

Cry j 1-induced Synthesis of Interleukin-5 and Interferon-Gamma by Peripheral Blood Mononuclear Cells of Patients with Seasonal Allergic Rhinitis due to Japanese Cedar Pollens

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This study comprised 130 adult patients with Japanese cedar pollen-specific IgE in the serum and 15 non-atopic individuals. Eighteen patients had no seasonal aggravation of nasal symptoms during the pollen season in 1998 (asymptomatic group). Forty-two patients had not been treated previously with immunotherapy and were treated with antihistamine tablets during the pollen season in 1998 (medication group). Sixty-one patients had undergone variable periods of immunotherapy using pollen extracts, and they were further divided into a good-IT group who responded markedly to immunotherapy and a poor-IT group who responded poorly to immunotherapy. The remaining nine patients had been treated with immunotherapy for more than 12 years and all of them had stopped immunotherapy by the end of May 1997 because they had no nasal symptoms for the last three pollen seasons and were considered to be cured of seasonal allergic rhinitis (cure group). Peripheral blood mononuclear cells (PBMCs) were collected from each subject during the cedar pollen season in 1998 and were stimulated for 96 h with 10 µg/ml Cry j 1. The concentrations of interleukin-5 (IL-5) and interferon-gamma (IFN-γ) in the culture supernatant were determined using an enzyme-linked immunosorbent assay. The levels of IFN-γ did not differ significantly among the non-atopic group, the asymptomatic group, the medication group, the poor-IT group and the good-IT group. The level of IL-5 in the asymptomatic group was not different from that in the non-atopic group. The levels of IL-5 in the medication group, the good-IT group and the poor-IT group were significantly higher than in the non-atopic group. The level of IL-5 in the good-IT group, but not in the poor-IT group, was significantly lower than in the medication group. The level of IL-5 in the cure group was not significantly different from in the non-atopic group, and the level of IFN-γ in the cure group was significantly lower than in the non-atopic group. In conclusion, immunotherapy can decrease the pollen allergen-induced synthesis of IL-5, but not of IFN-γ, and this immunological modulation is involved in the working mechanism of immunotherapy related to its clinical efficacy. A tolerance or anergy of both TH1 and TH2 cells under allergen stimulation may be an immunological indication of cure after the treatment of seasonal allergic rhinitis. Thus, the suppression of synthesis of IL-5 and IFN-γ by allergen-stimulated PBMCs is likely to be a reliable criterion for a possible cure of seasonal allergic rhinitis after immunotherapy. *Key words:* airway allergic disease, cytokine, immunotherapy.

INTRODUCTION

There is increasing evidence that airway allergic diseases such as allergic rhinitis and asthma represent a specialized form of cell-mediated immunity, in which CD4-positive T-helper (TH) cells and their products, various cytokines, play a central part in the orchestration and persistence of chronic eosinophil inflammation (1). Murine TH cells have been categorized into at least two distinct subsets, based on their profiles of cytokine secretion (2). One type of TH cells (TH1) secretes interleukin-2 (IL-2), interferon-gamma (INF-γ) and tumour necrosis factor-β (TNF-β), whereas the other type (TH2) produces IL-4, IL-5, IL-6, and IL-10, but not IL-2 and INF-γ. Other cytokines such as IL-3, granulocyte-macrophage-colony stimulating factor (GM-CSF) and TNF-α are produced by both types of TH cells. In humans, a clear functional dichotomy in CD4-positive T-cells

has not been documented. In a recent study, however, CD4-positive T-cells that exhibit TH1 or TH2 subsets could be isolated from peripheral blood or tissues in several diseases and conditions.

The phenotype of cytokine production by TH cells from peripheral blood and target tissues of atopic subjects has been extensively investigated. 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 (3, 4). Cytokines derived by TH2-like cells could mediate allergic inflammation through activating eosinophils, promoting mast cell development, regulating immunoglobulin isotype switching to IgE and modulating adhesion molecule expression (5–8). According to current thinking, atopic allergy might be defined as a TH2-like cell or TH2 response predominant inflammatory disease.

The Japanese cedar (*Cryptomeria japonica*) pollinates heavily during early spring, and is the main pollen causing seasonal allergic rhinitis in Japan. TH cells, especially TH2-like cells and cytokines secreted by them, are likely to play an important role in the pathogenesis of seasonal allergic rhinitis due to the pollen. Indeed, IL-5 synthesis by peripheral blood mononuclear cells (PBMCs) stimulated by the allergen is critically important for the clinical manifestation of seasonal allergic rhinitis in individuals sensitized to the pollen (9).

