Serologic Study of the Working Mechanisms of Immunotherapy for Children With Perennial Allergic Rhinitis

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Background: Recent double-blind placebo-controlled trials have clearly shown the efficacy of immunotherapy for perennial allergic rhinitis. However, the exact working mechanisms related to the clinical effect of immunotherapy remain unclear.

Objectives: To monitor the changes over time in immunologic parameters in children who received immunotherapy for perennial allergic rhinitis, and to elucidate the working mechanisms of immunotherapy related to its clinical efficacy.

Design: Nineteen children with perennial allergic rhinitis due to Dermatophagoides farinae enrolled in this prospective open study. Venous blood was collected to determine levels of specific IgE, specific IgG4, soluble interleukin 2 receptor, interleukin 4, soluble intercellular adhesion molecule 1, and soluble vascular cell adhesion molecule 1 at enrollment and 1, 2, 3, 5, and 10 years after enrollment.

Results: Immunotherapy affected serum levels of specific IgE, specific IgG4, soluble interleukin 2 receptor, interleukin 4, and soluble intercellular adhesion molecule 1, but not soluble vascular cell adhesion molecule 1. The rates of increase of levels of specific IgG4 and the rates of decrease of levels of soluble interleukin 2 receptor were correlated with the rates of decrease of symptom scores during the first 3 years of treatment, but not after 5 years. The rates of decrease in levels of soluble intercellular adhesion molecule 1 were correlated with the rates of decrease in symptom scores at 3 and 5 years after the beginning of the course of immunotherapy. The rates of decrease in levels of specific IgE and interleukin 4 were correlated with the rates of decrease in symptom scores after 5 and 10 years of treatment, but not during the first 3 years.

Conclusion: Each modulation in levels of specific IgE, specific IgG4, soluble interleukin 2 receptor, interleukin 4, and soluble intercellular adhesion molecule 1 contributed to the clinical effect of immunotherapy in particular phases of treatment for children with perennial allergic rhinitis.


ALLERGEN-SPECIFIC IMMUNOTHERAPY has been widely used to treat allergic diseases for more than 80 years, and recent double-blind placebo-controlled trials for perennial allergic rhinitis have clearly demonstrated its efficacy. Allergen-specific IgE and IgG, and especially the blocking antibody of IgG4, have been considered of key importance in allergic rhinitis, and for decades considerable attention has been devoted to the clinical role of specific IgE and IgG4 in serum in relation to clinical outcome. However, there has been no definite conclusion concerning the function of these antibodies because an increase in levels of specific IgG4 has been associated with the clinical efficacy of immunotherapy in some studies but not in others. Although there is evidence of some measure of clinical benefit as well as immunologic changes from short-term immunotherapy, it generally has to be administered for at least several years to sustain clinical efficacy. A serial follow-up study of specific IgE and IgG4 levels in serum during prolonged immunotherapy would improve our understanding of its working mechanism. The primary aim of this prospective study was therefore to investigate the changes over time in levels of specific IgE and IgG4 in children with perennial allergic rhinitis who received immunotherapy for 10 years, with a focus on clinical efficacy.

There is increasing evidence that airway allergic diseases represent a specialized form of cell-mediated immunity, in which CD4-positive helper T (T<sub>H2</sub>) cells and their products, various cytokines, play a central role in promoting airway inflammation. Cytokines derived from T<sub>H2</sub> cells could mediate allergic inflammation by ac-
PATIENTS AND METHODS

PATIENTS

Sixty children (26 girls and 34 boys; age range, 6-10 years) with severe perennial allergic rhinitis due to *Dermatophagoides farinae* were originally enrolled in this prospective open study. The guardians of all the children provided their informed consent. All the children were outpatients who satisfied all the following conditions before enrollment: (1) a well-documented history of typical perennial allergic rhinitis of severe grade according to the criteria of Okuda et al; with no aggravation during pollen season; (2) no history of asthma; (3) a positive skin test result and a positive reaction to nasal provocation with *D farinae* antigen according to the criteria of Okuda et al; (4) eosinophilia in nasal smears according to the criteria of Okuda et al; and (5) no previous immunotherapy or systemic corticosteroid treatment.

