

IgE Production after Four Routes of Injections of Japanese Cedar Pollen Allergen without Adjuvant: Crucial Role of Resident Cells at Intraperitoneal or Intranasal Injection Site in the Production of Specific IgE toward the Allergen

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Abstract: The production of specific IgE antibodies directed toward cedar pollen correlates well with the onset of allergic rhinitis; but the mechanisms of allergen recognition as nonself and Ig class switch to IgE by the immune system are still not fully understood. In the present study, we injected cedar pollen into mice through 4 different routes (intranasal (i.n.), intraperitoneal (i.p.), intravenous (i.v.), and subcutaneous (s.c.)) without adjuvant 1 to 3 times, and determined time-dependent changes in the total and specific serum IgE levels compared with those in the serum levels of other isotype Igs. After an i.p. or i.n. injection of allergen into the mice, they produced a 1.5- to 1.7-fold increase in total IgE, but none in IgG, IgM, or IgA antibodies in their serum, whereas an i.v. or s.c. injection of allergen was inactive as an inducer of total IgE antibodies. Upon a 2nd (s.c.) injection of the allergen into the i.p. or i.n. sensitized mice, a large amount of allergen-specific IgE antibodies was found in the serum. In the case of i.v. or s.c. sensitized mice, however, they produced total, but not specific, IgE antibodies; and a 3rd (s.c.) injection of the allergen resulted in a large amount of specific IgE antibodies in the serum. These results imply that resident cells at the i.p. or i.n. injection site may play a crucial role in the efficient production of total and specific IgE antibodies toward the allergen.

Key words: Rodent, IgE, Pollen, Rhinitis

Allergic rhinitis is one of the typical atopic diseases; and recently, the prevalence of the disease seems to be accelerating worldwide. In Japan, Japanese cedar pollenosis constitutes a growing problem for public health. Once inhaled into the human respiratory system, pollen releases allergens upon contact with the mucosa (15). The allergenic molecules can be stable enough to survive penetration through the epithelial layer, reaching the levels where they are recognized by the nonspecific or specific defense system (7). In a sensitized patient the production of specific IgE antibodies (Abs) directed toward an allergen is a prerequisite for the immunologic response leading to allergic rhinitis. These IgE Abs are bound to the effector cells in the submucosa via high-

affinity IgE receptors (16). Once the allergen is bound by specific IgE Abs cross-linking at least 2 IgE receptors, the signal for the release of mediators is transduced into the cell. Although the production of a large amount of specific IgE Abs directed toward an allergen is well correlated with the onset of allergic rhinitis, several questions regarding the mechanisms, have still not been fully answered. For example: What kinds of immune cells can recognize allergenic molecules as nonself? Are allergens recognized as nonself nonspecifically or specifically by the defense system? Why are IgE Abs rather than IgA, IgM, or IgG Abs produced toward an allergen?

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Abbreviations: Ab, antibody; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; i.n., intranasal; i.p., intraperitoneal; i.v., intravenous; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; s.c., subcutaneous.

In the present study, since injections of allergen with adjuvant obscure the characteristics of the injection sites, we injected Japanese cedar pollen without adjuvant into mice and explored the effects of 1, 2, or 3 injections of the allergen through 4 different routes (intranasal [i.n.], intraperitoneal [i.p.], intravenous [i.v.], and subcutaneous [s.c.]) on total and specific IgE production. In addition, to assess the level of specific IgE Ab, we prepared mouse cedar pollen-specific IgE Ab for use as a standard. The results were quite different depending upon the route of injection. Here, we report that the allergenic activity as an increase in total IgE Ab was induced by primary i.p. (or i.n.), but not i.v. (or s.c.), injection of cedar pollen extract and that 1 or 2 more s.c. injections of the same allergen into i.p. (or i.n.) or i.v. (or s.c.) allergen-sensitized mice, respectively, induced specific IgE Ab production.

Materials and Methods

Animals. There are lots of gene (e.g., IFN- γ and IL-4) knockout animals in BALB/c and C57BL/6 strains of mice; and their gene mapping has already been established. However, it has been reported that serum allergen-specific IgE levels in BALB/c mice were much higher than in C57BL/6 mice after allergen (e.g., ovalbumin and *Schistosoma mansoni* egg antigen) sensitization and exposure (2, 10); and we also found that BALB/c mice produced higher titers of serum IgE Ab than C57BL/6 mice after treatment with Japanese cedar pollen allergen (data not shown). Thus, in the present study, we used BALB/c mice as experimental animals. Specific pathogen-free male BALB/c mice (6–7 weeks of age) were purchased from Japan SLC (Hamamatsu, Japan). After injections of phosphate-buffered saline (PBS) or allergen, the mice were housed in our animal facility under specific pathogen-free conditions in an air-conditioned room at 23 ± 2 C and $\approx 50\%$ humidity for 1 to 2 weeks. The experiment was carried out in accordance with the Guidelines on Animal Experiments of Osaka Medical College and the Japanese Government Notification on Feeding and Safekeeping of Animals (Notification No. 6 of the Prime Minister's Office); the experimental protocol was approved by the Review Committee for Animal Experiments of Osaka Medical College.

Antigen. Japanese cedar, *Cryptomeria japonica*, pollen crude extract-cj was purchased from Cosmo Bio Co., Tokyo.

