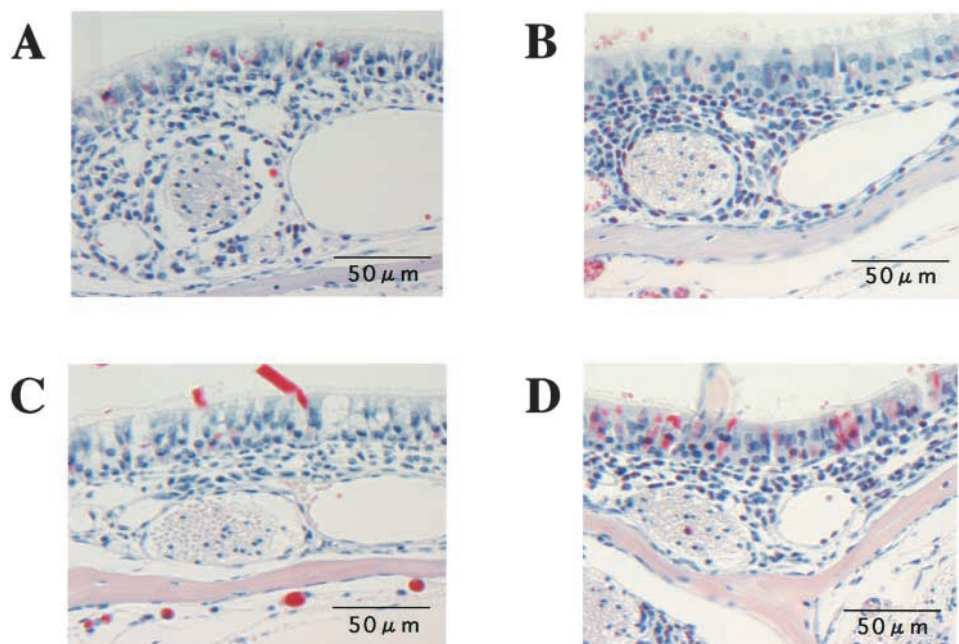


Figure 1. Nasal eosinophilia after nasal challenge in BALB/c (A and B) and C57BL/6 (C and D) mice. Wild-type BALB/c (A) and C57BL/6 (C) mice and counterpart Fc γ RIIB $-/-$ mice (B and D, respectively) were intranasally sensitized with *Schistosoma mansoni* egg antigen (SEA). At 12 hours after the final nasal challenge with SEA, mice were killed. Nasal sections were fixed, decalcified, and eosinophils in nasal mucosa were detected by Luna stain. Luna stain is specific for eosinophils and renders their cytoplasm red-brown on a blue background. (C) Peeled mucin and erythrocyte cluster were stained as bright red.

in a total volume of 200 μ l. Two weeks later, the mice were boosted in the same manner. Alternatively, mice were passively sensitized by an intravenous injection of 200 μ l of pooled sera from mice presensitized by a repeated intranasal application of SEA or from nonsensitized mice. One hour after passive sensitization, the mice were challenged intranasally with 20 μ l of 1- μ g SEA for 7 consecutive days.

SEA-specific Antibody Determination

Peripheral blood collected from the tail vein 12 hours after the final challenge was centrifuged at 200 $\times$ g, and the levels of SEA-specific antibody, including IgE, IgG1, and IgG2a, were measured by ELISA as described (6, 27). Titers are expressed as endpoint titers where the endpoint equals the final serum dilution yielding an absorbance equal to twice that of the background.

Histologic Examination

Twelve hours after the final nasal challenge, mice were killed with carbon dioxide. The heads were removed, fixed, and decalcified (6, 27). Coronal nasal sections were visualized by either hematoxylin/eosin or Luna stain. Luna stain is specific for eosinophils and renders their cytoplasm red-brown on a blue background (28). The number of infiltrating cells such as eosinophils and mononuclear cells in the posterior portion of nasal septum was determined microscopically in a blinded manner and was expressed as numbers per high-power field (10 $\times$ 40).

Detection of *In Vivo* Apoptosis in Nasal Mucosa

Detection of *in vivo* apoptosis in nasal mucosa was performed using the TdT-mediated dUTP-biotin nick labeling (TUNEL) method. In brief, the head of each mouse was cut, and skin, muscles, and brain tissues were removed. Each head was fixed in 10% phosphate-buffered formaldehyde, embedded in paraffin, and cut into 6- μ m thick sections in the coronal plane. Each selected section was deparaffinized and rehydrated. After incubation with 20 μ g/ml of proteinase K (Boehringer Mannheim, Mannheim, Germany), endogenous peroxidase was blocked by using 2% H₂O₂ in methanol for 30 minutes. TdT enzyme (Takara In Situ Apoptosis Detection Kit; Takara Bio Inc., Shiga, Japan) was dropped on the sections and incubated at 37°C for 60 minutes. Then antiluorescein isothiocyanate horseradish peroxidase conjugate (Takara In Situ Apoptosis Detection Kit) was dropped on the sections and incubated at 37°C for 30 minutes. The sections were stained with diaminobenzidine tetrahydrochloride (Sigma) for 10–15 minutes.