STUDY DESIGN

This study design followed the principles outlined in the Declaration of Helsinki. The study started in May 1985 and was completed by the end of March 1996. The aim of the study was originally to investigate the changes over time in levels of *D farinae*-specific IgE and IgG4 in children treated with immunotherapy for 10 years. At the time of enrollment, all the patients and their guardians were given a full explanation of immunotherapy and the study design. Thirty-three patients (14 girls and 19 boys) enrolled in the pharmacotherapy group, and 27 patients (12 girls and 15 boys) enrolled in the immunotherapy group. The patients in the pharmacotherapy group were treated with cromolyn sodium only. The patients in the immunotherapy group were treated with immunotherapy only and agreed not to take any concomitant medication that might affect their nasal symptoms during the study period. Venous blood was collected from each patient: on enrollment and 1, 2, 3, 5, and 10 years after enrollment. Venous blood was collected from all patients in the same months to avoid possible seasonal fluctuation in levels of house dust mites. Serum samples were separated and stored at −60°C until use in the present study.

tivating eosinophils, promoting mast cell development, regulating the transformation of immunoglobulin isotype to IgE, and modulating adhesion molecule expression. Currently, allergies might be characterized by Th2-like cells or a Th1/2 response leading to inflammatory disease. Recent in vitro and in vivo evidence suggests that immunotherapy alters T-cell cytokine profiles, which suppresses allergic inflammatory events.

In the present study, we also monitored changes in levels of interleukin 4 (IL-4), soluble interleukin 2 receptor (IL-2R), and soluble adhesion molecules in serum samples from children with perennial allergic rhinitis who were monitored during 10 years of immunotherapy, with a focus on clinical efficacy. Our preliminary studies suggest that immunotherapy could affect serum levels of IL-4, soluble IL-2R, and soluble adhesion molecules in patients with allergic rhinitis.

MODE OF IMMUNOTHERAPY

After providing their informed consent, the patients were each given subcutaneous injections of standardized *D farinae* antigen (Miles Inc, Spokane, Wash). Initial individual doses were determined based on the end point concentration revealed by the threshold skin test and serum concentrations of specific IgE. Doses were increased by 50% to 100% at weekly intervals until the highest tolerated dose or 3000 U was reached. The maximum tolerated dose (3000 U in 16 patients and 300 U in 3 patients) was attained in 4 to 8 months, after which weekly injections of this dose were continued for several months. The patients then received injections of the maximum tolerated dose on a biweekly basis for the next few months, followed by monthly injections. Finally, the interval between injections was gradually increased for up to 3 months in the absence of symptomatic relapse. After each injection, the patient was asked to remain under our supervision for a minimum of 30 minutes and to report any symptoms that they experienced. All the patients continued the treatment for 10 successive years to avoid possible relapse even if their nasal symptoms had resolved.

CLINICAL ASSESSMENT OF SYMPTOM SCORES

We evaluated the clinical effect of treatment based on our criteria using daily symptom diary cards at enrollment and 1, 2, 3, 5, and 10 years after enrollment. The details of the method used in this study have been described elsewhere. In brief, all the patients were asked to complete daily symptom diary cards for 14 days before we assessed their nasal symptom scores. The daily symptom cards included the number of sneezing attacks and nose blows, and the degree of nasal obstruction. Based on a careful survey of the diary cards, we graded the 3 nasal symptoms (sneezing, rhinorrhea, and obstruction) on a scale of 0 to 3 as follows:

<table>
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<td>1-5 sneezing attacks</td>
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<td></td>
<td>2</td>
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<td></td>
<td>3</td>
<td>More than 11 sneezing attacks</td>
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<table>
<thead>
<tr>
<th>Scoring of Rhinorrhea</th>
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<th>No nasal blowings</th>
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<tr>
<td></td>
<td>1</td>
<td>1-5 nasal blowings</td>
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<td>6-10 nasal blowings</td>
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<tr>
<td></td>
<td>3</td>
<td>More than 11 nasal blowings</td>
</tr>
</tbody>
</table>

PHARMACOTHERAPY GROUP

Changes in Symptom Scores Over Time

The symptom scores demonstrated a linear decrease as treatment proceeded (*Table 1*). The symptom scores after 1 year were significantly lower than those at enroll-
The levels of specific IgE did not change significantly for those at 2 years (\( P = .69 \); 2 years vs 3 years, \( P = .87 \)). The rates of decrease in levels of soluble IL-2R, IL-4, soluble ICAM-1, and soluble VCAM-1 were calculated according to the formula for the rate of decrease in levels of specific IgE.

### NUMBER OF PATIENTS IN THE PHARMACOTHERAPY GROUP USED FOR STATISTICAL ANALYSIS

Nine of the 33 patients discontinued the study by the end of the first year. In addition, another 12 patients stopped immunotherapy after 1 to 3 years. Only 6 patients remained in the trial by the end of 4 years after enrollment. Therefore, a total of 12 patients (3 girls and 7 boys; age range, 6-10 years) who remained in the study after 3 years were used for statistical analysis to compare with the results from the immunotherapy group.

### STABLE ANALYSIS

For comparisons between different groups, the Mann-Whitney \( U \) test was used. For comparisons of paired values, the Wilcoxon signed rank test was used, and differences were considered significant in each analysis when \( P < .05 \). The Spearman rank correlation coefficient (\( r_s \)) was calculated to determine the degree of correlation between 2 different parameters. Correlation was considered significant when \( P < .05 \).

