

Immunotherapy Affects the Seasonal Increase in Specific IgE and Interleukin-4 in Serum of Patients with Seasonal Allergic Rhinitis

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This study was designed to determine seasonal changes in cytokines, soluble CD23 and specific IgE in the serum of patients with seasonal allergic rhinitis, and the effect of immunotherapy on these seasonal changes. Fifty-four patients with seasonal allergic rhinitis caused by Japanese cedar pollens were divided into a medication group and an immunotherapy group. The patients of the medication group were treated with non-sedating antihistamines alone during the pollen season. The patients of the immunotherapy group had been treated for variable periods (mean, 5.0 ± 3.2 years) with immunotherapy using Japanese cedar pollen antigens. Serum samples were collected before and during the pollen season from each patient, to determine specific IgE, interleukin-4 (IL-4), interferon- γ (IFN- γ) and soluble CD23 levels in serum. A significant increase in specific IgE and IL-4 and a significant decrease in IFN- γ were observed during the pollen season in the medication group. In contrast, in the immunotherapy group, none of specific IgE, IL-4 and IFN- γ was significantly changed following natural exposure to pollens. However, these effects were not significant in patients undergoing immunotherapy for 3 or fewer years. Seasonal rates of increase in specific IgE and IL-4 differed significantly between good responders and poor responders to immunotherapy, but seasonal rates of decrease in IFN- γ did not. A seasonal rate of increase in soluble CD23 was significantly correlated with a seasonal rate of increase in specific IgE, in both the medication and the immunotherapy groups. The seasonal rate of increase in soluble CD23 was significantly smaller in the good responders than in the poor responders to immunotherapy. In conclusion, pollen immunotherapy reduces the seasonal increase in specific IgE, IL-4 and soluble CD23 in serum, and in addition switches the seasonal preferential activation of Th-2 cells to reciprocal activation of Th-1 cells with treatment over several years. It is likely that the mechanisms responsible for the clinically beneficial effects of immunotherapy principally involve the modulation of Th-2 rather than Th-1 cytokines.

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INTRODUCTION

Interleukin-4 (IL-4) plays an essential role in the switching of B lymphocytes to produce IgE [1, 2], and may therefore be of critical importance in the development of allergic diseases, although other cytokines may have synergistic effects. This critical role of IL-4 in isotype switching is reinforced by the observation that mice with germline disruption of the IL-4 gene fail to generate Th-2 cells *in vivo* and produce IgE antibodies [3]. On the other hand, interferon (IFN)-gamma inhibits the

production of IgE by B lymphocytes and inhibits the development of Th-2 clones [4, 5]. Therefore, imbalances between IL-4-producing and IFN- γ -producing helper T cells appear to be responsible for hyperproduction of IgE.

CD23 (Fc ϵ RII) is a low affinity receptor for IgE that is found on multiple types of cells including lymphocytes, monocytes, and eosinophils. Augmentation of the number of CD23-bearing cells and of the cellular density of CD23 has been documented in patients with allergic conditions [6]. Soluble CD23, which is also known as IgE-binding factor, is released after a series of

proteolytic steps, which is also known as IgE-binding factor [7]. There is increasing *in vitro* evidence that soluble CD23 is capable of potentiating the synthesis of human IgE [8] and that it probably involved in the expression of allergic diseases. Therefore, soluble CD23 is also probably involved in hyperproduction of specific IgE.

Immunotherapy is an effective form of treatment for pollen-induced seasonal allergic rhinitis [9, 10]. Although the mechanisms of pollen immunotherapy have not been fully established, this therapeutic method has been reported to blunt the usual rise in specific IgE that occurs during the pollen season. This attenuation of the seasonal increase in specific IgE by immunotherapy might be the result of altered T-cell cytokine profiles and/or decreased soluble CD23 levels during the pollen season. However, it remains to be determined whether immunotherapy can modulate T-cell cytokine profiles and soluble CD23 in serum. In the present study, we investigated the effect of natural exposure to pollens on cytokine profiles, soluble CD23 and specific IgE levels in the circulation, and whether these changes are modulated by pollen-specific immunotherapy.

MATERIALS AND METHODS

Patients. This study included 54 adult patients (28 females and 26 males, age range, 19–55 years) with seasonal allergic rhinitis due to Japanese cedar (*Cryptomeria japonica*) pollens who gave informed consent for participation. All the patients were selected from our outpatients who satisfied all of the following conditions before treatment: (1) well-documented history of typical Japanese cedar pollinosis but no history of asthma and no nasal symptoms outside of the cedar pollen season; (2) positive skin test to Japanese cedar pollens; (3) positive nasal provocation test to pollens; and (4) eosinophilia in nasal smears during the cedar pollen season before treatment. The patients were divided into a medication group (12 females and 11 males) and an immunotherapy group (16 females and 15 males) based on their treatment background. None of the patients of the medication group had previously been treated with immunotherapy or was receiving steroid treatment. The patients of the immunotherapy group had undergone variable periods (mean, 5.0 ± 3.2 years; range 1–11 years) of immunotherapy using Japanese cedar pollen (Torii Co, Ltd, Tokyo, Japan).

To serve as controls, 23 non-allergic healthy volunteers (age range, 25–64 years, nonatopic group) matched for age and sex were chosen on the basis of the following criteria: (1) no history of allergic disease; (2) no physical findings indicative of atopic disease; and (3) negative serum IgE antibodies specific to the major allergens in Japan, such as house dust mites, Japanese cedar pollen, ragweed, Japanese cypress pollen, and moulds.

Study design. Pollination of Japanese cedars occurred in 1995 from the first week of February to the second week of April. The patients of the medication group were treated with non-sedating antihistamines alone from the last week of January to the end of April. The 31 patients of the immunotherapy group continued to receive scheduled maintenance dose subcutaneous injections. The patients of the immunotherapy group were allowed to take rescue medicines (non-sedating antihistamine tablets) during the pollen season, if necessary.

