

Pollen Immunotherapy Inhibits T Helper 1 and 2 Cell Responses, but Suppression of T Helper 2 Cell Response Is a More Important Mechanism Related to the Clinical Efficacy

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Objectives: To investigate the allergen-induced IgE synthesis and cytokine production by peripheral blood mononuclear cells from patients with seasonal allergic rhinitis due to Japanese cedar (*Cryptomeria japonica*) pollens and to elucidate the immunological mechanisms related to the clinical efficacy of immunotherapy (IT) for seasonal allergic rhinitis.

Design: This study included 51 patients with seasonal allergic rhinitis due to the pollen and 8 nonatopic healthy volunteers (nonatopic group). Thirty-nine patients had been undergoing IT using the pollen extracts for various lengths of time (IT group). The remaining 12 patients had never been treated with IT (untreated group). Peripheral blood mononuclear cells (3.3×10^6 cells per milliliter) from each individual were cultured with Cry j 1, 4.17 $\mu\text{g/mL}$. After 96 hours, culture supernatants were harvested to determine the concentrations of IgE, interleukin (IL) 5, interferon γ (IFN- γ), and tumor necrosis factor α (TNF- α).

Results: The levels of IgE ($P = .02$), IL-5 ($P < .01$), and TNF- α ($P = .05$) were significantly higher in the untreated group than in the nonatopic group. The levels of IFN- γ did not differ significantly between the untreated and the nonatopic groups ($P = .19$). The levels of IgE, IL-5, and IFN- γ , but not of TNF- α , were inversely correlated with the duration (in years) of IT, and none of the levels of IgE ($P = .74$), IL-5 ($P = .15$), IFN- γ ($P = .61$), and TNF- α ($P = .55$) differed significantly between the nonatopic group and those who had been treated with IT for 10 years or more. The levels of IL-5 were significantly lower in the good responders than in the poor responders to IT ($P < .001$), whereas the levels of total IgE ($P = .20$), IFN- γ ($P = .16$), and TNF- α ($P = .14$) did not differ significantly between them.

Conclusion: The mechanisms responsible for the clinical efficacy of pollen IT are principally related to the tolerance or anergy of T helper 2 cells.

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CD4⁺ T HELPER (Th) cells and their products, various cytokines, play a central part in the orchestration and persistence of chronic eosinophil inflammation.¹ An accumulation of Th2-like cells at allergic inflammatory sites and increased levels of Th2 cell-derived cytokines in culture supernatants from peripheral blood lymphocytes have also been found.^{2,3} Cytokines derived by Th2-like cells could mediate allergic inflammation by activating eosinophilic leukocytes, promoting mast cell development, regulating immunoglobulin isotype switching to IgE, and modulating adhesion molecule expression.⁴ Allergy might be defined as a Th2-like cell predominant inflammatory disease.

The Japanese cedar (*Cryptomeria japonica*) pollinates heavily during early spring, and is the most important pollen-causing seasonal allergic rhinitis in Japan. T helper cells, especially Th2-like cells and Th2-type cytokines, are likely to play

an important role in the pathogenesis of seasonal allergic rhinitis due to the pollen, but the significance remains to be definitely established. The first aim of this study was to investigate the profile of cytokines produced by peripheral blood mononuclear cells (PBMCs) from patients with seasonal allergic rhinitis due to Japanese cedar pollens, in response to the cedar pollen allergen. During the pollen season, interleukin (IL) 4-stimulated PBMCs of atopic donors produced more IgE than IL-4-stimulated PBMCs of nonatopic donors, whereas out of the season no difference was observed between these 2 groups.⁵ In our study, PBMCs were, therefore, obtained in each subject during the cedar pollen season. Cry j 1 and Cry j 2 are known to be the major allergens of Japanese cedar pollens, but Cry j 1 is the more important allergen.⁶ Therefore, Cry j 1 was used to stimulate PBMCs in this study.

Allergen immunotherapy (IT) is recognized as a highly effective form of treat-

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PATIENTS AND METHODS

PATIENTS

This study included 51 adult patients with seasonal allergic rhinitis due to Japanese cedar 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 seasonal allergic rhinitis due to Japanese cedar pollens, (2) the severest grade according to Okuda criteria,⁷ (3) positive skin test result to Japanese cedar pollens according to Okuda criteria,⁷ (4) positive nasal provocation test result to the pollens according to Okuda criteria,⁷ and (5) eosinophilic leukocytosis in nasal smears during the cedar pollen season. Thirty-nine of the 51 patients had undergone variable periods (mean $\pm$ SD, 6.4 ± 5.2 years; range, 1-17 years) of IT using Japanese cedar pollen extracts (Torii Co Ltd, Tokyo, Japan) (IT group). Every patient in the IT group received maintenance injections of the same dose at the time of the present participation. The patients in the IT group were further divided into 3 groups according to the duration of IT: those who had been treated for 2 years or less (short IT group), those who had been treated for 3 to 9 years (medium IT group), and those who had been treated for 10 years or more (long IT group). The remaining 12 patients had not been treated previously with IT, and were not receiving corticosteroid treatment (untreated group).

