eluted from the carrier with ethyl acetate. Thromboxane B_2 antibody was added to the eluate to react with the antigen. The concentration of radioactivity in the antigen–antibody reaction product was used to determine concentration of TXB_2.

Statistical analysis

Statistical analysis was performed using the Wilcoxon signed-ranks test and all results are expressed as the mean ± SD.

RESULTS

Nasal symptoms

Changes in symptom scores during the three evaluation periods were assessed for each symptom in individual subjects (Fig. 1). Compared with the pretreatment period, the score for sneezing during the treatment period decreased in all subjects. In addition, except for one subject whose symptoms were aggravated due to a cold, in nine other subjects the same downward trend was observed for nasal obstruction and rhinorrhea scores during the treatment period.

After finishing treatment with ramatroban, in general, the symptoms that had been improved during the treatment period did not revert to levels seen during the pretreatment period, but remained as low as during the treatment period.

Nasal antigen challenge test

In eight of 10 patients, a lesser degree of reactivity to antigen challenge was recorded on day 32 compared with day 1. The volume of nasal fluid collected was also decreased or unchanged in the majority patients when results from day 32 and day 1 were compared. However, the resistance of the nasal cavity decreased in fewer patients than did reactivity and volume of nasal fluid (Table 4).

Concentration of mediators in nasal fluid

The concentrations of histamine and TXB_2 detected in the nasal fluid collected following antigen challenge tests in the post-treatment period were lower than concentrations in the nasal fluid during the pretreatment period in seven and eight patients, respectively (Fig. 2).

Table 4 Summary data for clinical efficacy and pre- and post-treatment nasal antigen challenge test

Patient no.	Age (years)	Sex	Clinical efficacy			Pre-treatment nasal antigen challenge test			Post-treatment nasal antigen challege test		
			Pre-treatment symptom score	Post-treatment symptom score	Evaluation	Reactivity	Volume nasal fluid (mL)	Resistance of nasal cavity (p : 50)	Reactivity	Volume nasal fluid (mL)	Resistance of nasal cavity (p : 50)
1	24	Male	2.0	1.9	–	++	0.4	Off scale	+	0.3	Off scale
2	29	Male	1.4	1.0	+	++	1.3	Off scale	+	0.3	0.11
3	22	Male	3.0	1.0	++	++	0.6	6.74	–	0.1	Off scale
4	23	Male	1.9	1.3	+	+	0.4	1.10	+	0.4	0.32
5	24	Male	1.0	1.0	–	++	0.3	Off scale	+	0.9	Off scale
6	32	Male	1.0	0.1	+	++	0.3	0.19	+++	0.6	0.28
7	25	Male	2.4	1.7	+	++	1.0	0.64	+	1.0	1.09
8	21	Male	2.1	1.9	–	+	0.2	0.26	–	0.1	Off scale
9	21	Male	2.9	1.6	++	++	1.3	Off scale	+	1.0	0.17
10	26	Male	1.9	3.0	–	++	0.4	0.12	+	0.2	0.12

Off scale indicates cases where no measurements were available due to excessive nasal obstruction with the resistance values p > 100 where p is Pascal.

The efficacy notations –, + and ++ corresponded to cases where the difference in symptom scores was <0.4, <1.0 and >1.0, respectively. For reactivity: +++, three symptoms (sneezing, nasal discharge and nasal obstruction) and six or more sneezing episodes; ++, three symptoms and five or fewer sneezing episodes; +, two symptoms and no counted sneezing episodes; –, no symptoms.

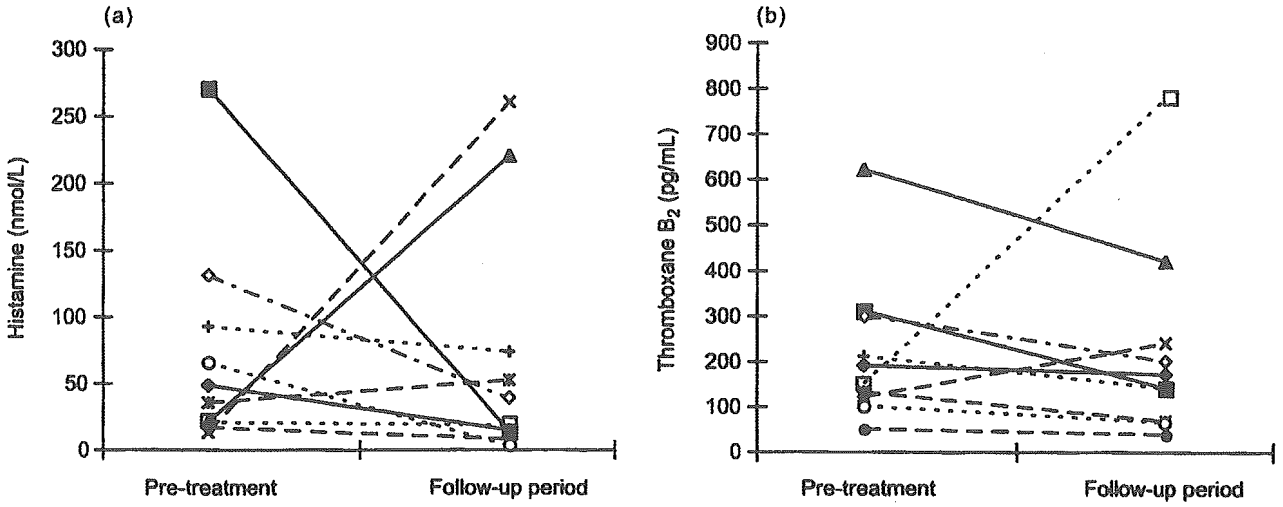

Fig. 2 (a) Histamine and (b) thromboxane B_2 concentrations in nasal discharge induced by nasal antigen challenge during the pretreatment and follow-up periods after ramatroban treatment.

The concentrations of histamine and TXB_2 in the nasal fluid sampled at two points are shown in Fig. 2. The concentrations of histamine and TXB_2 after treatment with ramatroban decreased from levels seen during the pretreatment period in eight and seven subjects, respectively. Median values of the concentration for both histamine and TXB_2 show a downward trend, but no significant difference was detected ($P = 0.492$ and $P = 0.332$, respectively).

Correlation of three individual nasal symptoms with the concentration of histamine and TXB_2 was also investigated during the pre- and post-treatment periods, independently. Among six analyses, as shown in Fig. 3, a positive correlation was shown only for nasal obstruction and TXB_2 concentration during the pretreatment period. Other analyses did not reach statistical significance.

Discussion

Ramatroban, a novel TXA_2 receptor antagonist, has been investigated in a series of clinical trials to confirm its efficacy in the treatment of patients with allergic rhinitis[12] and it is now available for daily use in Japan. The efficacy of ramatroban in improving nasal obstruction was of particular attraction to physicians, who often face difficulties in relieving patients from nasal obstruction.

Terada *et al.*,[6] who conducted antigen challenge tests with allergic rhinitis patients treated with ramatroban and measured changes in nasal cavity volume, reported that ramatroban significantly suppressed nasal obstruction within 2 h after administration. They also reported that the albumin concentration in the nasal fluid and a vascular permeability index were decreased following the administration of ramatroban. In another study,[13] which examined the migration of cells involved in nasal obstruction, it was confirmed that ramatroban lessened both the eosinophil count and the concentration of eosinophil cationic protein in nasal wash water. These results strongly support the clinical efficacy of ramatroban in improving nasal obstruction.

To further investigate the effects of ramatroban, we performed a clinical investigation with two major objectives. One of the objectives was to reconfirm the efficacy of ramatroban by comparing symptom levels during the pretreatment period with those after 4 weeks treatment with ramatroban. The other objective of the study was to determine the relationships between symptoms and the secretion of chemical mediators in the nasal fluid, namely histamine and TX, which are known to provoke allergic reactions in patients with allergic rhinitis.

