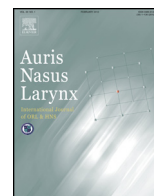




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Phase I/II study of oral immunotherapy with Cry j1–galactomannan conjugate for Japanese cedar pollinosis

Daisuke Murakami MD, PhD^{a,d,*}, Kazuhiko Kubo PhD^{a,e}, Motohiro Sawatsubashi PhD^a, Sayaka Kikkawa MD^a, Masayoshi Ejima MD^{a,f}, Akira Saito PhD^b, Akio Kato PhD^c, Shizuo Komune^a

^a Department of Otorhinolaryngology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan

^b Biobusiness Propulsion Group, Biobusiness Propulsion Division, Wako Filter Technology Co., Ltd., Ibaraki, Japan

^c Department of Biological Chemistry, Yamaguchi University, Yamaguchi, Japan

^d Department of Otorhinolaryngology, Saiseikai Fukuoka General Hospital, Fukuoka, Japan

^e Department of Otorhinolaryngology, Chidoriyoshi Hospital, Fukuoka, Japan

^f Department of Otorhinolaryngology, Kitakyushu Municipal Medical Center, Fukuoka, Japan

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ABSTRACT

Objective: Among many immunotherapeutic approaches, oral immunotherapy (OIT) is thought to be an effective route for desensitization against a variety of allergens. However, there is little evidence that OIT is effective for airway allergic diseases such as pollen allergy. Thus, in the present study, we assessed the safety, efficacy and immune response of OIT using the Cry j1–galactomannan conjugate for Japanese cedar pollen allergy.

Methods: An open trial was conducted over a period of 4 months. The OIT group comprised of 23 subjects. Treatment was initiated 1 month before the estimated pollen season and continued for 1 month. The control group (the pharmacological treatment group without OIT) comprised of 11 subjects. The symptoms and medication score, levels of allergen-specific serum antibodies, cellular components of lymphocytes and cytokine production from peripheral blood mononuclear cells (PBMCs) were evaluated throughout the pollen season.

Results: The participants receiving OIT treatment showed significant improvements in total symptom scores and symptom–medication scores during the pollen season compared with the control group. The levels of allergen-specific serum IgG4 and IL-10 production in PBMCs were significantly increased in the OIT group compared with that in the control group. Importantly, no severe adverse effects were observed in the participants receiving OIT treatment.

Conclusion: Short-term OIT using the Cry j1–galactomannan conjugate is effective, relatively safe and induces tolerant immune responses such as increased allergen-specific serum IgG4 and IL-10 production in PBMCs. These results suggest that OIT using allergen–galactomannan conjugates may provide a rapid, effective, and safe immunotherapy regimen for cedar pollen allergy.

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1. Introduction

Over the last few decades, many developed countries have experienced an increase in the prevalence of allergic rhinitis and other allergic disorders [1,2]. One of the most common allergens is tree pollen, such as Japanese cedar pollen (JCP) in Japan [3,4]. The

prevalence of allergic rhinitis caused by JCP is currently around 25% and increasing [3]. Although many participants with JCP allergy consequently suffer from heavy pollen exposure for almost 12 weeks, an effective therapy to reduce or cure the symptoms has not been established.

Subcutaneous immunotherapy (SCIT) is the only curative treatment for allergic rhinitis caused by JCP. It is effective for allergic rhinitis as supported by high-level evidence, including the results of meta-analyses [5]. However, its use in Japan has not become widespread because of various problems, including frequent medical visits for a few years, pain from injections, and the potential for severe side effects (e.g., anaphylaxis). To date,

* Corresponding author at: Department of Otorhinolaryngology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan. Tel.: +81 92 642 5668; fax: +81 92 642 5685.

E-mail address: muradai@qent.med.kyushu-u.ac.jp (D. Murakami).

most subjects are treated with antihistamine and corticosteroid agents to suppress allergic symptoms. Therefore, developing a fundamental immunotherapy that is less invasive, more convenient and safer for airway allergy is desirable. With regard to these parameters, the use of sublingual immunotherapy (SLIT) was recommended recently by the World Health Organization. This therapy is expected to induce tolerance to pollen allergens by the adsorption of allergen proteins through sublingual immune cells [6]. Similarly, oral immunotherapy (OIT) is a promising method for modulating immune responses [7–9], because many immune cells exist in the mucosa of the digestive tract. However, oral immunotherapy has a risk to cause anaphylaxis when the allergens are administered in a native form. Therefore, it is desirable to develop a new method to suppress anaphylaxis when allergens are administered in the gut.

Cry j1 is a major allergen of JCP [10], and we recently demonstrated that a Cry j1–galactomannan conjugate could mask the epitope sites of Cry j1, which completely inhibited the binding of sera IgE of the patient to these allergens [11], and traffic efficiently to dendritic cells in the gut lumen [12]. In addition, we observed that the production of antigen-specific IgE was reduced in mice intraperitoneally primed with allergen–polysaccharide conjugates [13]. These results show that galactomannan binding to Cry j1 is effective at reducing the risk of anaphylaxis, accelerating the uptake of antigen into gut dendritic cells, and reducing Th2 responses. Therefore, the Cry j1–galactomannan conjugate, which differs from naive Cry j1, may be effective and useful as a new oral immunotherapy agent. Thus, we conducted a clinical trial in adult participants with allergic rhinitis caused by JCP to evaluate the safety, efficiency and immune responses of OIT using a Cry j1–galactomannan conjugate.