To serve as controls, 8 healthy, adult volunteers (nonatopic group) matched for age and sex were chosen based on the following criteria: (1) no history of allergic disease; (2) no physical findings indicative of atopic diseases; and (3) negative serum IgE antibodies specific to the major allergens in Japan, such as house dust mites, Japanese cedar pollens, ragweed, and Japanese cypress pollens.

ment for allergic rhinitis, especially pollen-induced seasonal allergic rhinitis. Immunotherapy might alter T-lymphocyte cytokine profiles from the preferential Th2 cell response to the Th1 cell response, resulting in turning off the allergic inflammatory events. Therefore, the second aim of this study was to discuss the immunological mechanisms of pollen IT from a comparative study of the allergen-induced cytokine production among nonatopic control subjects, untreated patients, and patients undergoing IT, with special reference to the clinical effect.

RESULTS

BACKGROUND OF THE SUBJECTS

The ages at the PBMC sampling did not differ among the nonatopic, the untreated, the short IT, the medium IT, and the long IT groups. The serum levels of specific IgE at the PBMC sampling in the untreated group (33.6 ± 32.8 IU/mL) were not significantly different from those in the short IT (32.3 ± 34.9 IU/mL) ($P = .98$), those in the medium IT (43.2 ± 35.5 IU/mL) ($P = .51$), or those in the

MODE OF IT

The patients were given subcutaneous injections of Japanese cedar pollen extracts (Torii Co Ltd), which are only commercially available in Japan. These aqueous extracts were prepared from native pollens, and were demonstrated to contain 2.0 mg of protein per milliliter. The details of our IT have been described elsewhere.⁸ Immunotherapy was begun outside of the pollen season (between May and July). Individual initial doses were determined by threshold skin tests. Doses were increased by 50% to 100% at weekly intervals until the highest tolerated dose or 0.1 mL of 1:100 wt/vol was reached. 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. The patients 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.

STUDY DESIGN

This study design followed the principles outlined in the Declaration of Helsinki.⁹ Pollination of Japanese cedars occurred in 1997 from the second week of February to the third week of April. Peripheral blood was collected from each subject during the cedar pollen season, and serum concentrations of IgE and *Dermatophagoides farinae*-specific IgE were determined by the use of the lumiward immunoassay system.¹⁰ Peripheral blood mononuclear cells were isolated from heparinized blood samples by Ficoll density gradient centrifugation. Peripheral blood mononuclear cells were washed twice in phosphate-buffered saline (PBS) and resuspended in Roswell Park Memorial Institute 1640 (Flow Laboratories, Meckenheim, Germany), containing 10% inactivated fetal calf serum (JRH Biosciences, Lenexa, Kan); streptomycin sulfate, 100 μ g/mL (Sigma-Aldrich Corp, St Louis, Mo); penicillin G potassium, 100 IU/mL; and levo-glutamide, 2 mmol/L. Peripheral blood mononuclear cells

long IT (15.5 ± 15.5 IU/mL) ($P = .08$) groups. In addition, the serum levels of specific IgE in the untreated group were not significantly different from those before IT in the short IT (33.6 ± 29.5 IU/mL) ($P = .98$), medium IT (43.0 ± 29.0 IU/mL) ($P = .34$), or long IT (38.5 ± 20.7 IU/mL) ($P = .20$) groups.

IgE IN CULTURE SUPERNATANTS

The Kruskal-Wallis test demonstrated that the levels of IgE differed significantly among the nonatopic, untreated, short IT, medium IT, and long IT groups ($P = .03$). The Mann-Whitney test demonstrated that the levels of IgE in the untreated ($P = .02$) and the short IT ($P = .04$) groups, but not in the medium IT ($P = .17$) and the long IT ($P = .74$) groups, were significantly higher than those in the nonatopic group (**Figure 1**). The levels of IgE in the short IT ($P = .91$) and the medium IT ($P = .22$) groups were not significantly different from those in the untreated group, but the levels of IgE in the long IT group ($P = .01$) were significantly lower than those in the untreated group.

were cultured at a final concentration of 3.3×10^6 cells per milliliter in 12-well plates (Iwaki Glass, Tokyo) at 37°C in a fully humidified 5% carbon dioxide atmosphere. Peripheral blood mononuclear cells were stimulated with Cry j 1, 4.17 µg/mL. Culture supernatants were harvested after 96 hours by centrifugation, and stored at -80°C until measurement of IgE, IL-5, interferon γ (IFN- γ), and tumor necrosis factor α (TNF- α).

