

Serum levels of soluble interleukin-2 receptor in patients with perennial allergic rhinitis before and after immunotherapy

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Background: Interleukin-2 receptor (IL-2R) exists in soluble form in sera, and the rate of release of the soluble form of IL-2R (soluble IL-2R) reflects T cell activation in vivo. Since T lymphocytes play a central role in respiratory allergic disorders, the measurement of serum levels of soluble IL-2R may be useful in analyzing the disease state of allergic disorders.

Objective: To investigate the serum concentrations of soluble IL-2R in 48 patients with perennial allergic rhinitis and 14 nonatopic healthy controls, with special reference to the possible changes following long-term immunotherapy.

Methods: This retrospective study included 48 patients who had had variable periods of long-term immunotherapy with *Dermatophagoides farinae* extracts. The duration of immunotherapy ranged from 5 to 15 years. Serum samples were collected twice from each patient, before the initiation of immunotherapy and at the time of clinical assessment of immunotherapy. All the serum samples were simultaneously used for determination of soluble IL-2R concentrations, by the use of an enzyme-linked immunosorbent assay. To serve as controls, 14 nonallergic subjects of the same age range and sex were chosen.

Results: Patients with allergic rhinitis before immunotherapy had significantly higher serum levels of soluble IL-2R than nonatopic subjects. Elevated serum levels of soluble IL-2R decreased significantly following immunotherapy and the serum levels of soluble IL-2R in patients with allergic rhinitis after immunotherapy were not statistically different from those of nonatopic subjects. In addition, the percent decrease in serum soluble IL-2R correlated significantly with the duration of immunotherapy.

Conclusions: Hyperactivity of helper T cells of atopic patients is altered after long-term immunotherapy, and such immunoregulatory changes could be theoretically involved in the mechanisms of immunotherapy.

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INTRODUCTION

Through antigen binding and interaction with macrophage-derived interleukin-1, T cells synthesize and secrete interleukin-2 (IL-2) as a helper signal for clone expansion of activated T cells

with specific helper or cytotoxic/suppressor functions.^{1,2} Activation of T cells is also associated with the expression of high affinity interleukin-2 receptors (IL-2R), which are composed of at least three chains (α , β , γ), each interacting with distinct epitopes on the IL-2 molecule.³⁻⁵ Interleukin-2R exists in soluble form in sera. The rate of release of the soluble form of IL-2R (soluble IL-2R) reflects T cell activation in vivo.⁶ The measurement of serum levels of soluble IL-2R may therefore be useful in analyzing certain disease states associated with immune

activation. Indeed, in a variety of inflammatory as well as immunologic diseases with accompanying T cell dysregulation, including autoimmunity, AIDS, and allograft reaction, elevated levels of soluble IL-2R in serum and other secretions have been demonstrated.⁷⁻⁹ The levels of soluble IL-2R in these diseases correlate with immunologic activation and inflammation occurring in vivo.⁷⁻⁹

Various cells are associated with inflammatory events characteristic of allergic disorders in the respiratory system. Recent studies have suggested a central role for T lymphocytes in respiratory allergic disorders.¹⁰ Disturbance of T cell function in the immunoregulatory network might contribute to the pathogenesis of these diseases. In acute asthma, indeed, there is an increase in the number of circulating T lymphocytes that express IL-2R and concentration of soluble IL-2R in the peripheral blood, although there is no significant increase in total lymphocyte numbers compared with the control subjects.¹¹ Our study has aimed at investigating the serum concentrations of soluble IL-2R in patients with perennial allergic rhinitis, with special reference to the possible changes following long-term immunotherapy.

PATIENTS AND METHODS

Patients

This study included 14 nonallergic healthy volunteers and 48 patients with perennial allergic rhinitis due to *Dermatophagoides farinae* (*D. farinae*) who gave informed consent to participation in the present study. The 48 patients were selected from our outpa-

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tients who completely satisfied the following conditions: (1) typical symptoms of perennial allergic rhinitis before immunotherapy, (2) positive skin test and positive nasal provocation to *D. farinae* before immunotherapy, (3) eosinophilia in nasal smears before immunotherapy, (4) serum sample was obtained twice (before immunotherapy and at various times during the course of immunotherapy), stored at -60°C , and never retrieved before this study, (5) under treatment with immunotherapy using Hollister-Stier standardized *Dermatophagoides farinae* extracts (Miles Inc, USA) for more than 5 years. The duration of immunotherapy of the 48 patients who completely satisfied the above criteria ranged from 5 to 15 years, and the mean and the median values were 8.9 and 8.0 years. To serve as controls, 14 nonallergic healthy volunteers were chosen on the basis of the following criteria: (1) a negative history for allergy, (2) no physical findings consistent with atopic diseases; and (3) negative serum IgE antibodies specific to the common allergens.