2. Materials and methods

2.1. Participants

This study was conducted in two adjacent hospitals: Kyushu University Hospital and Chidoribashi General Hospital, Fukuoka, Japan. The participants for OIT were recruited from Kyushu University Hospital. Participants undergoing only pharmacological treatment without OIT were recruited from Chidoribashi General Hospital. As a result, 38 participants came to the clinics for screening (Fig. 1A). In two hospitals, the authors recruited participants using the same criteria as described below. The study population fulfilled the inclusion criteria and consisted of 34 Japanese participants (11 men and 23 women), ranging in age from 24 to 62 years, who were otherwise healthy but had moderate or severe rhinoconjunctivitis due to JCP allergy and received pharmacological treatment for at least the last three consecutive cedar pollen seasons, and lived in and around the city of Fukuoka in Japan, where a similar amount of pollen spread would be expected. The diagnosis of JCP allergy was based on participant clinical history and serum Cry j1-specific IgE levels of score 2 or greater using the CAP-RAST (SRL Inc., Tokyo, Japan). Exclusion criteria were as follows: severe asthma, chronic sinusitis, previous immunotherapy or ongoing immunotherapy with other allergens, treatment with β -blockers or participants on continuous corticosteroids, pregnancy or planned pregnancy, participation in another clinical trial, and the standard contraindications for immunotherapy [14]. Informed consent was obtained from all participants. The study was conducted according to the principles expressed in the Declaration of Helsinki and approved by the Institutional Ethics Committees of Kyushu University Hospital (number 21082) and Chidoribashi General Hospital. Moreover, cellular components of lymphocytes and cytokine production from peripheral blood mononuclear cells

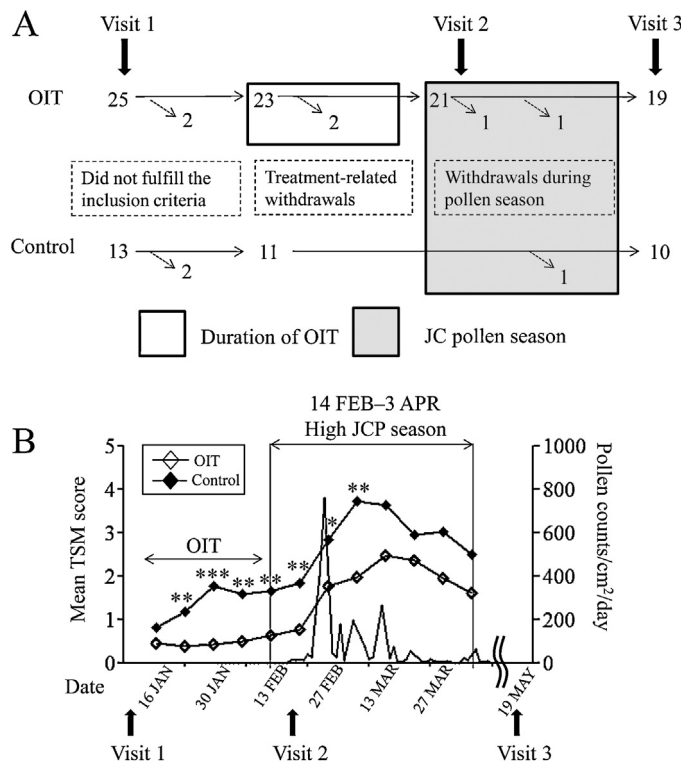


Fig. 1. Enrollment, participation demographics and daily JCP counts. (A) Number of individuals assessed for trial. Solid line: approval for the trial. Dotted line: withdrawal. Visit 1: end-to-mid December 2010, screening of potentially eligible subjects. Visit 2: mid-February 2011, the beginning of JCP season approximately 2 weeks after the end of OIT. Visit 3: early May 2011, the end of the JCP season. Duration of OIT: early January–early February 2011. JCP season: mid-February–late April 2011. (B) Mean JCP counts in Fukuoka City, Japan, 2011 and total symptom-medication (TSM) scores. Solid line: JCP counts. Open squares: TSM score in the OIT group ($n = 19$); solid squares: TSM score in the control group ($n = 10$). * $P < 0.05$, between control and OIT group.

(PBMcs) from 10 female participants of the OIT group and eight female participants from the pharmacological treatment group without OIT were analyzed.

2.2. Study design

In the OIT group, four capsules (total dose of Cry j1: 750 μ g) of Cry j1–galactomannan conjugate, divided into two capsules twice a day, were administered orally for 30 days from the beginning of January 2011 to the beginning of February 2011 before the JCP season. Participants in the pharmacological treatment group without OIT (control group) had no placebo capsules. The physicians (who belonged to the Nasal Allergy Study Group of the Department of Otorhinolaryngology, Graduate School of Medical Sciences, Kyushu University) examined the participants of both the groups in each hospital. The participants underwent pharmacological treatment for rhinoconjunctivitis due to JCP allergy throughout the JCP season according to the Japanese Guidelines for Allergic Rhinitis [15]. The mean annual amount of cedar pollen in Fukuoka was measured according to the Durham pollen samplers of three different areas: Kyushu University Hospital, Fukuoka City Medical Association Hospital and Fukuoka National Hospital. The participants carefully recorded the mean score regarding their nasal and ocular symptoms and the usage of rescue drugs (such as antihistamines) per week in their pollen paper diaries once a week during the JCP season. Data were collected and analyzed after the JCP season.

2.3. Cry j1–galactomannan conjugates

Standardized JCP antigen–galactomannan conjugates (Wako Filter Technology Co. Ltd., Ibaraki, Japan) were manufactured to ensure they were Good Manufacturing Practice grade and used [11]. A capsule of JCP antigen–galactomannan conjugates contained 187.5 µg of Cry j1, which is the major allergen of JCP.

2.4. Adverse events

Adverse events were monitored throughout the duration of OIT and were graded according to the Common Terminology Criteria for Adverse Event (CTCAE) v.4.0. Briefly, adverse events were graded as mild, grade 1; moderate, grade 2; severe, grade 3; life threatening, grade 4; and death, grade 5 according to the category for allergy/immunology in the CTCAE v.4.0 scoring system. Discontinuation criteria of OIT were grade ≥ 3 adverse events and even grade ≤ 2 adverse events if the participants wished to withdraw from the study.