DETERMINATION OF TOTAL IgE, IL-5, IFN- γ , AND TNF- α IN CULTURE SUPERNATANTS

IgE was determined by the use of the lumiward immunoassay system¹⁰; IL-5, IFN- γ , and TNF- α were measured by an enzyme-linked immunosorbent assay using commercially available kits (Otsuka Pharmaceutical Co Ltd, Tokyo). The details and justification of the methods used in this study have been described elsewhere.^{11,12}

In brief, microtitration plates were coated with anti-IL-5 monoclonal antibody (MoAb) (JES1-39D10), 2 µg/mL, and anti-IFN- γ MoAb (ANOC 1611), 10 µg/mL, or anti-TNF- α MoAb (ANOC 706), 2 µg/mL, in 100 µL per well of PBS, pH 7.4. Following overnight incubation at 4°C, the wells were blocked with 1% skim milk in PBS solution for at least 1 hour at room temperature and washed 3 times with PBS containing 0.05% polysorbate 20 (washing was always performed with this buffer). Samples or standard, 100 µL, in 0.1% bovine serum albumin (BSA)-PBS was added to the wells, and the plate was incubated at 4°C for 24 hours. The plates were then washed 3 times. Rabbit IL-5 antibodies (MoAb JES1-5A10) diluted to 1:500 with 0.1% BSA-PBS-polysorbate 20, rabbit anti-IFN- γ antibodies (MoAb OCT 1601) diluted to 1:2000 with 0.1% BSA-PBS-polysorbate 20, or biotin-labeled anti-TNF- α antibodies (MoAb ANOC 705) diluted to 1:5000 with 0.1% BSA-PBS-polysorbate 20, 100 µL, were added to each well. Finally, the plates were incubated for 2 hours at 23°C. Subsequently, the plates were washed, supplemented with horseradish peroxidase-labeled avidin (diluted to 1:10 000 with

0.1% BSA-PBS-polysorbate 20), 100 µL, for IL-5 assay and with horseradish peroxidase-labeled goat anti-rabbit IgG (diluted to 1:10 000 with 0.1% BSA-PBS-polysorbate 20), 100 µL, and incubated at 23°C for 2 hours. Finally, enzyme substrate (*O*-phenylenediamine dihydrochloride, 1 mg/mL, and 0.03% hydrogen peroxide in sodium citrate buffer, pH 5.0, 0.1 mol/L), 100 µL, was added to each well and the plates were incubated at room temperature for 10 minutes. The reaction was stopped by adding sulfuric acid, 100 µL, to each well, and the absorbance at 492 nm was determined using a multiscanner (Titertek Multiscan, MCC; Labsystems, Helsinki, Finland). The lowest sensitivity of the method used is 10 pg/mL for IL-5 and 5 pg/mL for IFN- γ and TNF- α , and does not measure the presence of other cytokines.

CLINICAL EVALUATION OF IT

The patients in the IT group were divided into the following 2 groups based on the clinical response to IT: good responders, no nasal symptoms without rescue medication; and poor responders, evident nasal symptoms or need for rescue medication to control nasal symptoms.

STATISTICAL ANALYSIS

If the level of IgE, IL-5, IFN- γ , or TNF- α in particular patients was below the sensitivity of the respective assay, the lowest detectable value (IgE, 0.3 IU/mL; IL-5, 10 pg/mL; IFN- γ , 5 pg/mL; and TNF- α , 5 pg/mL) obtained for that assay was arbitrarily used for statistical analysis. The Kruskal-Wallis test was used to compare the nonatopic, untreated, and 3 atopic groups. For comparisons between 2 different groups, the Mann-Whitney test was used, and differences were considered significant when $P < .05$. The Spearman correlation coefficient was calculated to determine the degree of correlation between 2 different variables, and significant correlation was accepted when $P < .05$ on Spearman rank correlation analysis. Data are given as the mean $\pm$ SD.

IL-5 IN CULTURE SUPERNATANTS

The Kruskal-Wallis test demonstrated that the levels of IL-5 differed significantly among the nonatopic, untreated, short IT, medium IT, and long IT groups ($P < .001$). The Mann-Whitney test demonstrated that the levels of IL-5 in the untreated ($P < .001$), short IT ($P = .03$), and medium IT ($P = .004$) groups were significantly higher than those in the nonatopic group (**Figure 2**). However, the levels of IL-5 did not differ significantly between the nonatopic and the long IT groups ($P = .15$). The levels of IL-5 in the short IT ($P = .11$) and medium IT ($P = .17$) groups were not significantly different from those in the untreated group, but the levels of IL-5 in the long IT group were significantly lower than those in the untreated group ($P < .001$) (Figure 2).

IFN- γ IN CULTURE SUPERNATANTS

The Kruskal-Wallis test demonstrated that the levels of IFN- γ did not differ significantly among the non-

atopic, untreated, short IT, medium IT, and long IT groups ($P = .09$). However, the Mann-Whitney test demonstrated that the levels of IFN- γ in the medium IT ($P = .02$) and long IT ($P = .009$) groups were significantly lower than those in the untreated group (**Figure 3**).