The backgrounds of the 14 nonatopic healthy volunteers and the 48 patients are summarized in Table 1.

Mode of Immunotherapy

Patients received subcutaneous injections with Hollister-Stier standardized *D. farinae* extracts (Miles Inc, USA) with prior informed consent. The initial doses were determined as indicated by the end-point concentration determined by the threshold skin test. Doses were increased by 50% at weekly intervals until the individual maximum tolerated dose was reached. The maximum tolerated dose was attained in 4 to 8 months with the once weekly pro-

gram. A weekly injection of this dose was administered for the first 3 to 5 months after attainment to the maintenance dose, and then they received injections of this dose on a biweekly basis for the next 3 to 5 months, followed by a monthly schedule. Finally, the interval between injections was gradually increased up to 2 months in the absence of an episode 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 experienced. No concomitant medication affecting nasal symptoms was admitted throughout the period, without prior informed consent. All patients intentionally continued the treatment to avoid possible relapse even though nasal symptoms resolved.

Clinical Evaluation

Clinical evaluation of symptoms of allergic rhinitis was scheduled twice in each patient, before the initiation of immunotherapy and at various times during immunotherapy. The clinical evaluation used in this study was based upon the criteria of Okuda et al¹² according to daily symptom diary cards. The patients were asked to complete daily symptom diary cards for the 14 days before each assessment. The daily symptom card includes the number of sneezing attacks, the amount of nose blowing, and the degree of nasal obstruction. A careful survey of the diary card graded the three nasal symptoms (sneeze, rhinorrhea, and obstruction) on a scale of 0 to 3, depending on severity (Table 2). The sum total of the three nasal symptom scores was used as symptomatic scores. The percent decrease in symptomatic scores from the pretreatment values following im-

munotherapy was calculated using the following formula.

$$\frac{\text{ss before IT} - \text{ss after IT}}{\text{ss before IT}} \times 100$$

where ss = symptomatic scores and IT = immunotherapy.

Determination of Soluble IL-2R

Serum samples were collected twice from each patient, before the initiation of immunotherapy and at the time of clinical assessment of immunotherapy. They were separated and stored at -60°C until used. All the serum samples were used simultaneously for determination of soluble IL-2R concentrations. The concentrations of soluble IL-2R were determined by the enzyme-linked immunosorbent assay (ELISA) as previously described by Chilosi et al,¹³ using two monoclonal antibodies (MoAb) recognizing different epitopes of the IL-2R molecules. In brief, soluble IL-2R available in the samples or standard binds to the polystyrene microtiter that was previously incubated with 100 mL anti-IL-2R MoAb (1 mg/mL). A horseradish peroxidase-conjugated anti-molecule binds to the IL-2R captured by the first antibody and completes the sandwich. After washing to remove the unbound enzyme-conjugated anti-IL-2R MoAb, a substrate solution is added to the wells. The reaction is then stopped and the absorbance determined at 490 nm. A standard curve was prepared by using different soluble IL-2R concentrations defined in arbitrary units per milliliter (considering 1000 U/mL the amount of soluble IL-2R present in 0.1 mL of a T Cell Science reference preparation of supernatant from phytohemagglutinin-stimulated lymphocytes).

Table 1. Sex and Ages of Nonatopic Healthy Volunteers and Patients with Perennial Allergic Rhinitis

	Sex		Age, yr					
	Female	Male	Mean	Standard Deviation	Standard Error	Youngest	Oldest	Median
Nonatopic controls	8	6	33.43	13.34	3.57	15	58	29
Allergic Rhinitics								
Before immunotherapy	28	20	28.00	13.59	1.96	13	61	24
After immunotherapy	28	20	36.81	13.56	1.96	18	67	32

Table 2. Scoring of Sneeze, Rhinorrhea, and Nasal Obstruction

Symptom	Score	Definition of Score
Sneeze	0	no sneezing attacks
	1	the number of sneezing attacks is 1–5
	2	the number of sneezing attacks is 6–10
	3	the number of sneezing attacks is over 11
Rhinorrhea	0	no nasal blowings
	1	the number of nasal blowings is 1–5
	2	the number of nasal blowings is 6–10
	3	the number of nasal blowings is over 11
Nasal obstruction	0	no nasal obstruction
	1	nasal obstruction without mouth breathing
	2	nasal obstruction with sporadic mouth breathing
	3	nasal obstruction with predominant mouth breathing

The ELISA measurements were run in duplicate. Minimum sensitivity for this assay was 80 U/mL of soluble IL-2R.