In Vitro Culture of Nasal Mononuclear Cells and Cytokine Determination

Mice were killed 12 hours after the final nasal challenge, and nasal mononuclear cells were isolated by enzymatic extraction using collagenase as described (6, 27). Cells were cultured in flat-bottomed 48-well plates (Corning, Corning, NY) with SEA (1 μ g/ml), Con A (2 μ g/ml) as a positive control, or supplemented medium as a negative control. After an incubation at 37°C for 72 hours in 5% CO₂, supernatants were collected and stored at -80°C. Levels of interleukin (IL)-4, IL-5, and IFN- γ production induced by stimulated (SEA and Con A), and unstimulated nasal mononuclear cells were measured by capture ELISA as described (6, 27). The detection limits for IL-4, IL-5, and IFN- γ in this system were 0.1 IU/ml, 10 pg/ml, and 0.1 IU/ml, respectively.

Statistical Analysis

Data are expressed as the mean $\pm$ SEM for each subject group. Statistical analysis was performed using Student's unpaired *t* test. Differences in antibody endpoint titers were determined using the Mann-Whitney *U* prime test.

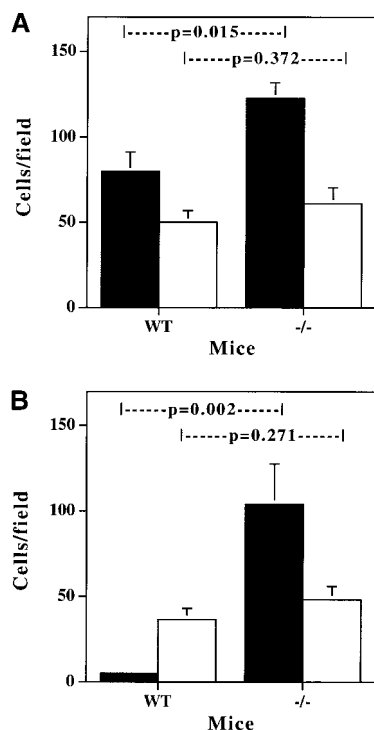


Figure 2. Quantitation of cellular infiltration into nasal mucosa after nasal challenge in BALB/c (A) and C57BL/6 (B) mice. Wild-type (WT) and Fc γ RIIB $-/-$ mice were intranasally sensitized and challenged with SEA, as described in Figure 1. The number of eosinophils (closed bar) and mononuclear cells (open bar) in the posterior portion of nasal septum was determined microscopically in high-power fields (10 $\times$ 40). Results show mean numbers of infiltrating cells per field $\pm$ SEM of six nasal sections from each group. Data are representative of two separate experiments.

RESULTS

Role of Fc γ RIIB in the Induction of Allergic Th2 Responses After Intranasal Sensitization with SEA in High-responder BALB/c Mice

After a nasal challenge with SEA, the nasal mucosa from wild-type BALB/c mice contained diffuse inflammatory infiltrate primarily comprised of eosinophils (6). Figures 1A and 1B show severe eosinophilia in the nasal mucosa in Fc γ RIIB $-/-$ BALB/c mice as compared with the wild type. The numbers of eosinophils infiltrating the nasal septum per field (10×40) were 80.0 ± 11.3 and 122.7 ± 9.2 in Fc γ RIIB $+/+$ and $-/-$ BALB/c, respectively (mean $\pm$ SEM, $n = 6$, $p = 0.015$). On the other hand, the magnitude of infiltration of mononuclear cells into nasal mucosa was similar between Fc γ RIIB $-/-$ mice and wild-type counterparts after the intranasal sensitization with SEA (50.3 ± 6.7 vs. 61.2 ± 9.2 , $p = 0.372$) (Figure 2). In addition, neutrophils and apoptotic cells were rarely seen in both mice (Figure 3).

Serum SEA-specific IgE, IgG1, and IgG2a levels were determined in Fc γ RIIB $+/+$ and Fc γ RIIB $-/-$ BALB/c mice after nasal challenge with SEA (Figure 4). Wild-type BALB/c mice predominantly expressed SEA-specific IgE and IgG1 after repeated intranasal sensitization with SEA (6). Fc γ RIIB $-/-$ BALB/c mice produced significantly less SEA-specific IgE ($p = 0.008$) but more IgG1 ($p = 0.115$) than the wild type. All groups produced similar amounts of IgG2a ($p = 0.515$; Figure 4).