2.5. Symptoms and medication use

During the cedar pollen season, the participants recorded their weekly symptoms of rhinoconjunctivitis, which were evaluated on a scale from 0 to 4 in accordance with the Japanese Allergic Rhinitis QOL Standard Questionnaire No. 1 (JRQLQ No1) [16]. The total symptom score was calculated as the sum of each component score as follows: none, 0; mild, 1; moderate, 2; severe, 3; and very severe, 4. Nasal and ocular symptoms covered by the questionnaire included runny nose, sneezing, nasal congestion, itchy nose, itchy eyes and watery eyes. The total medication score every week during the cedar pollen season was also calculated and recorded according to drug characteristics and duration of usage, according to the Practical Guideline for the Management of Allergic Rhinitis, Japan [15,16], as follows: antihistamines, leukotriene antagonists and topical ocular antihistamines were listed as 1, and topical nasal steroid sprays as 2. First, we treated participants using antihistamines or leukotriene antagonists as rescue medications for symptoms. If the symptoms did not improve and participants wished for more drugs to be prescribed, we prescribed nasal steroid sprays or/and ocular antihistamine drops according to the symptoms in addition to rescue medication. The total symptom medication score reflected the average total symptom score (which comprised of six types of scores, i.e., 4 points for the maximum value plus the total medication score).

2.6. Analysis of allergen-specific serum Abs

Antigen specific IgE, IgG4 and IgA in serum were confirmed with fluoro enzyme immunoassay (Phadia ImmunoCAP System, Phadia, Uppsala, Sweden) according to the manufacturer's instructions.

2.7. Blood samples, white blood cell (WBC) fractions and preparation of PBMCs

Peripheral blood was obtained from each participant before treatment (the last day of December 2010), during (the middle of February 2011) and after the JCP season (the beginning of May 2011). Total WBC and WBC fractions were measured using an automated hematology analyzer, XE-2100 (Sysmex, Kobe, Japan). Unless otherwise specified, PBMCs were isolated using BD Vacutainer® CPT™ Cell Preparation Tube with sodium heparin (BD Biosciences), according to the manufacturer's instructions.

2.8. Cell culture

PBMCs were cultured in RPMI 1640 medium (Wako Pure Chemical Industries, Ltd.) containing 10% heat-inactivated fetal bovine serum (FBS) (Japan BioSerum). All cell cultures were grown at 37 °C with 5% CO₂ atmosphere. Lymphocytes in PBMCs were adjusted to 1×10^6 cells/ml and plated in a 6-well plate containing 2 ml medium. Subsequently, cultured cells were transferred to new 6-well plates and 1 ml medium including recombinant IL-2 (600 IU) was added after 3 and 5 days. After 7 days, all culture media were centrifuged and the supernatant was isolated and frozen at –80 °C.

2.9. Flow cytometric analysis

PBMCs were isolated with BD Vacutainer® CPT™ Cell Preparation Tube with sodium heparin. Analysis of Forkhead box P3 (FoxP3)⁺ cells was performed by flow cytometry analysis (BD FACSCanto™ II) using FoxP3 Staining Kit (Becton Dickinson and Company) according to the manufacturer's recommendations [17].

2.10. Cytokine assay

All cytokines were measured from the culture media by enzyme-linked immunosorbent assay (ELISA). IL-4, IL-10, IL-13, and IFN-γ were determined using Human ELISA kits (Invitrogen) and IL-5 and TGF-β were determined using Quantikine Human ELISA kits (R&D Systems) according to the individual manufacturer's instructions.

2.11. Statistics

Statistical analysis was performed using GraphPad Prism 5. Comparisons between the control group and the OIT group at different time points were performed using the non-parametric Mann–Whitney *U*-test. Comparisons of paired samples of the participants before and at different time points during the pollen season were performed using the Wilcoxon signed-rank test. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Demographics

Fig. 1A describes the enrollment and participation demographics of the participants. Two of 23 participants during OIT were withdrawn from the study due to adverse effects and two of the 21 participants with OIT and one of the 11 participants without OIT were withdrawn from the study for personal reasons during pollen season. In the final analysis, 19 participants with OIT and 10 participants without OIT (control group) exhibited full compliance with the study protocol. The characteristics of each group are presented in Table 1. No differences were observed for the baseline characteristics except for sex.

3.2. Adverse effects

Seven subjects exhibited mild adverse effects during OIT as shown in Table 2. Five subjects exhibited adverse effects at grade 1 as described by the Common Terminology Criteria for Adverse Events. The adverse effects were resolved after 1 or 2 weeks following the beginning of OIT. Two participants complained of grade 2 urticaria of the face and breast. One was treated with antihistamines and steroids for 6 days. The other recovered without treatment 2 days later. Two of the 23 participants during OIT were withdrawn from the study because of adverse effects as

Table 1
Participant demographics.

Group	OIT	Pharmacological treatment	P value
Number of participants	19	10	
Sex (M/F)	9/10	0/10	0.009**
Age	39.7 ± 10.2	37.0 ± 10.6	0.475
Range	25–62	24–55	
Total IgE (IU/ml)	110 ± 147	138 ± 248	0.981
Range	20–595	16–835	
Cry j1-specific IgE (UA/ml)	12.1 ± 13.0	9.3 ± 5.8	0.945
Range	0.9–55.9	1.4–20.1	
Other allergies (%)			
House dust mite	7 (39.7%)	5 (50%)	0.384
Asthma	0 (0%)	1 (10%)	0.344
Atopic dermatitis	2 (10.5%)	0 (0%)	0.421
Food allergy	1 (5.2%)	0 (0%)	0.655
Total number of other allergies	10 (52.6%)	6 (60%)	0.507
Symptom score of pre-pollen season			
Total symptom score	2.0 ± 2.5	2.0 ± 2.1	0.756
Total nasal symptom score	1.5 ± 2.0	1.5 ± 1.5	0.749
Total ocular symptom score	0.4 ± 0.7	0.5 ± 0.9	0.955

Results of the PP (per protocol) population in percentages, ranges or means ± SDs. Total symptom score includes total nasal symptom score (sneezing, itchy nose, nasal congestion) and total ocular symptom score (watery eyes and itchy eyes). Total score = total of six individual symptom scores, each assessed on a 5-point scale (from 0 = absent to 4 = severe). Statistical analysis was performed using the Mann–Whitney U-test, Fisher's exact probability test.