The percent decreases from the pretreatment soluble IL-2R following immunotherapy (IT) were calculated using the following formula.

percent decrease in soluble IL-2R

$$\frac{\text{soluble IL-2R before IT} - \text{soluble IL-2R after IT}}{\text{soluble IL-2R before IT}} \times 100$$

Statistical Analysis

Differences between two different groups were analyzed statistically by the use of Mann-Whitney's U-test. Difference between the paired values of soluble IL-2R and symptomatic scores were analyzed by the use of Wilcoxon signed-ranks test. Significant differences were accepted in each analysis when $P < .05$. Pearson's correlation coefficient (r) was calculated to analyze possible correlation between two different parameters. Spearman's correlation coefficient (r_s) was calculated to analyze possible correlation

between two different parameters, and significant correlation was accepted when P was less than .05 according to the Spearman's rank correlation analysis.

RESULTS

Background of Subjects

The background of the sex and the age of the nonatopic healthy volunteers and patients is summarized in Table 1. There were no sex differences between the 48 patients and the 14 nonatopic healthy volunteers. The ages of the 48 patients before immunotherapy were not significantly different from those of the 14 nonatopic healthy volunteers ($P = .1189$). Also, the ages of the 48 patients at the second collection of the serum samples at various times in the courses of immunotherapy were not different from those of the 14 nonatopic healthy volunteers ($P = .3948$).

Clinical Evaluation

The symptomatic scores before immunotherapy and at various times after immunotherapy are summarized in Ta-

ble 3. The pretreatment symptom scores of the 48 patients studied ranged from 5 to 9 with a mean of 7.21. Every patient demonstrated some decrease in symptom scores following immunotherapy. The symptom scores of the 48 patients after immunotherapy varied from 0 to 5 with a mean of 1.38, significantly smaller than those before immunotherapy ($P < .0001$). The percent decrease in symptom scores following immunotherapy was $80.12 \pm 21.62\%$.

Soluble IL-2R

Table 4 and Figure 1 summarize the results of soluble IL-2R in the nonatopic healthy volunteers and in the patients with perennial allergic rhinitis before and after immunotherapy. Figure 2 demonstrates the serum concentration of soluble IL-2R before and after immunotherapy in the 48 patients studied. The serum level of soluble IL-2R in the 48 patients with perennial allergic rhinitis before immunotherapy was significantly higher than that of the nonatopic control group ($P = .0051$). The values of soluble IL-2R were above 600 U/mL in seven of the 48 patients. The level of soluble IL-2R in the 41 patients whose soluble IL-2R was less than 600 U/mL was also significantly higher than that of the nonatopic control group ($P = .0232$). The level of serum soluble IL-2R, however, in the patients with perennial allergic rhinitis decreased significantly after immunotherapy compared with the pretreatment level ($P < .0001$, Figure 2), and the serum level of soluble IL-2R after immunotherapy in the patients with allergic rhinitis was not significantly different from that of the nonatopic control group ($P = .7618$, Figure 1). Immunotherapy was associated with decreased serum concentrations of soluble IL-2R in 33 of the 48

Table 3. Symptom Scores of Patients with Allergic Rhinitis Before and After Immunotherapy

Allergic Rhinitis	Mean	Standard Deviation	Standard Error	Smallest	Largest	Median
Before immunotherapy	7.21	1.13	0.16	5	9	7
After immunotherapy	1.38	1.42	0.21	0	5	1

Table 4. Serum Soluble IL-2R (U/mL) in the Nonatopic Controls and the Patients with Allergic Rhinitis Before and After Immunotherapy

	Mean	Standard Deviation	Standard Error	Smallest	Largest	Median
Nonatopic controls	363.4	108.6	29.0	196	552	332.5
Allergic rhinitis						
Before immunotherapy	490.0	188.8	27.2	202	1300	444.5
After immunotherapy	351.1	96.8	14.0	209	649	322.0

patients studied by more than 10% from the pretreatment values. Five of the 48 patients, however, had more than 10% higher serum concentrations of soluble IL-2R after immunotherapy as compared with the pretreatment values. The percent decrease (mean $\pm$ standard deviation) in soluble IL-2R following immunotherapy was $20.78 \pm 30.28\%$.

