

The clinical role of specific IgE and IgG4 antibodies in patients having immunotherapy for seasonal allergic rhinitis

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This study was designed to investigate the clinical role of specific IgG4 and IgE responses in patients during immunotherapy for seasonal allergy. The study included 109 patients with seasonal allergic rhinitis due to Japanese cedar pollens. They were divided into the control group and the immunotherapy group. Serum samples were obtained at the start of immunotherapy, before the pollen season and during the season, to determine serum specific IgE and IgG4. In the control group specific IgE was significantly increased, but specific IgG4 was not changed during the pollen season. In the immunotherapy group specific IgE was not significantly increased, but specific IgG4 was significantly increased during the season. In the patients having immunotherapy for 2 years or less, the seasonal increase in specific IgG4 related to the magnitude of the clinical effect. In the patients having immunotherapy for 3 years or more, the seasonal increase in specific IgE related to the magnitude of the clinical effect. In conclusion, the specific IgG4 response and specific IgE response during the pollen season make a significant contribution to the clinical effect of immunotherapy. However, modulation of specific IgE and IgG4 responses out of the pollen season was unlikely to be an important phenomenon related to the clinical effect of immunotherapy.

Keywords *seasonal allergic rhinitis specific IgE specific IgG4 immunotherapy*

Allergen-specific immunotherapy has been widely used in the treatment of allergic diseases for more than 80 years but is still, in the opinion of many physicians, a controversial treatment. However, immunotherapy is an effective form of treatment for pollen-induced seasonal allergic rhinitis^{1,2} at least, although the mechanism underlying the clinical benefit of immunotherapy is still unclear.

Recent studies indicate that the allergic pathogenesis is triggered by a preferential activation of a special subset of T-helper lymphocytes (TH-2 cells) in predisposed atopic individuals.^{3,4} Recent work has also devoted increasing attention to the possible effect of immunotherapy on the T-cell cytokine profiles and suggests that the clinical improvement might be related to modulation of the hyperactivity of T-helper cells and an alteration from a predominant TH-2 response to TH-1 response, leading to reduced specific IgE.^{5–9}

Considerable attention had previously been devoted to

changes in allergen-specific antibodies, especially specific IgE and specific IgG4 in sera, in relation to the clinical effects of immunotherapy. Changes in specific IgE and IgG4 in sera are, at least in part, assumed to be involved in the working mechanisms of immunotherapy. Seasonal rises in specific IgE in patients with pollen allergy is well established. We have therefore investigated the seasonal dynamics of specific IgE and IgG4 in patients who were receiving immunotherapy for seasonal allergic rhinitis due to Japanese cedar (*Cryptomeria japonica*) pollens, with special reference to the clinical outcome.

Patients and methods

PATIENTS

This study included 109 adult patients (69 women and 40 men, age range: 17–58 years) with seasonal allergic rhinitis due to Japanese cedar (*C. japonica*) who gave informed consent for participation in the present study. All the patients were

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selected from our outpatients and completely satisfied the following conditions before treatment: (1) well-documented history of typical Japanese cedar pollinosis but no history of asthma and no nasal symptoms out of the cedar pollen season; (2) positive skin test to Japanese cedar pollens; (3) positive nasal provocation to the pollens; and (4) eosinophilia in nasal smears during the cedar pollen season of 1995. All the patients were recruited after a full explanation of the study protocol, by the end of December 1995. At the time of enrollment, 61 patients (39 women and 22 men) had been having immunotherapy using the Japanese cedar pollen antigens (Torii Co. Ltd, Tokyo, Japan) for various lengths of time (mean: 4.4 ± 4.3 years; range 1–16 years). They were further divided into the two groups, according to the duration of immunotherapy: those who had been treated for 2 years or less and those who had been treated for 3 years or more. Each patient from the immunotherapy group had started immunotherapy exclusively outside the pollen season. The remaining 48 patients (30 women and 18 men, control group) had not previously been treated with immunotherapy and were receiving steroid treatment.