Venous blood was collected twice from each patient, before the cedar pollen season of 1995 (between December 1994 and January 1995) and during the cedar pollen season 1995 (first week of April 1995). Also,

venous blood was collected from each subject of the nonatopic group, before the pollen season. Serum samples were separated, stored at -80°C , and never retrieved prior to use in the present study.

Mode of immunotherapy. The patients were given subcutaneous injections of Japanese cedar pollen extract (Torii Co, Ltd, Tokyo, Japan), which is only commercially available in Japan at present. The aqueous extracts were prepared from native pollens, and were demonstrated to contain 2.0 mg of protein per ml. Immunotherapy was begun outside the pollen season (between May and July). Individual initial doses were determined by threshold skin tests. Doses were increased by 50% at weekly intervals until the highest tolerated dose or 0.5 ml of 1 : 100 wt/vol was reached. The maximum tolerated dose was 0.5 ml of 1 : 100 wt/vol in 29 of the 31 patients, and 0.5 ml of 1 : 1000 wt/vol in the remaining two patients. The maximum tolerated dose was attained within 4 to 8 months, and weekly injection was continued for the first few months after attainment of the maintenance dose. They then received injections of this dose on a biweekly basis for the next few months, and then monthly injections. Finally, the interval between injections was gradually increased up to 2 months. During the pollen season, however, antigen injections were scheduled every 2 weeks for each patient. After each injection, the patient was asked to remain under our supervision for a minimum of 30 min and to report any symptoms that they may have experienced.

Determination of specific IgE, IL-4, IFN- γ , and soluble CD23 in sera. Specific IgE, IL-4, IFN- γ , and soluble CD23 in serum were determined at the same time. Specific IgE to Japanese cedar pollens was measured by the CAP system [11]. IL-4 was measured with our chemiluminescent enzyme-linked immunosorbent assay (ELISA) [12], and the justification of the method employed in this study has been described elsewhere [12, 13]. In brief, microtitre plates were coated with mouse monoclonal anti-human IL-4 antibody (R & D Systems, Minneapolis, MN, USA) and after incubation with serum samples or purified recombinant IL-4 (R & D System, Minneapolis, MN, USA) plates were developed with biotinylated mouse anti-human IL-4 antibody (Pharmingen, San Diego, CA, USA) followed by avidine-alkaline phosphatase conjugate (Tago, Camarillo, CA, USA). Then CSPD (Boehringer Tropic, Bedford, MA, USA) with enhancer (Emerald, Tago, Camarillo, CA, USA) was added to each well. The intensity of the chemiluminescence was determined by using micro luminater MR 1000 (Dynatech Laboratories, Inc., Chantilly, VA, USA). The method employed is capable of detecting 0.75–100 pg/ml of IL-4, and does not measure other cytokines.

IFN- γ was determined by an ELISA method described by Matsumori *et al.* [14], using an ELISA kit (Otsuka, Tokyo, Japan). The method employed is capable of detecting 0.2–100 pg/ml of IFN- γ , and does not measure the presence of other cytokines. In brief, microtitre plates were coated with anti-IFN- γ monoclonal antibody (MoAb ANOC 1611 at 10 $\mu\text{g/ml}$) in 100 μl /well of phosphate-buffered saline, pH 7.4 (PBS). Following overnight incubation at 4°C , the wells were blocked with a 1% skim milk in PBS solution for at least 1 h at room temperature and washed three times with PBS containing 0.05% Tween20 (Tween20–PBS; washing was always performed with this buffer). Samples or standard (100 μl of either) in 0.1% BSA–PBS were added to the wells and the plate was incubated at 4°C for 24 h. The plates were then washed three times, and 100 μl of rabbit anti-IFN- γ antibodies (OCT 1601, diluted to 1/2000 with 0.1% BSA–PBS–Tween20) were added to each well and plates were incubated for 2 h at 23°C . Subsequently, the plates were washed, supplemented with 100 μl of HRP-labelled goat anti-rabbit IgG (diluted to 1/10000 with 0.1% BSA–PBS–Tween20) and incubated at 23°C for 2 h. Finally, 100 μl of enzyme substrate (1 mg/ml *o*-phenylene diamine and 0.03% H_2O_2 in 0.1 M sodium citrate buffer, pH 5.0)

were added to each well and the plates incubated at room temperature for 10 min. The reaction was stopped by adding 100 μ l of H₂SO₄ to each well and the absorbance at 492 nm was determined, by using a Titertek Multiscan.

Serum concentrations of soluble CD23 were measured by a sandwich ELISA, with the use of two monoclonal antibodies which recognize a different epitope of CD23. The method employed in this study was a modified version of the method previously described by Yanagihara *et al.* [15]. The methodology and justification of the method and monoclonal antibodies employed in this study have been described elsewhere [15]. The minimum sensitivity of this assay was 40 U/ml of soluble CD23.

These methods were checked for the absence of interference with high IgE levels in the samples. The seasonal increase or decrease rates in specific IgE, IL-4, IFN- γ and soluble CD23 were calculated using the following formulae.

1. Seasonal rate of increase in specific IgE (%)

$$= \frac{\text{specific IgE during pollen season} - \text{specific IgE before pollen season}}{\text{specific IgE before pollen season}} \times 100$$

2. Seasonal rate of increase in IL-4 (%)

$$= \frac{\text{IL-4 during pollen season} - \text{IL-4 before pollen season}}{\text{IL-4 before pollen season}} \times 100$$

3. Seasonal rate of decrease in IFN- γ (%)

$$= \frac{\text{IFN-}\gamma \text{ before pollen season} - \text{IFN-}\gamma \text{ during pollen season}}{\text{IFN-}\gamma \text{ before pollen season}} \times 100$$

4. Seasonal rate of increase in soluble CD23 (%)

$$= \frac{\text{soluble CD23 during pollen season} - \text{soluble CD23 before pollen season}}{\text{soluble CD23 before pollen season}} \times 100$$

Statistical analysis. Soluble CD23 was quantitatively determined for every serum sample. However, concentrations of specific IgE, IL-4, and IFN- γ were not quantitatively determined for some serum samples because the values were below the lower limit of detection. As for each parameter, the patients whose serum concentrations were not detected in either of the determinations (before and during pollen season) were omitted from every statistical analysis. For comparisons between different groups, the Mann-Whitney *U*-test was used. For comparisons of paired values, Wilcoxon's signed-rank test was used. Differences were considered significant when $P < 0.05$. Spearman's correlation coefficient (r_s) was calculated to determine the degree of correlation between two different parameters, and significant correlation was accepted when $P < 0.05$ on Spearman's rank correlation analysis.