The percent decrease in soluble IL-2R following immunotherapy correlated significantly with the duration of immunotherapy ($r_s = .599$, $P < .0001$, Figure 3). However, there was no significant correlation between the percent decrease in soluble IL-2R and the percent decrease in symptom scores following immunotherapy ($r_s = .044$, $P = .9386$).

DISCUSSION

In a variety of inflammatory diseases with accompanying T cell dysregulation, elevated levels of soluble IL-2R in serum have been demonstrated.⁷⁻⁹ The levels of soluble IL-2R in these diseases correlate with immunologic activation and inflammation occurring in vivo. The measurement of serum levels of soluble IL-2R may be useful in analyzing certain disease states associated with immune activation.

Since T lymphocytes are considered to have a central role in allergic disorders, the measurement of serum soluble IL-2R has been reported by several investigators, resulting in contradictory results. Matsumoto et al demonstrated elevated serum levels of soluble IL-2R in children with eczema or food anaphylaxis.¹⁴ Guarnaccia et al failed to demonstrate an elevation of serum soluble IL-2R in patients with allergic disorders including atopic eczema and asthma.¹⁵ Brown et al reported elevated levels of soluble IL-2R in pa-

tients with asthma, independent of severity of the disease and of the presence of acute symptoms.¹⁶ On the other hand, Corrigan and Kay demonstrated increased concentrations of se-

rum soluble IL-2R in patients with acute asthma as compared with control subjects.¹⁷

Allergic rhinitis is also a type of allergic disorder like asthma and atopic

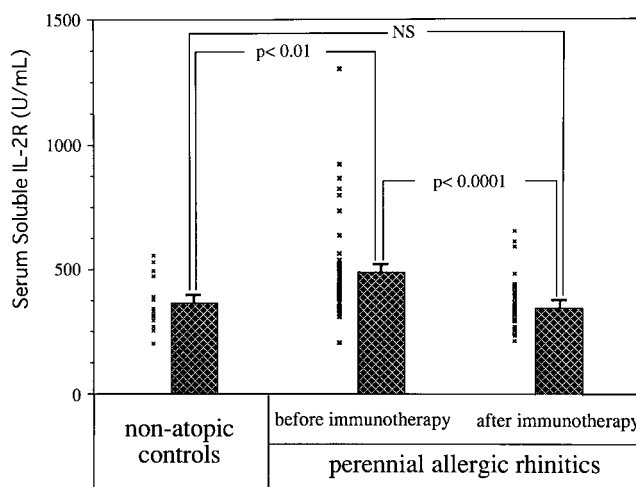


Figure 1. Serum levels (mean $\pm$ standard error) of soluble IL-2R in control subjects and in patients with perennial rhinitis before and after immunotherapy. The serum level of the patients with allergic rhinitis before immunotherapy is significantly higher than that of the control subjects. The serum level of the patients with allergic rhinitis after immunotherapy, however, is not different from that of the control subjects.

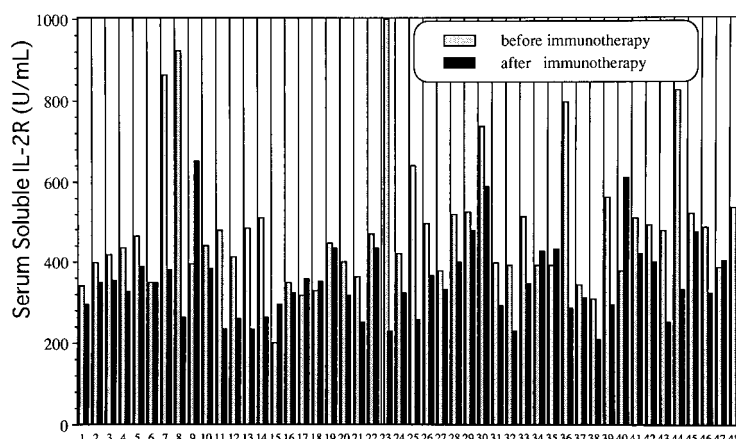


Figure 2. The change of serum concentrations of soluble IL-2R in 48 patients following immunotherapy. The serum concentrations of soluble IL-2R in the 48 patients before and after immunotherapy are shown.