Of 10 untreated male patients with moderate to severe perennial allergic rhinitis who were administered ramatroban for 28 days, nine experienced an improvement in nasal obstruction. An interesting observation was that the scores for nasal obstruction came were reduced at an earlier stage of the treatment period than expected (data not shown). In parallel with the improvement in nasal obstruction, scores for sneezing and a runny nose

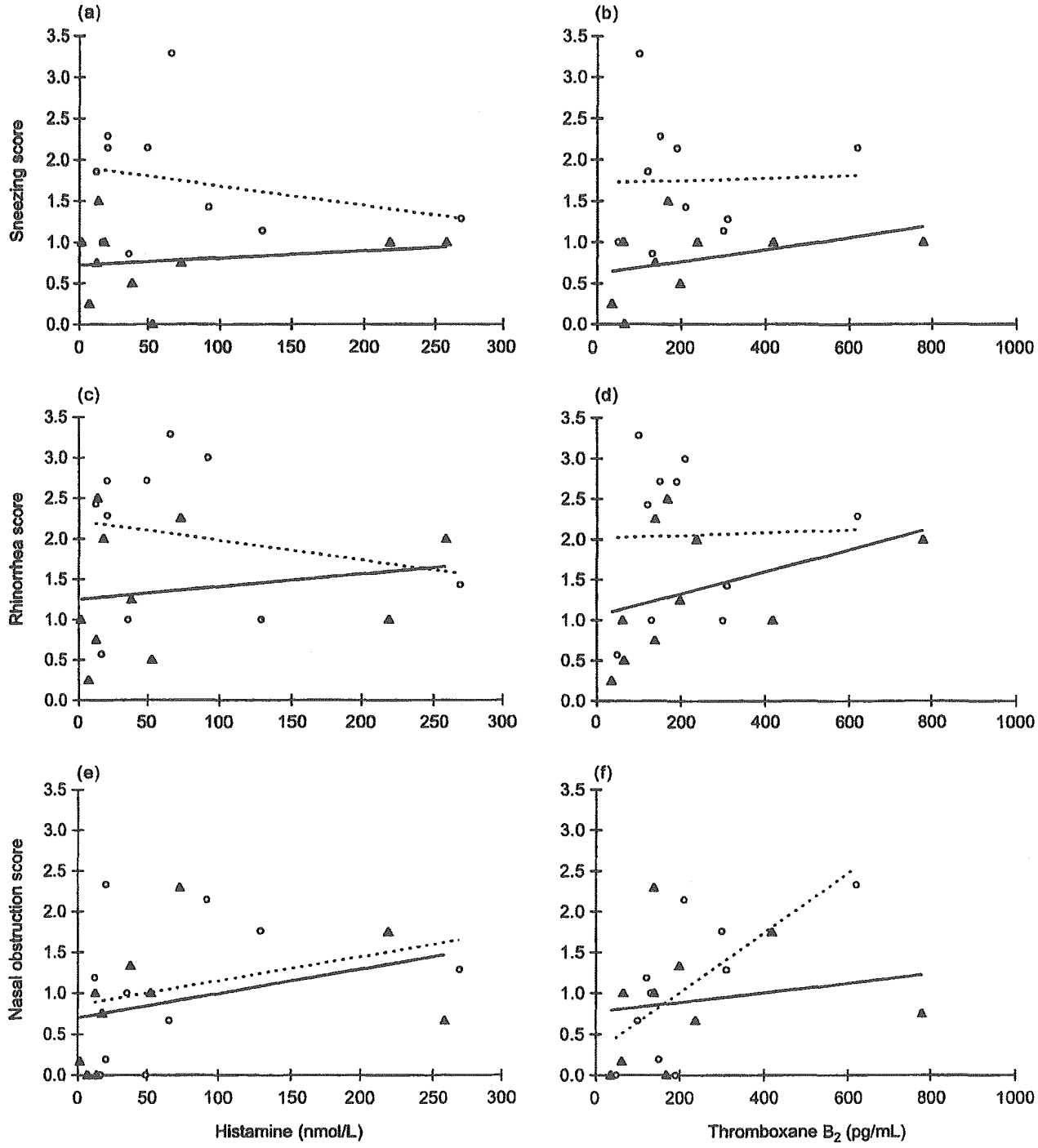

Fig. 3 Correlation of nasal symptoms, namely sneezing (a,b), rhinorrhea (c,d) and nasal obstruction (e,f), and concentrations of histamine (a,c,e) and thromboxane B_2 (b,d,f) in nasal discharge induced by nasal antigen challenge during the pretreatment period (O; -----) and during follow up (▲; ——). The regression equations for the pretreatment and follow-up periods are as follows: (a) $y = -0.0023x + 1.9084$ ($R^2 = 0.0623$) and $y = 0.0008x + 0.7158$ ($R^2 = 0.0332$), respectively; (b) $y = 0.0001x + 1.7121$ ($R^2 = 0.001$) and $y = 0.0007x + 0.6105$ ($R^2 = 0.1423$), respectively; (c) $y = -0.0024x + 2.2134$ ($R^2 = 0.0399$) and $y = 0.0016x + 1.2414$ ($R^2 = 0.034$), respectively; (d) $y = 0.0002x + 2.0073$ ($R^2 = 0.0008$) and $y = 0.0014x + 1.0426$ ($R^2 = 0.1512$); (e) $y = 0.0029x + 0.8488$ ($R^2 = 0.0758$) and $y = 0.003x + 0.6888$ ($R^2 = 0.1339$), respectively; and (f) $y = 0.0037x + 0.2575$ ($R^2 = 0.5003$) and $y = 0.0006x + 0.76448$ ($R^2 = 0.0299$), respectively.

also started decreasing at around day 3 and became stable around day 10. As a result, similar score changes in terms of time to onset and improvement tendency were seen across the three symptoms. Another finding was that ramatroban was effective not only in improving nasal obstruction, which was more or less expected from its mode of action and the results of clinical trials already reported,[12] but also in relieving sneezing and rhinorrhea and that the effect of ramatroban on these two symptoms was maintained even during the post-treatment period in the majority of patients.

To investigate the relationships between symptoms and the secretion of chemical mediators in the nasal fluid, an antigen challenge test was conducted before and after the treatment period. Thromboxane is thought to be involved in the process of developing nasal obstruction in patients with perennial allergic rhinitis and, because ramatroban is a TXA_2 receptor antagonist, it was predicted that TXB_2 would have been more sensitively influenced than histamine by treatment with ramatroban. However, the concentration of histamine in antigen-induced nasal discharge decreased to the same extent as did the concentration of TXB_2, which was in contrast with the prediction prior to the study.[5] With regard to the relationships of the concentrations of the mediators and clinical symptoms recorded in the patient diary, a statistical correlation was found only for nasal obstruction scores and TXB_2 concentration during the pretreatment period; no other combinations between any of the symptom scores and the concentration of histamine or TXB_2 during either the pre- or post-treatment period were correlated.

The results of the present study indicate that the TXA_2 receptor antagonist ramatroban can be used not only for allergic rhinitis patients with nasal obstruction, but also for those patients with sneezing and a runny nose in addition to nasal obstruction. These findings in perennial allergic rhinitis are in line with a report by Suzaki *et al.* that ramatroban effectively improved severe sneezing and rhinorrhea in patients with seasonal allergic rhinitis in a randomized study.[14] Based on the antigen challenge test results, it can be considered that the decrease in the concentration of TXB_2 produced by ramatroban may cause alterations in the nasal mucosa that resulted in a decrease in the total level of mast cell-related mediators and, consequently, an improvement in sneezing and rhinorrhea as well as nasal obstruction. It could be also suggested that ramatroban stabilizes mast cells or decreases the metabolism of arachidonic acid so that the amount of mediators released decreases. In contrast, there is a report that TXA_2 receptors may be present only in blood vessels.[2] If this is the case, another possible explanation for the results of the present study is that the broad improvement in symptoms following ramatroban could be a result of the inhibitory effects on vascular permeability and eosinophil infiltration, which is consistent with the overall results of the antigen challenge test. However, the decrease in histamine concentration in the same antigen challenge test cannot be fully explained by this speculation. Therefore, for the time being, no evident explanation can be given for the broad beneficial effects of ramatroban on the clinical symptoms of allergic rhinitis, as well as hypersensitivity of the nasal mucosa. The detailed pharmacological mechanism and clinical outcomes of ramatroban should be investigated further.