TNF- α IN CULTURE SUPERNATANTS

The Kruskal-Wallis test demonstrated that the levels of TNF- α did not differ significantly among the nonatopic, untreated, short IT, medium IT, and long IT groups ($P = .20$). The Mann-Whitney test demonstrated that the levels of TNF- α in the untreated group ($P = .05$), but not in the short IT ($P = .57$), medium IT ($P = .93$), and long IT ($P = .55$) groups, were significantly higher than those in the nonatopic group (**Figure 4**). The levels of TNF- α in the short IT ($P = .20$) and long IT ($P = .12$) groups were not significantly different from those in the untreated group, but the levels of TNF- α in the medium IT group ($P = .03$) were significantly lower than those in the untreated group (Figure 4).

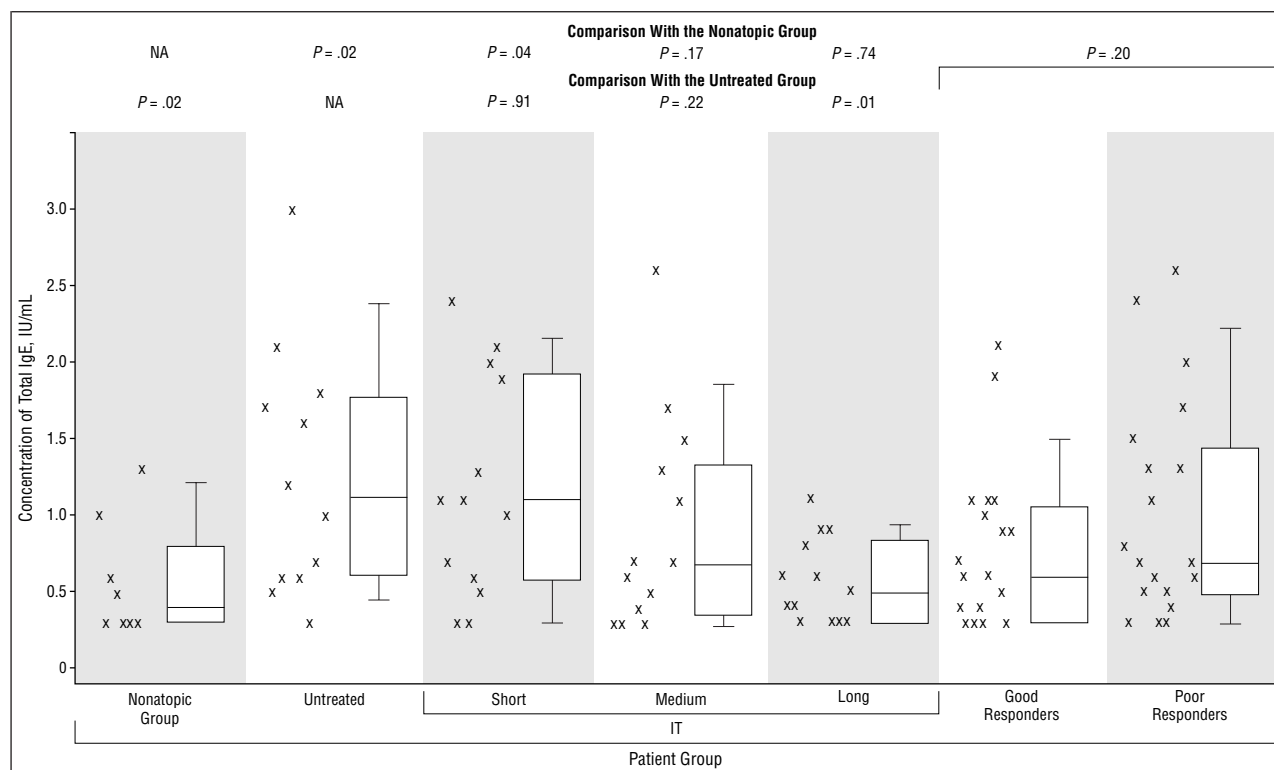


Figure 1. IgE levels in the culture supernatants. The levels of IgE in the untreated and the short immunotherapy (IT) groups were significantly higher than those in the nonatopic group, but the levels in the medium and long IT groups were not significantly different from those in the nonatopic group. The levels of IgE did not differ significantly between the good and poor responders. NA indicates not applicable. Boxes signify range of 25th to 75th percentile; vertical lines, median value; whiskers, range of 10th to 90th percentile; and x, value.

CORRELATION WITH THE DURATION OF IT

The levels of IgE ($r = -0.394$, $P = .003$), IL-5 ($r = -0.545$, $P < .001$), and IFN- γ ($r = -0.397$, $P = .004$), but not of TNF- α ($r = -0.190$, $P = .06$), were inversely correlated with the duration (in years) of IT.

CLINICAL EVALUATION OF IT

Twenty (51%) and 19 (49%) of the 39 patients exhibited good and poor responses, respectively, to IT. The duration of IT for the good responders (9.0 ± 5.6 years) was significantly longer than that for the poor responders (3.7 ± 2.9 years) ($P = .005$). The levels of IL-5 were significantly lower in the good responders than in the poor responders ($P < .001$). In contrast, none of the levels of total IgE, IFN- γ , and TNF- α differed significantly between the good and poor responders ($P = .20$, $P = .16$, and $P = .14$, respectively).