Abs. Abs used in this study were the following: mouse IgE, IgG, IgM, and IgA from Zymed (San Francisco, Calif., U.S.A.), Zymed, Bethyl (Montgomery, Tex., U.S.A.), and Zymed, respectively; rat anti-mouse

IgE, IgG, IgM, and IgA from Biosource (Camarillo, Calif., U.S.A.); and horseradish peroxidase (HRP)-labeled goat anti-mouse IgE, IgG, IgM, and IgA Abs from Nordic (Tilburg, the Netherlands), Cappel (Aurora, O., U.S.A.), Cappel, and Southern-Biotech (Birmingham, Ala., U.S.A.), respectively.

Preparation of high-titer IgE Ab toward Japanese cedar, *Cryptomeria japonica*, pollen crude extract-cj. To assess whether the serum contains total IgE or specific IgE Abs directed toward cedar pollen, we prepared mouse cedar pollen-specific IgE Ab. *Cryptomeria japonica* pollen crude extract-cj (100 μ g) in 0.8 ml of PBS was mixed with 0.2 ml of aluminum hydroxide gel (Sigma, St. Louis, Mo., U.S.A.) as an adjuvant; and the mixture was injected i.p. into BALB/c mice (7 weeks of age). Every 2 weeks after the 1st immunization, the mice were i.p. treated with the allergen in 0.8 ml of PBS and 0.2 ml of adjuvant. Ten days after the 4th immunization, the mice were i.p. treated with the allergen in 0.8 ml of PBS and 0.2 ml of adjuvant. Three days later, blood samples were taken by cardiac puncture under chloroform anesthesia. The whole blood was incubated in a CO₂ incubator at 37 C for 1 hr, left overnight at 4 C, and then centrifuged at $440 \times g$ for 20 min. The supernatant fraction was stored in microtubes at -80 C prior to use.

Serum preparation. Experimental schedules are shown in Fig. 1. Blood samples were taken by cardiac puncture under chloroform anesthesia at various time intervals after i.p., i.n., i.v., or s.c. injection of *Cryptomeria japonica* pollen crude extract-cj (100 μ g/0.2 ml of PBS/mouse) or PBS. The sera were prepared as described above.

Enzyme-linked immunosorbent assay (ELISA) for determination of cross-reactivity of anti-IgE Ab with IgA, IgG, or IgM. EIA/RIA plates (Costar #3591; Corning, New York, N.Y., U.S.A.) were coated with 100 μ l of rat anti-mouse IgE Ab (2 μ g/ml) at 4 C overnight. After 3 washes with PBS/Tween (PBS+0.5% Tween 20), the wells were filled with 400 μ l of 1% bovine serum albumin (BSA)/PBS and then incubated for 2 hr at room temperature to block unsaturated protein binding sites. The plates were next washed 3 times with PBS/Tween; and 100 μ l of Ig (IgG, IgE, IgM, or IgA; 0–250 μ g/ml) in 1% BSA/PBS was added to each of triplicate wells, after which the plates were incubated for 2 hr at room temperature. After 3 more washes with PBS/Tween, 100 μ l of HRP-labeled goat anti-mouse IgE Ab in 1% BSA/PBS was dispensed into each well, and the preparation was allowed to react for 1 hr at room temperature. The wells were washed thoroughly with PBS/Tween. The antigen-Ab complex was incubated with 100 μ l of tetramethylbenzidine (Sigma) for

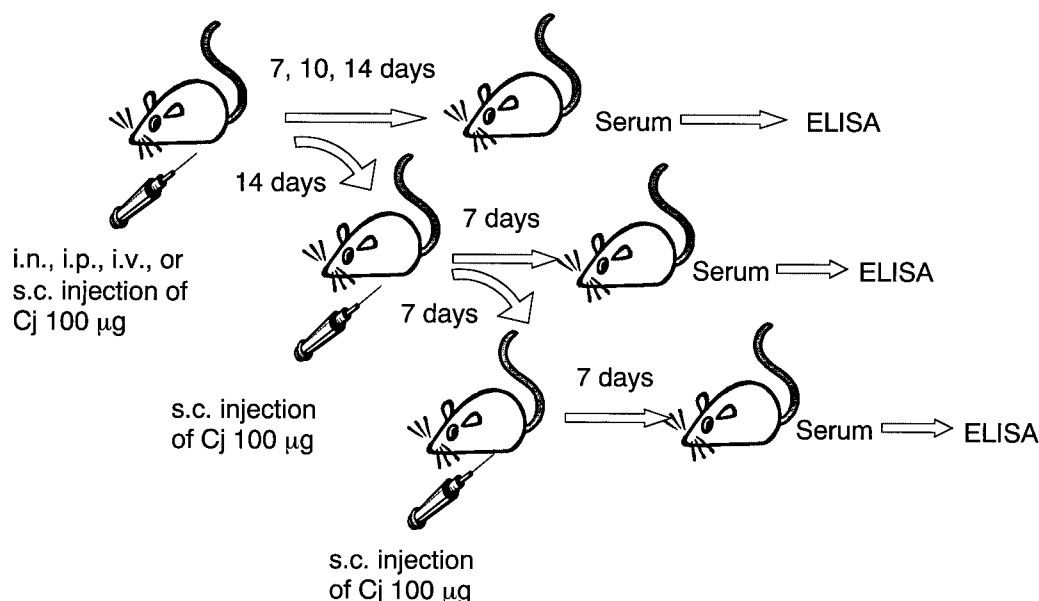


Fig. 1. Experimental schedules.