Allergen immunotherapy is recognized as a highly effective form of treatment for allergic rhinitis, especially pollen-induced seasonal allergic rhinitis (10–12) and interest in immunotherapy has increased during the last decade. However, the specific immunological mechanisms behind the clinical effect are unclear, although several possibilities have been proposed. Recent *in vitro* and *in vivo* evidence has suggested that immunotherapy alters T-cell cytokine profiles from the preferential TH2 response to TH1 response, turning off the allergic inflammatory events. We therefore investigated the effect of immunotherapy on the cytokine profiles produced by the pollen allergen-stimulated PBMCs of patients with seasonal allergic rhinitis due to Japanese cedar pollens.

It has been shown that IL-4-stimulated PBMC of atopic donors produce more IgE than IL-4-stimulated PBMC of non-atopic donors during the pollen season, whereas out of season no difference was observed between these two groups (13), which may suggest that natural pollen exposure might prime the reactivity of PBMC in patients with pollen-induced allergic rhinitis. In our study, PBMCs were therefore obtained from each subject during the cedar pollen season. Cry j 1 and Cry j 2 are known to be the major allergens of Japanese cedar pollen (14, 15). Cry j 1 is localized on the exine outside layer, and Cry j 2 is contained in the amyloplast inside pollen, and pollen extracts contain Cry j 1 and Cry j 2 at an approximate ratio of 4:1. Since Cry j 1 was recently reported to be the more important allergen (16), Cry j 1 was therefore used to stimulate PBMC in this study. The main aim of the study was to investigate the immunological mechanisms of pollen immunotherapy with special reference to its clinical effect.

PATIENTS AND METHODS

Patients

This study included 130 adult patients with levels of Japanese cedar pollen-specific IgE (> 0.34 U/ml) in serum detectable by CAP who gave informed consent for participation. Eighteen patients had no seasonal aggravation of nasal symptoms during the pollen

season in 1998 (asymptomatic group). Forty-two patients had not been treated previously with immunotherapy, and were treated with antihistamine tablets, such as 10 mg/day ebastine, during the pollen season in 1998 (medication group). Sixty-one patients had undergone variable periods (mean 41.5 ± 40.4 months; range 6–165 months) of immunotherapy using Japanese cedar pollen extracts (immunotherapy group). Every patient in the immunotherapy group received maintenance injections of the same dose at the time of participation. The remaining nine patients had been treated with immunotherapy for more than 12 years, and all of them had stopped immunotherapy by the end of May 1997, because they had no nasal symptoms for the last three pollen seasons and were considered to be cured of seasonal allergic rhinitis (cure group).

To serve as controls, 15 non-allergic healthy adult volunteers (non-atopic group) matched for age and sex were chosen on the basis of the following criteria: *i*) no history of allergic disease; *ii*) no physical findings indicative of atopic diseases; and *iii*) negative serum IgE antibodies specific to the major allergens in Japan, such as house dust mite, Japanese cedar pollen, ragweed and Japanese cypress pollen.

Mode of immunotherapy

The patients were given subcutaneous injections of Japanese cedar pollen extracts (Torii Co. Ltd, Tokyo, Japan), which are only commercially available in Japan at present. The aqueous extracts were prepared from native pollens, and were demonstrated to contain 2.0 mg/ml protein. The details of our immunotherapy are described elsewhere (12, 17). Individual initial doses were determined by threshold skin tests. Doses were increased by 50–100% at weekly intervals until the highest tolerated dose or 0.1 ml of 1:100 w/v was reached. The maximum tolerated dose was attained within 4–8 months, and weekly injections were continued for the first few months after attainment of the maintenance dose. They then received injections of this dose on a biweekly basis for the next few months, followed by monthly injections. Finally, the interval between injections was gradually increased up to 3 months.