## Determination of Parameter Levels

Serum concentrations of specific IgE, specific IgG4, soluble IL-2R, IL-4, and soluble adhesion molecules (soluble intercellular adhesion molecule 1 [ICAM-1] and soluble vascular cell adhesion molecule 1 [VCAM-1]) were determined on enrollment and 1, 3, 5, and 10 years after enrollment. Serum concentrations of specific IgE and specific IgG4 were also determined at 2 years after enrollment.

Levels of IgE specific to \( D. farinae \) were measured with the Kallestad Allercoat System (Shionogi Co Ltd, Tokyo, Japan), and the results were expressed as units per milliliter. Levels of IgG4 specific to \( D. farinae \) were determined with an enzyme-linked immunosorbent assay, the methodology and justification of which have been described elsewhere.

Levels of soluble IL-2R, soluble ICAM-1, and soluble VCAM-1 were also determined with an enzyme-linked immunosorbent assay from commercially available kits. Levels of IL-4 were measured with a chemiluminescent enzyme-linked immunosorbent assay. The details and the justification of each method used in the present study have been described elsewhere.

The minimum sensitivity of each assay was 40 U/mL of soluble IL-2R, 40 ng/mL of soluble ICAM-1 and soluble VCAM-1, and 0.75 pg/mL of IL-4.

The rate of decrease in levels of specific IgE and the rate of increase in levels of specific IgG4 were calculated using the following formulas:

\[
\text{Rate of Decrease (\%)} = \left( \frac{\text{Specific IgE at Enrollment} - \text{Specific IgE at x Years}}{\text{Specific IgE at Enrollment}} \right) \times 100
\]

\[
\text{Rate of Increase (\%)} = \left( \frac{\text{Specific IgG4 at x Years} - \text{Specific IgG4 at Enrollment}}{\text{Specific IgG4 at Enrollment}} \right) \times 100
\]

### Changes in Specific IgE Levels Over Time

The levels of specific IgE did not change significantly for 3 years (enrollment vs 1 year, \( P = .23 \); 1 year vs 2 years, \( P = .69 \); 2 years vs 3 years, \( P = .67 \) [Table 1]).

### Changes in Specific IgG4 Levels Over Time

The levels of specific IgG4 did not change significantly for 3 years (enrollment vs 1 year, \( P = .53 \); 1 year vs 2 years, \( P = .87 \); 2 years vs 3 years, \( P > .99 \) [Table 1]).

### Changes in Soluble IL-2R Levels Over Time

The levels of soluble IL-2R at 1 year were significantly lower than those at enrollment (\( P = .02 \)), but those at 3 years were not significantly different from those at 1 year (\( P = .84 \) [Table 1]).

### Changes in IL-4 Levels Over Time

The levels of IL-4 did not change significantly for 3 years (enrollment vs 1 year, \( P = .09 \); 1 year vs 3 years, \( P = .35 \) [Table 1]).
Changes in Soluble ICAM-1 Levels Over Time
The levels of soluble ICAM-1 did not change significantly for 3 years (enrollment vs 1 year, \(P = .07\); 1 year vs 3 years, \(P = .20\) [Table 1]).

Changes in Soluble VCAM-1 Levels Over Time
The levels of soluble VCAM-1 did not change significantly for 3 years (enrollment vs 1 year, \(P = .30\); 1 year vs 3 years, \(P = .06\) [Table 1]).

**IMMUNOTHERAPY GROUP**

**Correlation Between Symptom Score and Immunologic Parameters at Enrollment**
Levels of specific IgE (\(r_s = -0.27; P = .13\)), specific IgG4 (\(r_s = 0.07; P = .75\)), soluble IL-2R (\(r_s = 0.02; P = .84\)), IL-4 (\(r_s = 0.02; P = .87\)), and soluble ICAM-1 (\(r_s = 0.18; P = .59\)) at enrollment were not significantly correlated with the symptom scores at enrollment. However, the levels of soluble VCAM-1 at enrollment were significantly correlated with the symptom scores at enrollment (\(r_s = 0.75, P = .002\)).

**Changes in Symptom Scores Over Time**
The symptom scores demonstrated a linear decrease as treatment proceeded (Table 2). The symptom scores after enrollment became significantly lower year by year (enrollment vs 1 year, \(P < .001\); 1 year vs 2 years, \(P < .001\); 2 years vs 3 years, \(P = .06\); 3 years vs 5 years, \(P < .001\); 5 years vs 10 years, \(P < .001\)). The rate of decrease in symptom scores was significantly correlated with the duration (in years) of immunotherapy (\(r_s = 0.37; P < .001\)).