30 min at room temperature, and then the reaction was stopped by the addition of 100 µl of 1 M H₂SO₄. Thereafter OD_{450nm} was read by a microplate reader M-Emax (Molecular Devices Co., Sunnyvale, Calif., U.S.A.).

ELISA for total IgE, IgG, IgM, or IgA Ab. The procedure was the same as that for the measurement of the cross-reactivity of anti-IgE Ab with IgA, IgG, or IgM except that the wells were precoated with 100 µl of rat anti-IgE, anti-IgG, anti-IgM, or anti-IgA Ab and that HRP-labeled goat anti-mouse IgE, IgG, IgM, or IgA Ab, respectively, was used. For preparation of standard curves for Ig, the concentrations used in this study were as follow: IgG: 0.01 µg/ml, 0.02 µg/ml, 0.05 µg/ml, 0.1 µg/ml, and 0.2 µg/ml; IgE: 0.005 µg/ml, 0.01 µg/ml, 0.02 µg/ml, 0.05 µg/ml, and 0.1 µg/ml; IgM: 0.02 µg/ml, 0.05 µg/ml, 0.1 µg/ml, 0.2 µg/ml, and 0.5 µg/ml; IgA: 0.05 µg/ml, 0.1 µg/ml, 0.2 µg/ml, 0.5 µg/ml, and 1 µg/ml. To measure the total Ig concentration, we diluted serum samples 20, 50, and 100 times for IgE and 5,000, 10,000, and 20,000 times for IgG, IgM, and IgA, respectively. Based on the standard curve, determination of the amount of Ig was carried out by use of Microsoft Excel software. The lower limit of the Ab assayed in this system was 10 to 30 pg/ml. The SD of the triplicate assay was less than 30% at approximately 50 pg/ml and less than 15% at approximately 100 pg/ml.

ELISA for specific IgE Ab. The procedure was the same as that for the measurement of the total IgE, IgG, IgM, or IgA Ab concentration except that the wells of Immulon® 4 HBX flat-bottom microtiter plates (Thermo Labsystems, Franklin, Mass., U.S.A.) were used

after having been precoated with 10 µg of the Japanese cedar pollen crude extract in 100 µl of PBS. For preparation of the standard curve for specific IgE Ab, 80 to 10,240 times-diluted high-titer IgE Ab in 100 µl of 1% BSA/PBS was added to each of triplicate wells. Serum samples were diluted 160, 320, and 640 times; and the titers relative to the high-titer IgE Ab (=1.0) were determined based on the standard curve.

Results

Reactivity of IgE, IgA, IgG, or IgM with Anti-IgE Ab

The levels of reactivity of anti-IgE Ab with IgE and other Ig isotypes were explored by ELISA. As shown in Fig. 2, IgE revealed high reactivity with anti-IgE Ab, which reached a plateau at the approximate concentration of 0.2 µg/ml; whereas other Ig isotypes revealed only trace, if any, reactivity with anti-IgE Ab, even at the concentration of 200 µg/ml (Fig. 2, inset).

Time-Dependent Changes in Serum IgE Production after an i.p., i.n., i.v., or s.c. Injection of the Pollen Crude Extract into BALB/c Mice

Figure 3 shows time-dependent changes in serum IgE production after an i.p., i.n., i.v., or s.c. injection of cedar pollen, compared with those after PBS treatment. Either the i.p. or i.n. injection of the allergen resulted in an increase (1.53- to 1.72-fold of control) in the serum IgE production on day 10 (Fig. 3A) or on days 10 and 14 (Fig. 3B), respectively; whereas the serum IgG, IgM, and IgA concentration did not change significantly or rather actually decreased (Fig. 3, A and B). In contrast,

an i.v. or s.c. injection of the allergen did not induce serum IgE production on days 7, 10, and 14 after the treatment; and the concentrations of other Ig isotypes in the serum were also unchanged or somewhat decreased (Fig. 3, C and D).

Effects of the 2nd (s.c.) Injection of Pollen Crude Extract on Allergen-Sensitized BALB/c Mice

Next, we examined the effects of a 2nd (s.c.) injection of PBS or allergen on the serum IgE concentrations of allergen-sensitized or PBS-injected mice (Fig. 4). The 2nd (s.c.) injection of the same allergen into i.p., i.n., i.v., or s.c. allergen-sensitized animals produced a large amount of IgE Ab by the immune response toward the allergen; whereas other temporal combinations of PBS and allergen hardly affected the serum IgE concentration. In contrast, the serum IgG concentrations did not change significantly or were somewhat decreased; the IgM concentrations were retained at a constant level; and the IgA concentrations rather decreased (data not

shown).