Study design

The study design followed the principles outlined in the Declaration of Helsinki (18). Pollination of Japanese cedars occurred in 1998 from the second week of February to the second week of April. Peripheral blood was collected from each subject during the cedar pollen season in 1998 and serum concentrations of Japanese cedar pollen-specific IgE were determined by the use of CAP. PBMCs were isolated

from heparinized blood samples by Ficoll-density gradient centrifugation. The PBMCs were washed twice in phosphate buffered saline and resuspended in RPMI 1640 (Flow Laboratories, Meckenheim, Germany), containing 10% inactivated foetal calf serum (JRH Biosciences, Lenexa, USA), 100 µg/ml streptomycin (Sigma, St Louis, USA), 100 IU/ml penicillin and 2 mM L-glutamine. PBMCs were cultured at a final concentration of 1.0×10^6 cells/ml in 12-well plates (Iwaki glass, Tokyo, Japan) at 37°C in a fully humidified 5% CO₂ atmosphere. PBMCs were stimulated for 96 h with 10 µg/ml Cry j 1. Culture supernatants were harvested after 96 h by centrifugation, and stored at -80°C pending measurement of IL-5 and IFN-γ.

Determination of IL-5 and IFN-γ in culture supernatants

IL-5 and IFN-γ were measured using an enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Otsuka Pharmaceutical Co. Ltd, Tokyo, Japan). The details and justification of the methods used in this study have been described elsewhere (9, 19, 20).

In brief, IL-5 was measured using an ELISA kit (Otsuka Pharmaceutical Co. Ltd, Japan). Microtitre plates were coated with anti-IL-5 monoclonal antibody (MoAb JES1-39D10 at 2 µg/ml) in 100 µl/well of phosphate-buffered saline, pH 7.4 (PBS). Following overnight incubation at 4°C, the wells were blocked with a 1% skimmed milk in PBS solution for at least 1 h at room temperature and washed three times with PBS containing 0.05% Tween-20 (Tween-20-PBS, washing was always performed with this buffer). 100 µl of sample or standard in 0.1% BSA-PBS was added to the wells and the plate was incubated at 4°C for 24 h. The plates were then washed three times, and 100 µl rabbit IL-5 antibodies (MoAb JES1-5A10: diluted to 1/500 with 0.1% BSA-PBS-Tween-20) was added to each well and plates were incubated for 2 h at 23°C. Subsequently, the plates were washed, supplemented with 100 µl HRP-labelled avidin (diluted to 1/10,000 with 0.1% BSA-PBS-Tween-20) and incubated at 23°C for 2 h. Finally, 100 µl enzyme substrate (1 mg/ml O-phenylene diamine and 0.03% H₂O₂ in 0.1 M sodium citrate buffer, pH 5.0) was added to each well and the plates incubated at room temperature for 10 min. The reaction was stopped by adding 100 µl H₂SO₄ to each well and the absorbance at 492 nm was determined, using a Titertek Multiscan.

IFN-γ was determined using an ELISA kit (Otsuka Pharmaceutical Co. Ltd, Japan). Microtitration plates were coated with anti-IFN-γ monoclonal antibody (MoAb ANOC 1611 at 10 µg/ml) in 100 µl/well

of phosphate-buffered saline, pH 7.4 (PBS). Following overnight incubation at 4°C, the wells were blocked with a 1% skimmed milk in PBS solution for at least 1 h at room temperature and washed three times with PBS containing 0.05% Tween-20 (Tween-20-PBS, washing was always performed with this buffer). 100 µl of sample or standard in 0.1% BSA-PBS was added to the wells and the plate was incubated at 4°C for 24 h. The plates were then washed three times, and 100 µl rabbit anti-IFN-γ antibodies (OCT 1601: diluted to 1/2,000 with 0.1% BSA-PBS-Tween-20) was added to each well and plates were incubated for 2 h at 23°C. Subsequently, the plates were washed, supplemented with 100 µl HRP-labelled goat anti-rabbit IgG (diluted to 1/10,000 with 0.1% BSA-PBS-Tween-20) and incubated at 23°C for 2 h. Finally, 100 µl enzyme substrate (1 mg/ml O-phenylene diamine and 0.03% H₂O₂ in 0.1 M sodium citrate buffer, pH 5.0) was added to each well and the plates incubated at room temperature for 10 min. The reaction was stopped by adding 100 µl H₂SO₄ to each well and the absorbance at 492 nm was determined, using a Titertek Multiscan.

The lowest sensitivity of the method employed was 20 pg/ml for IL-5 and IFN-γ and it did not measure the presence of other cytokines.