COMMENT

The first aim of this study was to investigate whether the quantity and/or profiles of cytokines and IgE produced by PBMCs in response to the pollen allergen differed between nonatopic individuals and untreated patients with seasonal allergic rhinitis due to Japanese cedar pollens. The amounts of IgE produced by PBMCs were significantly larger in untreated patients than in nonatopic individuals, suggesting an enhanced IgE synthesis in patients with seasonal allergic rhinitis.

Of the cytokines secreted by activated Th2-like cells, IL-5 is particularly implicated in the pathogenesis of allergic diseases in the airway, because it selectively promotes the differentiation, priming, activation, and survival of eosinophilic leukocytes.¹³ Expression of messenger RNA for IL-5 has been detected in bronchial biopsy specimens of persons with asthma, and has also tended to increase in patients with severe diseases.¹⁴ In addition, treatment with anti-IL-5 MoAb could inhibit the development of airway hyperresponsiveness.¹⁵ In this study, the levels of IL-5 produced by PBMCs were significantly higher in the untreated group than in the nonatopic group, which suggested that allergen-stimulated Th2 cell response was more enhanced in patients with seasonal allergic rhinitis than in nonatopic individuals.

T helper 1 cell-type cytokines are also considered to be involved in the pathogenesis of allergic diseases. Interferon γ secreted by Th1-like cells plays reciprocal activities of Th2-type cytokines, because IFN- γ inhibits the IgE synthesis by B lymphocytes and the development of Th2 clones.¹⁶ Indeed, stimulated peripheral blood T lymphocytes from atopic individuals are reported to secrete significantly less IFN- γ than those from normal controls.¹⁷ However, some researchers^{18,19} demonstrated increased spontaneous and allergen-stimulated IFN- γ production in atopic individuals. In our present study, the levels of IFN- γ did not differ significantly between the nonatopic group and the untreated group, although the geometric mean values in the untreated group were 3.8-fold higher compared