STUDY DESIGN

The period for pollination of the Japanese cedar in 1996 was from the last week of February to the second week of April. The 48 patients in the control group were treated with non-sedating antihistamines alone from the last week of January to the end of April. The 61 patients in the immunotherapy group continued to receive a bi-weekly or a monthly maintenance dose by subcutaneous injection. The patients in the immunotherapy group were allowed to take medication (e.g. non-sedating antihistamine tablets) during the pollen season, if necessary.

Venous blood was collected twice from each patient: before the cedar pollen season of 1996 (January) for determination of pre-seasonal antibody levels and during the cedar pollen season of 1996 (first week of April) for determination of in-season antibody levels. An additional venous blood sample had been obtained from each patient in the immunotherapy group only, at the start of immunotherapy for the determination of baseline antibody levels (Figure 1). These serum samples were separated, stored at -80°C , and never retrieved prior to use for the present determination of cedar pollen-specific IgE and IgG4 in sera. All the serum samples were tested in the same batch, and all the assays were run in duplicate.

CLINICAL EVALUATION OF IMMUNOTHERAPY

The clinical evaluation of pollen immunotherapy was performed at the time of the in-season antibody determination and rated as follows: marked response = no nasal symptoms and no rescue medication; moderate response = mild nasal

symptoms but no need for medication; and poor response = need for medication to control nasal symptoms.

SPECIFIC IgE AND IgG4 DETERMINATION

Japanese cedar pollen-specific IgE in serum was determined by the CAP system and the results were expressed as U/mL. Japanese cedar pollen-specific IgG4 in serum was determined by the enzyme-linked immunosorbent assay (ELISA) as previously described by Engvall and Perlman¹⁰ with some modification. The methodology and justification of the method employed in this study have been described elsewhere.^{11,12} The result of specific IgG4 was expressed as arbitrary U/mL against a positive serum pool.

The seasonal increase in IgE, the seasonal increase in IgG4, the decrease in baseline IgE, and the increase in baseline IgG4 were calculated, using the formulae in Table 1.

STATISTICAL ANALYSIS

For comparison of data between the groups, the Mann-Whitney *U*-test was used. For comparison of the paired values within a group, the Wilcoxon's signed-rank test was used. Significant differences were accepted in each analysis when $P < 0.05$. Spearman's correlation coefficient (r_s) was calculated to analyse possible correlation between two different parameters, and significant correlation was accepted when P was less than 0.05.

Results

The results in terms of specific IgE and IgG4 are summarized in Tables 2 and 3. A statistical analysis of the changes in specific IgE and IgG4 is summarized in Table 4.

CONTROL GROUP

Specific IgE was significantly increased during the pollen season ($P < 0.0001$, Figure 2), but specific IgG4 was not significantly changed during the season ($P = 0.1170$, Figure 3).

IMMUNOTHERAPY GROUP

The pre-seasonal specific IgE level was not different from the baseline specific IgE level ($P = 0.5814$). On the other hand, the pre-seasonal specific IgG4 level was significantly higher than the baseline specific IgG4 level ($P < 0.0001$). The specific IgE level was not significantly increased during the pollen season ($P = 0.2474$), whereas the specific IgG4 level was significantly increased during the season ($P < 0.0001$). The respective numbers of patients in the immunotherapy group who showed marked, moderate, and poor responses were 28, 20, and 13.