**Changes in Specific IgE Levels Over Time**
The levels of specific IgE demonstrated a significant initial increase during the first 2 years of treatment (enrollment vs 1 year, \(P < .001\); enrollment vs 2 years, \(P = .02\), with a peak at 1 year. The levels of specific IgE did not differ significantly between 1 and 2 years (\(P = .19\)). After the peak, the levels of specific IgE decreased signifi-
cantly year by year (P < .001 for all comparisons). The levels of specific IgE at 3 years were not significantly different from those at enrollment (P = .78), but thereafter the levels of specific IgE were significantly lower than those at enrollment (5 years, P < .001; 10 years, P < .001 [Table 2 and Figure 1]).

**Changes in Specific IgG4 Levels Over Time**

The levels of specific IgG4 demonstrated a significant linear increase as treatment proceeded (enrollment vs 1 year, P < .001; 1 year vs 2 years, P = .003; 2 years vs 3 years, P = .003; 3 years vs 5 years, P = .006; 5 years vs 10 years, P = .002). In addition, the levels of specific IgG4 at any time after enrollment were significantly higher than those at enrollment (P < .001 for all comparisons [Table 2 and Figure 2]). The rates of increase in levels of specific IgG4 were significantly correlated with the duration (in years) of immunotherapy (r = 0.32; P = .003).

**Changes in Soluble IL-2R Levels Over Time**

The levels of soluble IL-2R demonstrated a linear decrease as treatment proceeded (enrollment vs 1 year, P < .001; 1 year vs 3 years, P = .004; 3 years vs 5 years, P < .001; 5 years vs 10 years, P < .001). In addition, the levels of soluble IL-2R at any time after enrollment were significantly lower than those at enrollment (P < .001 for all comparisons [Table 2 and Figure 3]). The rates of decrease in levels of soluble IL-2R were significantly correlated with the duration (in years) of immunotherapy (r = 0.44; P < .001).

**Changes in IL-4 Levels Over Time**

The levels of IL-4 at 1 year were not significantly different from those at enrollment (P = .44). Then, the levels of IL-4 demonstrated a significant decrease as immunotherapy proceeded (1 year vs 3 years, P = .001; 3 years vs 5 years, P = .03; 5 years vs 10 years, P = .005). In addition, the levels of IL-4 after 3 years were significantly lower than those at enrollment (P < .001 for all comparisons [Table 2 and Figure 4]). The rates of decrease in levels of IL-4 were significantly correlated with the duration (in years) of immunotherapy (r = 0.78; P < .001).

**Changes in Soluble ICAM-1 Levels Over Time**

The levels of soluble ICAM-1 demonstrated a linear decrease as treatment proceeded (enrollment vs 1 year,
The levels of soluble ICAM-1 at any time after enrollment were significantly lower than those at enrollment (1 year, \( P = .004 \); 3 years, \( P < .001 \); 5 years, \( P < .001 \); 10 years, \( P < .001 \) [Table 2 and Figure 5]). The rates of decrease in levels of soluble ICAM-1 were significantly correlated with the duration (in years) of immunotherapy \( (r_s = 0.73; P < .001) \).

**Changes in Soluble VCAM-1 Levels Over Time**

The levels of soluble VCAM-1 did not change significantly after 5 years of treatment (enrollment vs 1 year, \( P = .06 \); 1 year vs 3 years, \( P = .18 \); 3 years vs 5 years, \( P = .07 \)). However, the levels of soluble VCAM-1 at 10 years were significantly lower than those at 5 years \( (P = .03) \) [Table 2 and Figure 6]. The rates of decrease in levels of soluble VCAM-1 were not significantly correlated with the duration (in years) of immunotherapy \( (r_s = 0.24; P = .06) \).

**PHARMACOTHERAPY VS IMMUNOTHERAPY AT ENROLLMENT**

None of the symptom scores \( (P = .08) \), levels of specific IgE \( (P = .61) \), specific IgG4 \( (P = .78) \), soluble IL-2R \( (P = .73) \), IL-4 \( (P > .99) \), soluble ICAM-1 \( (P = .32) \), or soluble VCAM-1 \( (P = .34) \) at enrollment differed significantly between the pharmacotherapy and the immunotherapy groups.

**Rate of Decrease in Symptom Scores**

The rate of decrease in symptom scores at 1 year and 2 years did not differ significantly between the pharmacotherapy and the immunotherapy groups (1 year, \( P = .53 \); 2 years, \( P = .39 \)). However, the rate of decrease in symptom scores at 3 years was significantly higher in the immunotherapy group than in the pharmacotherapy group \( (P = .03) \).

**Rate of Decrease in Specific IgE Levels**

The rate of decrease in levels of specific IgE at 1 and 2 years was significantly higher in the pharmacotherapy group than in the immunotherapy group (1 year, \( P < .001 \); 2 years, \( P = .02 \)). However, the rate of decrease in levels of specific IgE at 3 years did not differ significantly between the 2 groups \( (P = .72) \).