ACKNOWLEDGMENT

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Original Article

Repeated antigen challenge in patients with perennial allergic rhinitis to house dust mites

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Abstract

Background: In allergic rhinitis, antigen–antibody reactions occur in the nasal mucosa through antigen exposure. A strong reaction occurs following exposure to a large amount of antigen, whereas no reaction occurs in the absence of antigens. In seasonal allergic rhinitis, nasal hypersensitivity increases during the pollen-dispersing season, which is known as the 'priming effect'. The purpose of the present study was to clarify whether repeated nasal challenges bring about increased nasal hypersensitivity in patients with perennial allergic rhinitis.

Methods: Fourteen patients with perennial allergic rhinitis to house dust mites were enrolled in the present study. Repeated challenge tests were performed once daily for 8 consecutive days with a fixed amount of antigen.

Results: Sneezing and nasal secretion were slightly enhanced by repeated challenges only on the 2nd and 3rd days, whereas nasal resistance remained unchanged. Increased sneezing and nasal secretion was marked in a group of subjects who were not sneezing at the first challenge, whereas changes in nasal reaction following repeated challenge were less obvious in subjects who were sneezing at the first challenge.

Conclusions: In contrast with pollinosis, nasal provocation reactions were not clearly enhanced by repeated provocation. To further understand nasal reactions induced by antigen challenge, studies should be performed under specified conditions (i.e. in an experimental room) with a prescribed quantity of antigens administered, as well as within a study environment.

Key words: nasal provocation test, perennial allergic rhinitis, priming effect, repeated antigen challenge.

Introduction

In type I allergic diseases, antigen–antibody reactions occur in the nasal mucosa, bronchus and/or skin through antigen exposure, leading to local allergic reactions and local sensitivity to antigens. The intensity of the allergic reaction depends on the quantity of antigen; a strong reaction occurs following exposure to a large amount of antigen, whereas no reaction occurs in the absence of antigens. In seasonal allergic rhinitis, Japanese cedar pollinosis, the most common pollinosis in Japan, antigen-specific nasal hypersensitivity increases during the pollen-dispersing season, which is known as the 'priming effect', but returns to a level comparable with that of non-allergic people during the off-season.[1]

Connell proposed the term 'priming effect' for the first time on the basis that repeated daily pollen challenges on 7 successive days decreased the nasal threshold for allergic rhinitis and that environmental exposure during the ragweed pollinating season primed ragweed-sensitive individuals.[1] Thereafter, Bacon *et al.*[2] and Borum *et al.*[3] confirmed this phenomenon. In contrast, Schumacher and Pain,[4] van Wijke *et al.*,[5] Grammer *et al.*,[6] Small *et al.*[7] and Doyle *et al.*[8] failed to demonstrate this phenomenon. In Japan, Konno[9] conducted repeated nasal challenges for 4 consecutive days in patients with Japanese cedar pollinosis using the paper disc method and revealed an increased manifestation of sneezing and discharge, but not nasal airway resistance, comparing provocation reactions on the 1st and

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4th days. Yoshida *et al.*[10,11] also performed repeated nasal challenges for 6 consecutive days in patients with Japanese cedar pollinosis using the paper disc method and demonstrated that the early phase reaction progresses following repeated challenge.

To our knowledge, studies of 'priming effects' have been performed by repeated challenge with pollen in patients with pollinosis but have not been documented in rhinitis patients with house dust mite allergy, the most common perennial allergy in Japan. This phenomenon, although it remains controversial, has practical importance in mite allergy compared with pollinosis, because allergen exposure is perennial and prolonged, leading to stronger priming of the nasal mucosa in mites allergy. For the first time, we have attempted to confirm whether this phenomenon occurs following repeated nasal challenge with discs containing dust mite allergen.

METHODS

Subjects

Fourteen patients with perennial allergic rhinitis to house dust mites, a major causative antigen in Japan, were enrolled in the present study. According to the *Japanese Practice Guideline for Allergic Rhinitis* (revised 3rd edn),[12] patients were diagnosed as having allergic rhinitis if they had allergy like symptoms together with positive results in two or more of the following three tests: (i) skin test or determination of serum antigen-specific IgE; (ii) nasal provocation test; and (iii) test for nasal eosinophilia.[12] Fourteen male patients with allergic rhinitis, having a class 3 or higher for serum antigen-specific IgE antibodies (CAP-RAST), and who were positive in the nasal provocation test (degree 2 or more severe) were enrolled in the present study (Table 1). These 14 subjects were also confirmed to have a negative response following nasal challenge with a control disc without allergen. Subjects ranged in age from 21 to 29 years, with a mean age of 24.2 years. None suffered from seasonal rhinitis and rhinoscopy did not reveal any gross anatomical abnormalities. There was a 1 month washout period before the present study and none of the subjects received astemizol in the 6 weeks prior to the study. None had received immunotherapy. All patients gave informed consent. The study was conducted under controlled conditions with hospitalization.

Table 1 Subjects' background

	n	Total
Type of disease		
Sneeze/secretion	1	14
Sneeze/secretion/obstruction	13	
Severity		
Mild	3	14
Moderate	6	
Severe	5	
Duration of disease		
5–< 10 years	1	14
10–< 20 years	12	
> 20 years	1	

Methodology

The nasal challenge test was performed using the paper disc method[13] for 8 consecutive days at 24 h intervals using an equal quantity of antigens at 19.00 h. Changes in nasal symptoms induced during the 5 min period after provocation were evaluated as follows: (i) sneezing or an itchy sensation of the nose; (ii) watery nasal secretion; and (iii) nasal mucosal swelling. Nasal secretions were collected by blowing the nose with a tissue after antigen provocation for 5 min and the tissue was then weighed. The difference in weight between tissues before and after nose blowing gave the quantity of secretion induced. The weight of the tissue just before provocation was also analyzed, because this indicates the natural secretion of nose. Nasal resistance (P = 100 Pa) was measured with a rhinoanemometer (anterior induction method) 20 min after antigen provocation (Fig. 1). Nasal resistance was hypothesized as 100 Pa/cm^3 per s when scaled out.

In the nasal challenge test, two discs (Allergen Disc 'Torii' House Dust; Torii Pharmaceutical, Tokyo, Japan) were placed on the anterior edge of the inferior turbinate mucosa on each side; each disc is a piece of small, round filter paper, 3 mm in diameter, that contained 250 μg crude allergen extract from house dust mites (33.3 ng Der f 1, a major allergic component). The concentrations of the major house dust mites allergens, Der p 1 and Der p 2, were measured in dust collected by vacuuming the hospital room. The allergen levels for both Der p 1 and Der p 2 were < 0.10 μg/g fine dust.

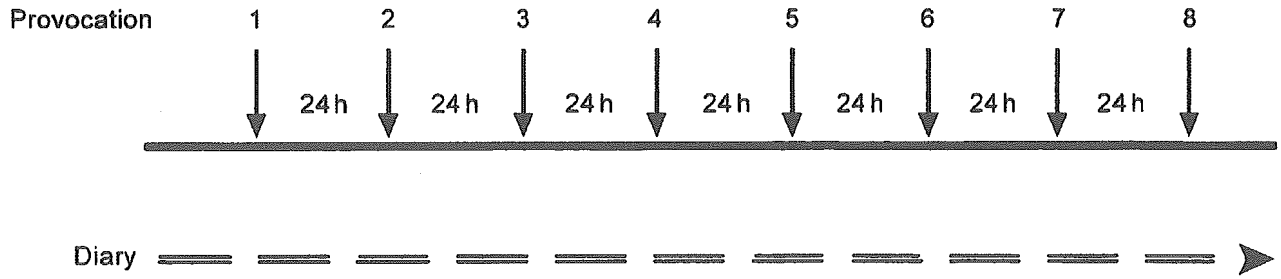

Fig. 1 Time-course of nasal antigen challenge. The arrows indicate nasal challenge with the house dust mites disc.