Preparation of High-Titer IgE Ab Reactive with the Pollen Crude Extract

An i.p. or i.n. injection of the allergen resulted in an increase in the serum IgE production on day 10 or on days 10 and 14, respectively (Fig. 3); and upon the 2nd (s.c.) injection of the allergen into allergen-sensitized mice, a large amount (2.22- to 3.57-fold of control) of IgE Abs increased in the serum of i.p., i.n., i.v., or s.c. allergen-sensitized mice (Fig. 4). To assess whether the sera contained total or specific IgE Abs against the allergen, we prepared high-titer IgE Ab reactive toward cedar pollen. As shown in Fig. 5, a significant amount of IgE Ab bound to cedar pollen (or the purified antigen, cryj-1) even after 10,240 times dilution to give a significant OD_{450nm} value (0.0409 ± 0.0163 [mean $\pm$ SD; $n=8$]).

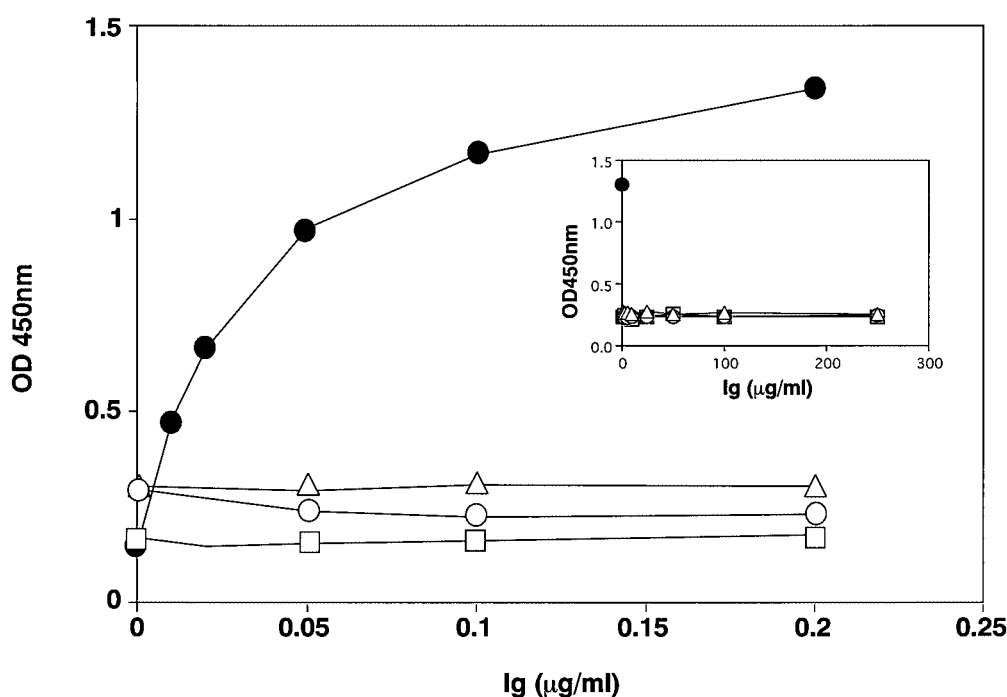
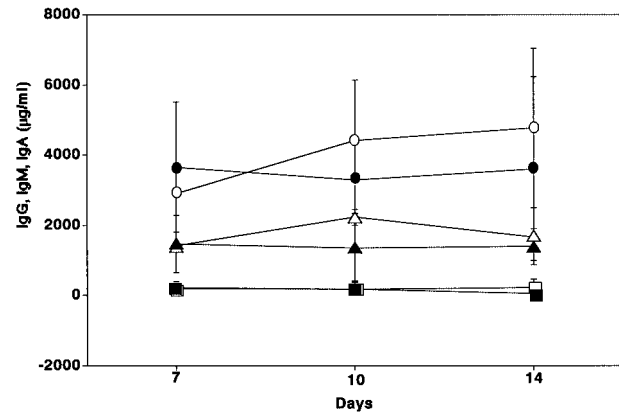
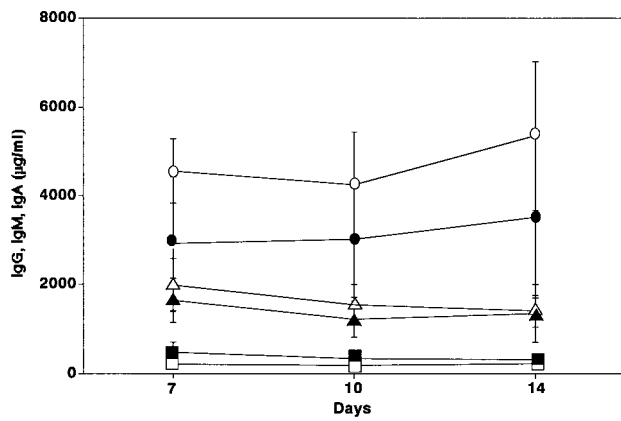
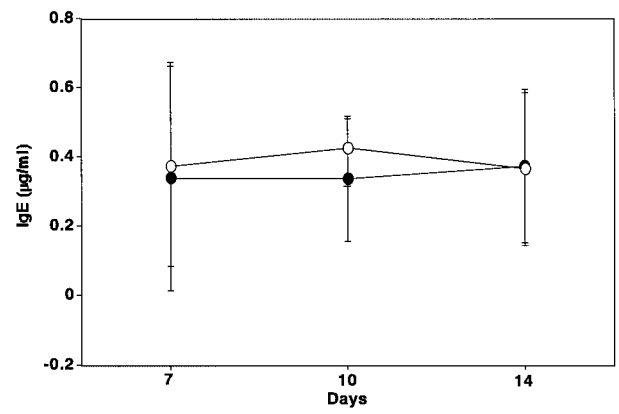
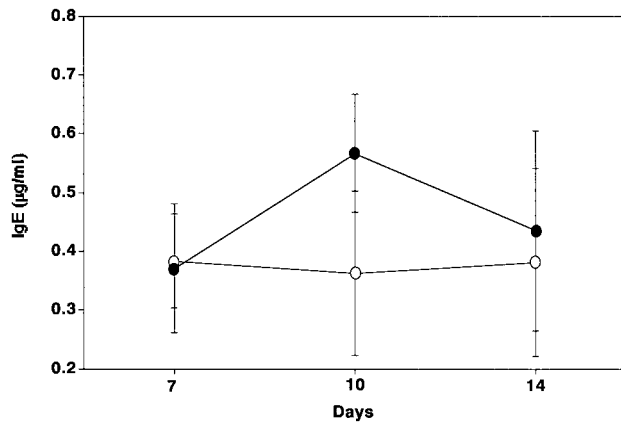