We examined cytokine production from nasal mononuclear cells after challenge. Nasal mononuclear cells isolated by enzyme extraction were stimulated with SEA or Con A for 72 hours, and then we assessed IL-4, IL-5, and IFN- γ production in the culture supernatants. SEA-stimulated nasal mononuclear cells from wild-type BALB/c mice produced detectable levels of both IL-4 and IL-5, whereas the cells with medium alone did not produce these cytokines. IL-4 production in those from Fc γ RIIB $-/-$ BALB/c mice was significantly decreased, whereas the levels of IL-5 were similar between the groups. In addition, IFN- γ was not detectable in either Fc γ RIIB $+/+$ or $-/-$ BALB/c mice in response to SEA. The findings were similar when the T cells were stimulated with Con A (Figures 5A–5C).

The Role of Fc γ RIIB in the Induction of Allergic Th2 Responses after Intranasal Sensitization with SEA in Low-responder C57BL/6 Mice

We examined whether the effect of Fc γ RIIB on the initiation of allergic rhinitis is dependent on strain using Fc γ RIIB $-/-$ C57BL/6 and the wild type, which has low responses to intranasal sensitization with SEA (6). Wild-type C57BL/6 mice under spe-

cific pathogen-free conditions produced detectable amounts of SEA-specific IgE and IgG1, whereas Fc γ RIIB $-/-$ C57BL/6 mice produced significantly more IgG1 ($p = 0.019$) and conversely less IgE ($p = 0.019$; Figure 6). In addition, eosinophil infiltration of the nasal mucosa was more apparent in Fc γ RIIB $-/-$ C57BL/6 mice than in the wild type after nasal challenge with SEA (Figures 1C and 1D). The numbers of eosinophils infiltrating the nasal septum per field (10×40) were 5.0 ± 1.6 and 103.8 ± 23.5 in Fc γ RIIB $+/+$ and $-/-$ C57BL/6, respectively (mean $\pm$ SEM, $n = 6$, $p = 0.002$) (Figure 2).

Detectable amounts of IL-4 and IL-5 were seen in nasal mononuclear cells from wild-type C57BL/6 mice intranasally sensitized with SEA in response to *in vitro* Con A stimulation (Figure 7h medium alone did not produce these cytokines). In nasal mononuclear cells from Fc γ RIIB $-/-$ C57BL/6 mice, IL-4 but not IL-5 production was significantly decreased as compared with that of the wild type after Con A stimulation (Figure 7).

Selective Suppression of IgE Production after Intraperitoneal Sensitization with SEA

We examined whether the role of Fc γ RIIB in specific antibody synthesis is restricted in intranasal sensitization. We intraperitoneally sensitized Fc γ RIIB $-/-$ and wild-type BALB/c mice with SEA adsorbed to alum. Like the results obtained after intranasal sensitization, SEA-specific IgE production was significantly decreased in Fc γ RIIB $-/-$ mice after an intraperitoneal boost with SEA compared with the wild type ($p = 0.005$; Figure 8A). Conversely, Fc γ RIIB $-/-$ mice produced more SEA-specific IgG1, although the difference between the groups was not significant ($p = 0.117$; Figure 8B). Neither mouse produced detectable amounts of IgG2a under these conditions (Figure 8C).

Role of Fc γ RIIB in the Exacerbation of Allergic Th2 Responses in Mice Passively Sensitized with SEA

We investigated the role of Fc γ RIIB in the exacerbation of allergic Th2 responses in passively sensitized mice. As described in METHODS, Fc γ RIIB $-/-$ and wild-type BALB/c mice passively sensitized with SEA by an intravenous injection of presensitized or nonsensitized sera were challenged intranasally with SEA daily for 7 consecutive days. Figure 9A shows that specific IgE synthesis was significantly decreased in Fc γ RIIB $-/-$ mice compared with the wild type ($p = 0.019$). Conversely, significantly more specific IgG1 was produced in Fc γ RIIB $-/-$ mice ($p = 0.031$; Figure 9B). On the other hand, IgG2a was undetectable in either Fc γ RIIB $-/-$ or in wild-type mice (Figure 9C). In addition, the degree of nasal eosinophilia was significantly higher in Fc γ RIIB $-/-$ mice than in the wild type, although the degree of eosinophilia was