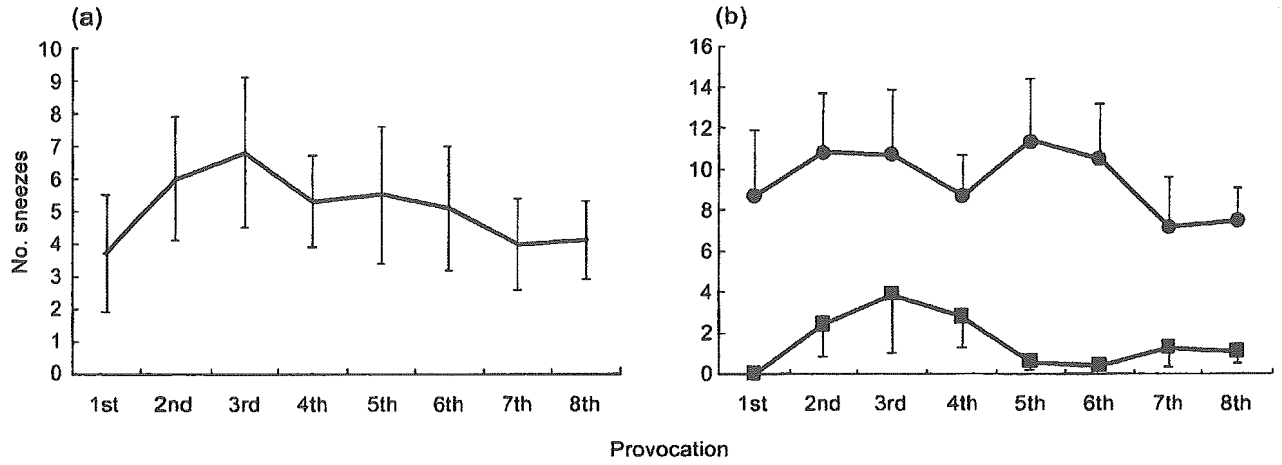

Fig. 2 Changes in the number of sneezes of (a) all subjects and (b) the low- (■; n = 7–8) and high-reaction (●; n = 6) groups. Data are the mean ± SEM.

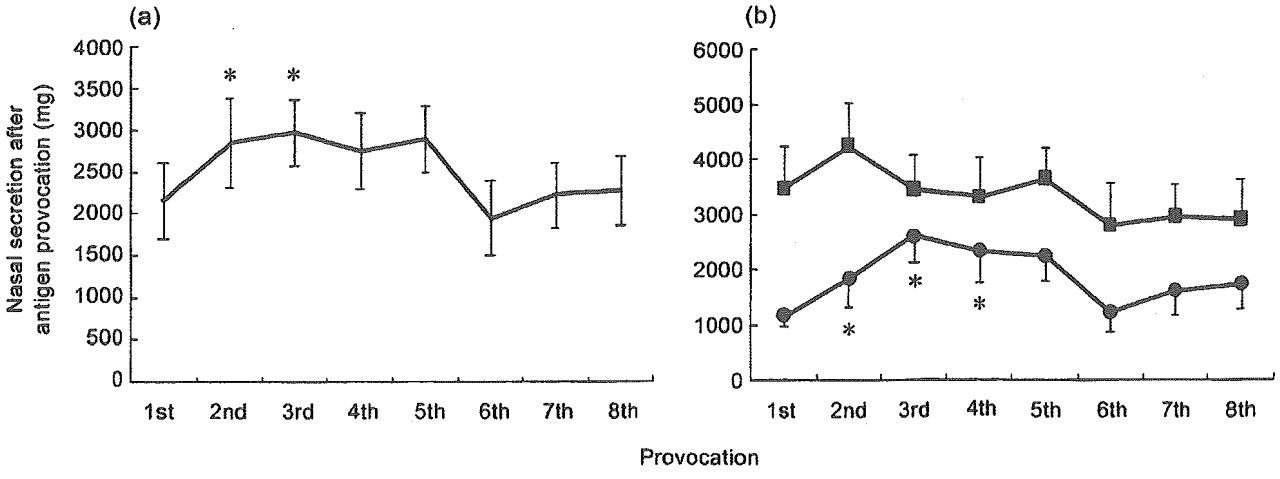

Fig. 3 Changes in the quantity of nasal secretion of (a) all subjects and (b) the low- (■; n = 7–8) and high-reaction (●; n = 6) groups. Data are the mean ± SEM. *P < 0.05 compared with the initial provocation.

Statistics

Nasal symptoms at each measurement point were compared with those observed at the first challenge with antigen. The Wilcoxon signed-rank test was used for statistical analysis and $P < 0.05$ was considered statistically significant.

RESULTS

Of 14 subjects enrolled, 13 completed the scheduled tests; one subject dropped out after the fourth provocation.

Number of sneezes

Sneezing tended to increase as the nasal challenges were repeated but gradually decreased after the forth challenge. However, the increase in sneezing was not statistically significant when compared with the initial challenge baseline.

Subjects were divided into groups depending on whether they were positive or negative for sneezing. The high-reaction group ($n = 6$) were positive for sneezing, whereas the low-reaction group ($n = 7$–8) did not sneeze at the initial challenge. There were no marked changes in the sneezing reaction in the high-reaction group during the course of the study, whereas sneezing tended to increase in the low-reaction group until the third provocation (Table 2; Fig. 2a,b).

Quantity of nasal secretion

The overall quantity of nasal secretion increased at the second and third antigen provocation compared with the initial challenge ($P < 0.05$). This change was more marked in the low-reaction group, like changes in the number of sneezes. Nasal secretion tended to decrease in the high-reaction group, but increased at the second–fourth provocations in the low-reaction group ($P < 0.05$; Table 2; Fig. 3a,b).

The quantity of nasal secretion just before each antigen challenge was followed up during the present study, but remained unchanged, even after the challenge had been repeated.

Nasal resistance

Changes in nasal resistance showed no clear tendency in response to repeated antigen challenge. Because

Table 2 Changes in induced nasal reactions

	Provocation							
	1st ($n = 14$)	2nd ($n = 14$)	3rd ($n = 14$)	4th ($n = 14$)	5th ($n = 13$)	6th ($n = 13$)	7th ($n = 13$)	8th ($n = 13$)
No. sneezes	3.7 ± 1.8	6.0 ± 1.9	6.8 ± 2.3	5.3 ± 1.4	5.5 ± 2.1	5.1 ± 1.9	4.0 ± 1.4	4.1 ± 1.2
Nasal secretion								
Before provocation (mg)	353.4 ± 97.1	307.6 ± 71.4	378.7 ± 127.6	233.6 ± 57.0	279.6 ± 93.1	157.4 ± 23.3	255.5 ± 70.7	194.8 ± 24.4
After provocation (mg)	2149.6 ± 456.6	2855.0 ± 537.9*	2976.0 ± 395.5*	2751.0 ± 452.7	2892.8 ± 404.4	1946.5 ± 450.7	2225.2 ± 390.1	2274.5 ± 422.5
Nasal resistance (Pa/cm³ per s)	28.81 ± 12.49	21.72 ± 11.34	0.27 ± 0.04	7.51 ± 7.12	7.95 ± 7.67	15.62 ± 10.39	7.99 ± 7.67	8.09 ± 7.66
Positivity score	2.1 ± 0.1	2.2 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	1.9 ± 0.2

Data are the mean ± SEM. *$P < 0.05$ compared with the initial provocation.
Thirteen subjects completed the schedule and only one subject dropped out after the fourth provocation.

nasal resistance scaled out in subjects with severe nasal obstruction, it was difficult to evaluate nasal resistance in all cases using the anterior induction method (Table 2; Fig. 4).

Overall positivity of nasal provocation reaction

Positivity of the nasal challenge reaction remained unchanged in the overall evaluation, even after antigen challenge had been repeated (Table 2; Fig. 5).

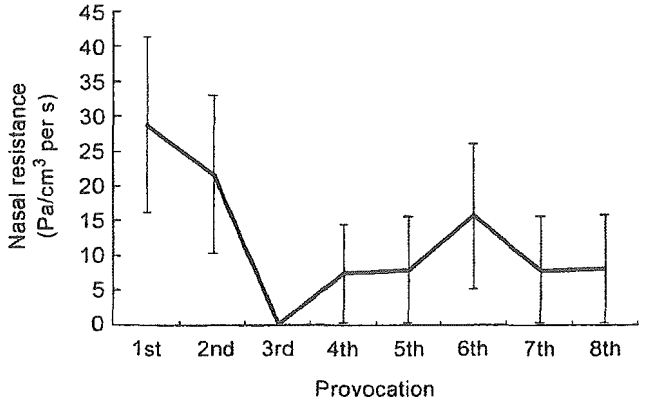

Fig. 4 Changes in nasal resistance. Data are the mean ± SEM. When scaled out, nasal resistance was hypothesized as 100 Pa/cm³ per s.