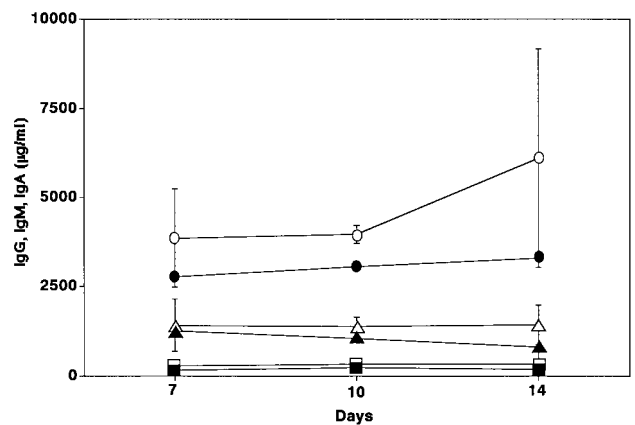
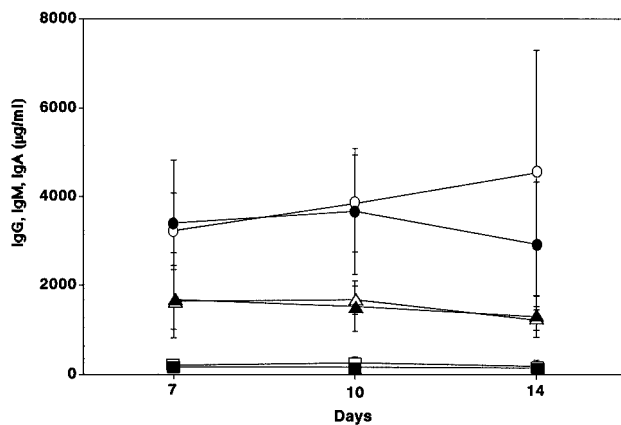
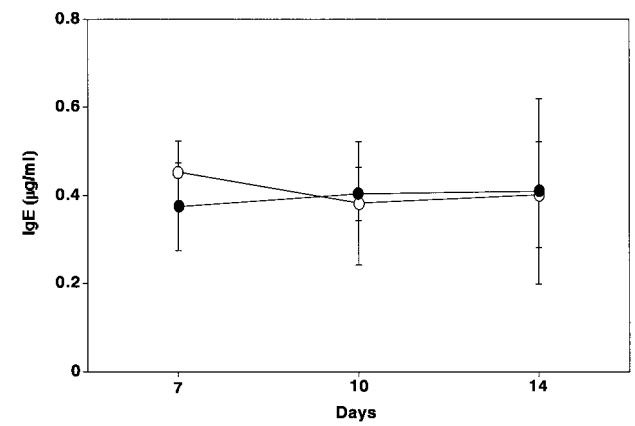
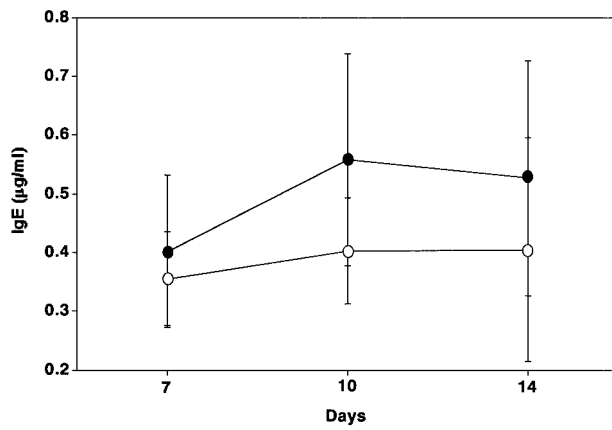
Fig. 2. Isotype specificity of anti-mouse IgE Ab. Vertical and horizontal axes represent the OD_{450nm} and the concentration of each Ig (IgE (●), IgA (△), IgG (□), or IgM (○)), respectively. Each value represents the mean $\pm$ SD ($n=3$); the SD bars are hidden by the symbols. (Inset) Isotype specificity of anti-mouse IgE Ab at high concentrations of Igs (IgA (△), IgG (□), or IgM (○)). Each value represents the mean $\pm$ SD ($n=3$); the SD bars are hidden by the symbols. ●, mean $\pm$ SD ($n=3$) of OD_{450nm} at 0.2 μ g/ml of IgE.