Clinical evaluation of immunotherapy. The patients of the immunotherapy group were divided into the following two subgroups based on their clinical response to immunotherapy: good responders = no nasal symptoms without rescue medication, and poor responders = evident nasal symptoms without rescue medication or need for rescue medication to control nasal symptoms.

RESULTS

Specific IgE, IL-4, IFN- γ and soluble CD23 in the nonatopic group

None of 23 subjects of the nonatopic group had serum concentrations of specific IgE above the lower limit of detection of the

CAP system. Serum concentrations of IL-4 above the lower limit of detection were obtained in the 18 subjects of the nonatopic group, and the mean values detected were 2.06 ± 1.76 pg/ml. Serum concentrations of IFN- γ above the lower limit of detection were obtained in 17 subjects, and the mean values of them were 2.50 ± 1.99 pg/ml. Soluble CD23 was quantitatively determined for every serum sample of the nonatopic group, and the mean values were 180.3 ± 46.2 U/ml.

Qualitative analysis

Qualitative seasonal changes in specific IgE, IL-4, IFN- γ and soluble CD23 in serum were divided into the following four criteria: (1) increase (value before season < value during season, or value before season was below the lower limit of detection and value during season was above the lower limit); (2) no change (value before season = value during season); (3) decrease (value before season > value during season, or value before season was above the lower limit and value during season was below the lower limit); and (4) unclassified (value before as well as during season was below the lower limit of detection). Qualitative seasonal changes in serum specific IgE, IL-4, IFN- γ and soluble CD23 are summarized in Table 1 (medication group), Table 2 (immunotherapy group) and Table 3.

Statistical analysis of seasonal changes

Seasonal changes in specific IgE. In the medication group, specific IgE levels during the pollen season were significantly higher than those before the pollen season ($n = 23$, $P < 0.0001$). In contrast, in the immunotherapy group, specific IgE levels during the pollen season were not significantly different from those before the pollen season ($n = 31$, $P = 0.1153$, Fig. 1). In the 13 patients (IT1, IT6, IT10, IT12–IT14, IT16, IT18, IT19, IT21, IT22, IT30 and IT31) treated for 3 or fewer years with immunotherapy, however, specific IgE was significantly increased during the pollen season (before season, 16.14 ± 9.26 U/ml; during season, 20.33 ± 11.82 U/ml, $P = 0.0033$).

Seasonal changes in IL-4. In the medication group, IL-4 levels during the pollen season were significantly higher than those before the pollen season ($n = 17$, $P = 0.0003$). In contrast, in the immunotherapy group, specific IgE levels during the pollen season were not significantly different from those before the pollen season ($n = 25$, $P = 0.9090$, Fig. 2). In the 8 patients (IT6, IT12–IT14, IT16, IT18, IT22 and IT30) treated for 3 or fewer years with immunotherapy, however, IL-4 was significantly increased during the pollen season ($P = 0.0117$).

Seasonal changes in IFN-gamma. In the medication group, IFN- γ levels during the pollen season were significantly lower than those before the pollen season ($n = 18$, $P = 0.0016$). In contrast, in the immunotherapy group, IFN- γ levels during the pollen season were not significantly different from those before the pollen season ($n = 27$, $P = 0.6938$, Fig. 3). However, 11 patients (IT1, IT6, IT12–IT14, IT16, IT19, IT21, IT22, IT30 and IT31) treated for 3 or fewer years with immunotherapy showed a

significant decrease in IFN- γ during the pollen season ($P = 0.0093$).

Seasonal changes in soluble CD23. An increase in soluble CD23 during the pollen season was significant in both the medication group ($n = 23$; before the season, 261.5 ± 109.1 U/ml, during the season, 335.7 ± 131.1 U/ml; $P < 0.0001$) and the immunotherapy group ($n = 31$; before the season, 197.9 ± 80.4 U/ml, during the season, 218.3 ± 97.5 U/ml; $P = 0.0289$).

Analysis of seasonal increase or decrease rates

Statistical analysis concerning seasonal rates of increase or decrease in specific IgE, IL-4, IFN- γ and soluble CD23 was performed for patients whose paired values (before the season and during pollen season) were above the lower limit of detection and could be quantitatively measured. For the group comparison of seasonal rate of increase in specific IgE, 22 patients (M1–M7 and M9–M23) of the medication group and 31 patients (IT1–IT31) of the immunotherapy group were used. For the group comparison of seasonal rate of increase in IL-4, 14 patients (M2, M4, M7, M9–M12, and M17–M23) of the medication group and 24 patients (IT2–IT9, IT11–IT17, and IT22–IT30) of the immunotherapy group were used. Group comparisons of seasonal rate of decrease in IFN- γ were performed in 14 patients

(M2, M4, M6, M8, M9, M12, M15, and M17–M23) of the medication group and 22 patients (IT3–IT5, IT7–IT9, IT11, IT13–IT15, IT17, IT19, and IT21–IT30) of the immunotherapy group. Group comparisons of seasonal rate of increase in soluble CD23 were performed in 23 patients (M1–M23) of the medication group and 31 patients (IT1–IT31) of the immunotherapy group.

The seasonal rate of increase in specific IgE in the medication group ($n = 22$, $156.0 \pm 242.8\%$) was significantly larger than that in the immunotherapy group ($n = 31$, $11.8 \pm 30.4\%$, $P < 0.0001$). The seasonal rate of increase in IL-4 was significantly larger in the medication group ($n = 14$, $114.8 \pm 149.7\%$) than in the immunotherapy group ($n = 24$, $28.1 \pm 94.6\%$, $P = 0.0059$). The seasonal rate of decrease in IFN- γ was significantly larger in the medication group ($n = 14$, $30.4 \pm 56.8\%$) than in the immunotherapy group ($n = 22$, $-100.6 \pm 268.7\%$, $P = 0.0149$). The seasonal rate of increase in soluble CD23 was significantly larger in the medication group ($n = 23$, $35.3 \pm 35.3\%$) than in the immunotherapy group ($n = 31$, $11.2 \pm 21.1\%$, $P = 0.0036$).