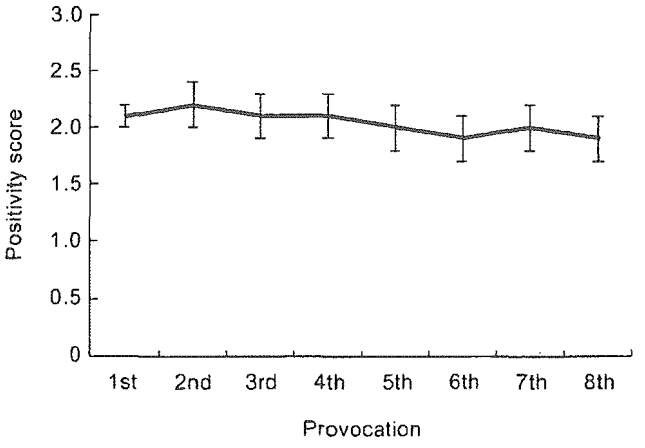

Fig. 5 Changes in positivity score. Data are the mean ± SEM.

Discussion

It has been reported that nasal hypersensitivity increases in patients with Japanese cedar pollinosis when antigen provocation is performed repeatedly.[9–11] In these studies, the quantity of antigens could be prescribed without inhalation of unexpected antigens from the natural environment because challenge tests were performed out of the pollen-dispersing season. In contrast, if patients with perennial allergic rhinitis are examined in a similar manner, it is impossible to strictly control the quantity of antigens, other than those used intentionally for provocation. Thus, the present study was conducted under controlled conditions with hospitalization, where the prevalence of both Der p 1 and Der f 1 was < 0.10 μg/g fine dust, to examine all subjects using the same quantity of antigens.

In the present study, the number and intensity of sneeze attacks tended to increase, peaking 3 days after the start of antigen challenge. The amount of nasal secretion after each challenge increased significantly on days 2–3 after the initial challenge. However, nasal resistance and overall intensity after the challenge test remained unchanged. Although nerve reflex-mediated changes, including changes in the number of sneezes and nasal secretion, were intensified by repeated challenge, no remarkable changes occurred in nasal resistance, which is mainly associated with vascular system reactions. Under the study schedule used in the present study, antigen provocation-induced nasal reactions, which involve the nervous system, increased, whereas no changes were observed in reactions such as nasal swelling or obstruction, in which the vascular system and migrating cell infiltration are thought to be deeply involved. It is suggested that individual symptoms may appear at different times according to the mechanisms of onset.

We failed to demonstrate clearly the priming effect in patients with house dust mite allergic rhinitis, unlike in ragweed pollinosis patients.[1] This controversial result may be dependent upon the types of allergen and the methods of provocation. Connell[1] challenged patients with pollen grains during both the pollen-dispersing and off seasons, whereas we did challenged patients with allergen discs in a mite-free environment and made baseline priming with mites the nasal challenge on the first day. Connell[1] and we repeated nasal allergen challenge on a daily basis for 7 consecutive days. Connell evaluated the priming effect by a decreased threshold of

allergen amount, whereas our evaluation examined changes in intensity of the overall response. Our method of evaluations is similar to that of Konno *et al.*, who examined patients with Japanese cedar pollinosis.[9] They showed a statistically significant difference in the degree of symptoms induced between the 2nd and 4th days. However, the changes reported by Konno *et al.*[9] were very small (i.e. 4.0 ± 0.74 *vs* 5.3 ± 3.5 sneezes; 1.95 ± 0.24 *vs* 3.05 ± 0.31 g nasal secretion; 80.7 ± 29.8 *vs* 90.7 ± 21.2% nasal airway resistance). These results, taken together with those of the present study and reports in the literature as mentioned in the Introduction, indicate that intensive prolonged exposure to pollen may induce the priming effect in patients with pollinosis, whereas acute short-time exposure to dust mites does not clearly cause the priming effect. This is a practical convenience if perennial exposure to dust mite allergen does not cause this phenomenon (i.e. increase in hypersensitivity, nasal provocation test can be repeated at short intervals for diagnosis and the duration of drug effects can be followed up by repeated provocation).

If it is true that the priming effect occurs in pollinosis but not in perennial dust mite rhinitis, what is the reason for this? The final conclusion needs further study regarding factors such as the quantity of allergen used, provocation in a hospital room, time intervals of antigen challenge and provocation methods.

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Roles of FcγRIIB in Nasal Eosinophilia and IgE Production in Murine Allergic Rhinitis

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The low-affinity IgG Fc receptor, FcγRIIB, 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 FcγRIIB 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. FcγRIIB gene-deficient (−/−) BALB/c mice displayed severe eosinophilia compared with that of wild-type counterparts. However, FcγRIIB −/− 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 FcγRIIB −/− mice, suggesting that the exacerbation of nasal eosinophila in FcγRIIB −/− mice is independent of the local IL-5 levels. The findings in low responder C57BL/6 mice were similar. In addition, nasal eosinophilia in FcγRIIB −/− mice passively sensitized with SEA was exacerbated, and conversely, specific IgE production was inhibited after a nasal challenge. These results suggest that FcγRIIB 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, FcγRI, FcγRIII, and FcεRI 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 binding antibody–antigen complexes (11–15). On the other hand, FcγRIIB 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 FcγRIIB gene (FcγRIIB −/−) develop enhanced immune-complex–mediated alveolitis, collagen-induced arthritis, systemic lupus erythematosus, and Goodpasture's syndrome (21–24). In addition, FcγRIIB −/− 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 FcγRIIB is involved in the initiation of atopic diseases such as allergic rhinitis (26). Moreover, the role of FcγRIIB 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 FcγRIIB 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 FcγRIIB 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 FcγRIIB −/− 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 (FcγRIIB +/+) mice purchased from Charles River Japan (Yokohama, Japan). Homozygous FcγRIIB gene-deficient (FcγRIIB −/−) BALB/c and C57BL/6 founder mice were gifts from Dr. Jeffery V. Ravetch (Rockefeller University, New York, NY) (24). FcγRIIB −/− 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 μg of SEA adsorbed to 1 mg of alum (Kyowa Kagaku, Kagawa, Japan)

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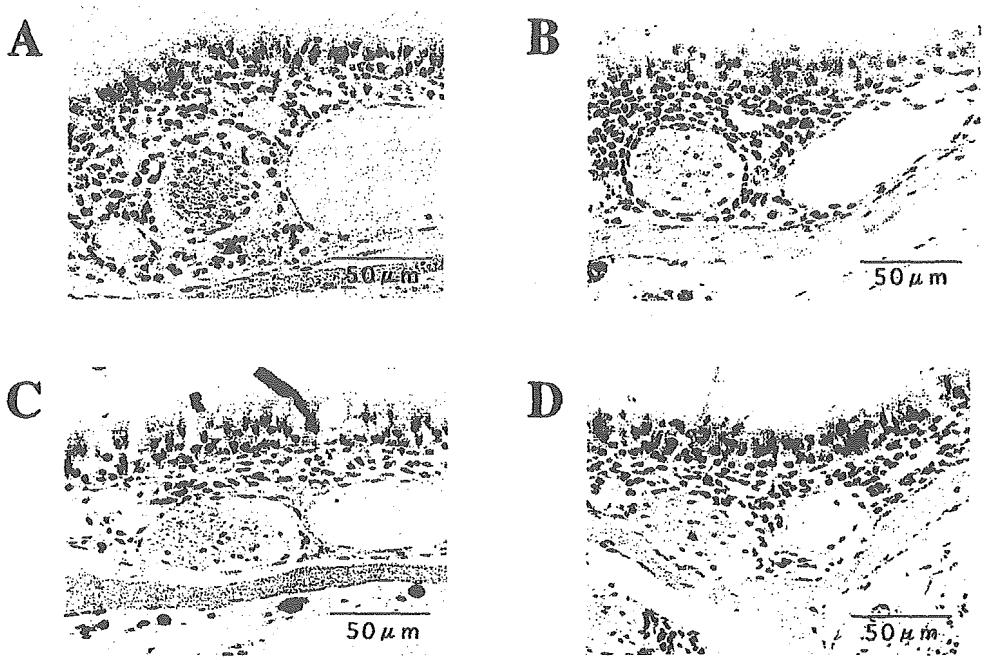

Figure 1. Nasal eosinophilia after nasal challenge in BALB/c (*A* and *B*) and C57BL/6 (*C* and *D*) mice. Wild-type BALB/c (*A*) and C57BL/6 (*C*) mice and counterpart FcγRIIB −/− mice (*B* and *D*, respectively) were intranasally sensitized with *Schistosoma mansoni* egg antigen (SEA). At 12 hours 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 μl. Two weeks later, the mice were boosted in the same manner. Alternatively, mice were passively sensitized by an intravenous injection of 200 μl 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 μl of 1-μg 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 × *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.