Fig. 3. Specific increase in IgE, but not IgG, IgA, or IgM, Ab in serum after i.p. or i.n., but not i.v. or s.c., injection of Japanese cedar, *Cryptomeria japonica*, pollen crude extract. Each value represents the mean $\pm$ SD (μ g/ml) of 5 serum samples from 5 mice. A, i.p.; B, i.n.; C, i.v.; D, s.c. Upper figures represent serum IgE levels after PBS (○) or allergen (●) treatment; and lower figures, the levels of other Igs in the serum after PBS (IgA: ○; IgM: △; IgG: □) or allergen (IgA: ●; IgM: ▲; IgG: ■) treatment. The difference in the serum levels of IgE Ab is not significant ($P>0.05$) according to Student's *t* test.



A

C



B

D

Fig. 3.

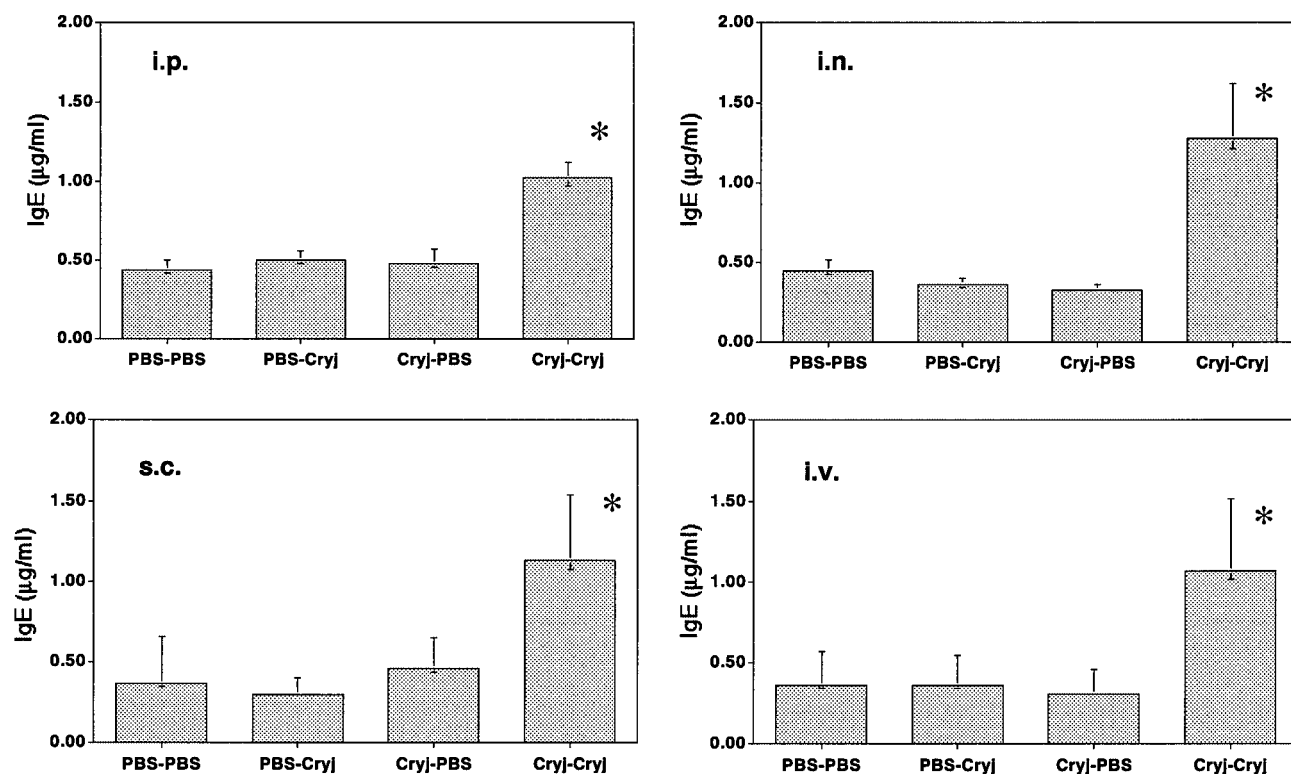


Fig. 4. Effects of a 2nd (s.c.) injection of PBS or pollen crude extract on PBS-injected or allergen-sensitized BALB/c mice. The levels of Igs in the serum were determined on day 7 after the 2nd injection. Each value represents the mean $\pm$ SD (μ g/ml) of 5 serum samples from 5 mice. The difference is significant (* $P < 0.05$) according to Student's t test.