Correlational analysis for the medication group

The results of correlational analysis between the seasonal rate of increase in specific IgE, seasonal rate of increase in IL-4,

Table 1. Qualitative estimation of the medication group (M)

Case no.	IgE			IL-4			IFN- γ			Soluble CD23		
	Before	During	Change	Before	During	Change	Before	During	Change	Before	During	Change
1	○	○	↑	●	●	?	○	●	▼	○	○	↑
2	○	○	↑	○	○	↑	○	○	▼	○	○	↑
3	○	○	↑	●	●	?	○	●	▼	○	○	↑
4	○	○	↑	○	○	↑	○	○	▼	○	○	↑
5	○	○	↑	●	○	↑	●	●	?	○	○	↑
6	○	○	↑	●	●	?	○	○	↑	○	○	↑
7	○	○	↑	○	○	↑	●	●	?	○	○	↑
8	●	○	↑	●	○	↑	○	○	↑	○	○	↑
9	○	○	↑	○	○	↑	○	○	▼	○	○	↑
10	○	○	↑	○	○	↑	○	●	▼	○	○	↑
11	○	○	↑	○	○	↑	●	●	?	○	○	↑
12	○	○	↑	○	○	↑	○	○	▼	○	○	↑
13	○	○	▼	●	●	?	●	●	?	○	○	▼
14	○	○	↑	●	○	↑	○	●	▼	○	○	↑
15	○	○	▼	●	●	?	○	○	↑	○	○	▼
16	○	○	↑	●	●	?	●	●	?	○	○	↑
17	○	○	↑	○	○	↑	○	○	▼	○	○	↑
18	○	○	↑	○	○	↑	○	○	▼	○	○	↑
19	○	○	↑	○	○	↑	○	○	▼	○	○	↑
20	○	○	↑	○	○	↑	○	○	▼	○	○	↑
21	○	○	↑	○	○	↑	○	○	▼	○	○	↑
22	○	○	↑	○	○	↑	○	○	▼	○	○	↑
23	○	○	↑	○	○	↑	○	○	▼	○	○	↑

○, Detectable (above the lower limit of detection); ●, undetectable (below the lower limit of detection); ↑, increase; ▼, decrease; ?, unclassified.

Table 2. Qualitative estimation of the immunotherapy group (IT)

Case no.	IgE			IL-4			IFN- γ			Soluble CD23			Years of therapy	Clinical outcome
	Before	During	Change	Before	During	Change	Before	During	Change	Before	During	Change		
1	○	○	↑	●	●	?	○	●	▼	○	○	↑	3	×
2	○	○	↑	○	○	↑	●	●	?	○	○	↑	8	⊙
3	○	○	▼	○	○	▼	○	○	↑	○	○	▼	10	⊙
4	○	○	▼	○	○	▼	○	○	↑	○	○	▼	4	×
5	○	○	↑	○	○	↑	○	○	↑	○	○	↑	6	×
6	○	○	↑	○	○	↑	○	●	↑	○	○	↑	1	×
7	○	○	▼	○	○	▼	○	○	▼	○	○	↑	9	×
8	○	○	↔	○	○	▼	○	○	↑	○	○	↑	11	×
9	○	○	▼	○	○	▼	○	○	↑	○	○	▼	10	⊙
10	○	○	↑	●	●	?	●	●	?	○	○	↑	3	×
11	○	○	↑	○	○	▼	○	○	↑	○	○	↑	7	×
12	○	○	↑	○	○	↑	○	●	↑	○	○	↑	2	×
13	○	○	↑	○	○	↑	○	○	▼	○	○	↑	2	×
14	○	○	↑	○	○	↑	○	○	▼	○	○	↑	1	×
15	○	○	▼	○	○	▼	○	○	↑	○	○	▼	10	⊙
16	○	○	↑	○	○	↑	○	●	↑	○	○	↑	1	×
17	○	○	▼	○	○	↔	○	○	↑	○	○	▼	8	⊙
18	○	○	▼	●	○	↑	●	○	?	○	○	▼	2	×
19	○	○	▼	●	○	?	○	○	↑	○	○	▼	3	×
20	○	○	↑	●	●	?	●	○	?	○	○	↑	4	⊙
21	○	○	↑	●	●	?	○	○	↔	○	○	↑	3	×
22	○	○	↑	○	○	↑	○	○	▼	○	○	↑	2	×
23	○	○	▼	○	○	▼	○	○	↑	○	○	↑	6	⊙
24	○	○	▼	○	○	▼	○	○	↑	○	○	↑	7	⊙
25	○	○	▼	○	○	▼	○	○	↑	○	○	↑	5	⊙
26	○	○	↑	○	○	↑	○	○	▼	○	○	↑	4	×
27	○	○	▼	○	○	▼	○	○	↑	○	○	↑	8	⊙
28	○	○	▼	○	○	▼	○	○	↑	○	○	↑	9	⊙
29	○	○	↑	○	○	▼	○	○	↑	○	○	↑	5	×
30	○	○	↑	○	○	↑	○	○	↑	○	○	↑	1	×
31	○	○	↑	●	●	?	○	●	▼	○	○	↑	1	×

○, Detectable (above the lower limit of detection); ●, undetectable (below the lower limit of detection); ↑, increase; ↔, no change; ▼, decrease; ?, unclassified; ⊙, good response; ×, poor response.