Clinical evaluation of immunotherapy

The patients in the immunotherapy group were further divided into the following two groups based on clinical response to immunotherapy: good-IT group (no nasal symptoms without rescue medication) and poor-IT group (evident nasal symptoms or need for rescue medication to control nasal symptoms). The good-IT group and the poor-IT group included 41 (67.2%) and 20 (32.8%) patients, respectively.

Statistical analysis

If the level of IL-5 or IFN-γ in particular patients was below the sensitivity of the respective assay, the lowest detectable value (20 pg/ml) was arbitrarily used for statistical analysis. For comparisons between different groups, the Mann-Whitney U test was used, and differences were considered significant when $p < 0.05$. Spearman's correlation coefficient (r_s) was used to determine the degree of correlation between two different parameters, and significant correlation was accepted when $p < 0.05$ on Spearman's rank correlation analysis.

RESULTS

Comparison of specific IgE in serum

No detectable levels of specific IgE were found in the serum of each individual in the non-atopic group.

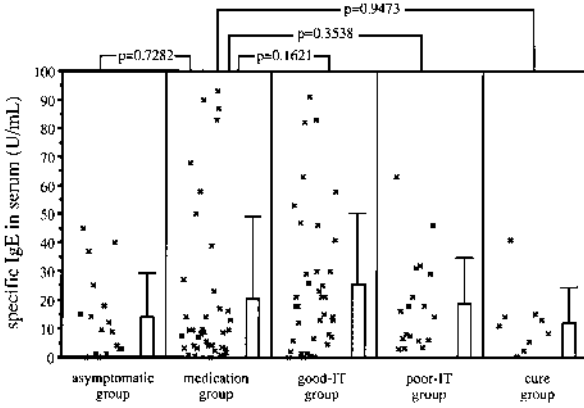


Fig. 1. Japanese cedar pollen-specific IgE in serum at the sampling of PBMC. Specific IgE was not detected in the serum of each individual in the non-atopic group. None of the levels of specific IgE in the asymptomatic group, the good-IT group, the poor-IT group and the cure group were significantly different from the levels of specific IgE in the medication group.

None of the levels of specific IgE in the asymptomatic group ($p = 0.7282$), the good-IT group ($p = 0.1621$), the poor-IT group ($p = 0.3538$) or the cure group ($p = 0.9473$) were significantly different from the levels of specific IgE in the medication group (Fig. 1).

Comparison with non-atopic group

Asymptomatic group. Neither the level of IL-5 nor the level of IFN- γ differed significantly between the non-atopic group and the asymptomatic group (IL-5: $p = 0.1364$, IFN- γ : $p = 0.2473$, Figs. 2 and 3).

Medication group. The level of IL-5 in the medica-

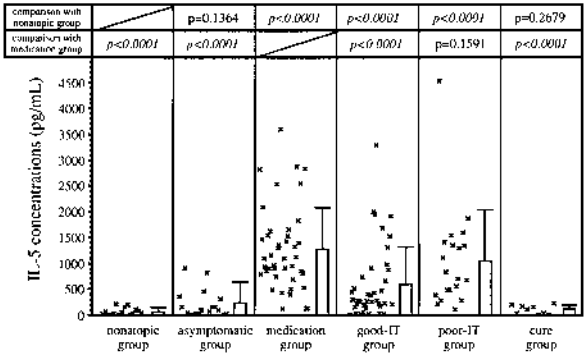


Fig. 2. Cry j 1-induced synthesis of IL-5. The levels of IL-5 in the medication group, the good-IT group and the poor-IT group were significantly higher than those in the non-atopic group, but the levels of IL-5 in the asymptomatic group and the cure group were not significantly different from those in the non-atopic group. The levels of IL-5 in the poor-IT group were not significantly different from those in the medication group, but the levels of IL-5 in the remaining groups were significantly lower than those in the medication group.