**Rate of Increase in Specific IgG4 Levels**

The rate of increase in levels of specific IgG4 in the immunotherapy group was significantly higher than that in the pharmacotherapy group at all times examined (1 year, \( P < .001 \); 2 years, \( P < .001 \); 3 years, \( P < .001 \)).

**Rate of Decrease in Soluble IL-2R Levels**

The rate of decrease in levels of soluble IL-2R in the immunotherapy group was significantly larger than that in the pharmacotherapy group at all times examined (1 year, \( P = .001 \); 3 years, \( P < .001 \)).

**Rate of Decrease in IL-4 Levels**

The rate of decrease in levels of IL-4 at 1 year did not differ significantly between the 2 groups \( (P = .08) \), but the rate of decrease in levels of IL-4 at 3 years in the immunotherapy group was significantly larger than that in the pharmacotherapy group \( (P < .001) \).

**Rate of Decrease in Soluble ICAM-1 Levels**

The rate of decrease in soluble ICAM-1 at 1 year did not differ significantly between the 2 groups \( (P = .57) \), but the rate of decrease in soluble ICAM-1 at 3 years in the immunotherapy group was significantly larger than that in the pharmacotherapy group \( (P = .04) \).

**Rate of Decrease in Soluble VCAM-1 Levels**

The rate of decrease in levels of soluble VCAM-1 did not differ significantly between the 2 groups at any time examined (1 year, \( P = .39 \); 3 years, \( P = .54 \)).
CORRELATIONAL ANALYSIS FOR RATE OF DECREASE IN SYMPTOM SCORES IN THE IMMUNOTHERAPY GROUP

Specific IgE

The rates of decrease in levels of specific IgE were significantly correlated with the rates of decrease in symptom scores at 5 and 10 years, but not at 1, 2, and 3 years (1 year: \( r_s = 0.32, P = .17 \); 2 years: \( r_s = -0.06, P = .77 \); 3 years: \( r_s = -0.13, P = .56 \); 5 years: \( r_s = 0.66, P = .006 \); 10 years: \( r_s = 0.72, P = .003 \)).

Specific IgG4

The rates of increase in levels of specific IgG4 were significantly correlated with the rates of decrease in symptom scores at 1, 2, and 3 years, but not at 5 and 10 years (1 year: \( r_s = 0.78, P < .001 \); 2 years: \( r_s = 0.68, P = .004 \); 3 years: \( r_s = 0.79, P < .001 \); 5 years: \( r_s = 0.36, P = .14 \); 10 years: \( r_s = 0.34, P = .56 \)).

Soluble IL-2R

The rates of decrease in levels of soluble IL-2R were significantly correlated with the rates of decrease in symptom scores at 1 and 3 years, but not at 5 and 10 years (1 year: \( r_s = 0.67, P = .005 \); 3 years: \( r_s = 0.71, P = .003 \); 5 years: \( r_s = 0.17, P = .52 \); 10 years: \( r_s = 0.17, P = .64 \)).

Interleukin 4

The correlation between the rates of decrease in levels of IL-4 and the rates of decrease in symptom scores was significant at 5 and 10 years (5 years: \( r_s = 0.74, P = .004 \); 10 years: \( r_s = 0.74, P = .008 \)), but not at 1 and 3 years (1 year: \( r_s = 0.28, P = .29 \); 3 years: \( r_s = -0.30, P = .23 \)).

Soluble ICAM-1

The correlation between the rates of decrease in levels of soluble ICAM-1 and the rates of decrease in symptom scores was significant at 3 and 5 years (3 years: \( r_s = 0.65, P = .006 \); 5 years: \( r_s = 0.67, P = .005 \)), but not at 1 and 10 years (1 year: \( r_s = -0.12, P = .60 \); 10 years: \( r_s = 0.43, P = .27 \)).

Soluble VCAM-1

At all times examined, the rates of decrease in levels of soluble VCAM-1 were not significantly correlated with the rates of decrease in symptom scores (1 year: \( r_s = -0.45, P = .05 \); 3 years: \( r_s = -0.36, P = .11 \); 5 years: \( r_s = 0.07, P = .82 \); 10 years: \( r_s = 0.37, P = .45 \)).

CORRELATIONAL ANALYSIS BETWEEN LEVELS OF SPECIFIC IgE AND IL-4 IN THE IMMUNOTHERAPY GROUP

At all times examined, the levels of specific IgE were significantly correlated with the levels of IL-4 (at enrollment: \( r_s = 0.62, P = .02 \); 1 year: \( r_s = 0.52, P = .05 \); 3 years: \( r_s = 0.66, P = .009 \); 5 years: \( r_s = 0.84, P = .001 \); 10 years: \( r_s = 0.70, P = .008 \)). At 1 year, the rates of decrease in levels of specific IgE were not significantly correlated with the rates of decrease in levels of IL-4 (\( r_s = -0.15, P = .55 \)), but after 3 years, the rates of decrease in levels of specific IgE were significantly correlated with the rates of decrease in levels of IL-4 (3 years: \( r_s = 0.64, P = .01 \); 5 years: \( r_s = 0.85, P = .001 \); 10 years: \( r_s = 0.67, P = .009 \)).

