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Letter to the Editor

Long-term immunological effects of Japanese cedar pollen-based subcutaneous immunotherapy

Dear Editor,

Japanese cedar pollen (JCP)-induced allergic rhinitis is the most common allergic disease, and its prevalence continues to increase.^{1,2} Allergen-specific immunotherapy (AIT) is an established treatment for pollen allergy.³ The efficacy of JCP-based AIT for JCP allergy has been documented.^{4,5} Although AIT should ideally be continued for 3–5 years, the immunological and clinical effects of long-term JCP-based AIT remains unclear. Herein, we investigated immunological changes including JCP-specific IgE and IgG4 levels and basophil activation (BA) in patients with JCP-induced allergic rhinitis 5 years after JCP-based SCIT.

We retrospectively evaluated data for patients who received JCP-based SCIT for 5 years at the pediatric department of Fujita Health University. We evaluated the immunological results for 19 patients (male, 13; female, 6; median age: 10.41; range 5–44 years). Their median JCP-specific IgE level was 41.3 UA/mL (range: 3.81–1709.0 UA/mL). The procedures used and the possible risks were explained to all patients and their parents; written informed consent was obtained. This study conformed to the guidelines established by the Declaration of Helsinki and was approved by the Research Ethics Committee of Fujita Health University, Aichi, Japan (No. 10-224).

All patients received a SCIT injection containing a standardized JCP extract (Torii Pharmaceutical, Tokyo, Japan). The up-dosing protocol was administered as a rush SCIT protocol (17 patients) or conventional SCIT protocol (2 patients) at the following doses: 0.1, 0.2, 0.6, 1, 2, 6, 10, 20, 40, 60, 60, 60, 100, 100, 140, 200, and 200 Japanese allergy units (JAU). Under the rush SCIT protocol, patients were injected several times a day, and under the conventional SCIT protocol, once or twice a week. The maintenance dose was 200 JAU or the maximum tolerated dose. Maintenance doses were administered at 4-week intervals for 5 years. The 200 JAU dose of the extract contained 0.06–0.25 µg/mL of Cry j 1 and 0.08–0.31 µg/mL of Cry j 2, which are the major JCP allergens. SCIT was initiated during summer. A baseline immunologic evaluation, which included specific IgE and IgG4 levels and BA, was performed before initiating SCIT. Re-evaluations were performed 5 months, 17 months, and 5 years after SCIT.

Patients' serum-specific IgE and IgG4 antibody levels were determined using the ImmunoCAP® System. The assay was performed at Thermo Fisher Scientific (Uppsala, Sweden). The cutoff levels were 0.35 UA/mL for specific IgE and 0.07 mgA/L for specific IgG4. BA was determined using an Allergen Kit (Beckman

Coulter, Fullerton, CA, USA), according to the manufacturer's instructions. Phosphate-buffered saline and anti-IgE antibodies (10 µg/mL) were used as negative and positive controls, respectively. Samples were analyzed using a FACSCalibur™ cell analyzer with CellQuest™ software (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Basophils were identified based on their forward and side scatter properties and on the absence of CD3 expression and presence of CRTH2 expression. JCP extracts were prepared using our previously reported methods.⁶ To assess allergen-specific BA, we used a series of JCP extract concentrations (five concentrations, ranging 10–0.001 µg/mL of total protein). Data acquisition generally involved 500 basophils; we excluded samples for which data acquisition involved <200 cells.

We established dose–response curves for BA based on 10-fold decreasing concentrations of the respective extracts. We used two-way analysis of variance (ANOVA) to construct the dose–response curve. Furthermore, BA was assessed from the area under the dose–response curve (AUC). Results were compared using the Dunn's multiple comparisons test. *P*-values <0.05 were considered significant. Patient data were excluded if paired before and after treatment blood sample sets were unavailable. Statistical analysis was performed using GraphPad Prism 6.01 (GraphPad Software, San Diego, CA, USA).

JCP-specific IgE levels decreased significantly after 17 months of SCIT (median values: 41.30, 36.50, 27.70, and 14.30 UA/mL, *P* < 0.05; Fig. 1A). JCP-specific IgG4 levels increased significantly after 5 months of SCIT (median values: 0.26, 0.96, 2.00 and 5.24 mgA/mL, *P* < 0.05; Fig. 1B). The BA by the JCP extract was dose-dependent; the dose–response curves shifted downward after SCIT (Fig. 2A). Two-way ANOVA showed a significant effect of treatment [*F* (3, 36) = 22.67; *P* < 0.0001] and concentration [*F* (4, 48) = 109.6; *P* < 0.0001]. The treatment-by-concentration interaction was significant [*F* (12, 144) = 11.72; *P* < 0.0001; Fig. 2A]. The AUC for BA was markedly reduced after 5 months of SCIT [median values: 143.1, 52.13, 39.75, and 29.95; *P* < 0.05; Fig. 2B].