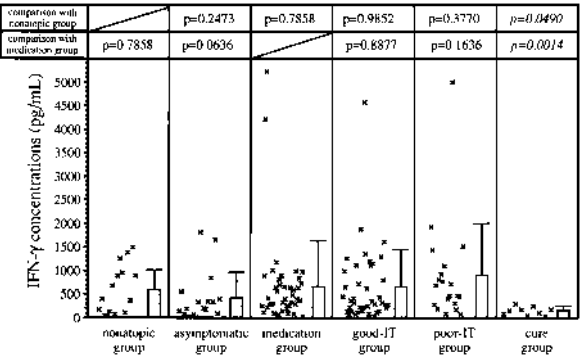


Fig. 3. Cry j 1-induced synthesis of IFN- γ . The levels of IFN- γ in the cure group were significantly lower than those in the non-atopic group, but the levels of IFN- γ in the remaining group were not significantly different from those in the non-atopic group. The levels of IFN- γ in the cure group were significantly lower than those in the medication group, but the levels of IFN- γ in the remaining groups were not significantly different from those in the medication group.

tion group was significantly higher than in the non-atopic group ($p < 0.0001$, Fig. 2). However, the level of IFN- γ did not differ significantly between the medication group and the non-atopic group ($p = 0.7858$, Fig. 3).

Immunotherapy group. The level of IL-5 in the immunotherapy group was significantly higher than in the non-atopic group ($p < 0.0001$). However, the level of IFN- γ did not differ significantly between the immunotherapy group and the non-atopic group ($p = 0.7197$).

Good-IT group. The level of IL-5 in the good-IT group was significantly higher than in the non-atopic group ($p < 0.0001$, Fig. 2). However, the level of IFN- γ did not differ significantly between the good-IT group and the non-atopic group ($p = 0.9852$, Fig. 3).

Poor-IT group. The level of IL-5 in the poor-IT group was significantly higher than in the non-atopic group ($p < 0.0001$, Fig. 2). However, the level of IFN- γ did not differ significantly between the poor-IT group and the non-atopic group ($p = 0.3770$, Fig. 3).

Cure group. The level of IL-5 did not differ significantly between the non-atopic group and the cure group ($p = 0.2679$, Fig. 2), but the level of IFN- γ in the cure group was significantly lower than in the non-atopic group ($p = 0.0490$, Fig. 3).

Comparison with medication group

Asymptomatic group. The level of IL-5 in the asymptomatic group was significantly lower than that in the medication group ($p < 0.0001$, Fig. 2). However, the level of IFN- γ did not differ significantly between the asymptomatic group and the medication group ($p = 0.0636$, Fig. 3).

Immunotherapy group. Neither the level of IL-5 nor the level of IFN- γ differed significantly between the immunotherapy group and the medication group (IL-5: $p = 0.1591$, IFN- γ : $p = 0.5151$).

Good-IT group. The level of IL-5 in the good-IT group was significantly lower than that in the medication group ($p < 0.0001$, Fig. 2). However, the level of IFN- γ did not differ significantly between the good-IT group and the medication group ($p = 0.8877$, Fig. 3).

Poor-IT group. Neither the level of IL-5 nor the level of IFN- γ differed significantly between the poor-IT group and the medication group (IL-5: $p = 0.1591$, IFN- γ : $p = 0.1636$, Figs. 2 and 3).

Cure group. Both the level of IL-5 and the level of IFN- γ in the cure group were significantly lower than those in the medication group (IL-5: $p < 0.0001$, IFN- γ : $p = 0.0014$, Figs. 2 and 3).

Comparison between the good-IT group and the poor-IT group

The length (months) of immunotherapy was significantly longer in the good-IT group than in the poor-IT group (good-IT group: 47.3 ± 42.6 months; poor-IT group: 29.4 ± 33.4 months, $p = 0.0479$). The level of specific IgE in serum did not differ significantly between the good-IT group and the poor-IT group (good-IT group: 25.2 ± 24.8 U/ml; poor-IT group: 18.4 ± 16.5 U/ml, $p = 0.6090$). The levels of IFN- γ did not differ significantly between the good-IT group and the poor-IT group ($p = 0.1621$), but the level of IL-5 in the good-IT group was significantly lower than in the poor-IT group ($p = 0.0075$).

Correlation with the duration of immunotherapy. The level of IL-5 in the immunotherapy group was inversely correlated with the length (months) of immunotherapy ($r_s = -0.353$, $p = 0.0060$, Fig. 4). However, the level of IFN- γ was not significantly correlated with the length of immunotherapy ($r_s = -0.207$, $p = 0.1054$, Fig. 5).