CORRELATIONAL ANALYSIS FOR RATE OF DECREASE IN SYMPTOM SCORES AT 10 YEARS IN THE IMMUNOTHERAPY GROUP

The rates of decrease in symptom scores at 10 years were not significantly correlated with any of the symptom scores (\( r_s = 0.37, P = .70 \)), levels of specific IgE (\( r_s = 0.19, P = .73 \)), specific IgG4 (\( r_s = -0.02, P = .26 \)), soluble IL-2R (\( r_s = 0.36, P = .50 \)), IL-4 (\( r_s = 0.47, P = .19 \)), soluble ICAM-1 (\( r_s = 0.19, P = .74 \)), or soluble VCAM-1 (\( r_s = 0.42, P = .29 \)) at enrollment. The rates of decrease in symptom scores at 10 years were not significantly correlated with any of the rates of decrease in symptom scores after 1 year of immunotherapy (\( r_s = 0.31, P = .72 \)), levels of specific IgE (\( r_s = 0.37, P = .46 \)), specific IgG4 (\( r_s = 0.38, P = .44 \)), soluble IL-2R (\( r_s = 0.35, P = .55 \)), IL-4 (\( r_s = 0.08, P = .50 \)), soluble ICAM-1 (\( r_s = 0.22, P = .87 \)), or soluble VCAM-1 (\( r_s = 0.44, P = .26 \)).

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 and other types of cells involved in allergic inflammatory events, our study focused exclusively on several immunologic parameters in serum. This study began in 1985, when considerable attention was focused on changes in allergen-specific antibodies in relation to the clinical effects of immunotherapy, based on the “blocking” specific IgG4 theory. The original aim of this prospective study was therefore to investigate the changes over time in levels of specific IgE and IgG4 in children treated with immunotherapy for perennial allergic rhinitis. However, increased attention has recently focused on the possibility that the clinical efficacy of immunotherapy might be related to altered T cell activity and cytokine profiles. Thus, the role of specific IgE and IgG4 in immunotherapy had not been addressed by the recent hypothesis of the working mechanisms of immunotherapy when we completed the collection of the serum samples in 1996. We therefore decided to extend the protocol to simultaneously determine levels of soluble IL-2R, IL-4, soluble ICAM-1, and soluble VCAM-1, together with levels of specific IgE and specific IgG4 in serum samples from our patients with perennial allergic rhinitis.

Our study design is an open prospective trial but not a double-blind placebo-controlled trial, because for ethical reasons it is not possible in Japan to perform such a time-consuming clinical trial. Although this study has no control group, our previous study established that serum levels of specific IgE and IgG4, soluble IL-2R, IL-4, soluble ICAM-1, and soluble VCAM-1 did not fluctuate significantly in untreated adult patients for 10 years.
However, no studies have demonstrated natural changes in these immunologic parameters in children with perennial allergic rhinitis. We also failed to recruit children with perennial allergic rhinitis to participate as untreated controls; however, 33 children with perennial allergic rhinitis who were enrolled in the pharmacotherapy group were treated with cromolyn sodium but not immunotherapy. They were originally scheduled to be treated with cromolyn sodium only for the same period of 10 years. Many children withdrew from the study, and 12 and 6 children remained to participate in the study after 3 and 4 years, respectively. Thus, we were obliged to suspend our observations of the pharmacotherapy group after 3 years and study the statistical changes in levels of specific IgE, specific IgG4, soluble IL-2R, IL-4, soluble ICAM-1, and soluble VCAM-1 in serum samples from children not treated with immunotherapy for 3 years.

The levels of specific IgE, specific IgG4, IL-4, soluble ICAM-1, and soluble VCAM-1 did not fluctuate significantly by the end of 3 years in the pharmacotherapy group. Therefore, the changes over time in specific IgE, specific IgG4, IL-4, and soluble ICAM-1 observed in the immunotherapy group were likely caused by immunotherapy. However, the levels of soluble IL-2R in the pharmacotherapy group at 1 year were significantly lower than those at enrollment. This decrease in levels of soluble IL-2R in the pharmacotherapy group is not likely to be age-related; rather, it is probably due to the pharmacological treatment, because antiallergy medication can significantly affect the serum levels of IL-4. Even though serum levels of soluble IL-2R decrease with time, the rate of decrease in levels of soluble IL-2R at 1 year (P = .001) and 3 years (P < .001) of treatment was significantly higher in the immunotherapy group than in the pharmacotherapy group. Thus, the decrease in levels of soluble IL-2R in the immunotherapy group was probably due mainly to the action of immunotherapy.

