
Clinical and immunologic changes after allergen immunotherapy with Hop Japanese pollen

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Background and Objective: Hop Japanese (Hop J) pollen has been reported as one of the major causative pollen allergens in the autumn season. There have been no published data regarding the clinical and immunologic effects of Hop J pollen immunotherapy in sensitized patients. In this study, we evaluated clinical and immunologic effects of Hop J immunotherapy.

Patients and Methods: Pollens were collected in our area, and “Depo-Hop J” was prepared in the laboratory of Allergopharma (Reinbek, Germany). Fifteen asthmatic patients who had Hop J immunotherapy for >1 year were enrolled. Their clinical parameters, such as asthma symptom scores, were monitored. Skin reactivity to Hop J and degree of airway hyperresponsiveness to methacholine were measured before and 1 year after the immunotherapy. Sera were collected before the immunotherapy, at the end of initial therapy, and 1 year after the therapy. Serum total IgE levels were compared by radioimmunoassay. Serum-specific IgE, IgG1, and IgG4 levels to Hop J were compared by ELISA. To evaluate the changes of cellular mechanisms, soluble CD30 (sCD30), soluble interleukin (IL)-2 receptor (sIL-2R), soluble CD23 (sCD23), and IL-10 levels were measured by ELISA.

Results: Specific IgG1 and IgG4 levels began to increase at the end of the initial therapy ($P < 0.05$) with significant decreases in symptom scores ($P < 0.05$), whereas total and specific IgE levels showed variable responses during the immunotherapy with no statistical significance ($P > 0.05$). Serum sIL-2R and sCD30 levels decreased significantly ($P < 0.05$) 1 year after immunotherapy. No significant changes were noted in sCD23, IL-10, skin reactivity to Hop J, or airway responsiveness to methacholine ($P > 0.05$).

Conclusions: We are certain that Hop J allergen immunotherapy, if carried out properly according to suitable indications, can favorably influence asthma. Thus, an increase in specific IgG4 and IgG1 antibodies and reduction of a possible Th2 lymphocyte marker (sCD30) may be associated with symptomatic improvements.

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INTRODUCTION

Guidelines and indications for immunotherapy with inhalant allergens and/or venoms were proposed in 1987¹ and have been published within the past sev-

eral years by the World Health Organization,^{2,3} the European Academy of Allergy and Clinical Immunology,^{1,4} the International Consensus Report on Asthma,⁵ the Global Strategy for Asthma Management and Prevention,⁶ the International Consensus Report on Rhinitis,⁷ the British Society for Allergy and Clinical Immunology,⁸ the American Academy of Allergy, Asthma, and Immunology, and the American College of Allergy, Asthma, and Immunology.⁹ Physicians and scientists from several countries convened at the World Health Organization headquarters in Geneva in

January 1997, to review the science and indications for allergen immunotherapy for treating allergic diseases. They published their position papers in major journals, including *Journal of Allergy and Clinical Immunology*, *Annals of Allergy, Asthma and Immunology*, and *Allergy* in 1998^{10,11} and emphasized that allergen immunotherapy should be considered an effective treatment modality for allergic asthma, if carried out properly according to suitable indications. Hop Japanese (Hop J) belongs to a weed of the Cannabinaceae family, and the Urticales order is widespread in both rural and urban areas of Korea. It has been considered one of the major causative pollens of autumn pollinosis in this country.¹² To the best of our knowledge, this is the first study to test Hop J immunotherapy and to observe the clinical and immunologic effects during 1 year of immunotherapy.

MATERIALS AND METHODS

Preparation of Hop J Pollen Extract and Administration Method of Hop J Immunotherapy

To prepare immunotherapy extract, an adequate amount of pollen was collected in the Suwon area during the last 2 weeks of September 1997. The flowers were cut and air dried. When the pollens were dropped, they were collected through a sieve. Then they were defatted with ethyl ether, dried, and sent to Dr. O. Cromwell (Reinbek, Germany) for “Depo-Hop J” preparation. The pollens were extracted with a buffered salt solution, adsorbed on aluminum hydroxide suspended in a physiologic saline solution, and preserved with 0.4% phenol. Standardization was carried out in protein nitrogen units

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Table 1. Clinical Characteristics of the Study Patients*