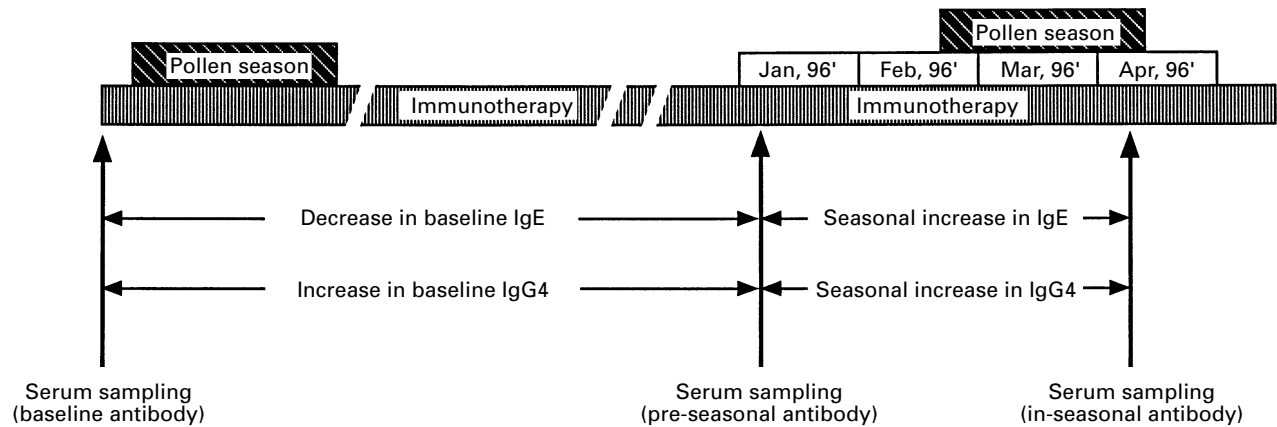


Figure 1. Schematic presentation of the study design. Serum samples were obtained twice from each patient of the control group, before the pollen season (January 1996) and during the pollen season (the first week of April 1996), to determine pre-seasonal specific IgE and IgG4 and in-seasonal specific IgE and IgG4, respectively. Additional serum samples were obtained at the initiation of immunotherapy from the patients on immunotherapy.

Table 1. Formulae for calculating seasonal increases and decreases in IgE and IgG4

$$\begin{aligned} \text{decrease in baseline IgE} &= \frac{\text{baseline IgE} - \text{pre-seasonal IgE}}{\text{baseline IgE}} \times 100 \\ \text{increase in baseline IgG4} &= \frac{\text{pre-seasonal IgG4} - \text{baseline IgG4}}{\text{baseline IgG4}} \times 100 \\ \text{seasonal increase in IgE} &= \frac{\text{in-seasonal IgE} - \text{pre-seasonal IgE}}{\text{pre-seasonal IgE}} \times 100 \\ \text{seasonal increase in IgG4} &= \frac{\text{in-seasonal IgG4} - \text{pre-seasonal IgG4}}{\text{pre-seasonal IgG4}} \times 100 \end{aligned}$$

Table 2. Serum specific IgE

	Baseline IgE (U/mL)	Pre-seasonal IgE (U/mL)	In-seasonal IgE (U/mL)	Decrease in baseline IgE (%)	Seasonal increase in IgE (%)
Control group (<i>n</i> = 48)		24.0 ± 23.0	33.6 ± 26.6		67.3 ± 81.0
IT group (<i>n</i> = 61)	20.1 ± 16.5	21.1 ± 19.2	22.6 ± 20.4	1.2 ± 32.9	9.5 ± 35.8
Marked response (<i>n</i> = 28)	20.5 ± 19.6	20.2 ± 21.5	19.6 ± 20.9	5.5 ± 29.1	−3.8 ± 14.2
Moderate response (<i>n</i> = 20)	21.9 ± 11.5	23.8 ± 15.1	23.6 ± 13.6	−7.2 ± 41.5	2.4 ± 20.3
Poor response (<i>n</i> = 13)	16.7 ± 16.0	19.0 ± 20.6	27.7 ± 27.5	−6.4 ± 23.9	49.1 ± 55.7
Short-IT group (<i>n</i> = 34)	18.0 ± 13.4	20.8 ± 17.3	24.4 ± 19.8	−14.8 ± 34.2	22.1 ± 43.0
Marked response (<i>n</i> = 8)	11.2 ± 5.2	12.9 ± 6.3	14.2 ± 7.1	−19.9 ± 29.6	10.5 ± 15.4
Moderate response (<i>n</i> = 14)	22.5 ± 12.5	26.1 ± 16.1	26.3 ± 14.4	−17.9 ± 43.8	3.9 ± 22.9
Poor response (<i>n</i> = 12)	17.3 ± 16.6	19.8 ± 21.3	29.1 ± 28.2	−7.7 ± 24.6	51.1 ± 57.7
Long-IT group (<i>n</i> = 27)	22.8 ± 19.6	21.4 ± 22.0	20.3 ± 21.3	15.9 ± 21.5	−6.3 ± 12.0
Marked response (<i>n</i> = 20)	24.2 ± 22.1	23.0 ± 24.8	21.7 ± 24.2	15.7 ± 22.4	−9.4 ± 9.0
Moderate + poor (<i>n</i> = 7)	18.9 ± 9.9	16.9 ± 11.2	16.3 ± 9.3	16.5 ± 20.4	2.7 ± 15.4

IT = immunotherapy.