It was previously postulated that immunotherapy might suppress IgE synthesis, but this suppression did not occur within a few years after immunotherapy. Our previous study also failed to show any early decrease in specific IgE levels even in patients who responded well to immunotherapy. In the present study, the levels of specific IgE showed a significant initial increase during the first 2 years, then decreased and reverted to the pretreatment levels at 3 years, and further decreased significantly after 5 years as the therapy proceeded. Our correlational analysis showed a significant relationship between the rates of decrease in levels of specific IgE and those in symptom scores at 5 and 10 years, but not during the first 3 years of immunotherapy. Our results thus suggest that active suppression of specific IgE synthesis after 5 years is probably involved in the working mechanisms of immunotherapy related to clinical efficacy, which is in agreement with the clinical role of specific IgE in adult patients treated with immunotherapy.

In the present study, the levels of specific IgG4 were significantly higher during immunotherapy, and the rates of increase were significantly correlated with the duration of the treatment. In addition, the rates of increase in levels of specific IgG4 were significantly correlated with the rates of decrease in symptom scores during the first 3 years, but not after 5 years. Therefore, we conclude that an increase in levels of specific IgG4 plays an important role in the clinical efficacy of immunotherapy during the first 3 years, and that a decrease in levels of specific IgE makes a more significant contribution once specific IgE titers decrease with several years of treatment.

The activation of T cells is associated with the expression of high-affinity IL-2. Interleukin 2 receptor exists in soluble form (soluble IL-2R) in serum, and the rate of release of soluble IL-2R reflects T-cell activation in vivo. Serum levels of soluble IL-2R in atopic patients were reported to be elevated. In our previous study, elevated serum levels of soluble IL-2R in patients with perennial allergic rhinitis decreased with variable periods of immunotherapy, although the rate of decrease in soluble IL-2R was not correlated with the clinical effect of immunotherapy. In the present study, serum levels of soluble IL-2R were significantly lower after immunotherapy and the rates of decrease in soluble IL-2R were significantly correlated with the duration of the treatment. In addition, the rates of decrease in soluble IL-2R were significantly correlated with the rates of decrease in symptom scores at 1 and 3 years, but was not at 5 and 10 years after immunotherapy. Our present findings thus suggest that immunotherapy could affect serum levels of soluble IL-2R or T-cell activation, and that this immunologic modulation is at least partly involved in the working mechanisms of immunotherapy linked to symptomatic improvement during the few years after the start of treatment.

Interleukin 4 plays a key role not only in inducing and increasing the generation of primary polyclonal and secondary specific IgE responses by B lymphocytes but also in developing Th2-like cells. In addition, IL-4 has been shown to up-regulate the expression of adhesion molecules such as VCAM-1 on endothelial cells, which are involved in the selective infiltration of eosinophils in allergic inflammation. Thus, IL-4 may be the most important cytokine involved in allergic pathogenesis. Several studies have documented a significant elevation of serum IL-4 levels in atopic individuals compared with nonatopic controls. We previously demonstrated that serum levels of IL-4 were elevated in adult patients with perennial allergic rhinitis, and that the elevation was down-regulated by prolonged immunotherapy. In the present study, the levels of IL-4 at any time after 2 years were significantly lower than pretreatment levels, and the rate of decrease in levels of IL-4 was significantly correlated with the duration of immunotherapy. At all times examined during 10 years of immunotherapy, levels of IL-4 were correlated with levels of specific IgE, and rates of decrease in IL-4 were well correlated with rates of decrease in specific IgE. These findings are likely to mean that immunotherapy affects T-cell cytokine production and alters the regulation of IgE synthesis, leading to a decrease in levels of specific IgE. The rates of decrease in levels of IL-4 were significantly correlated with the
rates of decrease in symptom scores after 5 years, but not during the first 3 years. Therefore, it is likely that a decrease in levels of IL-4 might not play an important part in the clinical efficacy of immunotherapy during the first several years of treatment, but probably contributes to the clinical efficacy of prolonged immunotherapy.