Patient	Sex/age, yr	Clinical manifestation	Skin reactivity (A/H ratio)	Asthma severity	Bronchoprovocation test result
CJ	M/28	AR + BA	3+	III	Dual
CK	M/20	AR + BA	2+	III	Early
KY	F/17	AR + BA	3+	I	Early
YK	M/13	AR + BA	2+	I	Early
JJ	M/38	BA	3+	II	Early
YI	F/42	AR + BA	3+	I	Early
KY	F/33	AR + BA	3+	III	Early
KS	F/35	AR + BA	3+	III	Early
OC	F/44	AR + BA	2+	I	Dual
KJ	F/44	AR + BA	2+	III	Early
MJ	M/40	BA	3+	III	Early
CH	M/39	AR + BA	5+	I	Dual
LJ	M/45	AR + BA	4+	II	Early
LT	F/33	AR + BA	5+	I	Dual
KYY	F/39	AR + BA	3+	II	Dual

* A/H ratio; allergen (Hop J to histamine wheal ratio), AR, allergic rhinitis; BA, bronchial asthma. Asthma severity: I, mild intermittent; II, mild persistent; III, moderate.

(PNU). The initial preparation was composed of three serial vials with strengths 1 (25 PNU), 2 (250 PNU), and 3 (2,500 PNU). For the maintenance treatment, vial 3 (2,500 PNU) was used, and was administered monthly for 1 year. The initial dose was 0.1 mL, and the maximum tolerable dose was decided on an individual basis.

Fifteen patients with mild to moderate asthma who suffered from seasonal aggravation of asthmatic symptoms were enrolled. All the patients had high serum-specific IgE antibody to Hop J, as well as positive [$>3+$ airway hyperresponsiveness (A/H) ratio] responses to skin prick tests and positive results to Hop J bronchoprovocation tests, which were performed according to the previously described method.¹² To exclude allergen exposure effect, Hop J immunotherapy was started within 1 month after the pollen season. Patients' sera were collected three times: before the immunotherapy, at the end of initial therapy, and 1 year after immunotherapy. The clinical characteristics of the study patients are summarized in Table 1. All the patients gave their informed consent, which was regulated by Ajou University Medical Center, Suwon, Korea.

Changes of Symptom and Medication Scores, Skin Reactivity to Hop J, and A/H to Methacholine

Symptom scores were monitored three times: before the treatment, at the end of initial therapy, and 1 year after immunotherapy. Symptom scores ranged from 0 to 3 depending on the severity of dyspnea, coughing, sputum discharge, limitation of activity, and nocturnal symptoms based on Korean guidelines for asthma treatment.¹³

Degree of A/H to methacholine was measured two times out of season (before and 1 year after the immunotherapy) using a modification of Chai's method.¹⁴

Changes of Serum Total and Specific IgE Antibodies

Serum total IgE level was measured by AlaSTAT (DPC, Los Angeles, CA) according to the manufacturer's instructions. A change of specific IgE antibody was determined by ELISA according to the previously described method.¹² Briefly, microtiter plates (Costar, Corning, NY) were first coated with 100 μ L of Hop J (1 μ g/well) extract and left at 4° C overnight. Each well was washed three times with 0.05% Tween-phosphate-buffered saline (PBS-T), and the remaining binding sites were blocked by incubation with 350 μ L of 5% bovine serum albumin for 1 hour at room temperature. Each well was washed three times and then incubated for 2 hours at room temperature with 50 μ L of either the patients' sera (1:2 dilution) or control sera from 15 patients who showed negative skin prick test responses to common inhalant allergens and the Hop J pollen. After the wells were washed three times with PBS-T, 50 μ L of 1:1,000 (vol/vol) biotin-labeled goat anti-human IgE antibody (Sigma, St.

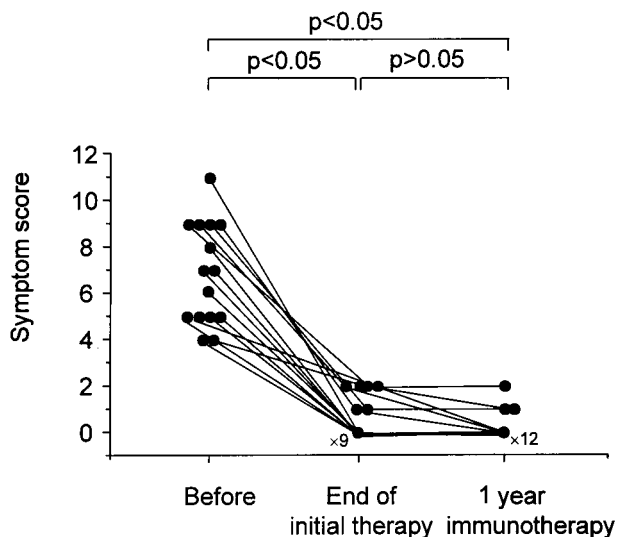


Figure 1. Changes of symptom scores after 1 year of immunotherapy with Hop J pollen. A significant difference was noted in symptom score ($P < 0.05$).