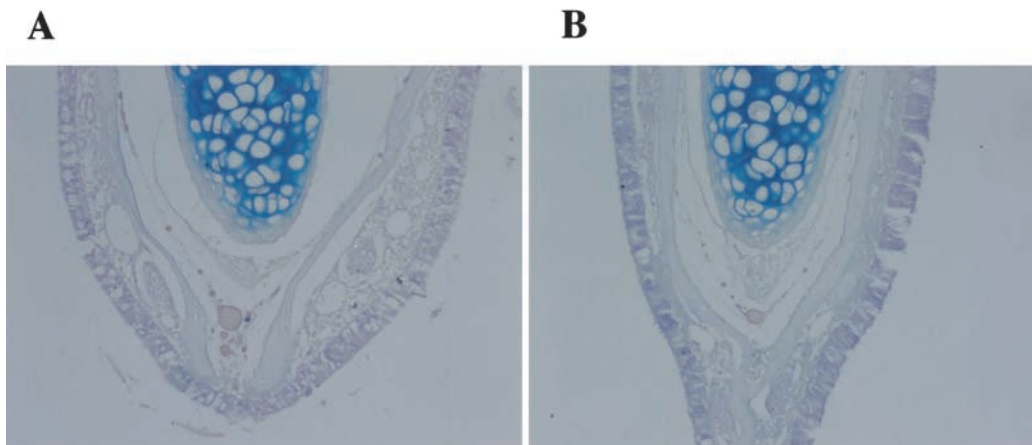


Figure 3. Detection of *in vivo* apoptosis in nasal mucosa. WT (A) and Fc γ RIIB $-/-$ (B) BALB/c mice were intranasally sensitized with SEA. At 12 hours after the final nasal challenge, mice were killed. Detection of *in vivo* apoptosis in nasal mucosa was performed using TdT-mediated dUPT-biotin nick labeling (TUNEL) method.

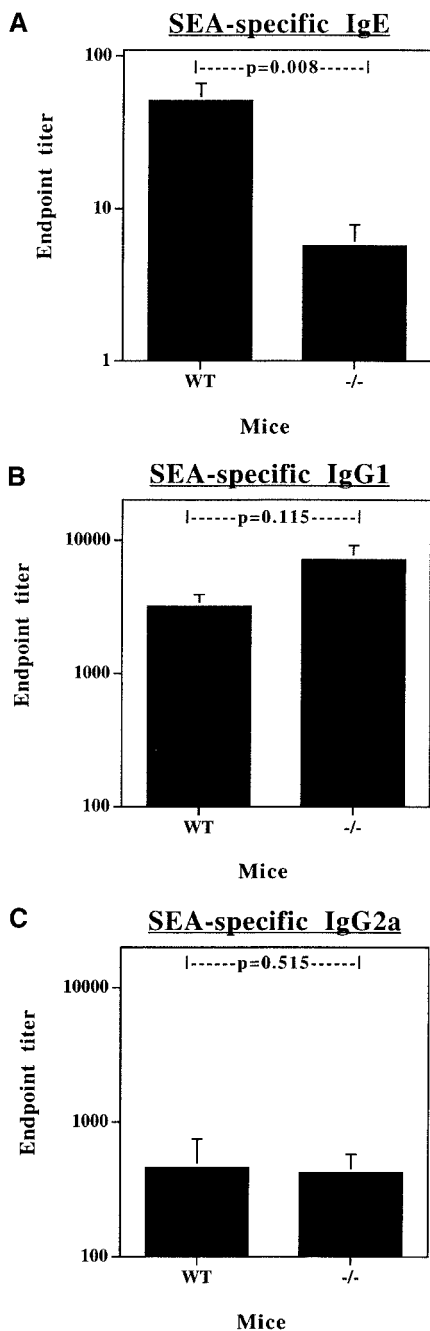


Figure 4. Antibody production in high-responder BALB/c mice after intranasal sensitization with SEA. After final nasal challenge, blood was sampled from WT and Fc γ RIIB $-/-$ ($-/-$) mice, and levels of serum SEA-specific IgE (A), IgG1 (B), and IgG2a (C) were determined by ELISA. Results show mean endpoint titer $\pm$ SEM of six serum samples from each group. Data are representative of three separate experiments.

lower than that observed in mice actively sensitized with SEA (Figure 1). The numbers of eosinophils infiltrating into nasal septum per field (10×40) were 11.8 ± 2.8 and 25.2 ± 3.6 in Fc γ RIIB $+/+$ and $-/-$ BALB/c mice, respectively (mean $\pm$ SEM, $n = 6$, $p = 0.016$). Furthermore, either SEA-specific IgE, IgG1, or IgG2a was not detected in serum from mice passively sensitized with sera from SEA nonsensitized mice and subsequently challenged intranasally with SEA.