Table 3. Qualitative seasonal change in IgE, IL-4, IFN- γ , and soluble CD23

Change	Specific IgE		IL-4		IFN- γ		Soluble CD23	
	Medication group	Immunotherapy group	Medication group	Immunotherapy group	Medication group	Immunotherapy group	Medication group	Immunotherapy group
Increase	21	17	17	11	3	15	21	21
No change	0	1	0	1	0	1	0	0
Decrease	2	13	0	13	15	11	2	10
Unclassified*	0	0	6	6	5	4	0	0

* Unclassified, not detected before or during the season, because results were below the sensitivity of the test.

seasonal rate of decrease in IFN- γ and seasonal rate of increase in soluble CD23 are summarized in Table 4. The correlations between the seasonal rate of increase in specific IgE, seasonal increase in IL-4 and seasonal rate of increase in IFN- γ were significant. The seasonal rate of increase in soluble CD23 was significantly correlated with the seasonal rate of increase in specific IgE and the seasonal rate of increase in IL-4, but not with the seasonal rate of decrease in IFN- γ .

Correlational analysis for the immunotherapy group.

The results of correlational analysis between the seasonal rate of increase in specific IgE, seasonal rate of increase in IL-4, seasonal rate of decrease in IFN- γ and seasonal rate of increase in soluble CD23 are summarized in Table 5. The seasonal rate of increase in specific IgE was significantly correlated with the rates of seasonal increase in IL-4 and the seasonal increase in soluble CD23, but not with the seasonal rate of decrease in IFN- γ . The seasonal rate of increase in IL-4 was significantly correlated with the rates of seasonal increase in specific IgE, seasonal decrease in IFN- γ and seasonal increase in soluble CD23. The seasonal rate

of decrease in IFN- γ was significantly correlated with the seasonal rate of increase in IL-4, but not with the rate of seasonal increase in specific IgE or seasonal increase in soluble CD23.

The duration (years) of immunotherapy was inversely correlated with the rates of seasonal increase in specific IgE ($n = 31$, $r_s = -0.736$, $P < 0.0001$), seasonal increase in IL-4 ($n = 24$, $r_s = -0.820$, $P < 0.0001$, Fig. 4), seasonal decrease in IFN- γ ($n = 22$, $r_s = -0.507$, $P = 0.0207$) and seasonal increase in soluble CD23 ($n = 31$, $r_s = -0.571$, $P = 0.0015$).

Ratio of seasonal rate of increase in specific IgE to seasonal rate of increase in IL-4

The ratio of seasonal rate of increase in specific IgE to seasonal rate of increase in IL-4 was significantly smaller in the immunotherapy group ($n = 24$, 0.47 ± 0.43) than in the medication group ($n = 14$, 2.25 ± 1.65 , $P < 0.0001$). The seasonal rate of increase in specific IgE was significantly correlated with seasonal rate of increase in IL-4 in both the medication group and the immunotherapy group (medication group, $r_s = 0.925$, $P = 0.0008$; immunotherapy group, $r_s = 0.796$, $P = 0.0001$), but the regression coefficient of the medication group was much larger than

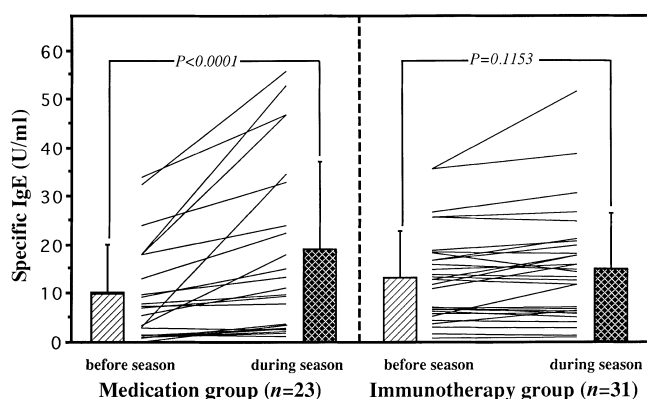


Fig. 1. Seasonal change in specific IgE. In the medication group, specific IgE during the pollen season was significantly higher than that before the pollen season. In contrast, in the immunotherapy group, specific IgE during the pollen season did not differ significantly from that before the pollen season.

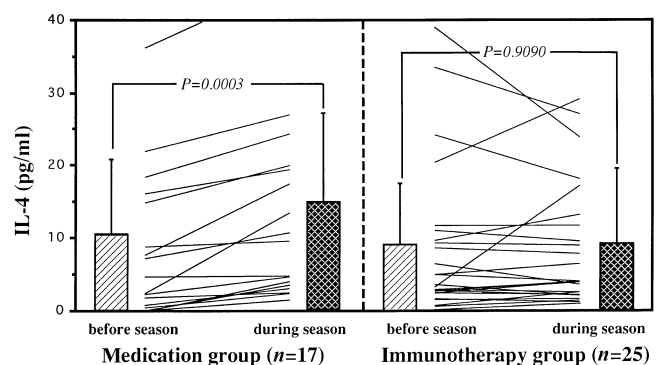


Fig. 2. Seasonal change in IL-4. In the medication group, serum IL-4 during the pollen season was significantly higher than that before the pollen season. In contrast, serum IL-4 during pollen season did not differ significantly from the preseasonal baseline in the immunotherapy group.

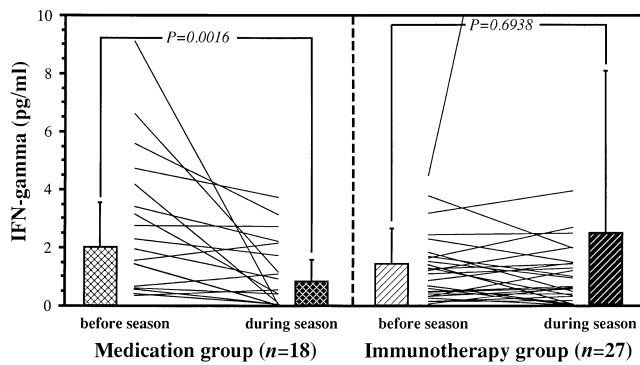


Fig. 3. Seasonal change in IFN- γ . Serum IFN- γ significantly decreased following natural exposure to pollens in the medication group, whereas IFN-gamma in the pollen season was not significantly decreased in the immunotherapy group.

that of the immunotherapy group (medication group, 1.821; immunotherapy group, 0.148, Fig. 5).