** $P < 0.01$, between control and oral immunotherapy (OIT) group. M: male; F: female.

follows: one had grade 2 urticaria of the face and breast and the other had grade 1 laryngopharyngeal dysesthesia. It was possible for these participants with adverse effects to continue OIT, but they chose to interrupt the present study.

3.3. Pollen counts

In 2011, the Japanese cedar pollen season started in the middle of February and extended to the beginning of April in Fukuoka City, Japan (Fig. 1B). The mean annual amount of cedar pollen was 2621 cm⁻² in Fukuoka.

3.4. Clinical efficacy

The calculation of clinical efficacy was based on weekly symptom and medication scores. The total symptom–medication scores and pollen counts in the community during the JCP season

are shown in Fig. 1B. The temporal profiles of total symptom–medication scores were lower in the OIT group compared with that in the control group at three timepoints from 14 February to 20 February, from 28 February to 6 March, and from 21 March to 27 March, 2011, during the high JCP season ($P = 0.005$, 0.025 and 0.007, respectively). Thus, the mean score of the following symptoms, runny nose, nasal congestion, itchy nose, and itchy eyes; total nasal symptom score, total ocular symptom score; total symptom score and total symptom–medication scores were also lower in the OIT group compared with that in the control group during the high JCP season as shown in Fig. 2A, B, C, D ($P = 0.038$, 0.036, 0.021, 0.042, 0.027, 0.043 and 0.041, respectively).

3.5. Allergen-specific serum Abs

To investigate the protective mechanisms shown by OIT, we evaluated immune responses in the participants and controls. There was no significant difference in the levels of total IgE, Cry j1-specific IgE, IgG4, and IgA in serum samples collected before the JCP season and OIT (Fig. 3A). During the JCP season (visit 2), the levels of Cry j1-specific IgE and IgG4 were increased in the OIT group compared with that in the control group ($P = 0.033$ and 0.038, respectively). However, the levels of specific IgE and IgG4 in the OIT group returned to control levels at visit 3. The levels of total IgE and Cry j1-specific IgA did not change throughout the JCP season in both the groups. Fig. 3B shows the change in serum levels of Cry j1-specific IgE and IgG4 in individuals from the OIT and control groups during the JCP season. The levels of Cry j1-specific IgE and IgG4 increased in individuals from the OIT group at visit 2 (the beginning of JCP season and 2 weeks after the end of OIT), compared with those at visit 1 ($P < 0.001$ and 0.001, respectively). The levels remained the same at visit 3 after the JCP season. In contrast, the levels of Cry j1-specific IgE at visit 2 decreased compared with those at visit 1 ($P = 0.004$) and increased at visit 3 in individuals from the control group ($P = 0.003$). The individual levels of Cry j1-specific IgG4 were increased at visit 3 compared with visit 2 in the control group ($P = 0.024$). These results suggested that OIT-induced levels of Cry j1-specific IgE and IgG4 were not affected by JCP exposure.

3.6. The percent rate and number of cellular components from PBMCs

The rates of CD4⁺ T cells, CD4⁺CD25⁺FoxP3⁺ T cells and CD4⁺CD25⁺FoxP3⁺ T cells in PBMCs were similar between the two groups throughout the JCP season (Fig. 4A). In addition, the

Table 2
Adverse events (AEs) during oral immunotherapy (OIT) using the Cry j1–galactomannan conjugate.

No.	M/F	AEs	Severity (grade)	Treatment days	Duration (days)
OIT group					
1	M	Rhinitis	1	2	10
		Sneezing	1	2	10
		Pruritus ^b	1	2	10
2	M	Abdominal pain	1	2	5
3	M	Pruritus ^b	1	2	10
4 ^a	F	Pruritus ^b	2	13	2
		Urticaria	2	13	2
		Eyelid edema	2	13	2
		Nausea	1	13	0.1
		Vomiting	1	13	0.1
5	F	Laryngopharyngeal dysesthesia	1	7	7
		Malaise	1	7	7
6 ^a	F	Laryngopharyngeal dysesthesia	1	1	2
7	F	Urticaria	2	7	2
		Eyelid edema	2	7	2

AEs were graded according to Common Terminology Criteria for Adverse Event (CTCAE) v.4.0. Total AEs (%) = 7/23 (30.4%).

^a Treatment-related withdrawal = 2/23 (8.6%).

^b Pruritus of any part of the body. M: male; F: female.