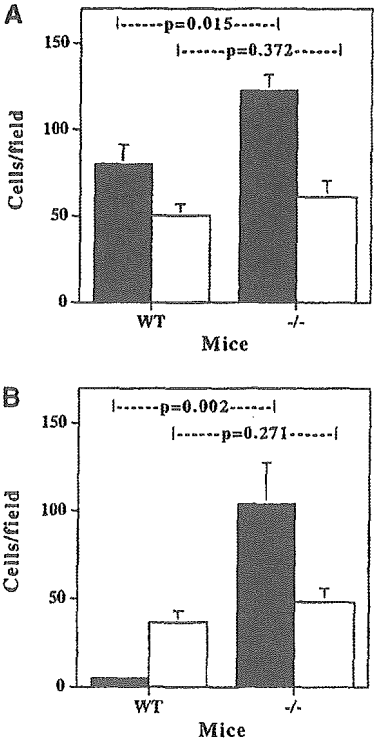

Figure 2. Quantitation of cellular infiltration into nasal mucosa after nasal challenge in BALB/c (*A*) and C57BL/6 (*B*) mice. Wild-type (WT) and FcγRIIB −/− mice were intranasally sensitized and challenged with SEA, as described in Figure 1. The number of eosinophils (*closed bar*) and mononuclear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

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 × 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-μm thick sections in the coronal plane. Each selected section was deparaffinized and rehydrated. After incubation with 20 μg/ml of proteinase K (Boheringer Mannheim, Mannheim, Germany), endogenous peroxidase was blocked by using 2% H_2O_2 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 antifluorescein 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 diaminobenzidene 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 μg/ml), Con A (2 μg/ml) as a positive control, or supplemented medium as a negative control. After an incubation at 37°C for 72 hours in 5% CO_2, 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 ± 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.

RESULTS

Role of FcγRIIB 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 FcγRIIB −/− 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 FcγRIIB +/+ and −/− BALB/c, respectively (mean ± SEM, n = 6, p = 0.015). On the other hand, the magnitude of infiltration of mononuclear cells into nasal mucosa was similar between FcγRIIB −/− 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 FcγRIIB +/+ and FcγRIIB −/− 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). FcγRIIB −/− 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 FcγRIIB −/− 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 FcγRIIB +/+ 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 FcγRIIB in the Induction of Allergic Th2 Responses after Intranasal Sensitization with SEA in Low-responder C57BL/6 Mice

We examined whether the effect of FcγRIIB on the initiation of allergic rhinitis is dependent on strain using FcγRIIB −/− C57BL/6 and the wild type, which has low responses to intranasal sensitization with SEA (6). Wild-type C57BL/6 mice under specific pathogen-free conditions produced detectable amounts of SEA-specific IgE and IgG1, whereas FcγRIIB −/− 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 FcγRIIB −/− 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 FcγRIIB +/+ and −/− C57BL/6, respectively (mean ± 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 FcγRIIB −/− 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 FcγRIIB in specific antibody synthesis is restricted in intranasal sensitization. We intraperitoneally sensitized FcγRIIB −/− 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 FcγRIIB −/− mice after an intraperitoneal boost with SEA compared with the wild type (p = 0.005; Figure 8A). Conversely, FcγRIIB −/− 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 FcγRIIB in the Exacerbation of Allergic Th2 Responses in Mice Passively Sensitized with SEA

We investigated the role of FcγRIIB in the exacerbation of allergic Th2 responses in passively sensitized mice. As described in METHODS, FcγRIIB −/− 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 FcγRIIB −/− mice compared with the wild type (p = 0.019). Conversely, significantly more specific IgG1 was produced in FcγRIIB −/− mice (p = 0.031; Figure 9B). On the other hand, IgG2a was undetectable in either FcγRIIB −/− or in wild-type mice (Figure 9C). In addition, the degree of nasal eosinophilia was significantly higher in FcγRIIB −/− mice than in the wild type, although the degree of eosinophilia was

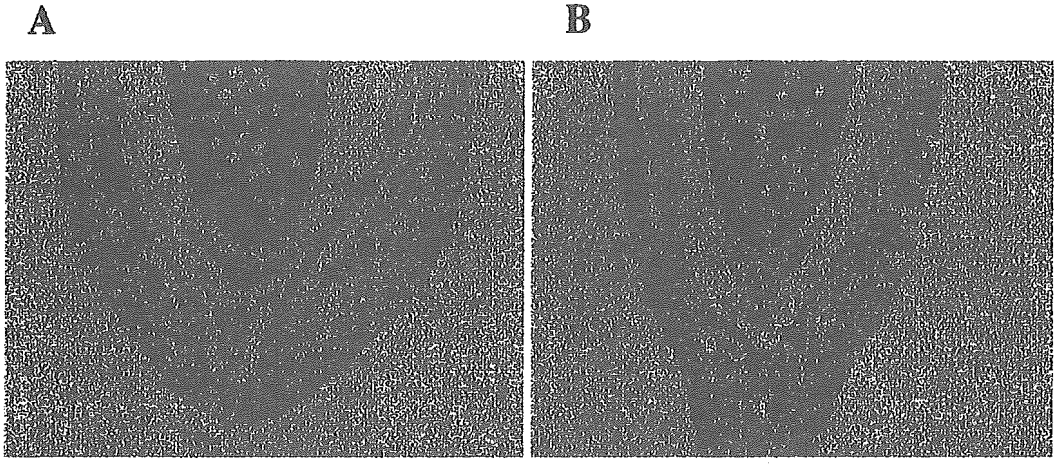

Figure 3. Detection of *in vivo* apoptosis in nasal mucosa. WT (*A*) and FcγRIIB −/− (*B*) BALB/c mice were intranasally sensitized with SEA. At 12 hours after the final nasal challenge, mice were killed. Detection of *in vivo* apoptosis in nasal mucosa was performed using TdT-mediated dUPT-biotin nick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

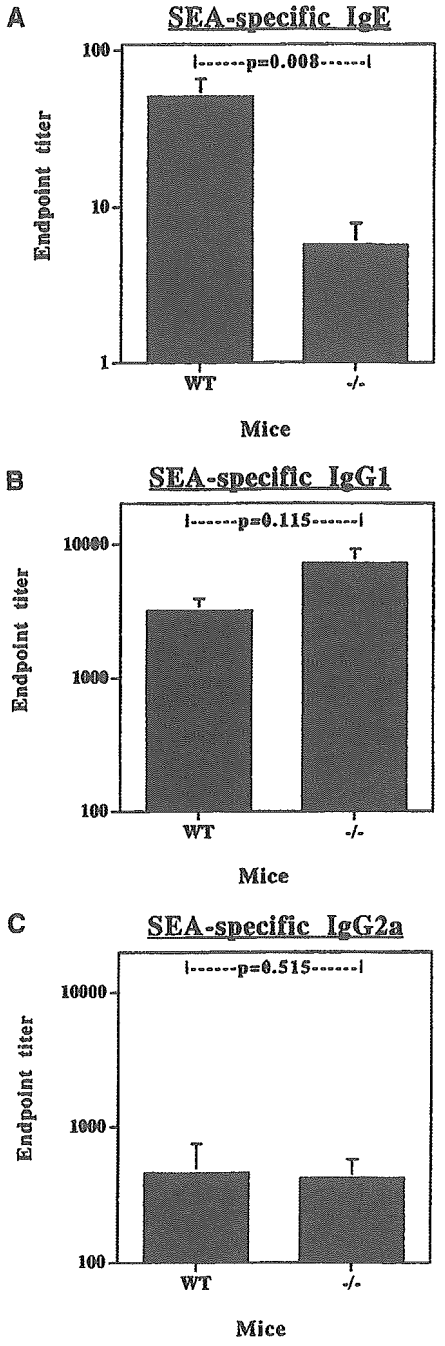

Figure 4. Antibody production in high-responder BALB/c mice after intranasal sensitization with SEA. After final nasal challenge, blood was sampled from WT and FcγRIIB −/− (−/−) mice, and levels of serum SEA-specific IgE (*A*), IgG1 (*B*), and IgG2a (*C*) were determined by ELISA. Results show mean endpoint titer ± 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 FcγRIIB +/+ and −/− BALB/c mice, respectively (mean ± 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