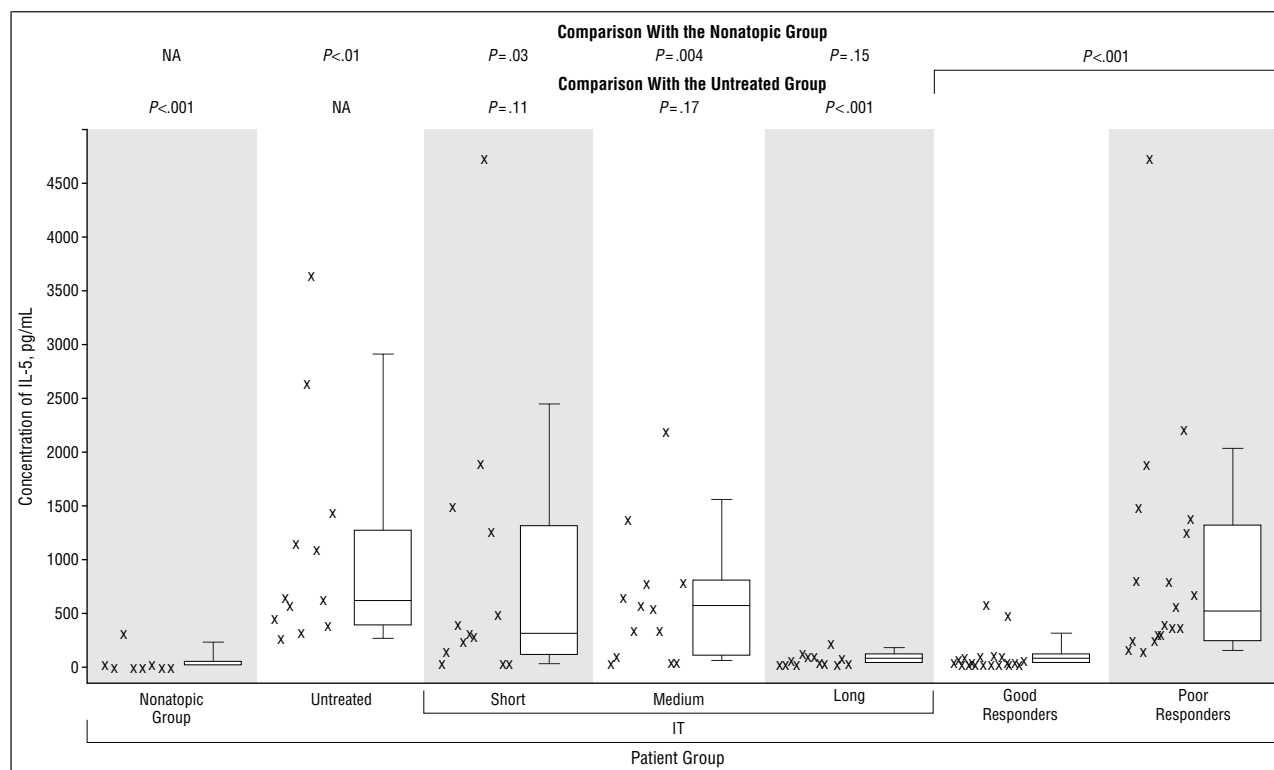


Figure 2. Interleukin (IL) 5 levels in the culture supernatants. The levels of IL-5 in the untreated, short immunotherapy (IT), and medium IT groups were significantly higher than those in the nonatopic group, but the levels of IL-5 did not differ significantly between the nonatopic and the long IT groups. The levels of IL-5 in the short and medium IT groups were not significantly different from those in the untreated group, but the levels of IL-5 in the long IT group were significantly lower than those in the untreated group. The levels of IL-5 in the good responders were significantly lower than those in the poor responders. NA indicates not applicable. For an explanation of the diagram, see the legend to Figure 1.

with those in the nonatopic group. Our data, therefore, suggest that the Th1 cell response is never suppressed in patients with seasonal allergic rhinitis compared with nonatopic individuals.

Tumor necrosis factor α is produced by Th1- and Th2-like cells, which are reported to be involved in switching B lymphocytes from IgG to IgE production.²⁰ In our study, the levels of TNF- α produced by PBMCs were significantly higher in the untreated group than in the nonatopic group. The increase in TNF- α production in the patients in the untreated group is likely involved in their allergic manifestation.

Thus, our study demonstrated that Cry j 1 stimulation resulted in enhancements of IgE synthesis and Th1 and Th2 cell responses, which was in agreement with the report by Ohashi et al.⁸ It is also of clinical interest whether the increased IgE synthesis and enhanced Th1 and Th2 cell responses in the cedar pollen-sensitive patients could be modulated by IT. To the best of our knowledge, however, this is the first study to investigate possible effects of IT on the production of IgE and cytokines by Cry j 1-stimulated PBMCs.

The concentrations of IgE produced by PBMCs were inversely correlated with the duration of IT. The levels of IgE in the long IT group were significantly lower than those in the untreated group and were not significantly different from those in the nonatopic group. However, the levels of IgE did not differ significantly between the good and poor responders. Our findings, therefore, suggest that pollen IT suppresses

IgE synthesis but that this suppression is not closely related to the clinical efficacy of IT.

The amounts of IL-5 were inversely correlated with the duration of IT. Although the levels of IL-5 in the short and medium IT groups were not significantly different from those in the untreated group and were significantly higher than those in the nonatopic group, the levels of IL-5 in the long IT group were significantly lower than those in the untreated group and were not significantly different from those in the nonatopic group. These findings suggest that IT suppresses the allergen-stimulated Th2 cell response in patients with seasonal allergic rhinitis with treatment over several years. Since the levels of IL-5 were significantly lower in the good responders compared with the poor responders to IT, it is likely that the suppression of Th2 cell response is linked to the clinical efficacy of IT.

In our study, the amounts of IFN- γ were inversely correlated with the duration of IT. Indeed, the levels of IFN- γ in the medium and long IT groups were significantly lower than those in the untreated group. Therefore, our study suggests that IT does not enhance, but rather suppresses, the allergen-stimulated Th1 cell response in patients with seasonal allergic rhinitis with treatment over several years. However, it is unlikely that the suppression of Th1 cell response is a working mechanism of IT related to the clinical efficacy, because the levels of IFN- γ did not differ between the good and poor responders. The