Table 3. Serum specific IgG4

	Baseline IgG4 (U/mL)	Pre-seasonal IgG4 (U/mL)	In-seasonal IgG4 (U/mL)	Increase in baseline IgG4 (%)	Seasonal increase in IgG4 (%)
Control group (<i>n</i> = 48)		55.0 ± 43.6	60.8 ± 53.5		9.9 ± 28.1
IT group (<i>n</i> = 61)	50.4 ± 38.6	244.6 ± 310.0	342.8 ± 447.5	454.6 ± 841.6	43.7 ± 34.7
Marked response (<i>n</i> = 28)	49.1 ± 40.2	343.4 ± 387.9	495.2 ± 565.7	712.7 ± 1069.7	56.2 ± 38.7
Moderate response (<i>n</i> = 20)	56.5 ± 40.8	200.8 ± 221.0	267.0 ± 295.0	330.0 ± 634.6	41.3 ± 22.7
Poor response (<i>n</i> = 13)	43.8 ± 32.8	99.2 ± 125.2	128.3 ± 173.9	90.2 ± 63.9	20.3 ± 29.6
Short-IT group (<i>n</i> = 34)	57.8 ± 46.9	145.6 ± 199.9	196.6 ± 244.1	99.7 ± 89.0	44.3 ± 34.6
Marked response (<i>n</i> = 8)	60.5 ± 66.0	165.2 ± 233.5	233.5 ± 322.6	75.0 ± 32.6	90.0 ± 96.1
Moderate response (<i>n</i> = 14)	67.4 ± 44.6	171.7 ± 205.1	230.6 ± 248.3	113.5 ± 104.9	47.5 ± 22.9
Poor response (<i>n</i> = 12)	44.8 ± 34.1	101.9 ± 130.4	132.3 ± 181.0	89.4 ± 66.6	20.1 ± 30.9
Long-IT group (<i>n</i> = 27)	41.1 ± 22.1	369.3 ± 376.9	527.0 ± 568.5	901.5 ± 1119.1	42.9 ± 35.6
Marked response (<i>n</i> = 20)	44.6 ± 24.8	414.6 ± 407.7	599.9 ± 613.2	961.4 ± 1181.0	48.7 ± 39.2
Moderate + poor (<i>n</i> = 7)	31.1 ± 3.4	239.7 ± 250.7	318.7 ± 376.3	730.1 ± 981.8	26.4 ± 14.5

IT = immunotherapy.

Table 4. Dynamics of specific IgE and IgG4

	Change in baseline IgE	Change in baseline IgG4	Seasonal change in IgE	Seasonal change in IgG4
Control group			↑↑↑↑	NS
Immunotherapy group	NS	↑↑↑↑	NS	↑↑↑↑
Marked response patients	NS	↑↑↑↑	NS	↑↑↑↑
Moderate response patients	NS	↑↑↑↑	NS	↑↑↑↑
Poor response patients	NS	↑↑	↑↑	↑
Short-immunotherapy group	↑	↑↑↑↑	↑↑	↑↑↑↑
Long-immunotherapy group	NS	↑↑↑↑	↓	↑↑↑↑

NS: no significant change; ↑: significant increase ($P < 0.05$); ↑↑: significant increase ($P < 0.01$); ↑↑↑↑: significant increase ($P < 0.0001$); ↓: significant decrease ($P < 0.05$).