Clinical evaluation of immunotherapy

Eleven (35.5%) and 20 (64.5%) patients exhibited, respectively, good response and poor response to immunotherapy. The duration of immunotherapy for the good responders was significantly longer than that for the poor responders ($P = 0.0006$, Table 6). None of the specific IgE, IL-4, IFN- γ and soluble CD23 before the pollen season differed significantly between the good and poor responders (specific IgE, $P = 0.4955$; IL-4, $P = 0.4641$; IFN- γ , $P = 0.5890$; soluble CD23, $P = 0.5087$, Table 6). Seasonal rates of increase in specific IgE, IL-4 and soluble CD23 for the good responders were significantly smaller than those for the poor responders (IgE, $P = 0.0002$, IL-4, $P = 0.0139$; soluble

CD23, $P = 0.0053$, Table 6). However, seasonal rates of decrease in IFN- γ did not differ significantly between the good and poor responders ($P = 0.2167$, Table 6).

Systemic adverse reaction during immunotherapy

Systemic adverse reactions were experienced in two cases (IT2 and IT28) during immunotherapy. In one patient (IT2), anaphylactic reactions such as hypotension and dyspnoea were observed 20 min after the initial subcutaneous injection. In the other patient (IT28), similar anaphylactic reactions were observed 15 min after the 6th subcutaneous injection. In either case, anaphylactic reactions occurred outside the pollen season. Clinical responses to immunotherapy were judged as good responses in both cases.

DISCUSSION

Seasonal increases in serum specific IgE in pollen-sensitive patients are documented. Since Th-2 cell derived IL-4 plays a key role in inducing and increasing the generation of both primary polyclonal and secondary specific IgE responses by B lymphocytes [16], IL-4 appears to be involved in seasonal increases in specific IgE in seasonal allergic rhinitis. It has recently been reported that *in vitro* IgE production by peripheral blood mononuclear cells (PBMC) in the presence of IL-4 does not differ significantly between atopic and non-atopic donors [17]. More recently, however, Gagnon *et al.* [18] demonstrated that, during the pollen season, IL-4-stimulated PBMC of atopic donors produced more IgE than did IL-4-stimulated PBMC of non-atopic donors, while outside the pollen season no difference was observed between these two groups.

Table 4. Correlation analysis in the medication group

	Seasonal increase rate in specific IgE	Seasonal increase rate in IL-4	Seasonal increase rate in IFN- γ	Seasonal increase rate in soluble CD23
Seasonal increase rate in specific IgE		$r_s = 0.925^*$ $P = 0.0008$ ($n = 14$)	$r_s = 0.698^*$ $P = 0.0156$ ($n = 13$)	$r_s = 0.759^*$ $P = 0.0005$ ($n = 22$)
Seasonal increase rate in IL-4	$r_s = 0.925^*$ $P = 0.0008$ ($n = 14$)		$r_s = 0.655^*$ $P = 0.0385$ ($n = 11$)	$r_s = 0.736^*$ $P = 0.0079$ ($n = 14$)
Seasonal increase rate in IFN- γ	$r_s = 0.698^*$ $P = 0.0156$ ($n = 13$)	$r_s = 0.655^*$ $P = 0.0385$ ($n = 11$)		$r_s = 0.471^\dagger$ $P = 0.0881$ ($n = 14$)
Seasonal increase rate in soluble CD23	$r_s = 0.759^*$ $P = 0.0005$ ($n = 22$)	$r_s = 0.736^*$ $P = 0.0079$ ($n = 14$)	$r_s = 0.471^\dagger$ $P = 0.0881$ ($n = 14$)	

* Significant correlation.

† Insignificant correlation.

Table 5. Correlation analysis in the immunotherapy group

	Seasonal increase rate in specific IgE	Seasonal increase rate in IL-4	Seasonal increase rate in IFN- γ	Seasonal increase rate in soluble CD23
Seasonal increase rate in specific IgE		$r_s = 0.796^*$ $P = 0.0001$ ($n = 24$)	$r_s = 0.382^\dagger$ $P = 0.0798$ ($n = 22$)	$r_s = 0.774^*$ $P < 0.0001$ ($n = 31$)
Seasonal increase rate in IL-4	$r_s = 0.796^*$ $P = 0.0001$ ($n = 24$)		$r_s = 0.499^*$ $P = 0.0295$ ($n = 20$)	$r_s = 0.575^*$ $P = 0.0058$ ($n = 24$)
Seasonal increase rate in IFN- γ	$r_s = 0.382^\dagger$ $P = 0.0798$ ($n = 22$)	$r_s = 0.499^*$ $P = 0.0295$ ($n = 20$)		$r_s = 0.302^\dagger$ $P = 0.1663$ ($n = 22$)
Seasonal increase rate in soluble CD23	$r_s = 0.774^*$ $P < 0.0001$ ($n = 31$)	$r_s = 0.575^*$ $P = 0.0058$ ($n = 24$)	$r_s = 0.302^\dagger$ $P = 0.1663$ ($n = 22$)	

* Significant correlation.

† Insignificant correlation.