DISCUSSION

Fc γ RIIB plays an inhibitory role in both IgE- and IgG-mediated anaphylactic responses during the effector phase of allergy (14). This study examined the role of Fc γ RIIB during the induction phase of allergy using a murine model of allergic rhinitis (6, 27). To date, the involvement of Fc γ RIIB in the production of IgE has not been investigated. In terms of pre-B-cell receptor-positive pre-B cells, recent study demonstrated that Fc γ RIIB negatively regulates pre-B-cell receptor-mediated signaling for apoptosis (29). We found that SEA-specific IgE production in Fc γ RIIB $-/-$ mice was significantly decreased after intranasal sensitization with SEA as compared with that of wild-type BALB/c mice (Figure 4). This result suggests that signals through Fc γ RIIB play an important role in increasing antigen-specific IgE synthesis in this model. The decreased production of IL-4 by nasal mononuclear cells from Fc γ RIIB $-/-$ mice in response to SEA restimulation (Figure 5) supports this result as IL-4 plays a critical role in *in vivo* IgE synthesis (30). Although IL-4 displays the inhibitory effect on the expression and function of Fc γ RII (31), the mechanisms governing why a deficiency of Fc γ RIIB leads to the inhibition of IL-4 production remain to be elucidated.

After intranasal sensitization with SEA, Fc γ RIIB $-/-$ BALB/c mice developed a severe eosinophilia in the nasal mucosa as compared with wild-type BALB/c (Figure 1). Several investigators have demonstrated that Fc γ RIIB plays an inhibitory role in the induction of tissue inflammation (14, 21–23). For example, Fc γ RIIB $-/-$ mice with immune complex-mediated alveolitis developed enhanced alveolar hemorrhage, increased interstitial neutrophil infiltration, and perivascular edema as compared with wild-type mice in (21). Fc γ RIIB $-/-$ mice immunized with collagen type IV develop massive pulmonary hemorrhage with neutrophil and macrophage infiltration and crescent glomerulonephritis (23). In addition, hemorrhage in ileum villi is increased in Fc γ RIIB $-/-$ mice during IgE-mediated systemic anaphylaxis (15). Our results are consistent with these findings and suggest that Fc γ RIIB inhibits eosinophilic inflammation.

Although it is unknown whether the functions of human eosinophils are mediated by Fc γ RIIB, Fc γ RIIA/C, or both, human and mouse eosinophils express Fc γ RII, and murine Fc γ RIIB is involved in the survival, degranulation, or apoptosis of eosinophils (17, 32, 33). In addition, ligation of Fc γ RIIB on not only developing but also mature eosinophils isolated from hepatic granulomas from *S. mansoni*-infected mice induces their destruction through Fas-mediated apoptosis (17). More recently, Kim and colleagues demonstrated that Fc γ RII ligation pivotally regulates both the survival and death of eosinophils (33). This study found that nasal mononuclear cells from both Fc γ RIIB $+/+$ and $-/-$ mice produced similar amounts of IL-5 after intranasal sensitization with SEA (Figure 5). One explanation of why nasal eosinophilia was exacerbated in Fc γ RIIB $-/-$ mice irrespective of local IL-5 production is a lack of direct ligation of *S. mansoni*-specific IgG on Fc γ RIIB on eosinophils. In addition, TUNEL-positive cells in the nasal mucosa were rarely seen in both wild type and Fc γ RIIB $-/-$ mice after the intranasal challenge with SEA (Figure 3), suggesting that *in vivo* apoptosis of eosinophils triggered by Fc γ RIIB less contributes to the suppression of nasal eosinophilia in this model.

The production of both antigen-specific IgE and IgG1 is positively regulated by Th2 responses in mice, and antigen-specific IgE and nasal eosinophilia are two of the major indicators of the initiation of allergic rhinitis. However, recent investigations have demonstrated that the production of antigen-specific IgE and of specific IgG1 and/or local eosinophilia is not definitely correlated (30, 34). IL-4 appears not to be essential for IgG1 class switching or nasal eosinophilia but plays a crucial role in