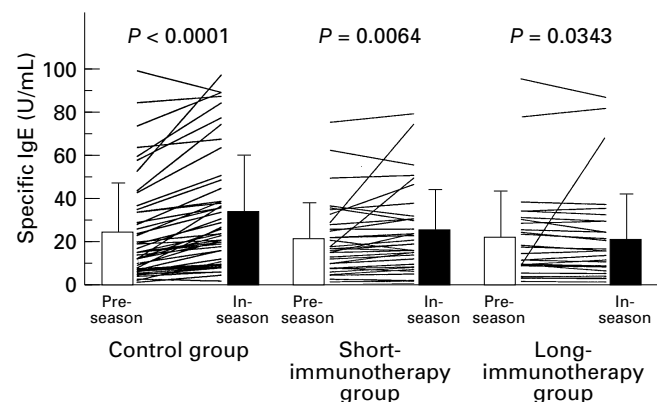


Figure 2. Seasonal changes in specific IgE. The in-seasonal specific IgE level is significantly higher than the pre-seasonal specific IgE level in the control group ($P < 0.0001$) as well as in the short-immunotherapy group ($P = 0.0064$), whereas the in-seasonal specific IgE is significantly lower than the pre-seasonal specific IgE in the long-immunotherapy group ($P = 0.0343$). □ pre-season; ■, in-season.

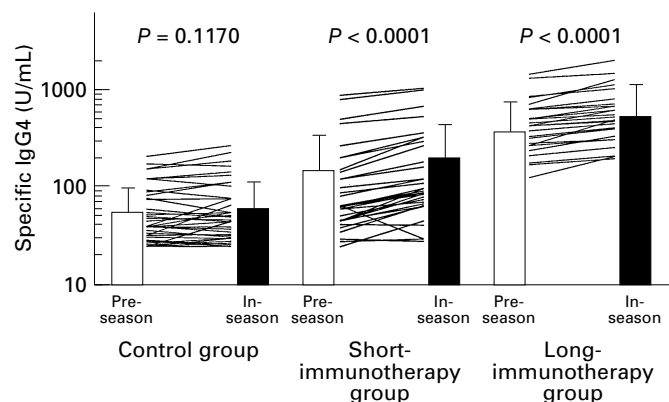


Figure 3. Seasonal changes in specific IgG4. In the control group, no significant differences are observed between the pre-seasonal specific IgG4 level and the in-seasonal specific IgG4 ($P = 0.1170$). In both the short-immunotherapy group and the long-immunotherapy group, the in-seasonal specific IgG4 level is significantly higher than the pre-seasonal specific IgG4 ($P < 0.0001$). □, pre-season; ■, in-season.

Marked response

The pre-seasonal specific IgE level was not significantly different from the baseline specific IgE level ($P = 0.6113$), whereas the pre-seasonal specific IgG4 level was significantly higher than the baseline specific IgG4 level ($P < 0.0001$). The seasonal increase in specific IgE was not significant ($P = 0.2885$), whereas the seasonal increase in specific IgG4 was significant ($P < 0.0001$).

Moderate response

The pre-seasonal specific IgE level was not significantly different from the baseline specific IgE level ($P = 0.5621$), whereas the pre-seasonal specific IgG4 level was significantly higher than the baseline specific IgG4 level ($P < 0.0001$). The seasonal increase in specific IgE was not significant ($P = 0.9651$), but the seasonal increase in specific IgG4 was significant ($P < 0.0001$).

Poor response

The pre-seasonal specific IgE level was not significantly different from the baseline specific IgE level ($P = 0.2486$), whereas the pre-seasonal specific IgG4 level was significantly higher than the baseline specific IgG4 level ($P = 0.0022$). The seasonal increase in specific IgE was significant ($P = 0.0055$), and the seasonal increase in specific IgG4 was weak but significant ($P = 0.0302$). However, the seasonal increase in specific IgG4 in this group was significantly smaller than that in the marked response patients ($P < 0.0001$) or in the moderate response patients ($P < 0.0001$).

Short-immunotherapy group

The pre-seasonal specific IgE and IgG4 levels were significantly higher than the corresponding baseline levels (IgE: $P = 0.0123$; IgG4: $P < 0.0001$). Specific IgE and IgG4 levels were significantly increased during the pollen season (IgE: $P = 0.0064$; IgG4: $P < 0.0001$). However, the seasonal increase in specific IgE in this group was significantly smaller than that in the control group ($P < 0.0001$).