Thus, reduced allergen-specific BA and an early increase in JCP-specific IgG4 was recorded; subsequently, JCP-specific IgE titers decreased during the JCP-based SCIT; this trend continued for 5 years thereafter. A 1-year JCP-based SCIT study showed a JCP-specific BA decrease and JCP-specific IgG4 increase 1 month after initiating SCIT, and this tendency continued throughout the study. However, changes in JCP-specific IgE titers were not reported.⁷ Another pollen (*Parietaria*)-based SCIT study showed an early increase and late decrease in specific IgE titers, and a continuous increase in specific IgG4 titers.⁸ Our study showed that JCP-based SCIT reduced JCP-specific IgE titers in long-term SCIT. The

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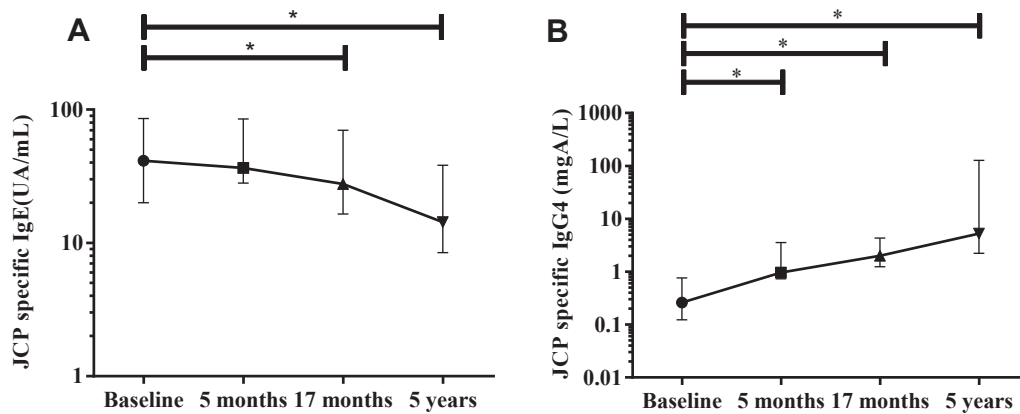


Fig. 1. The median values and interquartile range for (A) Japanese cedar-specific immunoglobulin (Ig) E levels, (B) Japanese cedar specific IgG4 levels before and after subcutaneous immunotherapy. * $P < 0.05$, Dunn's multiple comparisons test.

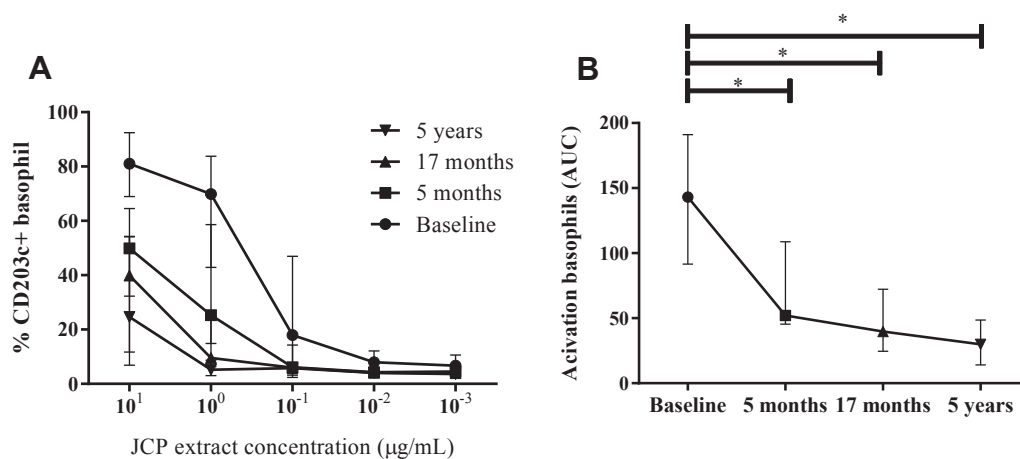


Fig. 2. The median values and interquartile ranges of allergen-specific basophil activation by Japanese cedar pollen extract before and after subcutaneous immunotherapy. (A) Dose–response curves before and after allergen-specific basophil activation by Japanese cedar pollen extract. The curves shifted downward after SCIT ($P < 0.0001$) per the two-way analysis of variance (ANOVA) for the dose–response curve. (B) The area under the dose–response curve (AUC) of the basophil activation. * $P < 0.05$, Dunn's multiple comparisons test.

immunological effects of SLIT are weaker than those of SCIT.⁹ A 2-year JCP-based SLIT trial showed that JCP-specific IgE and IgG4 titers increased at the end of the first and second JCP season after SLIT compared to at baseline.⁵

Our study had some limitations. First, we evaluated the immunologic efficacy of JCP-based SCIT, but not the clinical efficacy. The counts of pollen count differ each year, and the placebo effect of SCIT is strong, making it difficult to evaluate the clinical efficacy without comparison with the placebo treatment.¹⁰ We did not establish a placebo control group for comparison of the efficacy of SCIT. Second, the study was retrospective and contained a small sample. We evaluated the patients who underwent JCP-based SCIT at our hospital, but not those who underwent SCIT at other hospitals. There was only one patient who opted out of the SCIT after 2 years.

JCP-based SCIT reduced JCP-specific IgE and BA and elevated JCP-specific IgG4 levels over the long term. JCP-based SCIT should be administered for >17 months.

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Conflict of interest

The authors have no conflict of interest to declare.

Chisato Inuo^{a,b,c,*}, Hitoshi Ando^d, Kenichi Tanaka^a, Yoichi Nakajima^a, Ikuya Tsuge^a, Atsuo Urisu^b, Yasuto Kondo^a

^a Department of Pediatrics, School of Medicine, Fujita Health University, Toyoake, Japan

^b Department of Pediatrics, Second Teaching Hospital, Fujita Health University, Nagoya, Japan

^c Department of Pediatrics, University Hospital St. Marianna University School of Medicine, Kawasaki, Japan

^d Department of Pediatrics, Toyokawa City Hospital, Toyokawa, Japan

* Corresponding author. Department of Pediatrics, University Hospital St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, Kanagawa 216-8511, Japan.

E-mail address: inuo@mbh.nifty.com (C. Inuo).

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