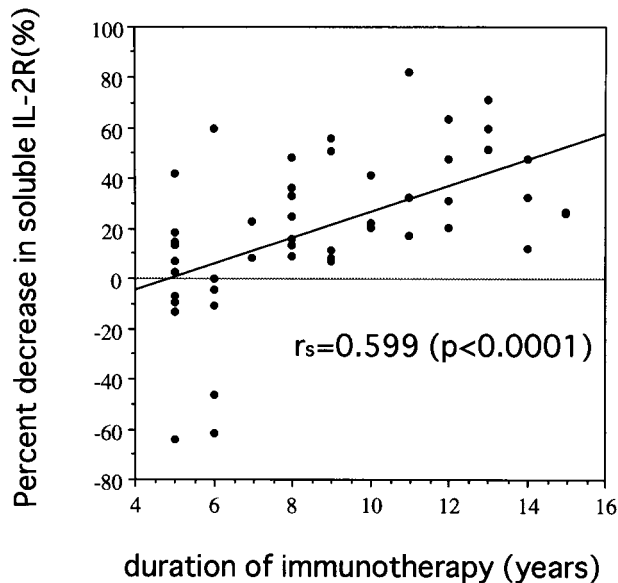


Figure 3. Correlation between the percent decrease in soluble IL-2R and the duration of immunotherapy. The percent decrease in soluble IL-2R significantly correlates with the duration of immunotherapy ($r_s = .599$, $P < .0001$).

eczema. The serum levels of soluble IL-2R in patients with allergic rhinitis remains to be explained. The primary aim of this study was to determine the serum levels of soluble IL-2R in patients with perennial allergic rhinitis due to *D. farinae* compared with those of nonatopic controls. Our results have demonstrated that patients with allergic rhinitis before immunotherapy had significantly higher serum levels of soluble IL-2R than nonatopic subjects, although there was a considerable overlap between the serum concentrations in patients with allergic rhinitis and nonatopic subjects. We interpret this significant elevation of serum soluble IL-2R levels to represent immunologic activation and inflammation occurring in allergic rhinitis.

Immunologic activation and inflammation in respiratory allergic diseases could be modulated by appropriate treatment. Indeed, Corrigan and Kay have demonstrated that concentrations of serum soluble IL-2R decreased as patients with acute asthma improved clinically during the first 3-day period of hospital treatment.¹⁷ Meanwhile, immunotherapy is an active treatment for allergic rhinitis and might modulate

immunologic activation and inflammation in allergic diseases to decrease the serum levels of soluble IL-2R. In an in vitro study, a decrease in the production of IL-2R from house-dust-antigen-stimulated lymphocytes of patients treated with immunotherapy has been demonstrated.¹⁸

The second aim of this study was to elucidate whether immunotherapy is capable of decreasing the elevated serum levels of soluble IL-2R in allergic rhinitis. Tsai et al discussed a possible decrease in elevated serum levels of soluble IL-2R in asthmatic patients following immunotherapy, based on comparing the serum levels of soluble IL-2R after variable periods of immunotherapy.¹⁹ They found a significant decrease in the serum levels of soluble IL-2R following immunotherapy, but the significance was weak ($P < .05$). Such discussion based on group comparisons could lead to confusing conclusions, because pretreatment values are considerably different in individual patients and there was a considerable overlap between the serum concentrations in atopic patients and nonatopic subjects. Although there is evidence of some clinical benefit as well as immu-

nologic change during the early phase of immunotherapy, a sustained clinical response is only possible with prolonged therapy. Immunotherapy must be administered for 3 to 5 years for such sustained clinical efficacy.²⁰ We therefore investigated a possible decrease in the serum levels of soluble IL-2R in patients with allergic rhinitis following more than 5 years of immunotherapy from a comparison of pretreatment and post-treatment values in individual patients.

Our data demonstrate that elevated serum levels of soluble IL-2R decreased following immunotherapy in 33 of 48 patients, and that the serum levels of soluble IL-2R in patients with allergic rhinitis after immunotherapy were not statistically different from those of nonatopic subjects. In addition, the percent decrease in serum soluble IL-2R correlated significantly with the duration of immunotherapy. These results indicate that hyperactivity of helper T cells of atopic patients is gradually modulated as immunotherapy proceeds and returns to normal after long-term immunotherapy. Such immunoregulatory changes could be theoretically involved in the mechanisms of immunotherapy. Our study, however, failed to find significant correlations between the percent decrease rate in soluble IL-2R and the percent decrease in symptom scores. Other unknown mechanisms could be involved in symptomatic relief during immunotherapy for allergic rhinitis.

Also, we examined serum soluble IL-2R before immunotherapy and at various times during the course of immunotherapy. To elucidate completely the role of serum soluble IL-2R, serial determination of soluble IL-2R may be necessary during immunotherapy. Further investigations might be needed to explain completely the therapeutic role of soluble IL-2R in immunotherapy of patients with allergic disorders.

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