The respective numbers of patients in this group who showed marked, moderate, and poor responses were eight, 14, and 12. None of the levels of the baseline specific IgE ($P = 0.8167$), baseline specific IgG4 ($P > 0.9999$), pre-seasonal specific IgE ($P = 0.7871$), and pre-seasonal specific IgG4 ($P = 0.8166$) was significantly different between the marked response patients and the poor response patients. Neither the decrease in baseline specific IgE ($P = 0.3159$) nor the increase in baseline specific IgG4 ($P = 0.8772$) was significantly different between them. The seasonal increase in specific IgG4 was significantly larger in the marked response patients than the poor response patients ($P = 0.0043$), but the seasonal increase in specific IgE was not significantly different between them ($P = 0.0758$).

Long-immunotherapy group

The pre-seasonal specific IgE level was not significantly different from the baseline specific IgE level ($P = 0.0692$). However, the pre-seasonal specific IgE level was significantly lower than the baseline specific IgE level in 18 patients treated with immunotherapy for 5 years or more (baseline IgE: 35.4 ± 11.1 U/mL; pre-seasonal IgE: 15.8 ± 11.2 U/mL, $P = 0.0095$). The pre-seasonal specific IgG4 level was significantly higher than the baseline specific IgG4 level ($P < 0.0001$). Unexpectedly, the specific IgE level was significantly reduced during the pollen season ($P = 0.0343$). The specific IgG4 level was significantly increased during the season ($P < 0.0001$).

The respective numbers of patients in the long-immunotherapy group who showed marked, moderate, and poor responses were 20, six, and one. None of the levels of baseline specific IgE ($P = 0.9779$), baseline specific IgG4 ($P = 0.4043$), pre-seasonal specific IgE ($P = 0.9118$), and pre-seasonal specific IgG4 ($P = 0.1143$) was significantly different between the marked response patients and the remaining patients. Neither the decrease in baseline specific IgE ($P = 0.9119$) nor the increase in baseline specific IgG4 ($P = 0.2806$) was significantly different between them. In addition, the seasonal increase in specific IgG4 was not significantly different between them ($P = 0.1148$), but the seasonal increase in specific IgE was significantly smaller in the marked response patients than the remaining patients ($P = 0.0355$).

CORRELATION ANALYSIS OF IMMUNOTHERAPY PATIENTS

The seasonal increase in specific IgG4 was not significantly correlated with the duration of immunotherapy ($r_s = -0.200$, $P = 0.0761$), whereas the seasonal increase in specific IgE was inversely correlated with duration of immunotherapy ($r_s = -0.462$, $P < 0.0001$). The decrease in baseline specific IgE was significantly correlated with the duration of immunotherapy ($r_s = 0.531$, $P < 0.0001$), and the increase in baseline specific IgG4 was also significantly correlated with the duration of immunotherapy ($r_s = 0.837$, $P < 0.0001$).

Discussion

The clinical role of allergen-specific IgG4 in allergic respiratory diseases has been investigated over the last two decades, but still remains controversial. Two opposite roles, an anaphylactic^{13,14} and a blocking antibody, for specific IgG4 have been suggested. It has been suggested that a rise in specific IgG4 was strongly associated with unsuccessful immunotherapy and an early increase in specific IgG4 could predict a poor clinical outcome of immunotherapy.¹⁵

However, a number of studies directed attention to the role of IgG4 as a protective factor. IgG4 myeloma proteins and