FcγRIIB 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 FcγRIIB during the induction phase of allergy using a murine model of allergic rhinitis (6, 27). To date, the involvement of FcγRIIB in the production of IgE has not been investigated. In terms of pre–B-cell receptor–positive pre-B cells, recent study demonstrated that FcγRIIB negatively regulates pre–B-cell receptor–mediated signaling for apoptosis (29). We found that SEA-specific IgE production in FcγRIIB −/− 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 FcγRIIB play an important role in increasing antigen-specific IgE synthesis in this model. The decreased production of IL-4 by nasal mononuclear cells from FcγRIIB −/− 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 FcγRII (31), the mechanisms governing why a deficiency of FcγRIIB leads to the inhibition of IL-4 production remain to be elucidated.

After intranasal sensitization with SEA, FcγRIIB −/− 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 FcγRIIB plays an inhibitory role in the induction of tissue inflammation (14, 21–23). For example, FcγRIIB −/− 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). FcγRIIB −/− 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 FcγRIIB −/− mice during IgE-mediated systemic anaphylaxis (15). Our results are consistent with these findings and suggest that FcγRIIB inhibits eosinophilic inflammation.

Although it is unknown whether the functions of human eosinophils are mediated by FcγRIIB, FcγRIIA/C, or both, human and mouse eosinophils express FcγRII, and murine FcγRIIB is involved in the survival, degranulation, or apoptosis of eosinophils (17, 32, 33). In addition, ligation of FcγRIIB 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 FcγRII ligation pivotally regulates both the survival and death of eosinophils (33). This study found that nasal mononuclear cells from both FcγRIIB +/+ and −/− mice produced similar amounts of IL-5 after intranasal sensitization with SEA (Figure 5). One explanation of why nasal eosinophilia was exacerbated in FcγRIIB −/− mice irrespective of local IL-5 production is a lack of direct ligation of *S. mansoni*–specific IgG on FcγRIIB on eosinophils. In addition, TUNEL-positive cells in the nasal mucosa were rarely seen in both wild type and FcγRIIB −/− mice after the intranasal challenge with SEA (Figure 3), suggesting that *in vivo* apoptosis of eosinophils triggered by FcγRIIB 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

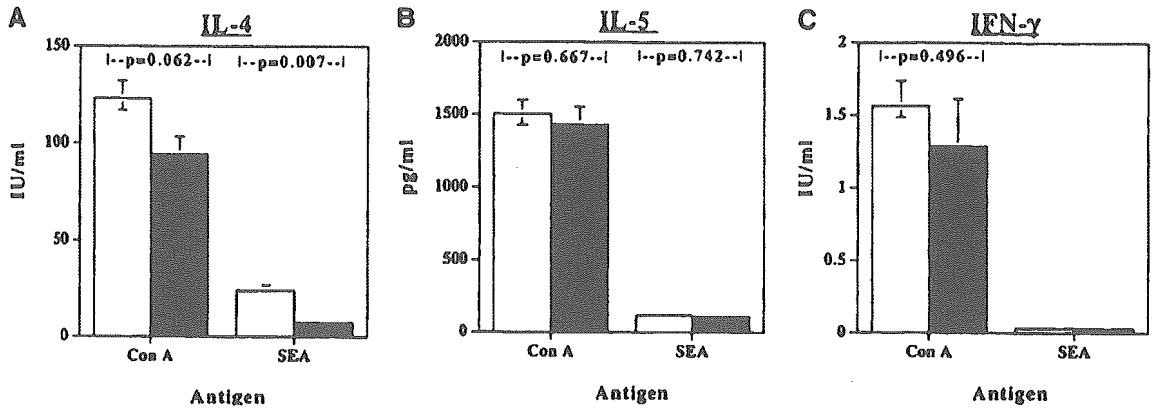

Figure 5. Production of interleukin (IL)-4 (*A*), IL-5 (*B*), and IFN-γ (C) by nasal mononuclear cells from BALB/c wild type (*open bars*) and FcγRIIB −/− (*closed bars*) mice. Mice were intranasally sensitized and subsequently challenged with SEA. At 12 hours after final nasal challenge with SEA, nasal 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 ± SEM of three individual pools. Data are representative of two separate experiments.

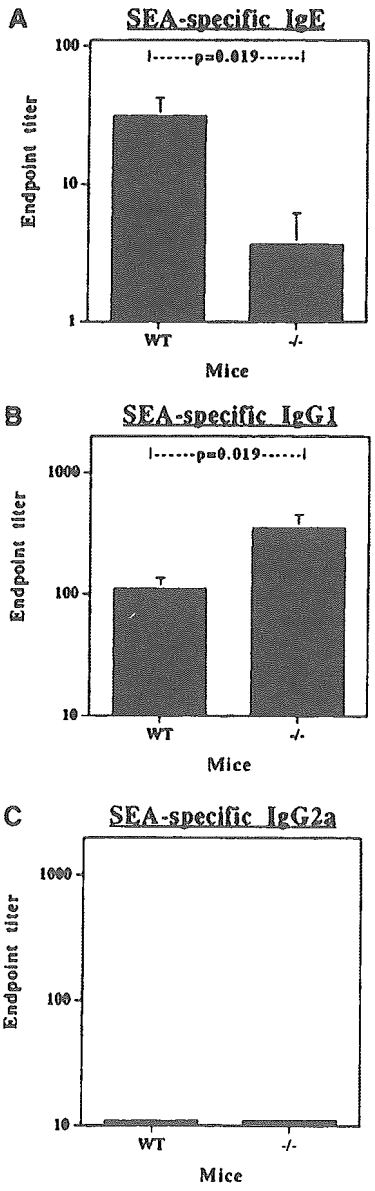

Figure 6. Antibody production in low-responder C57BL/6 mice after intranasal sensitization with SEA. WT and FcγRIIB −/− C57BL/6 mice were intranasally sensitized with SEA under a specific pathogen-free (SPF) condition. After final nasal challenge, blood was sampled, and levels of serum SEA-specific IgE (*A*), IgG1 (*B*), and IgG2a (C) were determined by ELISA. Results show mean endpoint titer ± 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 significant 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 FcγRIIB −/− 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 FcγRIIB −/− 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 FcγRIIB 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 FcγRIIB −/− C57BL/6 mice. IgM and IgG production was augmented in FcγRIIB −/− 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 FcγRIIB −/− mice after immunization with bovine type II collagen (22). In addition, FcγRIIB −/− 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 FcγRIIB plays an inhibitory role in the production of antigen-specific IgG1 (the dominant IgG isotype