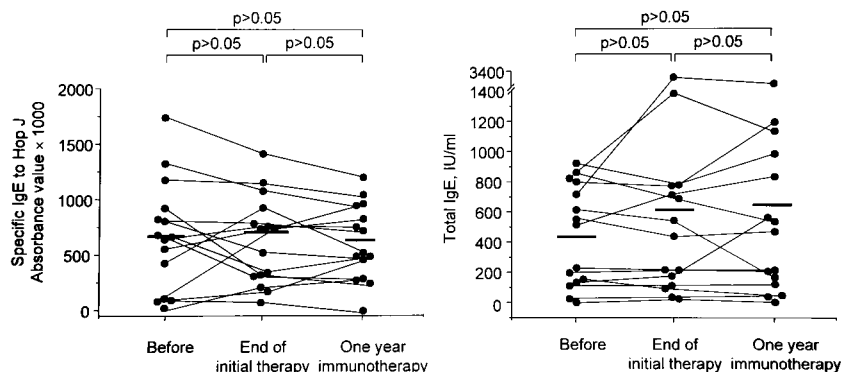


Figure 2. Changes of total (a) and specific IgE (b) antibody to Hop J pollen. There were no significant changes during the 1 year of immunotherapy ($P > 0.05$). Horizontal bars indicate mean values.

Louis, MO) was added to the wells and incubated for 1.5 hours at room temperature. The wells were then washed three times with PBS-T and incubated with 1:1,000 (vol/vol) streptavidin peroxidase (Sigma) for 30 minutes before another washing step, which was followed by incubation with 100 μ L of 3,3',5,5'-tetramethylene benzidine in 0.05 mol/L phosphate citrate buffer, pH 5.0, and 10 mL of 30% H_2O_2 in a phosphate citrate buffer for 10 minutes at room temperature. The reaction was stopped by the addition of 100 μ L of 2.5 N sulfuric acid, and the absorbance was read at 450 nm by an automated reader.

ELISA for Specific IgG1 and IgG4 Antibodies to Hop J Pollen Extract

Diluted patient serum or negative control serum (1:10 for soluble IgG1 and 1:2 for soluble IgG4 in diluent buffer; PBS-T containing 5% bovine serum albumin; 100 μ L) was added to each well coated with Hop J pollen. After incubation for 2 hours at 25°C, the wells were washed three times with PBS-T. After they were washed three times with PBS-T, 50 μ L of 1:1,000 (vol/vol) biotin-labeled goat anti-human IgG1 or IgG4 antibody (Sigma) were added to the wells and incubated for 1.5 hours at room temperature. The wells were then washed three times with PBS-T and incubated with 1:1,000 (vol/vol) streptavidin peroxidase (Sigma) for 30 minutes before another washing step, which was followed by incubation with 100 μ L of 3,3',5,5'-tetramethylene benzidine in

0.05 M phosphate citrate buffer, pH 5.0, and 10 mL of 30% H_2O_2 in a phosphate citrate buffer for 10 minutes at room temperature. The reaction was stopped by the addition of 100 μ L of 2.5 N sulfuric acid, and the absorbance was read at 450 nm by an automated reader. The antibody titer was expressed as absorbance values. The positive cut-off value was determined as 0.14 from the mean absorbance + 2 standard deviations (SD) of 30 controls. All the samples were run on the same day.

ELISA for Soluble (s)CD30, Soluble Interleukin-2 Receptor (sIL-2R), sCD23, and IL-10 Level

To evaluate the changes of soluble cell marker and cytokine, the levels of sCD30, sIL-2R, sCD23, and IL-10 were measured by ELISA kits (Benger Med

Systems, Vienna, Austria) according to the manufacturer's guidance.

Statistical Analysis

Wilcoxon signed rank tests using SPSS (Chicago, IL) version 7.0 were applied to evaluate the statistical differences between two values. A P value of 0.05 or less was regarded as significant.

RESULTS

Changes of Skin Reactivity to Hop J, Symptom Scores, and A/H

Figure 1 shows the changes of asthma symptom scores during the 1 year of immunotherapy. Symptom scores decreased significantly at the end of initial therapy ($P < 0.05$) and then were maintained for up to 1 year after immunotherapy with no statistically significant differences ($P > 0.05$). Although skin reactivity to Hop J tended to decrease after 1 year of immunotherapy, there was no statistical significance ($P > 0.05$, data not shown). There was no significant change in A/H to methacholine during the 1 year of immunotherapy ($P > 0.05$, data not shown).