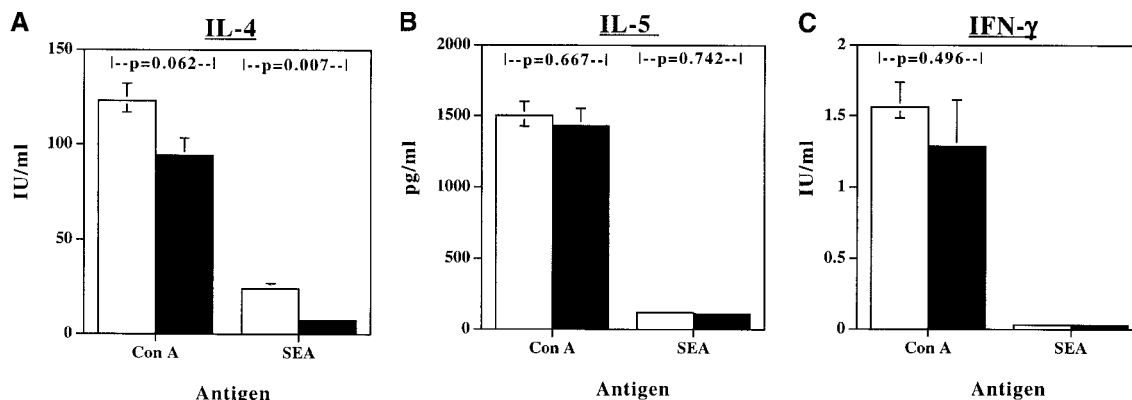


Figure 5. Production of interleukin (IL)-4 (A), IL-5 (B), and IFN- γ (C) by nasal mononuclear cells from BALB/c wild type (open bars) and Fc γ RIIB $-/-$ (closed bars) mice. Mice were intranasally sensitized and subsequently challenged with SEA. At 12 hours after final nasal challenge with SEA, nasal mononuclear cells were isolated and cultured *in vitro* for 72 hours with Concanavalin (Con) A, SEA, or medium alone. Cytokines were measured by ELISA. Results show means $\pm$ SEM of three individual pools. Data are representative of two separate experiments.

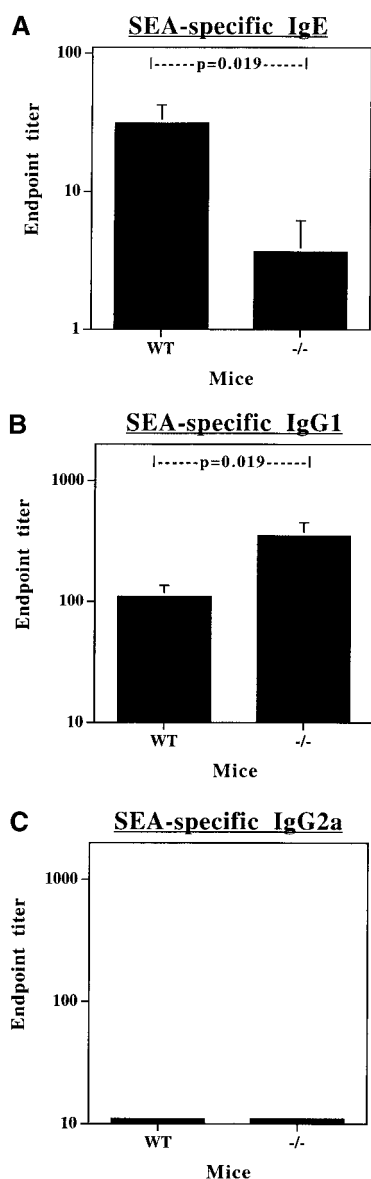


Figure 6. Antibody production in low-responder C57BL/6 mice after intranasal sensitization with SEA. WT and Fc γ RIIB $-/-$ C57BL/6 mice were intranasally sensitized with SEA under a specific pathogen-free (SPF) condition. After final nasal challenge, blood was sampled, and levels of serum SEA-specific IgE (A), IgG1 (B), and IgG2a (C) were determined by ELISA. Results show mean endpoint titer $\pm$ SEM of six serum samples from each group. Data are representative of two separate experiments.

IgE production (30). For example, we recently reported that titers of antigen-specific IgE in IL-4 gene-deficient (IL-4 $-/-$) BALB/c mice sensitized with SEA are negative, whereas those of specific IgG1 are significantly lower but not abolished compared with wild-type control mice (30). We also identified sex-related differences in the production of IgE but not of IgG1 in CBA/J mice after intranasal sensitization (34).

In addition, antibody production and the development of nasal eosinophilia were seen in C57BL/6 mice after intranasal sensitization with SEA under specific pathogen-free conditions. SEA-specific IgG1 levels were elevated. Nasal eosinophilia was exacerbated, and conversely, levels of specific IgE were decreased in Fc γ RIIB $-/-$ C57BL/6 mice compared with wild-type C57BL/6 mice (Figures 1 and 6). The genetic background of these mice has potential for modulating the initiation of Th2 responses (6, 35). In fact, SEA-specific IL-4 production by nasal mononuclear cells was not detected in either wild-type or Fc γ RIIB $-/-$ C57BL/6 mice despite that both of the mice could produce the detectable amounts of SEA-specific IgE after the intranasal sensitization. As described by Bix and colleagues, IL-4 production was less in C57BL/6 mice as compared with BALB/c mice (36). Thus, this discrepancy may arise from a possibility that SEA-specific IL-4 production by C57BL/6 mice was too small to detect above the detection limit (0.1 IU/ml). This difference in IL-4 production may lead to the ability in SEA-specific IgE production between the two strains (Figures 4 and 6). These results suggest that the regulatory effect of Fc γ RIIB on the initiation of allergic rhinitis after intranasal sensitization with SEA is not restricted to specific mouse strains as it appears in both high- and low-responder strains.