Adhesion molecules such as ICAM-1 and VCAM-1 play an important role early in the cascade of events leading to the development of airway allergic inflammation. In particular, VCAM-1 is a key adhesion molecule implicated in eosinophil accumulation in atopic diseases, because neutrophils cannot use VCAM-1–dependent pathways.27 Soluble forms of ICAM-1 and VCAM-1 have been identified in peripheral blood, and serum levels of these adhesion molecules were also reported to be higher in patients with allergic conditions.8,16,28 Higher levels of these adhesion molecules in the serum of atopic individuals may reflect the up-regulation of cell-surface ICAM-1 and VCAM-1 expression in allergic inflammation. In the present study, immunotherapy significantly decreased levels of soluble ICAM-1. The rates of decrease in soluble ICAM-1 were significantly correlated with the duration of immunotherapy, and were also correlated with the rates of decrease in symptom scores at 3 and 5 years, but not at 1 and 10 years. By contrast, the rates of decrease in soluble VCAM-1 were not correlated with the duration of immunotherapy or the rates of decrease in symptom scores throughout the observation period. In our previous studies, immunotherapy did not significantly decrease levels of soluble VCAM-1 in adult patients with perennial allergic rhinitis.32 The decrease in soluble VCAM-1 observed in children receiving immunotherapy for 5 to 10 years is not likely the result of immunotherapy. Therefore, our results suggest that the decrease in soluble ICAM-1 induced by immunotherapy is implicated in its clinical efficacy 3 and 5 years after treatment. However, the changes in levels of soluble VCAM-1 during immunotherapy do not seem to play an important part in its clinical efficacy.

Since the symptom scores and levels of specific IgE, specific IgG4, soluble IL-2R, IL-4, soluble ICAM-1, and soluble VCAM-1 before treatment were not correlated with the rates of decrease in symptom scores at 10 years, it is impossible to predict the future clinical efficacy of treatment before the start of immunotherapy. In addition, since no rates of decrease or increase in symptom scores, levels of specific IgE, specific IgG4, soluble IL-2R, IL-4, soluble ICAM-1, or soluble VCAM-1 at 1 year were correlated with the rates of decrease in symptom scores at 10 years, it is also impossible to predict the future clinical efficacy of treatment even at 1 year after the start of immunotherapy.

CONCLUSIONS

In our study, immunotherapy affected serum levels of specific IgE, specific IgG4, soluble IL-2R, IL-4, and soluble ICAM-1 in children with perennial allergic rhinitis. This immunologic effect of immunotherapy is likely involved in the working mechanism linked to its clinical efficacy. However, the modulation of different immunologic parameters contributed to the clinical effect at different phases of treatment. An increase in levels of specific IgG4 and a decrease in levels of soluble IL-2R reduced symptoms of perennial allergic rhinitis during the first 3 years of immunotherapy, a decrease in levels of soluble ICAM-1 did so from the third to the fifth year, and a decrease in levels of specific IgE and IL-4 did so after the fifth year.

The primary aim of this study was to investigate over time the changes in several immunologic parameters in the serum of children receiving immunotherapy for perennial allergic rhinitis, and to elucidate the working mechanism of immunotherapy related to its clinical efficacy. However, our study also compared the clinical efficacy of immunotherapy with that of pharmacological treatment using cromolyn sodium. The rate of decrease in symptom scores at 1 and 2 years did not differ significantly between the 2 groups, but the rate of decrease in symptom scores at 3 years was significantly higher in the immunotherapy group. This finding suggests that immunotherapy is not always more effective than cromolyn sodium in the short-term, but that it is substantially more effective in the long-term. This study is the first to document that immunotherapy is more effective than pharmacological treatment in the long-term, which could impact long-term therapeutic strategies of treating perennial allergic rhinitis.

The possible long-term efficacy of immunotherapy for patients with asthma and perennial allergies was previously evaluated in 3 studies.29–31 Two studies demonstrated that after 1 year of immunotherapy for patients with perennial allergies, most if not all exhibited asthmatic relapse within 1 year of discontinuing treatment.29,30 In the most recent study, immunotherapy for perennial asthma using Dermatophagoides pteronyssinus extract was stopped after 1 to 5 years, and patients were followed up every 6 months for the next 3 years.31 The efficacy of immunotherapy lasts longer after treatment is discontinued in patients who received immunotherapy for a longer period: 3 years after stopping immunotherapy, there was no relapse in 52% of the patients who had received immunotherapy for more than 36 months, compared with 38% of those who had received immunotherapy for less than 35 months.

However, the long-standing efficacy of immunotherapy for perennial allergic rhinitis is yet to be investigated. All the patients in our study were treated with immunotherapy for 10 years. Most patients (15/19) were completely free of nasal symptoms at the end of the study when they terminated immunotherapy. For the past year after the discontinuation of therapy, no symptomatic relapses have been reported from any of the patients. Therefore, our study suggests that 10 years of immunotherapy can provide long-term clinical efficacy and may have the potential for a long-term cure even after therapy is discontinued. However, a shorter period of immunotherapy might be sufficient to provide long-term clinical efficacy after discontinuation. A multicenter prospective study involving a larger number of patients will be required to satisfactorily address the questions of when to discontinue successful immunotherapy and what immunologic parameters might predict when to safely terminate treatment.
Accepted for publication June 4, 1998.

This study was supported by Grant-in-Aid for Scientific Research 07671878 from the Ministry of Education, Science and Culture of Japan, Tokyo.

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