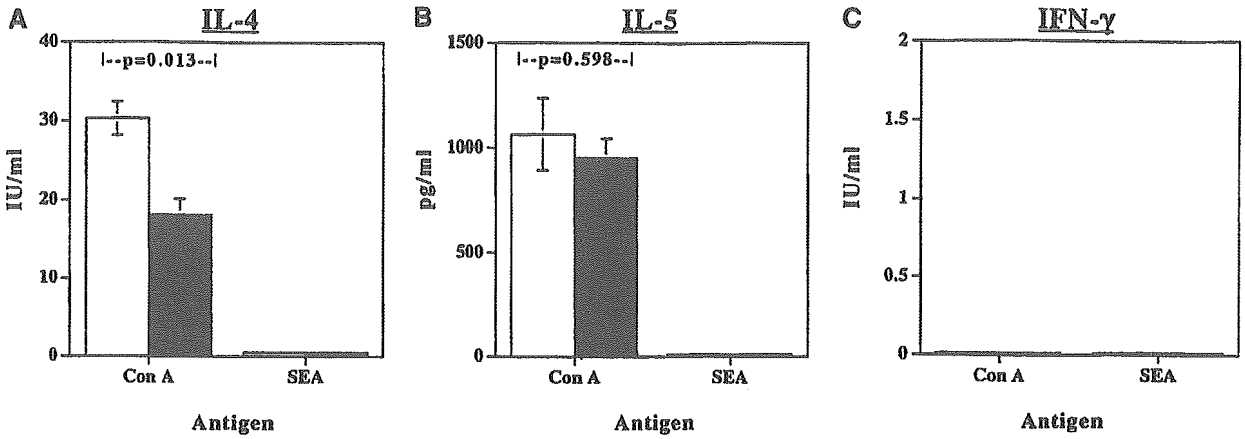

Figure 7. Production of IL-4 (*A*), IL-5 (*B*), and IFN-γ (C) by nasal mononuclear cells from C57BL/6 wild-type (*open bar*) and FcγRIIB −/− (*closed bar*) mice under SPF conditions. Mice were intranasally sensitized and subsequently challenged with SEA. Twelve hours after final nasal challenge with 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 ± 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 FcγRIIB −/− and wild-type mice, suggesting that FcγRIIB 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 FcγRIIB +/+ 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 FcγRIIB −/− mice was significantly decreased (Figure 8A). These results suggest that the role of FcγRIIB in the regulation of specific IgE production was the same regardless of the route of SEA sensitization.

Furthermore, when FcγRIIB −/− 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 FcγRIIB in local eosinophilia and IgE synthesis is the same between active and passive sensitization with SEA.

In conclusion, we found that nasal eosinophila is exacerbated, whereas the production of specific IgE is decreased in FcγRIIB −/− mice after intranasal sensitization with SEA. Moreover, the involvement of FcγRIIB 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 FcγRIIB. These results suggest that the regulation of expression and/or function of FcγRIIB 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; 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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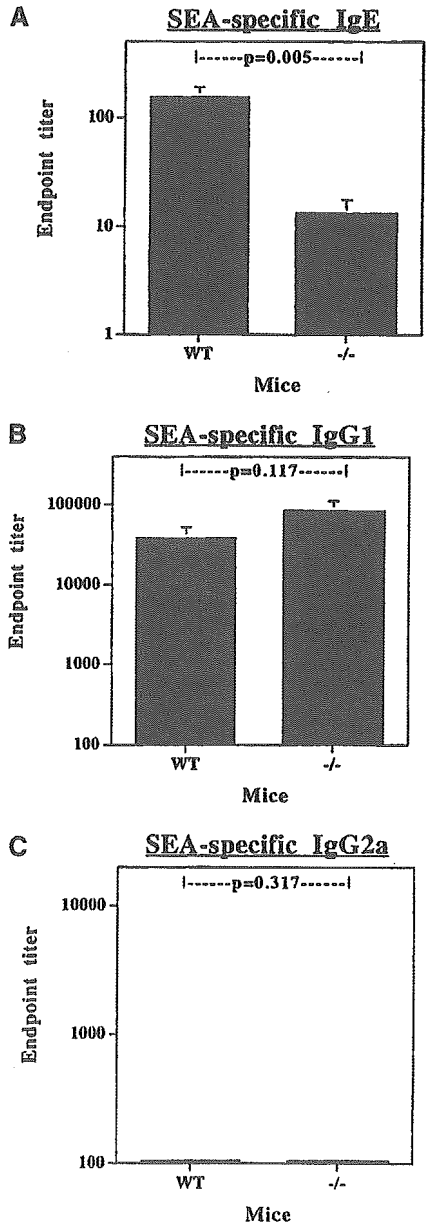

Figure 8. Antibody production after intraperitoneal sensitization with SEA. WT and FcγRIIB −/− BALB/c mice were primed and intraperitoneally boosted with SEA adsorbed to alum. At 1 week after boosting, blood was sampled, and levels of serum SEA–specific IgE (*A*), IgG1 (*B*), and IgG2a (C) were determined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

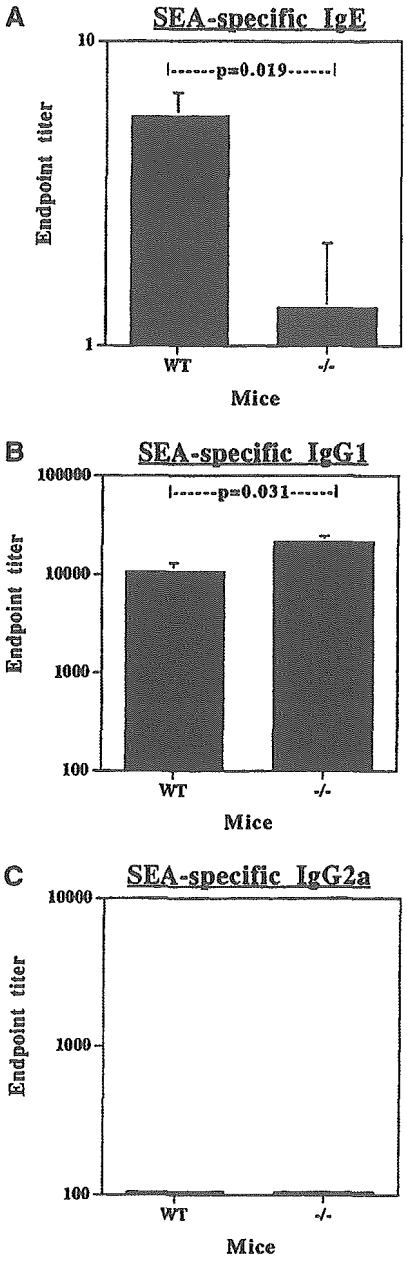

Figure 9. Antibody production after passive sensitization with SEA. WT and FcγRIIB −/− BALB/c mice were passively sensitized with SEA by intravenous injection of pooled sera from presensitized or nonsensitized mice. One hour after passive sensitization, the mice were challenged intranasally with 20 μl of 1 μg 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 ± SEM of six serum samples from each group. Data are representative of two separate experiments.

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Analysis of Natural History of Japanese Cedar Pollinosis

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Key Words
Japanese cedar pollinosis · Japanese cedar pollen · Remission, spontaneous · Aging · Serum IgE antibody · Cross-sectional study · Vertical-sectional study

Abstract
Background: The marked increase in the incidence of Japanese cedar (*Cryptomeria japonica;* JC) pollinosis is a social problem in Japan. Elucidation of its natural history is, therefore, essential. ***Methods:*** Cross-sectional and vertical-sectional studies were performed regarding the effects of aging on sensitization to Japanese cedar pollen (JCP) and development of JC pollinosis by measuring serum IgE titers to JCP and by oral examination of residents of the Maruyama Town, Chiba, Japan from 1995 to 2001. We also studied the incidence of its spontaneous remission and the background factors. ***Results:*** In a vertical-sectional study, the serum IgE titer to JCP was strongly influenced by the amounts of pollen scattered. An increase in age by 6 years did not reduce the IgE titer to JCP in subjects in their 40s. However, in subjects aged 60 or more, annual differences in the JCP count did not affect serum IgE titer to JCP, which remained low even after a season with a high pollen count. In subjects with JC pollinosis aged over 40 showing a CAP RAST score of more than 2 to JCP in 1995, spontaneous remission of JC pollinosis was observed in 16.1% over a period of 6 years. Factors affecting spontaneous remission include sex, age at the time, serum IgE titer to JCP and age at first onset of JC pollinosis. ***Conclusions:*** The CAP RAST score was strongly associated with spontaneous remission in the multivariable model.

Introduction

Allergic diseases are increasing worldwide [1–5]. In particular, increases in Japanese cedar (JC) pollinosis are becoming a social problem in Japan. Accordingly, we conducted cross-sectional research on sensitization to Japanese cedar pollen (JCP) and prevalence of JC pollinosis among residents including children and adults in a town located on the Boso Peninsula of the Chiba Prefecture. Subjects were followed up over a period of 6 years from 1995 to 2001 to study the effect of aging on the serum IgE antibody titer to JCP, the development of JC pollinosis in those who had a positive serum IgE antibody titer to JCP, the incidence of spontaneous remission and the background factors. Because only 40 years have passed since the first report on JC pollinosis, there has been almost no report on the effect of aging on sensitization to JCP and the development of JC pollinosis. To take long-term measures against JC pollinosis, it is necessary to understand the natural history of the disease.

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