DISCUSSION

The first aim of the study was to investigate whether the profiles of cytokines produced by PBMCs differed between non-atopic subjects and patients with seasonal allergic rhinitis due to Japanese cedar pollens. In this study, we determined IL-5 as a representative of TH2-type cytokines and IFN- γ as a representative of TH1-type cytokines. First, we compared the level of IL-5 produced by PBMCs between patients with seasonal allergic rhinitis and non-atopic individuals. Our study design failed to provide an untreated control group with seasonal allergic rhinitis for ethical restrictions. Each patient in the medication group

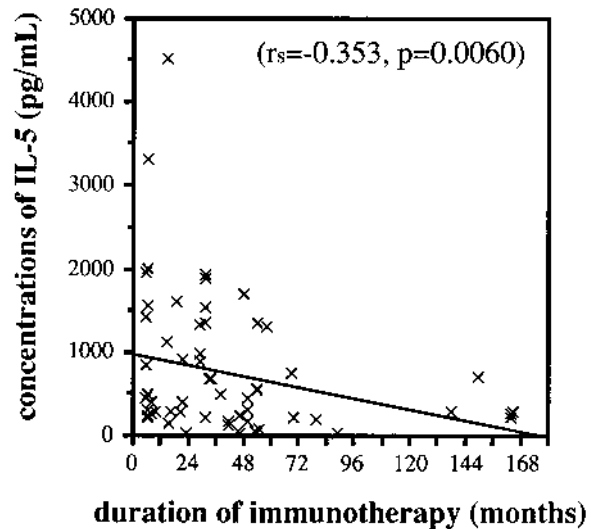


Fig. 4. Correlation of IL-5 synthesis and duration of immunotherapy. The level of IL-5 was inversely correlated with the length of immunotherapy.

was treated with oral administration of antihistamine tablets during the pollen season, but was not treated with immunotherapy or systemic steroids. Strictly speaking, the patients in the medication group were not untreated controls. The synthesis of IL-5 in the medication group might be somewhat decreased by the anti-allergenic treatment. However, the level of IL-5 in the medication group was significantly higher than that in the non-atopic group. In addition, the level of IL-5 in the asymptomatic group was significantly lower than that in the medication group and was not significantly different from that in the non-atopic group.

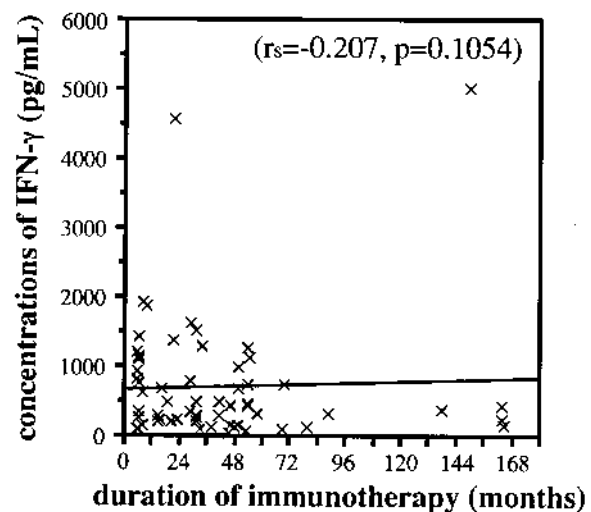


Fig. 5. Correlation of IFN- γ synthesis and duration of immunotherapy. The level of IFN- γ was not significantly correlated with the length of immunotherapy.

Mori et al. demonstrated that T-cells of both atopic and non-atopic patients with asthma produced significantly higher amounts of IL-5 in response to non-specific stimulation compared with those of non-atopic individuals (21). In another study, allergen-induced IL5 production by PBMC from sensitized atopic subjects with symptoms (asthma or rhinitis) is elevated compared with that in atopic subjects with no symptoms and non-atopic normal control subjects, suggesting that elevated IL-5 synthesis appears not to be a feature of atopic disease *per se* but is observed only in the presence of ongoing atopic diseases (22). Taking these lines of evidence together, the level of IL-5 synthesized by PBMCs is likely to be related to the clinical manifestation of individuals sensitized to the pollen, which is in agreement with our previous study (9).

The fundamental mechanisms of synthesis of TH1-type cytokines and TH2-type cytokines are considered to differ between atopic and non-atopic individuals. IFN- γ secreted by TH1-like cells is generally considered to play the opposite role to TH2-type cytokines, because IFN- γ inhibits the IgE synthesis by B lymphocytes and the development of TH2 clones (23). In our previous study, Der f 1-stimulated peripheral blood T lymphocytes of house dust mite-sensitive individuals are reported to secrete significantly less IFN- γ than those from normal controls (24). We thus determined the concentrations of IFN- γ produced by PBMC cultures. The level of IFN- γ synthesized by PBMCs did not differ significantly among the non-atopic group, the medication group and the asymptomatic group. This finding may suggest that the Cry j 1-induced synthesis of IFN- γ is not substantially involved in the sensitization and clinical manifestation of seasonal allergic rhinitis due to Japanese cedar pollens and that Cry j 1 and Der f 1 have quite different effects on the synthesis of IFN- γ .