At least four studies have documented a significant elevation of serum IL-4 in atopic individuals compared with non-atopic controls [12, 19–21]. These findings suggest that seasonal increases in serum IL-4 might be responsible for seasonal increases in specific IgE in patients with seasonal allergic rhinitis. However, little is known concerning seasonal changes in serum IL-4 and their relationship to seasonal increases in

specific IgE. The primary purpose of this study was to determine the seasonal changes in serum IL-4 and specific IgE levels in pollen-sensitive patients. In our study, the seasonal increase in IL-4 was significant and was significantly related to the seasonal

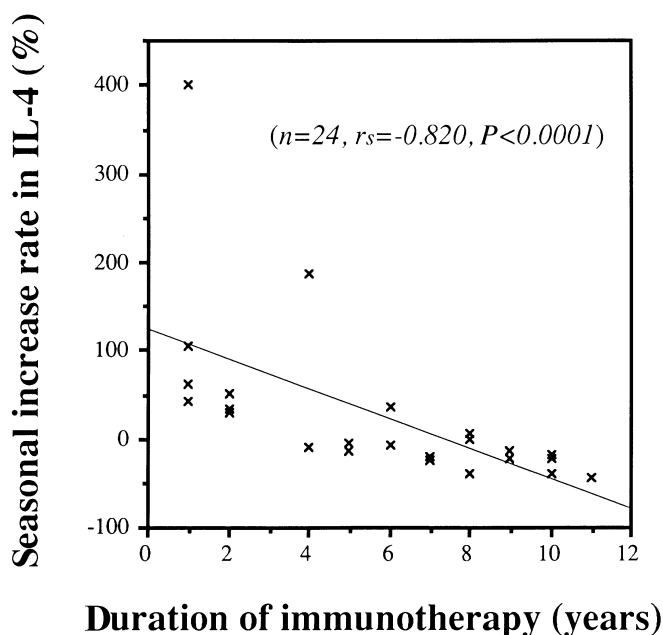


Fig. 4. Diagram of correlation between seasonal increase in IL-4 and duration of immunotherapy. The seasonal rate of increase in IL-4 was inversely correlated with the duration (years) of immunotherapy.

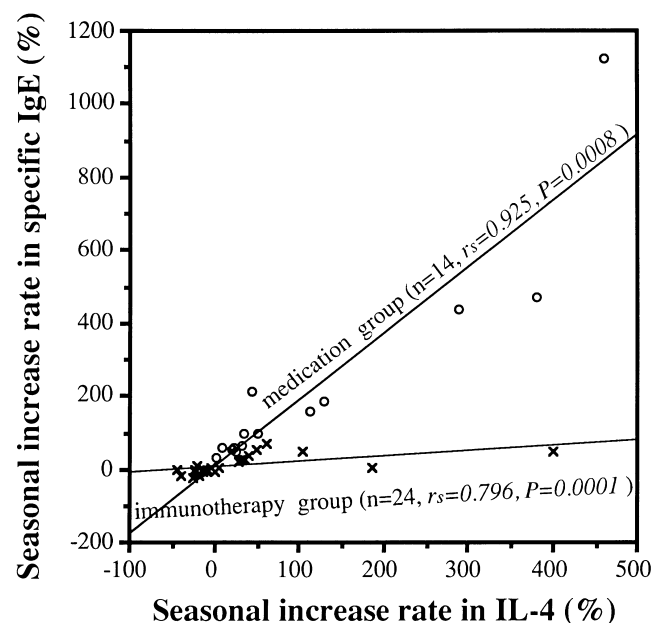


Fig. 5. Diagram of correlation between seasonal increase in specific IgE and IL-4. The seasonal rate of increase in specific IgE was significantly correlated with the seasonal rate of increase in IL-4 in both the medication group (open circle) and the immunotherapy group (cross). The regression coefficient for the medication group (1.821) was much larger than that for the immunotherapy group (0.148).

Table 6. Comparison of good responders and poor responders

Parameter compared	Good responders	<i>n</i>	Poor responders	<i>n</i>	Difference <i>P</i>
Duration of immunotherapy (years)	7.3 ± 2.1	11	3.6 ± 2.8	20	0.0006*
Specific IgE before the pollen season (U/ml)	11.8 ± 10.0	11	14.0 ± 9.5	20	0.4955
IL-4 before the pollen season (pg/ml)	10.7 ± 12.2	10	7.9 ± 9.2	14	0.4641
IFN-γ before the pollen season (pg/ml)	1.2 ± 1.1	9	1.6 ± 1.36	13	0.5890
Soluble CD23 before the pollen season (U/ml)	211.5 ± 78.6	11	190.4 ± 82.3	20	0.5087
Seasonal increase rate in specific IgE (%)	-8.8 ± 9.9	11	23.0 ± 32.0	20	0.0002*
Seasonal increase rate in IL-4 (%)	-17.1 ± 14.8	10	60.3 ± 114.0	14	0.0139*
Seasonal decrease rate in IFN-γ (%)	-51.6 ± 45.7	9	-134.6 ± 349.2	13	0.2167
Seasonal increase rate in soluble CD23 (%)	-2.4 ± 12.0	11	18.7 ± 21.5	20	0.0053*

* Significant difference.

increase in specific IgE in patients without immunotherapy. This is the first *in vivo* study to clearly demonstrate that an increase in IL-4 is related to the seasonal increase in specific IgE in patients with pollen allergy.

Immunotherapy is an effective form of treatment for seasonal allergic rhinitis [9, 10]. We previously demonstrated that elevation of serum IL-4 in perennial allergic rhinitis could be down-regulated by prolonged immunotherapy, and that the extent of this decrease was significantly correlated with that of decrease in specific IgE in serum [12]. Similarly, pollen immunotherapy might modulate seasonal changes in serum IL-4 and specific IgE. The second purpose of this study was therefore to elucidate the effect of pollen immunotherapy on seasonal changes in serum IL-4 and specific IgE.

In the present study, serum levels of neither IL-4 nor specific IgE significantly changed in immunotherapy patients, whereas both IL-4 and specific IgE significantly increased during the pollen season in patients untreated with immunotherapy. In addition, the seasonal rates of increase in IL-4 and specific IgE were significantly larger in patients untreated with immunotherapy than in those on immunotherapy. Our results thus suggest that pollen immunotherapy can inhibit the seasonal increase in serum IL-4 and specific IgE. However, this inhibitory effect on seasonal increase in IL-4 and specific IgE was not significant in patients undergoing immunotherapy for 3 or fewer years, and was significantly correlated with the duration of immunotherapy. Thus, immunotherapy is likely to suppress the seasonal increase in serum IL-4 and specific IgE with treatment over several years. In addition, the seasonal rate of increase in IL-4 and specific IgE was significantly smaller in those with good response to immunotherapy than in those with poor response to it. These findings could be interpreted to mean that the inhibitory effect of immunotherapy on the seasonal increase in IL-4 and specific IgE plays a role in mediating its clinical effect.