In contrast, specific IgG1 production was significantly increased in Fc γ RIIB $-/-$ C57BL/6 mice. IgM and IgG production was augmented in Fc γ RIIB $-/-$ mice after immunization with both thymus-dependent and thymus-independent antigens (25). In a murine model of collagen-induced arthritis, more IgG1, IgG2a, IgG2b, and IgM antibodies specific for bovine type II collagen were produced in Fc γ RIIB $-/-$ mice after immunization with bovine type II collagen (22). In addition, Fc γ RIIB $-/-$ mice produced more IgG1, IgG2a, IgG2b, IgG3, and IgM but not IgA specific for bovine type IV collagen after immunization with bovine type IV collagen (23). Our results appear to be consistent with the findings of the reports described previously here and suggest that Fc γ RIIB plays an inhibitory role in the production of antigen-specific IgG1 (the dominant IgG isotype

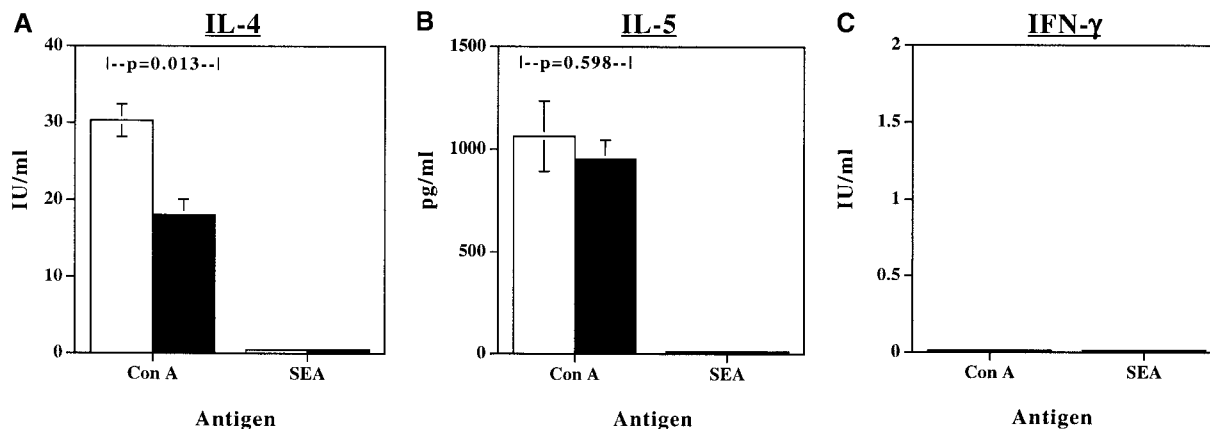


Figure 7. Production of IL-4 (A), IL-5 (B), and IFN- γ (C) by nasal mononuclear cells from C57BL/6 wild-type (open bar) and Fc γ RIIB $-/-$ (closed bar) mice under SPF conditions. Mice were intranasally sensitized and subsequently challenged with SEA. Twelve hours after final nasal challenge with SEA, nasal mononuclear cells were isolated and cultured *in vitro* for 72 hours with Con A, SEA, or medium alone. Cytokines were measured by ELISA. Results show mean $\pm$ SEM of three individual pools. Data are representative of two separate experiments.

in this model) after intranasal sensitization with SEA (6). On the other hand, similar amounts of SEA-specific IgG2a were seen in Fc γ RIIB $-/-$ and wild-type mice, suggesting that Fc γ RIIB does not play an inhibitory role in the production of minor IgG isotypes in this model.

The route of sensitization and/or the use of adjuvants can influence the initiation of Th2 responses (35, 37). We therefore sensitized Fc γ RIIB $+/+$ and $-/-$ mice intraperitoneally with SEA adsorbed to alum, an adjuvant that is frequently used to induce experimental allergy in mice (38). Compared with wild-type BALB/c mice, specific IgE production in Fc γ RIIB $-/-$ mice was significantly decreased (Figure 8A). These results suggest that the role of Fc γ RIIB in the regulation of specific IgE production was the same regardless of the route of SEA sensitization.

Furthermore, when Fc γ RIIB $-/-$ mice were passively sensitized with SEA by intravenous injection of presensitized sera, nasal eosinophilia increased, and conversely, IgE production decreased after a nasal challenge with SEA (Figure 9). Passive sensitization together with local antigen challenge results in allergic inflammation characterized by local eosinophilia (39). These results suggested that the role of Fc γ RIIB in local eosinophilia and IgE synthesis is the same between active and passive sensitization with SEA.

In conclusion, we found that nasal eosinophilia is exacerbated, whereas the production of specific IgE is decreased in Fc γ RIIB $-/-$ mice after intranasal sensitization with SEA. Moreover, the involvement of Fc γ RIIB in the initiation of allergic rhinitis seems not to be restricted to a specific mouse strain. In addition, the route and type of sensitization did not alter the effect of Fc γ RIIB. These results suggest that the regulation of expression and/or function of Fc γ RIIB will be a useful tool with which to control allergic inflammation characterized by nasal eosinophilia.