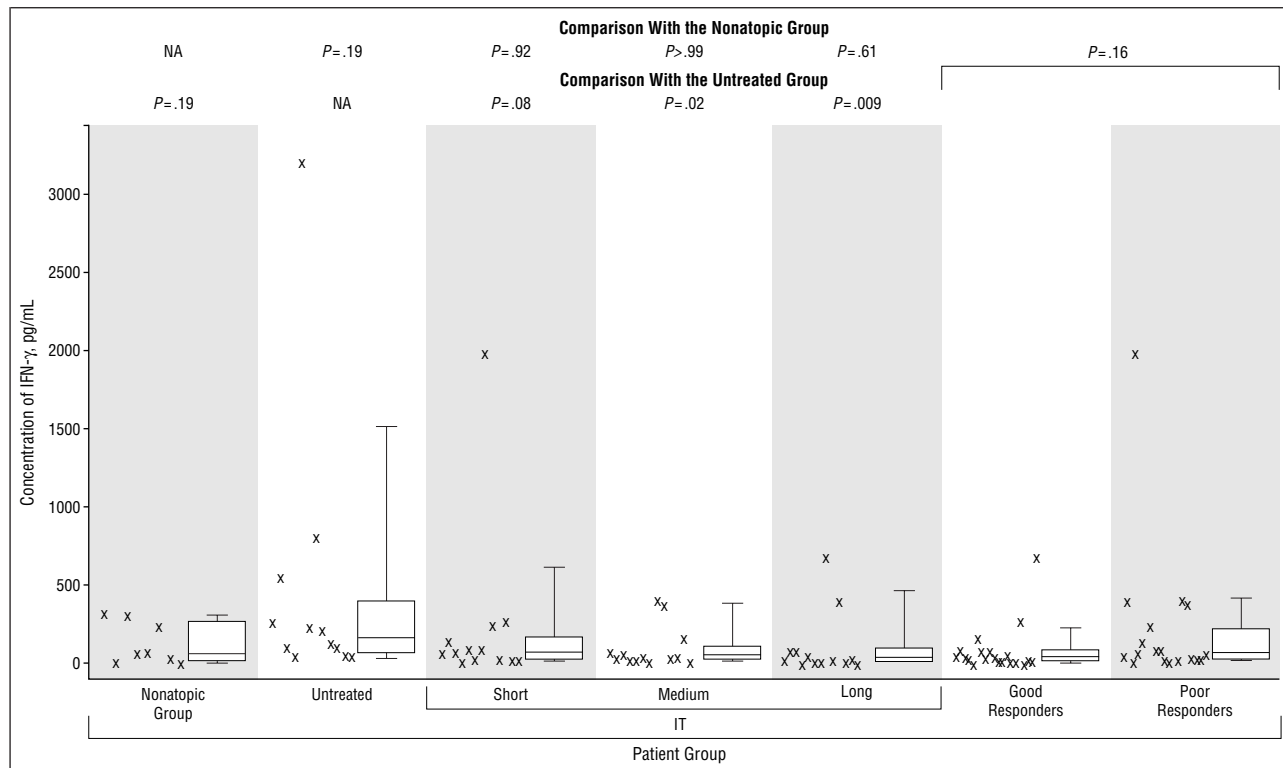


Figure 3. Interferon γ (IFN- γ) levels in the culture supernatants. The levels of IFN- γ in the untreated and the short, medium, and long immunotherapy (IT) groups were not significantly different from those in the nonatopic group. The levels of IFN- γ in the short IT group were not significantly different from those in the untreated group, but the levels of IFN- γ in the medium and long IT groups were significantly lower than those in the untreated group. The levels of IFN- γ did not differ significantly between the good and poor responders. NA indicates not applicable. For an explanation of the diagram, see the legend to Figure 1.

levels of TNF- α were not correlated with the duration of IT, and did not differ between the good and poor responders to IT. Our findings thus suggest that IT may decrease TNF- α production, but that the decrease in TNF- α production is probably not related to the clinical efficacy of IT.

The effect of IT on the Th1 and Th2 cell responses has recently been studied by several investigators. Secrist et al²¹ have demonstrated that production of IFN- γ was not affected in subjects allergic to grass pollen and mites who were undergoing IT, whereas IL-4 production was reduced by IT; also, the levels of IL-4 are inversely related to the length of time undergoing IT. On the other hand, an important role of IFN- γ in the mechanism of IT has also been demonstrated. In a previous serologic study¹² of patients allergic to Japanese cedar pollen, IT switched the seasonal preferential activation of Th2 cells to reciprocal activation of Th1 cells with treatment over several years. Varney et al²² and Durham et al²³ reported an insignificant change in IL-4 expression but a significant increase in IFN- γ expression in experiments using *in situ* hybridization to inflammatory cells in the skin or nasal mucosa in patients who underwent grass pollen IT. Conversely, MacDonald et al,²⁴ studying cytokine release from PBMCs in patients undergoing ragweed IT, showed a highly significant reduction in the production of IFN- γ but no significant changes in IL-4. O'Brien et al²⁵ have most recently demonstrated that IT resulted in a reduction in expression of IL-4 and IFN- γ in circulating lymphocytes. Our results are completely in agreement with

their findings, in suggesting that IT suppresses Th1 and Th2 cell responses during allergen stimulation. Thus, there is no agreement among different investigators regarding the effect of IT on the reactivity of Th cells or cytokine production. The exact reasons for these different results are unclear, but some differences such as types of allergies (seasonal or perennial), length of IT, target organs (peripheral blood or inflamed tissue), and experimental protocols (*in vitro* or *in vivo*) among different studies are likely to underlie the contradictory results.