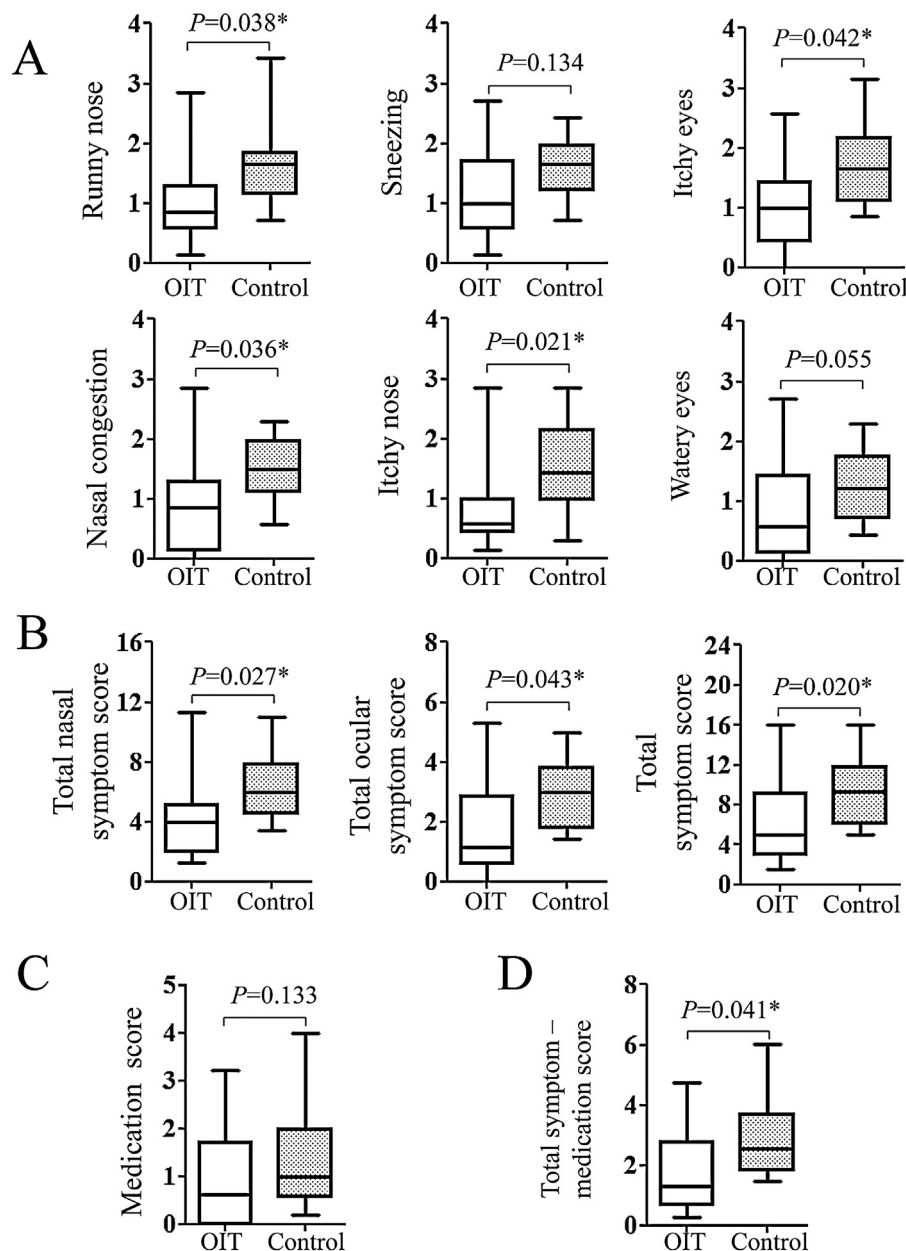


Fig. 2. Symptoms and medication use. (A) Symptom scores, (B) total nasal, ocular symptom scores and total symptom scores, (C) medication score, and (D) TSM scores. Open squares: OIT group ($n = 19$); solid square: control group ($n = 10$). Each score shows mean weekly score during peak pollen season (14 February–3 April, 2011). Data are depicted as box-and-whisker plots. * $P < 0.05$, between control and OIT group.

numbers of $CD4^+$ T cells, $CD4^+CD25^+FoxP3^-$ T cells and $CD4^+CD25^+FoxP3^+$ T cells from peripheral blood were also similar between the two groups through the JCP season (Fig. 4B). However, the rate but not the number of individual $CD4^+$ T cells at visit 3 was decreased compared with that at visit 2 in each group as shown in Fig. 4A (OIT group: $P = 0.003$; control group: $P = 0.007$). The number and the rate of individual $CD4^+CD25^+FoxP3^-$ T cells increased at visit 3 compared with those at visit 2 in each group (OIT group: $P = 0.007$ and 0.003 , respectively; control group: $P = 0.007$ and 0.007 , respectively). However, neither lymphocytes nor $CD4^+CD25^+FoxP3^+$ T cell numbers or rates changed at visit 3 compared with those at visit 2 in each group.

3.7. Cytokine production from PBMCs

Fig. 5 shows the change in serum cytokine levels between visits of both the groups. The level of IL-10 production was

increased at visit 2 in the OIT group, compared with that in the control group ($P = 0.031$). Importantly, this difference was also observed at visit 3 ($P = 0.038$). Individual IL-10 levels at visit 2 were retained at visit 3 in subjects from each group. The level of IL-4 production from PBMCs in the OIT group decreased at visit 2, compared with that in the control group ($P = 0.025$). In contrast, the levels of IL-5 production in the OIT group increased at visit 2, compared with that in the control group ($P = 0.001$). The levels of IL-4 and IL-5 production were similar between the two groups at visit 3. The levels of IL-13, IFN- γ , and TGF- β production were similar between the two groups throughout the JCP season. However, the levels of individuals' IL-4, IL-13 and TGF- β production increased at visit 3 compared with those at visit 2 in each group (OIT group: $P = 0.009$, 0.007 and 0.039 , respectively; control group: $P = 0.031$, 0.015 and 0.015 , respectively), whereas individuals' IL-5 and IFN- γ production at visit 3 were similar compared with those at visit 2 in the OIT group,