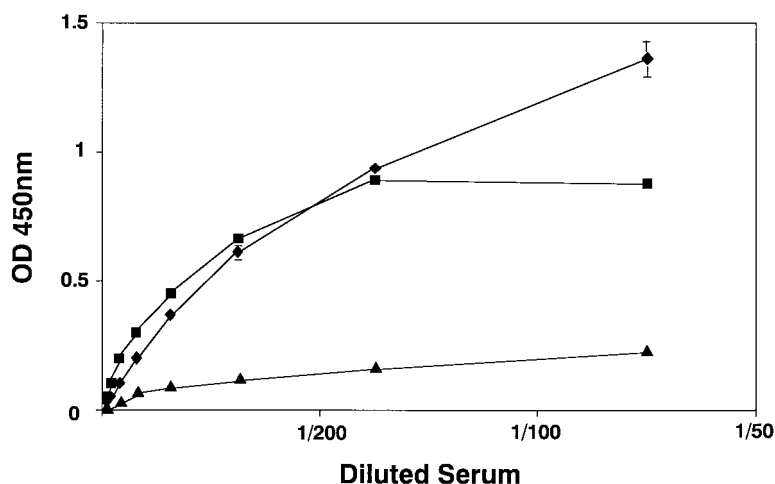


Fig. 5. Preparation of high-titer IgE Ab reactive with the pollen crude extract. Test serum samples (80 to 10,240 times diluted with PBS) were applied to wells precoated with 10 μ g/ml (▲) or 100 μ g/ml (■) of pollen crude extract or 100 μ g/ml of purified *Cryptomeria japonica* pollen, Cryj-1 (◆). Vertical and horizontal axes represent the OD_{450nm} and dilution rate, respectively. Each value represents the mean $\pm$ SD ($n = 3$); and all but one SD bars are hidden by the symbols.

Allergen-Specific IgE Production by the 2nd (s.c.) Injection of Pollen Crude Extract to i.p. or i.n. Allergen-Sensitized BALB/c Mice

As we had obtained high-titer IgE Ab specific for cedar pollen (Fig. 5), we next assessed the levels of

total IgE Abs and allergen-specific IgE Abs (Table 1). The animals that had been once i.p. or i.n. treated with allergen produced a significant amount of IgE Ab (Fig. 3), but hardly reacted with the allergen, suggesting the increase to be due to nonspecific IgE Ab in the serum.

Upon the 2nd (s.c.) injection of the same allergen to the i.p. or i.n. allergen-sensitized animals, they produced a large amount of IgE Ab (Fig. 4); and most of them did react with the allergen (Table 1), suggesting an increase in the allergen-specific IgE Ab in the serum. In contrast, a primary i.v. or s.c. injection of the allergen did not enhance IgE Ab production (Fig. 3); the 2nd (s.c.) injection of the allergen into the i.v. or s.c. allergen-sensitized mice induced IgE Ab (Fig. 4); but most of them did not react with the allergen (Table 1), suggesting that the increase was due to that in nonspecific IgE Ab in the serum. However, the 3rd (s.c.) sensitization with the same allergen produced a large amount of allergen-specific IgE Ab in the serum of these mice (Table 1).

Discussion

In most previous animal models of pollen-induced allergic rhinitis, the allergic reaction was induced by repetitive pollen inhalation challenges to animals that had been sensitized by repeated instillation of the pollen extract plus adjuvant including aluminum hydroxide gel into their nostrils (1, 9, 12). Under these conditions, however, edema is induced in the nasal mucosa; and leukocytes, especially eosinophils, migrate into the cavity. Therefore, we cannot understand pre-

cisely the first reaction of the immune system to the allergen. In the present study, we injected cedar pollen into mice through 4 different routes without adjuvant. After a primary i.p. or i.n. injection of allergen into mice they produced a significant amount of total IgE Ab in their serum, whereas the i.v. or s.c. injection of allergen was inactive as an inducer of total IgE Ab. Upon the 2nd (s.c.) injection of the allergen into the allergen-sensitized mice, they produced a large amount of IgE Ab regardless of the injection route; however, the level of the allergen-specific IgE was increased only in the i.p. or i.n. sensitized mice. These results imply that the presence of resident cells including macrophages for reaction with cedar pollen in the i.p. or i.n., but not in the s.c. or i.v., injection site may be crucial for the production of total and specific IgE Abs toward the allergen.

In Fig. 5 or Table 1, a standard curve was obtained by use of the serum containing IgE Ab specific toward cedar pollen. The OD_{450nm} was determined for the test serum sample; and the specific binding activity was expressed relative to the OD_{450nm} of the high-titer IgE Ab taken as 1.0. The OD_{450nm} after the control (1st PBS [i.n.] and 2nd allergen [s.c.]) treatment was 0.169–0.192 (mean=0.178) (Table 1); 1st i.n. and 2nd s.c. treatments with allergen caused a 3.57-fold increase

Table 1. Allergen-specific IgE production by an s.c. injection of *Cryptomeria japonica* pollen crude extract into i.p. or i.n. allergen-sensitized BALB/c mice

Treatment ^{a)}			Relative to high-titer IgE Ab ^{b)} (mean±SD; n=9)
1st	2nd	3rd	
i.p. PBS	s.c. PBS		0.172±0.111
i.p. PBS	s.c. pollen		0.180±0.123
i.p. pollen	s.c. PBS		0.177±0.112
i.p. pollen	s.c. pollen		0.698±0.205*
i.n. PBS	s.c. PBS		0.192±0.106
i.n. PBS	s.c. pollen		0.173±0.107
i.n. pollen	s.c. PBS		0.169±0.115
i.n. pollen	s.c. pollen		0.730±0.198*
i.v. PBS	s.c. PBS		0.208±0.139
i.v. PBS	s.c. pollen		0.207±0.136
i.v. pollen	s.c. PBS		0.206±0.138
i.v. pollen	s.c. pollen		0.277±0.057
i.v. pollen	s.c. pollen	s.c. pollen	0.769±0.194*
s.c. PBS	s.c. PBS		0.192±0.126
s.c. PBS	s.c. pollen		0.192±0.127
s.c. pollen	s.c. PBS		0.199±0.134
s.c. pollen	s.c. pollen		0.278±0.166
s.c. pollen	s.c. pollen	s.c. pollen	1.474±0.233**

^{a)} 2nd: PBS or pollen treatment on day 14 after the 1st treatment. 3rd: pollen treatment on day 7 after the 2nd treatment. On day 7 after the 2nd or 3rd treatment, serum was prepared.