Immunotherapy is a clinically effective treatment for most atopic patients, but is not effective for all of them. Nevertheless, only a few studies have referred to a possible correlation between clinical efficacy and modification of Th cell responses by IT. In our previous study,¹² the effect on Th2 cell response but not on Th1 cell response was exclusively related to the clinical efficacy, although pollen IT affected Th1 and Th2 cell responses during the pollen season. In our present study, similarly, the suppression of Th2 cell response but not of Th1 cell response was exclusively linked to the clinical efficacy, although IT affected Th1 and Th2 cell responses during the allergen stimulation. Taking this evidence together, suppression of Th2 cell response might be a key mechanism of action underlying the clinical efficacy of IT for seasonal allergic rhinitis due to Japanese cedar pollens, and modulation of Th1 cell response is likely to be an epiphenomenon unrelated to the clinical efficacy. O'Brien et al²⁵ have also discussed a correlation between cytokine produc-

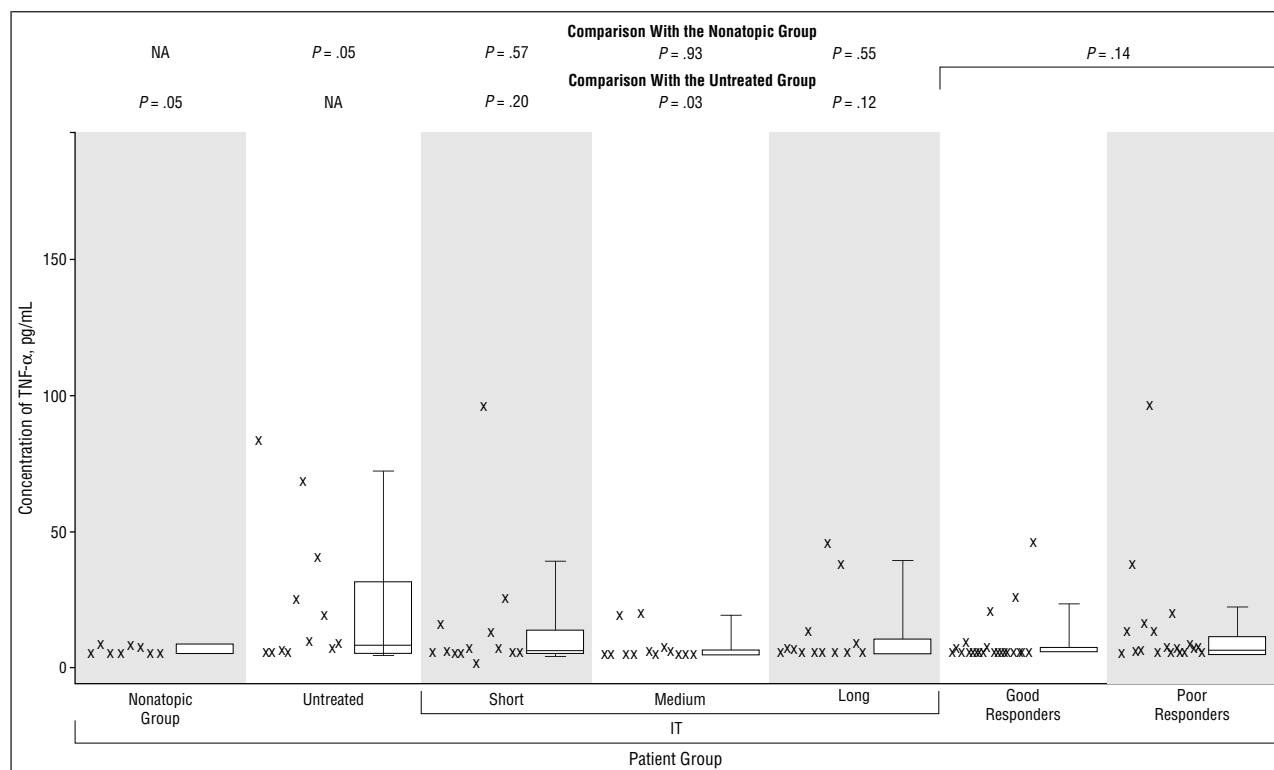


Figure 4. Tumor necrosis factor α (TNF- α) levels in the culture supernatants. The levels of TNF- α in the untreated group were significantly higher than those in the nonatopic group. However, the levels of TNF- α in the short, medium, and long immunotherapy (IT) groups were not significantly different from those in the nonatopic group. The levels of TNF- α in the short IT group were not significantly different from those in the untreated group, but the levels of TNF- α in the medium and long IT groups were significantly lower than those in the untreated group. The levels of TNF- α did not differ significantly between the good and poor responders. NA indicates not applicable. For an explanation of the diagram, see the legend to Figure 1.

tion and clinical efficacy of IT. Although their study was not quantitative, suppression of Th1 and Th2 cell responses tended to be related to the clinical efficacy of IT for perennial allergic rhinitis and asthma due to house dust mites.

CONCLUSIONS

Our study has demonstrated that IT for seasonal allergic rhinitis may possibly be working through induction of tolerance or anergy of Th1 and Th2 cells. However, our study is likely to support a view that mechanisms responsible for the clinical efficacy of IT principally involve the tolerance of Th2 rather than Th1 cells. In addition, suppression of IgE synthesis is not likely to be linked to the clinical efficacy of IT for seasonal allergic rhinitis. Incidentally, our study design is not a double-blind placebo-controlled trial, because it is not possible in Japan to perform such a time-consuming clinical trial for ethical reasons. Some factors other than IT might possibly affect the result, although this is extremely unlikely.

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