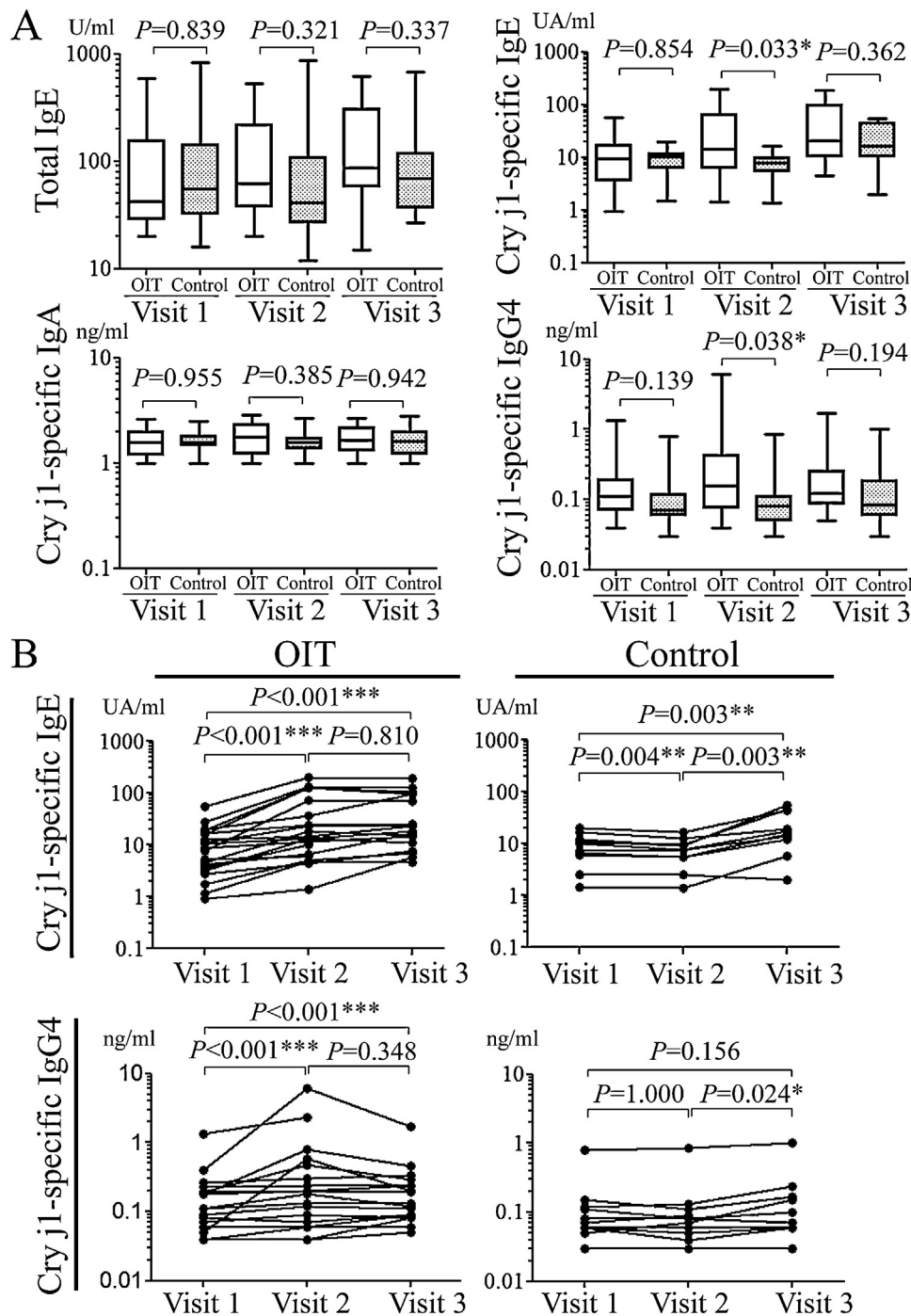


Fig. 3. Measurement of serum total IgE and Cry j1-specific IgE, IgG4 and IgA. (A) Serum immunoglobulins. Open squares: OIT group; solid squares: control group. Data are depicted as box-and-whisker plots. NS: not significant; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, between control and OIT group. V1: OIT group ($n = 23$); control group ($n = 11$), V2: OIT group ($n = 20$); control group ($n = 11$), V3: OIT group ($n = 19$); control group ($n = 10$). (B) Transition of individual serum Cry j1-specific IgE and IgG4. Open squares: OIT group; solid squares: control group. NS: not significant; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. V1: OIT group ($n = 20$); control group ($n = 11$), V2: OIT group ($n = 20$); control group ($n = 11$), V3: OIT group ($n = 19$); control group ($n = 10$).

but increased in the control group ($P = 0.031$ and 0.031 , respectively).

4. Discussion

OIT is a promising treatment option for modulating immune responses [7–9]. However, there is a little evidence that OIT is effective for participants with pollen allergy. In the present study, we show for the first time that short-term OIT using the Cry j1–galactomannan conjugate is effective, relatively safe and can

induce tolerant immune responses in participants sensitized to JCP allergen. Compared with that in the control group, participants in the OIT group showed significant improvements for total symptom scores and symptom–medication scores during the peak pollen season. Moreover, the levels of serum Cry j1-specific IgG4 and IL-10 production in PBMCs were significantly increased in the OIT group compared with the control group. In addition, IL-4 production from PBMCs in the OIT group was significantly decreased at the beginning of the pollen season compared with the control group. PBMC IL-5 production and serum Cry j1-specific