We next studied the effect of pollen-immunotherapy on the Cry j 1-induced synthesis of IL-5 and IFN- γ . The level of IL-5 in the immunotherapy group was significantly higher than in the non-atopic group and was not significantly different from that in the medication group. The level of IFN- γ in the immunotherapy group was not significantly different from that in the non-atopic group or in the medication group. These findings alone may indicate that pollen-immunotherapy does not affect the synthesis of IL-5 or IFN- γ by Cry j 1-stimulated PBMCs.

Immunotherapy is a clinically effective treatment for patients with Japanese cedar pollinosis, but it is not effective for all of them. The synthesis of IL-5 and IFN- γ in the immunotherapy group was therefore analysed based on clinical efficacy. The level of IFN- γ did not differ between those who responded

well to immunotherapy (the good-IT group) and those who responded poorly to immunotherapy (the poor-IT groups). In addition, the level of IFN- γ did not differ significantly among the good-IT group, the poor-IT group, the non-atopic group and the medication group. Furthermore, the level of IFN- γ synthesis was not correlated with the length (months) of immunotherapy. Therefore, immunotherapy is not likely to affect the synthesis of IFN- γ by Cry j 1-stimulated PBMCs.

On the other hand, the level of IL-5 synthesized by Cry j 1-stimulated PBMCs in the good-IT group was significantly lower than in the poor-IT group. The level of IL-5 in the good-IT group was significantly lower than that in the medication group, whereas the level of IL-5 in the poor-IT group was not different from that in the medication group. In addition, the level of IL-5 in the immunotherapy group was significantly correlated with the length of immunotherapy. All these lines of evidence suggest that immunotherapy could decrease the synthesis of IL-5 by Cry j 1-stimulated PBMCs and that this effect on the synthesis of IL-5 is significantly related to the clinical efficacy of immunotherapy. To summarize our present study shows that immunotherapy can decrease the pollen allergen-induced synthesis of IL-5, but not of IFN- γ , and this immunological modulation is involved in the working mechanisms of immunotherapy linked to its clinical efficacy (Fig. 6).

The effect of immunotherapy on cytokine synthesis has been evaluated by several authors. Secrist et al. have demonstrated that production of IFN- γ by allergen-specific CD4⁺ T-cells was not affected in grass pollen and mite allergic subjects on immunotherapy whereas IL-4 production was reduced by immunotherapy and that the levels of IL-4 produced are inversely related to the length of time on immunotherapy (25). A similar effect of immunotherapy on allergen-induced T-cell reactivity was reported by Söderlund et al. They analysed allergen-induced IL-4 and IFN- γ mRNA expression in peripheral blood mononuclear cells (PBMCs) of birch allergic patients, using reverse transcriptase-polymerase chain reaction (RT-PCR). The patients receiving immunotherapy expressed less IL-4 mRNA than the patients receiving placebo injections during the pollen season, whereas no significant difference in the IFN- γ mRNA expression could be observed between them (26). On the other hand, the important role of IFN- γ in the mechanism of immunotherapy has also been demonstrated. Varney et al. and Durham et al. reported a significant increase in IFN- γ expression without a significant changes in IL-4 expression in experiments using *in situ* hybridization to inflammatory cells in the skin or nasal mucosa in patients who received