Conflict of Interest Statement: T.W. has no declared conflict of interest; M.O. has no declared conflict of interest; H.H. has no declared conflict of interest; T.Y. has no declared conflict of interest; N.O. has no declared conflict of interest; Y.S. has no declared conflict of interest; Y.O. has no declared conflict of interest; T.T. has no declared conflict of interest; K.N. has no declared conflict of interest.

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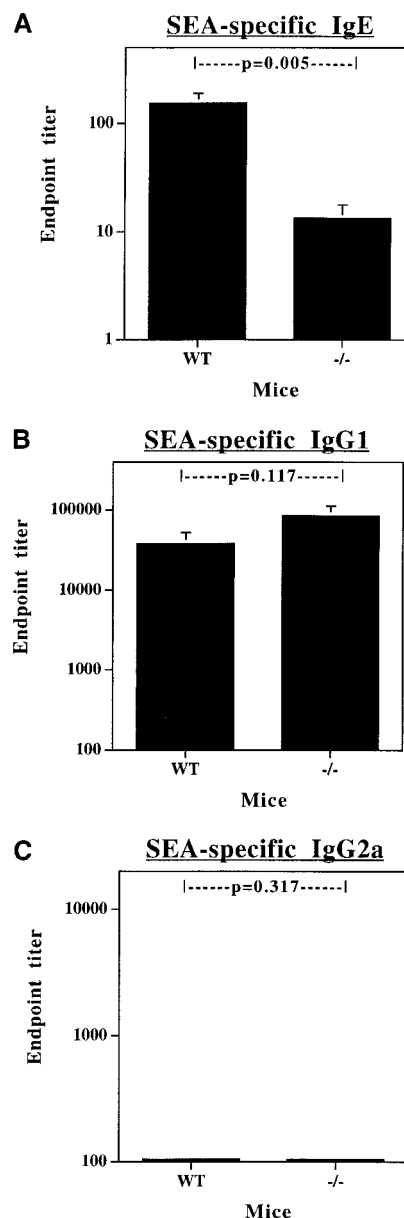


Figure 8. Antibody production after intraperitoneal sensitization with SEA. WT and Fc γ RIIB $-/-$ BALB/c mice were primed and intraperitoneally boosted with SEA adsorbed to alum. At 1 week after boosting, blood was sampled, and levels of serum SEA-specific IgE (A), IgG1 (B), and IgG2a (C) were determined by ELISA. Results show mean endpoint titer $\pm$ SEM of six serum samples from each group. Data are representative of two separate experiments.

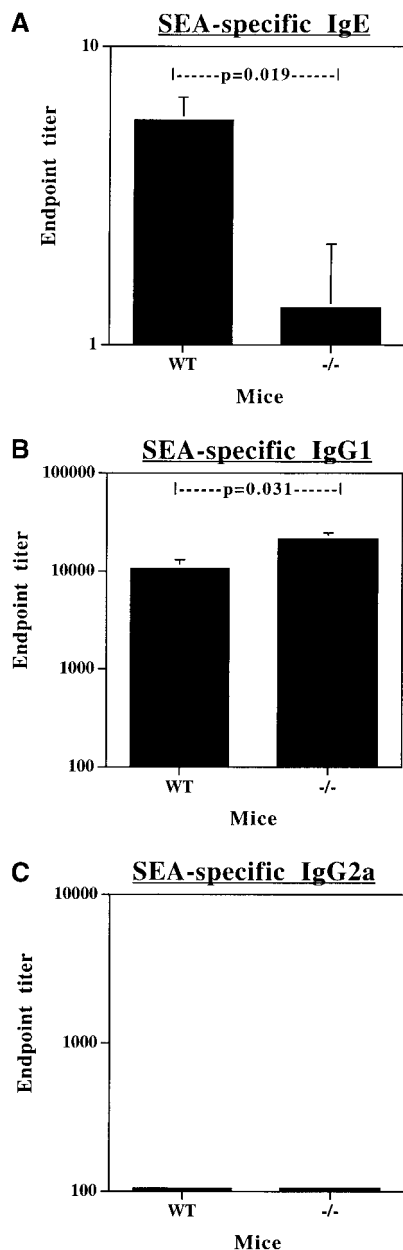


Figure 9. Antibody production after passive sensitization with SEA. WT and FcγRIIB $-/-$ BALB/c mice were passively sensitized with SEA by intravenous injection of pooled sera from presensitized or nonsensitized mice. One hour after passive sensitization, the mice were challenged intranasally with 20 μ l of 1 μ g SEA for 7 consecutive days. Blood was sampled, and levels of serum SEA-specific IgE (A), IgG1 (B), and IgG2a (C) were determined by ELISA. Results show mean endpoint titer $\pm$ SEM of six serum samples from each group. Data are representative of two separate experiments.

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