Changes of Serum Total and Specific IgE and Specific IgG1 and IgG4 Antibodies

Figure 2 shows the changes of serum total IgE (Fig 2A) and specific IgE antibody levels during 1 year of immunotherapy (Fig 2B). There were no significant changes in either total or spe-

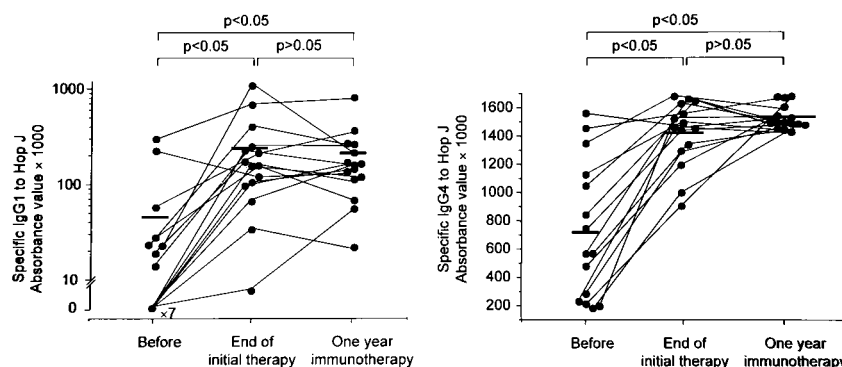


Figure 3. Changes of specific IgG1 (A) and IgG4 (B) antibodies to Hop J pollen during 1 year of immunotherapy. They increased significantly at the end of initial therapy ($P < 0.05$) and increased further for up to 1 year. Horizontal bars indicate mean values.

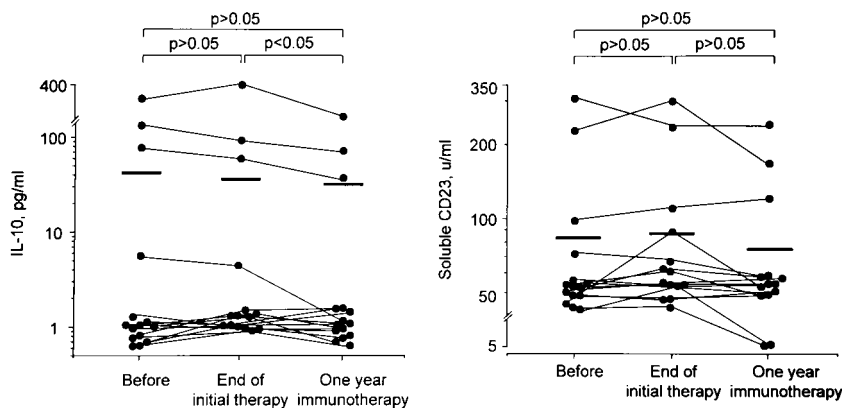


Figure 4. Changes of IL-10 (A) and sCD23 (B) levels during the 1 year of immunotherapy. There were no significant changes in serum IL-12 and sCD23 levels ($P > 0.05$). Horizontal bars indicate mean values.

cific IgE antibody levels during the 1 year of immunotherapy ($P > 0.05$).

Figure 3 shows the changes of serum-specific IgG1 (Fig 3A) and IgG4 (Fig 3B) antibodies to Hop J pollen during the 1 year of immunotherapy. Specific IgG1 and IgG4 levels began to increase at the end of the initial therapy and were statistically significant ($P < 0.05$); they increased further 1 year after the therapy in all patients.

Changes of IL-10, sCD23, sIL-2R, and sCD30 Levels

Figure 4 shows the changes of serum IL-10 (Fig 4A) and soluble CD23 (Fig 4B) levels during the 1 year of immunotherapy. There were no significant changes in serum IL-10 and sCD23 levels ($P > 0.05$).

Figure 5 shows the changes of sIL-2R (Fig 5A) and sCD30 levels (Fig 5B) during the 1 year of immunotherapy. The levels began to decrease at 6 months and decreased further after the 1 year of immunotherapy ($P < 0.05$).