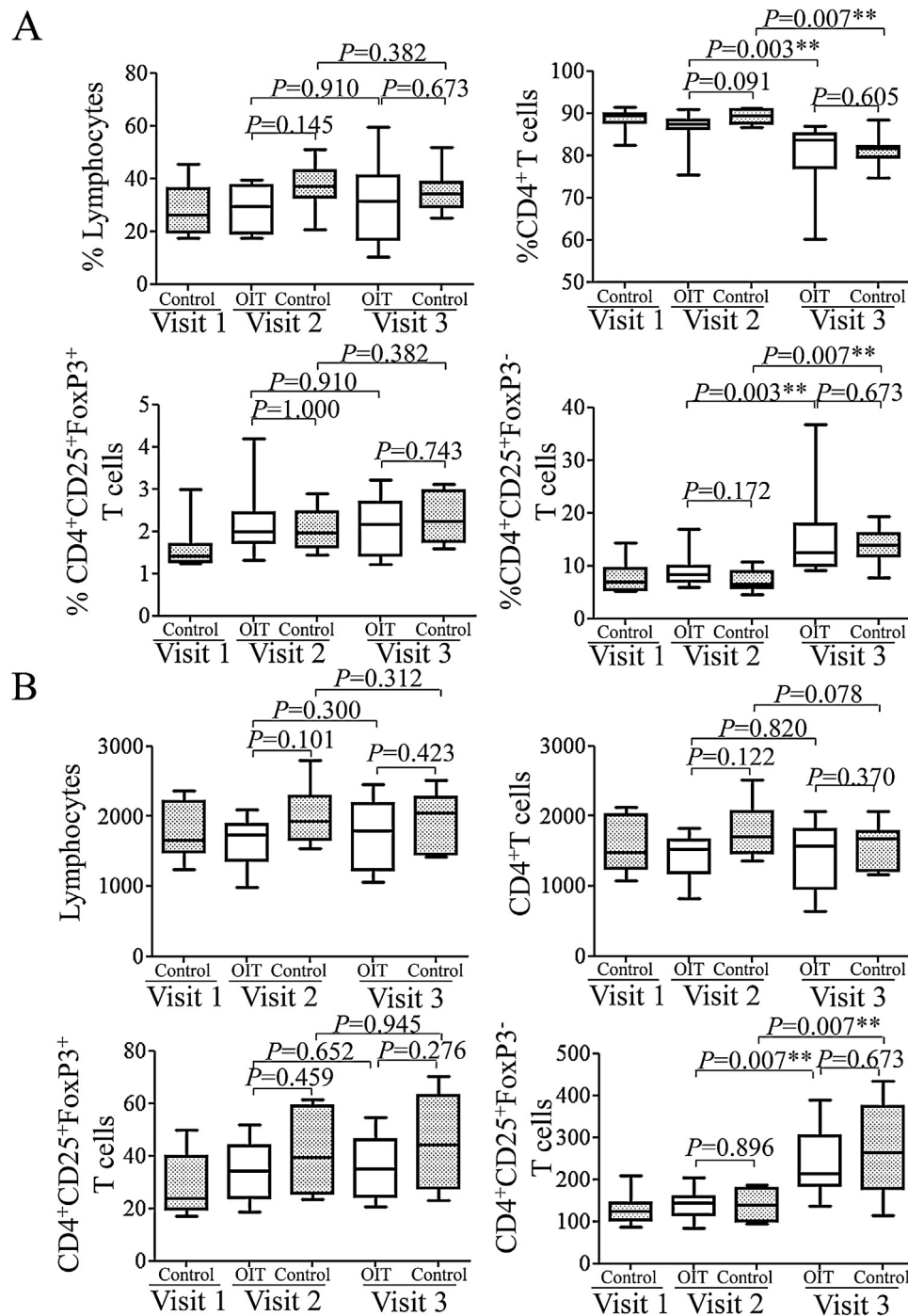


Fig. 4. Percent rate and number of cellular components from PBMCs. (A) Cell percentages and (B) numbers. Open squares: OIT group; solid squares: control group. (%) Lymphocytes represent the percent rate of cells in PBMCs. % other cells show the percent rate in the gated lymphocytes based on the forward scatter/side scatter plot. Data were depicted as box-and-whisker plots. ** $P < 0.01$. V1: control group ($n = 8$), V2: OIT group ($n = 10$); control group ($n = 8$), V3: OIT group ($n = 9$); control group ($n = 8$).

IgE levels in the OIT group at the end of the pollen season were similar to those at the beginning of the pollen season. In contrast, PBMC IL-5 production and serum Cry j1-specific IgE levels in the control group at the end of the pollen season were significantly increased compared with those at the beginning of the pollen season. Importantly, no severe adverse effects were observed in participants receiving OIT.

The results in this study regarding the transition of serum Cry j1-specific IgG4 and IgE levels during the pollen season are very similar to that of previous studies of OIT for birch pollen allergy [18] and peanut allergy [19]. The levels of serum IgG4 and IgE in the

OIT group were markedly increased for a month after OIT and the increased levels lasted throughout the JCP season. Serum Cry j1-specific IgE levels in the control group, but not OIT group, were remarkably increased at the end of the JCP season. This suggests that OIT using Cry j1-galactomannan conjugates prevented increased serum Cry j1-specific IgE during the JCP season and induced immune tolerance for allergen exposure. Moreover, the increased level of serum IgG4 suggested that OIT with the Cry j1-galactomannan conjugate might also be an effective treatment along with SLIT and SCIT [20]. In addition, we observed that IL-4 production from PBMCs in the OIT group was significantly