A few studies have discussed the possibility of modifying cytokine secretion by T cells from atopic subjects by immunotherapy [22–24]. Reduced IL-4 production with bee venom immunotherapy has been reported [22, 23]. Secrist *et al.* [24]

have documented that immunotherapy reduces IL-4 production by allergen-specific CD4⁺ T cells from grass pollen and mite allergic subjects to levels observed with T cells from nonallergic subjects, or to levels induced with nonallergic antigens such as tetanus toxoid, and that in most cases the levels of IL-4 produced are inversely related to the length of time on immunotherapy [24]. Our *in vivo* results are in agreement with the conclusion derived from *in vitro* study by Secrist *et al.* [24], in that immunotherapy accomplishes its clinical effects together with reduction in seasonal enhancement of IL-4 synthesis in response to allergen stimulation, and that the degree of this reduction is related to the duration of immunotherapy. Other interesting findings of the present study suggestive of the mechanism of immunotherapy were that the ratio of seasonal increase in specific IgE to seasonal increase in IL-4 was significantly smaller in the immunotherapy group than in the medication group and that the regression coefficient was much smaller in the immunotherapy group than in the medication group. These findings together indicate that immunotherapy could also reduce the reactivity of B cells to IL-4.

Since the regulation of IgE synthesis essentially depends on the reciprocal activities of IL-4 and IFN-γ, we also studied the seasonal changes in serum IFN-γ levels. A significant correlation between seasonal rate of decrease in IFN-γ and seasonal rate of increase in IL-4 was observed in both the medication group and the immunotherapy group. IFN-γ was significantly decreased following natural exposure to pollens in the medication group, whereas IFN-γ was not significantly decreased in the immunotherapy group. The seasonal rate of decrease in IFN-γ differed significantly between the two groups. These findings suggest that immunotherapy can affect the seasonal attenuation of serum Th-1 cytokines in response to natural exposure to allergens, which might also be involved in the mechanisms of its action.

Another interesting finding of our study was that the correlation between seasonal changes in IFN-γ and seasonal changes in specific IgE was not significant in the immunotherapy group, suggesting that IL-4 production may be inversely related to IFN-γ production but that the effect of immunotherapy on Th-1

cytokines is not directly linked to suppression of seasonal increase in specific IgE. In this respect, our study does not support an important part of Th-1 cytokine enhancement in a reduction of specific IgE. In an *in vitro* study by Secrist *et al.* [24], production of IFN- γ by allergen-specific CD4⁺ T cells was not affected in allergic subjects on immunotherapy, although IL-4 production was reduced by immunotherapy. Our results are consistent with those by Secrist *et al.* [24], in suggesting that modification of Th-2 cytokines, but not of Th-1 cytokines, is a key mechanism linked to suppression of specific IgE during immunotherapy.

Intramuscular administration of IFN- γ has been tested as a treatment for seasonal allergic rhinitis, but found to be of little clinical benefit [25]. In the present study, the seasonal rate of decrease in IFN- γ did not differ significantly between the good and poor responders to immunotherapy. Our findings therefore do not suggest that increase in Th-1 cytokines is a major mechanism of immunotherapy linked to its clinical effect. Suppression of seasonal increases in Th-2 cytokines is more likely to be related to the clinical benefit of pollen immunotherapy.

On the other hand, an important role of IFN- γ in the mechanism of immunotherapy has been demonstrated. Varney *et al.* [26] studied the effect of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. Nine months of immunotherapy was associated with lower median cell counts for allergen-induced mRNA expression for the Th-2 pattern cytokines such as IL-4, but the degree of reduction was not significant. In contrast, immunotherapy induced a shift in the pattern of allergen-induced cytokine mRNA expression toward the induction of Th-1 type cytokines such as IFN- γ in half of the patients. Their study supports the view that immunotherapy does not modulate expression of Th-2 pattern cytokines in response to allergen exposure, but that it may exert its effects through induction of T cell tolerance.

Since immunotherapy is a form of systemic treatment and its clinical benefit is likely to be, at least in part, a consequence of its systemic effects on T cells as well as other types of cells involved in the allergic inflammatory process, our study focused exclusively on specific IgE and cytokines in serum. Varney *et al.* [26] investigated the effect of nine months of pollen immunotherapy on the cytokine profiles in the cells recruited following allergen-induced late-phase cutaneous responses. Also, in our study, modulation of serum IL-4 levels was not detected in patients under short-term immunotherapy. The possibility cannot be excluded that only longer-term immunotherapy may affect the expression of Th-2 type cytokines in inflamed tissues in response to allergen exposure.

Finally, we also determined serum soluble CD23 levels in patients with seasonal allergic rhinitis, since soluble CD23 is capable of potentiating human IgE synthesis [8] and elevated serum levels of soluble CD23 have been reported in atopic patients [20, 21, 27, 28]. A seasonal rate of increase in soluble CD23 was significantly correlated with a seasonal rate of increase in specific IgE in both the medication group and the

immunotherapy group. Soluble CD23 was significantly increased during the pollen season in both the medication group and the immunotherapy group, but the seasonal rate of increase in soluble CD23 was significantly smaller in the immunotherapy group than in the medication group. In addition, the seasonal rate of increase in soluble CD23 in those with good response to immunotherapy was significantly smaller than in those with poor response to immunotherapy. Therefore, our findings suggest that the suppression of a seasonal increase in soluble CD23 by immunotherapy is probably involved in the suppression of seasonal increase in specific IgE, and is also related to the clinical effect of immunotherapy.

In conclusion, the seasonal increase in serum IL-4 in response to pollen exposure was related to the seasonal increase in serum specific IgE. Pollen immunotherapy reduced the seasonal increase in serum IL-4, soluble CD23 and specific IgE levels, and in addition switched the seasonal preferential Th-2 cytokine to the reciprocal Th-1 cytokine. Immunotherapy probably exerts its clinical effect mainly through the control of Th-2 type cytokines and in addition, through a shift in cytokine profiles, but such effects only become apparent after considerable years of treatment.

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