DISCUSSION

A recent clinical study of 10 Hop J-sensitive asthmatic subjects,¹⁴ in which their bronchial sensitivity was proven by specific bronchoprovocation test with homemade Hop J pollen extract, showed that they all had high serum-specific IgE antibody on ELISA. They had suffered from seasonal aggravation of respiratory symptoms in the autumn season. Intrinsic asthma with influenza-like illness in

the autumn season had been diagnosed. The results of skin prick testing throughout the year in patients with respiratory allergy¹² revealed that 79 (6.1%) of 1,287 patients showed more than a 2+ A/H ratio to this pollen. When we classified 404 patients suffering from autumn pollinosis, 14.4% had more than a 2+ A/H response to this pollen. These results suggested that Hop J pollen should be considered one of the major causative pollens in Korea.

Large-scale studies of efficacy of subcutaneous immunotherapy with inhalant allergens have been undertaken in patients with pollen asthma. Double-blind placebo-controlled studies using standardized vaccines have shown that immunotherapy has a beneficial effect on bronchial symptoms and/or decreases

the need for asthma medications in both pollen and house dust mite asthma.^{15,16}

This is the first study to test Hop J pollen immunotherapy in sensitized patients. All the patients showed positive responses to Hop J bronchoprovocation tests. Pollens were collected from this area and sent to Allergopharma, one of the major immunotherapy-producing companies. They prepared alum-precipitated immunotherapy extract according to their standardized laboratory guidelines. In this study, after 1 year of immunotherapy with Hop J pollen extract, symptom scores decreased significantly in all subjects, although skin reactivity to Hop J or A/H to methacholine did not change significantly after 1 year of immunotherapy.

Regarding the mechanism of allergen immunotherapy, earlier studies of the mechanisms of allergen immunotherapy were focused on circulating antibodies and effector cells, such as decreases in specific IgE antibody level and increases in specific IgG levels.^{15,16} However, recent studies, which demonstrated immunomodulation from Th2 to Th1 responses, cytokine regulation of the immune responses, and specific inhibition or ablation of immune responses by means of tolerance induction,¹⁶⁻¹⁹ have reinforced the importance and value of allergen immunotherapy. In this study, specific IgG1 and IgG4 levels increased at the end of the initial therapy and were maintained at 1 year after the therapy in all patients,

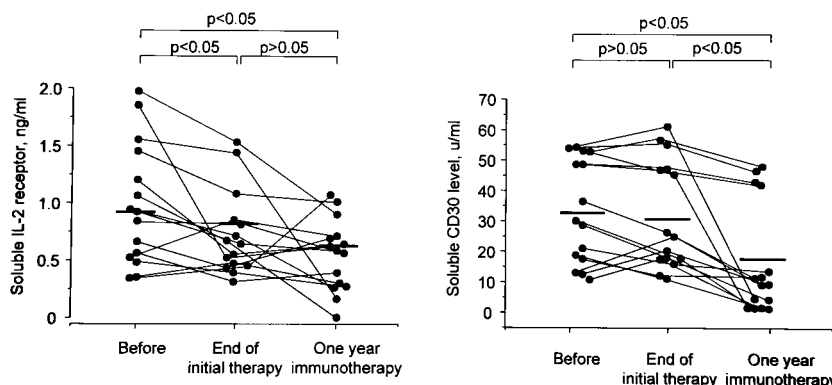


Figure 5. Changes of sIL-2R and sCD30 levels during the 1 year of immunotherapy. They decreased significantly after 1 year of immunotherapy ($P < 0.05$). Horizontal bars indicate mean values.

whereas total and specific IgE levels showed variable responses with no significant changes during the immunotherapy.

Regarding the changes of cellular markers, there have been several reports of changes of soluble cell markers in association with the effect of allergen immunotherapy.^{20–22} We looked at changes in levels of IL-10, which is produced by Th2 cells, sCD23 (low-affinity IgE receptor), sCD30, which is known to be expressed on Th2 cytokine-producing cells,^{23,24} and sIL-2 receptor. In this study, IL-2R levels decreased significantly after 1 year of immunotherapy, which may suggest the reduction of the CD4+ T-cell response, and sCD30 levels decreased significantly, which may suggest a possible decrease of Th2 response, whereas no significant changes were noted in IL-10 and sCD23 levels, although there have been a few reports showing a significant decrease of sCD23 as well as sCD30 levels during allergen immunotherapy with house dust mite.^{22,25} These findings suggest that 1 year of immunotherapy with Hop J pollen may reduce the expansion of Th2-derived cells as well as increase the production of allergen-specific IgG1 and IgG4 antibodies.

IN CONCLUSION

We are certain that Hop J immunotherapy, if carried out properly according to suitable indications, can favorably influence asthmatic symptoms. Thus, the increase of specific IgG4 and IgG1 antibodies and reduction of possible Th2-lymphocyte markers may be associated with symptomatic improvements.

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