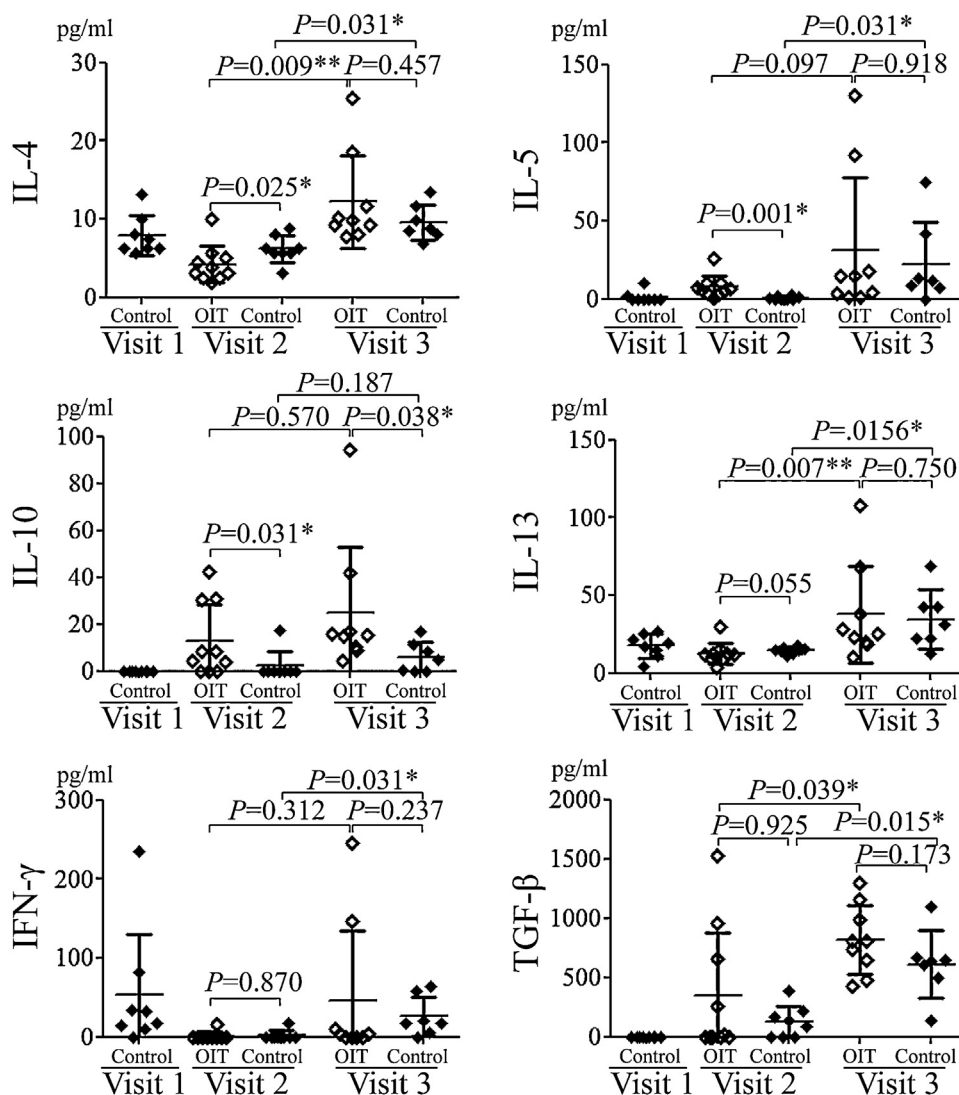


Fig. 5. Cytokine production from PBMCs. Cytokine levels were determined by ELISA. Open squares: OIT group; solid squares: control group. Data are mean $\pm$ SD. $^{*}P < 0.05$; $^{**}P < 0.01$. V1: control group ($n = 8$), V2: OIT group ($n = 10$); control group ($n = 8$), V3: OIT group ($n = 9$); control group ($n = 7$).

decreased and conversely, IL-10 production from PBMCs was significantly increased at the beginning of the pollen season compared with that in the control group. These results show that OIT using the Cry j1–galactomannan conjugate immediately may induce tolerant immune responses at the beginning of the JCP season, which lasted until 2 weeks after the end of OIT, at which time the participants are relatively free of JCP allergen.

IL-10 is a key cytokine for immunological tolerance and down-regulates T cell functions and B-cell switching to IgE and potentiates B-cell switching to IgG4 [21,22]. Thus, increased IL-10 production at the beginning of the JCP season may be related to decreased IL-4 and increased IgG4 production. After 3 months of OIT IL-10 production from PBMCs, but not other cytokines, was significantly increased at the end of the JCP season compared with that in the controls. Furthermore, the number of mononuclear cells cultured from peripheral blood of the OIT group was significantly decreased compared with controls at the end of the pollen season (data not shown). These results including the inhibitory effect on IgE production of OIT as described above may suggest regulatory effects of IL-10.

Past studies have shown a significant decrease in FoxP3 $^{+}$ CD25 $^{+}$ CD4 $^{+}$ T cells, naturally occurring regulatory T cells, in PBMCs from participants with atopic dermatitis or bronchial

asthma [23]. A recent study showed the number of IL-10-producing regulatory T (Tr1) cells but not FoxP3 $^{+}$ CD25 $^{+}$ CD4 $^{+}$ T cells was significantly increased in the SLIT group compared with the untreated group and healthy subjects [24]. Our data also show that the percentage and number of naturally occurring regulatory T cells (FoxP3 $^{+}$ CD25 $^{+}$ CD4 $^{+}$ T cells) in the OIT group remained unchanged compared with that in the controls throughout the JCP season. In this study, we did not evaluate the source of IL-10 production from PBMCs. Further studies to identify whether naturally occurring regulatory T cells, Tr1 cells or other cell types produce IL-10 are required.

The safety of OIT for pollen allergy had been confirmed in many trials, and no severe systemic side effects were reported, although many discomforting effects (not life-threatening) were observed [25]. These effects were mainly gastrointestinal and tended to increase with increased dosage of allergen [26]. Because of these adverse events OIT has been not used except to treat food allergy in recent years [16,27]. In this study, no severe adverse events were reported during treatment, but 7 of the 23 participants with OIT had grade 1 or grade 2 adverse events, which were present only for 2 weeks from the start of OIT. Gastrointestinal disorders such as stomach pain and diarrhea have often been reported, but only 2 of 7 participants in this study with adverse events developed

gastrointestinal disorders, which may have been caused by IgE epitopes of the Cry j1 that are masked by galactomannans [11]. Galactomannans are polysaccharides that form highly soluble dietary fiber, and are extracted from natural guar and protect the intestinal tract [28,29]. Therefore, the administration of the Cry j1–galactomannan conjugate may impair antigen–antibody reactions in the intestinal tract and prevent gastrointestinal disorders. In addition, the short term schedule of OIT using the Cry j1–galactomannan conjugate may reduce the development of side effects as the treatment schedule was 30 days compared with that of SLIT or SCID, which takes years to finish [30]. Thus, OIT with the Cry j1–galactomannan conjugate was accepted by all participants (except for the two who withdrew with adverse events), and therefore may be a potent novel treatment for JCP allergy instead of SLIT or SCID.

There were several limitations in our study: the study cohort was small; the baseline characteristics of the sex of the participants; the study did not use a placebo arm; and the trial was not a randomized control study. With regard to the limitation of the baseline characteristics of the sex of the participants, clinical efficacy and allergen-specific serum Abs were similar even in comparison with women only (data not shown). Further research is required to determine whether OIT is a universally effective method for the radical treatment of airway allergy.

In summary, here we report that short-term OIT using the Cry j1–galactomannan conjugate is effective, relatively safe without severe adverse effects, and can induce tolerant immune responses. Our findings suggest that OIT using allergen–galactomannan conjugates may provide a rapid, effective, and safe immunotherapy regimen for cedar pollen allergy.

Conflict of interest

None declared.

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