sera containing bee venom-specific IgG4 passively sensitize human basophils and thereafter suppress IgE-mediated basophil degranulation triggered by bee venom antigen when IgG4 antibodies are present in excess on the surface.^{16,17} Also, inhibition of passive sensitization by specific IgE of baboon and monkey skin and of human basophils could be induced by IgG4 subclass myeloma proteins and by sera containing a high level of specific IgG4.¹⁸ These investigations suggest that specific IgG4 could compete with specific IgE at sites on the surface of mast cells as a so-called blocking antibody to inhibit IgE-mediated immunological reactions. The protective role of specific IgG4, as a blocking antibody, has also been supported by increasing evidence that specific IgG4 is significantly increased in patients receiving immunotherapy for bee venom and house dust mite allergies.^{11,12,19–21} However, critics are sceptical of the clinical role of specific IgG4, which is based on the idea that the increase in specific IgG4 is simply a marker of antigen exposure following subcutaneous injection and probably represents an epiphenomenon unrelated to the underlying working mechanism of clinically successful immunotherapy.²² To establish the clinical significance of specific IgG4, a significant correlation should be established between clinical response to immunotherapy and changes in specific IgG4. Few studies have paid attention to the role of specific IgG4 in seasonal allergy due to pollens, although specific IgG4 has been studied in been venom- and house dust mite-immunotherapy.

The Japanese cedar pollinates heavily during early spring, and there is no significant interference by other pollens. Therefore, Japanese cedar allergy provides a supreme *in vivo* model to investigate the effect of immunotherapy on natural changes in specific IgE and IgG4 in response to natural exposure to the allergen. Our study therefore aimed at elucidating the clinical role of specific IgE and specific IgG4 during immunotherapy for Japanese cedar pollen allergy.

In patients untreated with immunotherapy, specific IgE was significantly increased in response to natural exposure to the pollen but specific IgG4 did not change. In the contrary, specific IgE did not change and specific IgG4 was significantly increased in the immunotherapy group during the pollen season. Therefore, our data suggest that immunotherapy is capable of attenuation of the usual rise in specific IgE during the pollen season and may additionally change seasonal preferential IgE production to IgG4 production. This antibody switch may be due in part to an immunotherapy-induced decrease in serum IL-4, because IL-4 plays a critical role in switching B lymphocytes from IgG to IgE production²³ and immunotherapy can reduce serum IL-4 in the long term.⁵ The seasonal changes in specific IgE were completely different between the short- and the long-immunotherapy groups. The seasonal rise in specific IgE was significant in the short-immunotherapy group ($P = 0.0064$), although the seasonal increase in specific IgE in this group was significantly smaller than that in the control group ($P < 0.0001$). By contrast, specific IgE

level was significantly decreased during the pollen season in the long-immunotherapy group ($P = 0.0343$). This discrepancy might be attributed to the phenomenon that a decline of serum IL-4 is only possible with prolonged immunotherapy.⁵

In our patients having immunotherapy, the specific IgE level before the pollen season of 1996 was not significantly different from the serum specific IgE level at the start of immunotherapy ($P = 0.5814$). A reduction in specific IgE was previously postulated as a working mechanism of immunotherapy in 1970s, but a review of the recent literature indicates that IgE antibody response shows an initial increase followed by gradual decrease after several years of immunotherapy or that the IgE antibody is not affected by immunotherapy.^{11,12,24–26} Our results are in agreement with the literature in that specific IgE in the short-immunotherapy group was significantly increased before the pollen season of 1996 than at the start of immunotherapy ($P = 0.0123$), that the decrease rate in baseline specific IgE was significantly correlated with the duration of immunotherapy ($r_s = 0.531$, $P < 0.0001$), and that the pre-seasonal specific IgE level was significantly lower than that at the start of treatment in the patients who received immunotherapy for 5 years or more ($P = 0.0095$).

Short-term immunotherapy induced attenuation of the seasonal increase in specific IgE, and longer-term immunotherapy led to a decrease in baseline specific IgE as well as control of the seasonal increase in specific IgE. A decrease in specific IgE seems theoretically to be related to a good clinical course. A seasonal rise in specific IgE was not significant in the marked response patients ($P = 0.2885$) and moderate response patients ($P = 0.9651$), whereas a seasonal rise in specific IgE was significant in the poor response patients ($P = 0.0055$). Therefore, our study has confirmed that attenuation of the seasonal rise in specific IgE by immunotherapy is one of the working mechanisms related to clinical improvement. Several studies have argued against an important role for specific IgE reduction in house dust mite immunotherapy for perennial allergic rhinitis.^{5,11,12,26} It is therefore assumed that pollen immunotherapy and mite immunotherapy may produce clinical improvement through different mechanisms or that the nature of the diseases may be somewhat different between perennial allergic rhinitis and seasonal allergic rhinitis, because the former is a disease caused by perennial exposure to small amounts of allergens, whereas the latter is caused by vast mounts of allergens for a restricted period.