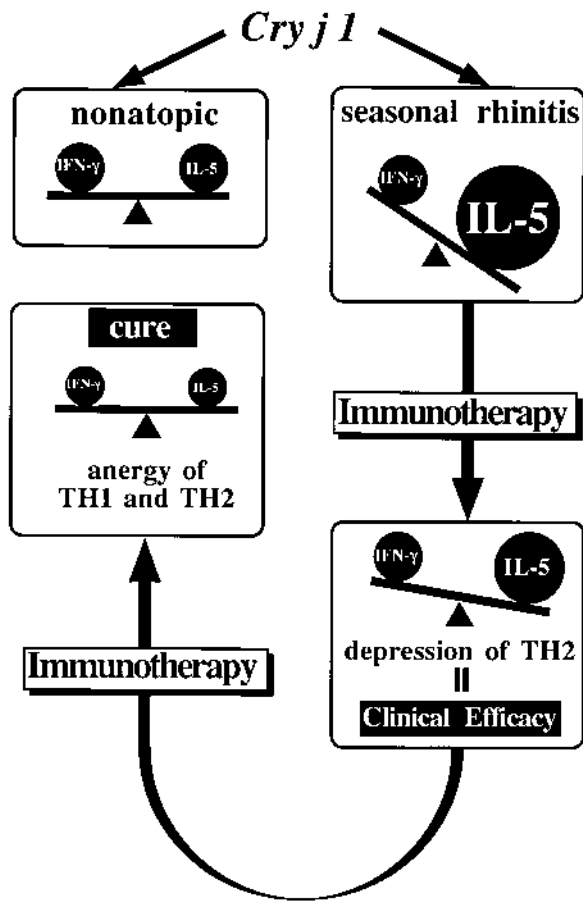


Fig. 6. Schematic presentation of the working mechanism of immunotherapy. Immunotherapy can decrease the pollen allergen-induced synthesis of IL-5, but not of IFN- γ , and this immunological modulation is involved in the working mechanisms of immunotherapy linked to the clinical efficacy. A suppression of IFN- γ synthesis and IL-5 synthesis is apparent in patients who were cured of seasonal allergic rhinitis by immunotherapy.

grass pollen immunotherapy (27, 28). Conversely, MacDonald et al., studying cytokine release from PBMCs in patients on ragweed immunotherapy, showed a highly significant reduction in the production of IFN- γ , but no significant changes in IL-4 (29). O'Brien et al. stimulated PBMCs from patients sensitive to house dust mite with Der p 2, and measured cytokine production by the use of polymerase chain reaction (30). They have demonstrated that immunotherapy results in a reduction in expression of both IL-4 and IFN- γ in circulating lymphocytes. In our previous study, mite-immunotherapy suppressed both IL-5 and IFN- γ synthesis, but the suppression of IL-5 was exclusively linked to the clinical efficacy of immunotherapy (9).

Thus, there is no general agreement among different investigators regarding the effect of im-

muno-therapy on TH responses or cytokine profiles. The exact reason for these different results is unclear, but some factors, such as different types of allergies (seasonal or perennial), different length of immunotherapy, different target organs (peripheral blood or inflamed tissue) and different experimental protocols (*in vitro* or *in vivo*) among different studies, are likely to underlie the contradictory results.

The most outstanding advantage of immunotherapy is that it may have the potential to alleviate more or less permanently abnormal immunological responses and to cure nasal symptoms in the long-term, even after discontinuation of the injection course, whereas drug treatment is effective only while being administered. The long-term clinical efficacy of immunotherapy for seasonal allergic rhinitis due to pollens was previously evaluated in several studies. In some studies, clinical efficacy was detectable for up to 6 years after discontinuing pollen immunotherapy for seasonal allergic rhinitis (31, 32). Our present study included a cure group composed of nine patients who had been treated for more than 12 years with immunotherapy and who had had no nasal symptoms during the last three pollen seasons. They stopped immunotherapy by the end of May in 1997, and they had no nasal symptoms with any specific treatments during the pollen season in 1998, although their level of specific IgE was not different from those in the other groups. The level of IL-5 in the cure group was as low as that in the non-atopic group, and the level of IFN- γ in the cure group was significantly lower than that in the non-atopic group. An inference can be drawn from the evidence that neither TH1 cells nor TH2 cells in cured patients respond to the allergen to synthesize IL-5 or IFN- γ . In other words, a tolerance or anergy of both TH1 and TH2 cells under allergen stimulation is an immunological hallmark of the cure after the treatment of seasonal allergic rhinitis (Fig. 6). Prolonged mite-immunotherapy frequently results in a disappearance of mite-specific IgE in serum, whereas a disappearance of pollen-specific IgE can be hardly expected in patients with pollen-allergy even after prolonged pollen-immunotherapy. Thus, the suppression of synthesis of both IL-5 and IFN- γ by allergen-stimulated PBMCs is likely to be a reliable criterion for a possible cure of seasonal allergic rhinitis after immunotherapy.

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