^{b)} Relative to the OD_{450nm} of high-titer IgE Ab, which was assessed as 1.0. The difference is significant (* $P<0.05$; ** $P<0.01$) according to Student's *t* test.

in the serum IgE Ab concentrations in the serum (Fig. 4); and the relative OD_{450nm} value was 0.730 (Table 1). If the difference ($0.178 \times 2.57 = 0.458$) was due to an increase in the serum allergen-specific IgE Ab, the high-titer IgE Ab might contain $\approx 63\%$ ($=0.458 \div 0.730$) of allergen-specific IgE Ab and $\approx 37\%$ of total IgE Ab. Unexpectedly, 3 successive s.c. allergen treatments without adjuvant could induce allergen-specific IgE Ab ($63\% \times 1.474 = 92.9\%$) most efficiently (Table 1).

When allergen was primarily injected i.p. or i.n., total IgE Ab increased in the serum of the immunized mice (Fig. 3 and Table 1); and an s.c. injection of the same antigen to the immunized mice caused an increase in the amount of allergen-specific IgE Ab in the serum (Table 1). Furthermore, primary i.v. or s.c., followed by an s.c. injection of allergen into mice produced a significant amount of total IgE Ab (Fig. 4 and Table 1); and primary i.v. or s.c. followed by 2 s.c. injections of allergen into the immunized mice produced a large amount of allergen-specific IgE Ab in the serum (Table 1). These results indicate that the production of total IgE Ab without a change in the IgG, IgM, or IgA concentration is a prerequisite for the production of allergen-specific IgE Abs. Extensive studies on the surface Ig on mouse B cells by many investigators showed that resting B cells bear IgM and IgD and that the B cells bearing the other isotypes are derived from the virgin B cells (19). It is generally accepted that lymphocytes bearing IgG or IgA are already committed to the synthesis of their respective isotype after differentiation. Therefore, it has been assumed that same principle may apply to the precursors of IgE-forming cells. However, an i.p. or i.n. injection of the pollen crude extract of pollen from *Cryptomeria japonica* into mice resulted in an increase in the amount of total, but not specific, IgE Ab in their serum (Figs. 3 and 4, and Table 1), implying the presence of resting B cells bearing surface IgE. In this respect, resident cells at the i.p. or i.n. injection site may play a crucial role in the formation of the precursors of IgE-forming cells bearing nonspecific surface IgE.

An i.p. or i.n. injection of cedar pollen crude extract into the mice resulted in an increase in the amount of total IgE, but not one in that of IgM, IgG, or IgA, in their serum (Figs. 3 and 4). Also after the 2nd (s.c.) injection of the allergen to i.v. or s.c. sensitized mice, total IgE Ab increased in the serum; whereas the serum IgG, IgM, or IgA concentration did not change significantly. Upon the 2nd (1st: i.p. or i.n.) or 3rd (1st: i.v. or s.c.; 2nd: s.c.) s.c. injection of the allergen into these allergen-sensitized mice, they produced a large amount of allergen-specific IgE Ab in the serum (Table 1).

Thus, these results indicate that the production of total IgE Ab without a change in the IgG, IgM, or IgA Ab concentration is a prerequisite for the production of allergen-specific IgE Abs directed toward an allergen. These results are consistent with the dissociation between the IgE Ab response and IgG Ab response after various treatments such as low-dose X-irradiation (17), cyclophosphamide injection (3), repeated injections of complete Freund's adjuvant (18), and infection by certain nematodes (13).

It is well known that stimulation of mouse B cells with lipopolysaccharide (LPS) results in differentiation of B cells and the formation of a variety of Ig isotypes other than IgE (11). However, culture of the same B cells for 5 days with LPS together with 100 to 500 units/ml of IL-4 resulted in the formation of IgE and selective enhancement of IgG₁ formation (5), which was accompanied by a decrease in IgG_{2b} and IgG₃ formation. The effect of IL-4 on B cells was counteracted by IFN- γ (4, 6, 8). It was also found that treatment of cultures of pure B cells with LPS and IL-4 resulted in the appearance of surface IgE⁺ B cells (14) and that depletion of surface IgE⁺ B cells in the cell preparation abolished IgE formation. Thus, the major effect of IL-4 in this system appears to be switching from surface IgM⁺/IgD⁺ B cells to surface IgG₁ and/or surface IgE.

These results imply that the resident cells (e.g., dendritic cells and macrophages, but not monocytes) at an allergen injection site may be crucial as an antigen-presenting cell for total IgE production in B cells and that the interaction of resident cells in the nasal mucosa or peritoneal cavity with an allergen may result in the appearance of surface IgE⁺ B cells through the induction of some cytokine. Investigations to assess these possibilities are presently underway in our laboratory.

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