The seasonal increase in specific IgG4 was significant in the immunotherapy group ($P < 0.0001$), unlike the control patients ($P = 0.1170$). The increase in baseline specific IgG4 as well as a seasonal increase in specific IgG4 was observed in all the immunotherapy subgroups, even in the poor response patients. The increase rate in baseline specific IgG4 was correlated with the duration of immunotherapy ($P < 0.0001$). Therefore, there is a possibility that specific IgG4 elevation is

an epiphenomenon due to subcutaneous antigen exposure, and is not related to the working mechanisms of immunotherapy. In the short-immunotherapy group, the increase in baseline specific IgG4 was not significantly different between the marked response patients and the poor response patients. In the long-immunotherapy group, similarly, the increase in baseline specific IgG4 was not significantly different between the marked response patients and the remaining patients. Thus, it is unlikely that an elevation in specific IgG4 out of the pollen season is involved in the working mechanism of immunotherapy related to clinical improvement.

However, our analysis included some evidence to support the clinical relevance of a specific IgG4 response during the pollen season. First of all, the seasonal increase in specific IgG4 was significant even in the poor response patients, but the significance level was much lower in the poor response patients ($P = 0.0302$) than in the marked or moderate response group ($P < 0.0001$). In addition, the seasonal increase in the poor response group was significantly smaller than in the marked or moderate response group ($P < 0.0001$). This suggests that the magnitude of the seasonal increase in specific IgG4 might be at least partially involved in the clinical effect of immunotherapy. Besides, in the short-immunotherapy group, the seasonal increase in specific IgE was not significantly different between the marked response patients and the poor response patients ($P = 0.0758$), but the seasonal increase in specific IgG4 was significantly different between them ($P = 0.0043$). These data could be interpreted to mean that a specific IgG4 response during the pollen season was the only parameter to differentiate the magnitude of the clinical effect during short-term immunotherapy.

By contrast, in the long-immunotherapy group, no significant difference was observed in the seasonal increase in specific IgG4 between the marked response patients and the remaining patients ($P = 0.1148$). Therefore, the seasonal increase in specific IgG4 is unlikely to be a major working mechanism related to the clinical effect of long-term immunotherapy. On the other hand, in the long-immunotherapy group, the seasonal increase in specific IgE was significantly smaller in the marked response patients than the remaining patients ($P = 0.0355$), suggesting that the magnitude of the clinical improvement in patients having long-term immunotherapy is mainly influenced by the magnitude of seasonal changes in specific IgE.

In conclusion, the present study has demonstrated that both specific IgE and specific IgG4 are important in pollen immunotherapy. However, modulation of specific IgE and IgG4 responses out of the pollen season is unlikely to be an important phenomenon related to the clinical effect of immunotherapy. Specific IgG4 and specific IgE responses during the pollen season make a significant contribution to the clinical effect of short-term immunotherapy and long-term immunotherapy, respectively.

However, there are anomalies in that the onset of clinical

improvement usually occurs well before changes in specific IgE or IgG4.²² Thus changes in specific IgE or IgG4 may occur only after a considerable period of immunotherapy and can hardly explain the very early clinical effects. In the opinion of the authors, allergic rhinitis is an extremely complex disease, in which a variety of immunological as well as other reactions are involved. It appears that no single pathogenic phenomenon can account for the whole nature of the disease. It is also probable that immunotherapy modulates and influences many different non-immunological²⁷ as well as immunological^{5-9,11,12,19-21,24-26} phenomena to produce clinical effects and that clinical improvement is a consequence of different mechanisms with time. It is no wonder that changes in specific IgE or IgG4 cannot account for the very early clinical improvement. A clinically important role of specific IgE and IgG4 during immunotherapy should not be ignored for this reason alone.

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