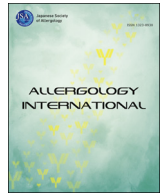




Contents lists available at ScienceDirect

Allergology International

journal homepage: <http://www.elsevier.com/locate/alit>

Original article

Safety and efficacy of a new regimen of short-term oral immunotherapy with Cry j 1-galactomannan conjugate for Japanese cedar pollinosis: A prospective, randomized, open-label study



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

^a Department of Otorhinolaryngology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, 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, Kitakyushu Municipal Medical Center, Fukuoka, Japan

^f Department of Otorhinolaryngology, Saitama Medical University Hospital, Saitama, Japan

ARTICLE INFO

Article history:

Received 31 July 2014

Received in revised form

4 October 2014

Accepted 30 October 2014

Available online 12 January 2015

Keywords:

Oral immunotherapy

Japanese cedar pollinosis

Cry j1-galactomannan conjugate

Clinical efficacy

Adverse effects

Abbreviations:

OIT, oral immunotherapy; JCP, Japanese

cedar pollinosis; SCIT, subcutaneous

immunotherapy; SLIT, sublingual

immunotherapy; CTCAE, the Common

Terminology Criteria for Adverse Event;

TS, total symptom; TSM, total symptom

medication; ITT, intention-to-treat;

AEs, adverse events

ABSTRACT

Background: Short-term oral immunotherapy (OIT) using the Cry j1-galactomannan conjugate for Japanese cedar pollinosis may be effective and relatively safe. However, a treatment regimen has not been established. In the present study, we examined a new OIT regimen with a build-up phase and extended the maintenance phase of OIT to the peak period of the pollen season to enhance the therapeutic effect and safety of OIT.

Methods: A prospective, randomized, open-label trial was conducted over a period of 4 months. Participants were randomly divided into two groups. The OIT group comprised 23 subjects. The build-up phase was initiated 1 month before the expected pollen season. The maintenance phase was continued for 51 days during the peak pollen season. The control group comprised 24 subjects. The symptoms and medication score, levels of allergen-specific serum antibodies throughout the pollen season, and adverse effects with OIT were evaluated.

Results: Participants receiving OIT showed significant improvements in total symptom scores, medication score, and total symptom-medication scores throughout the pollen season compared with the control group. The levels of allergen-specific serum IgG4 were significantly increased in the OIT group but not in the control group throughout the cedar pollen season. Importantly, no severe adverse effects were observed with OIT.

Conclusions: The new regimen of short-term OIT using the Cry j1-galactomannan conjugate for Japanese cedar pollinosis is effective, relatively safe and induces immune tolerance. Thus, OIT using allergen–galactomannan conjugates may provide a rapid, effective, and thus convenient immunotherapy for pollinosis instead of SLIT or SCIT.

Copyright © 2014, Japanese Society of Allergy. Production and hosting by Elsevier B.V. All rights reserved.

Introduction

In the past few decades, the prevalence of allergic disease has been increasing in many developed country.^{1,2} In Japan, the prevalence of allergic rhinitis caused by Japanese cedar pollinosis (JCP)

has been increasing, and is currently around 25%.³ It is a significant health problem.⁴ To date, most subjects with JCP are treated with antihistamines and corticosteroid nasal sprays to suppress allergic symptoms. However, the effect of pharmacological treatment is temporary. Therefore, long-term symptomatic control using immunotherapy is desirable.

Subcutaneous immunotherapy (SCIT) is a curative treatment for JCP. It is effective for allergic rhinitis and is supported by high-level evidence that includes 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

* Corresponding author. Department of Otorhinolaryngology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1 Higashi-ku, Fukuoka 812-8582, Japan.

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

Peer review under responsibility of Japanese Society of Allergy.

injections, and the potential of severe side effects including anaphylaxis. Just recently, sublingual immunotherapy (SLIT) for JCP was approved by the Japanese Ministry of Health, Labour and Welfare as a safe form of immunotherapy compared with SCIT.^{5,6} SLIT is expected to be a widely accepted treatment for JCP. However, SLIT also has some problems. A few years of treatment is required before the therapeutic effect is apparent,⁷ so it takes longer time before its therapeutic efficiency can be judged, and the effect is thought to be somewhat weaker than SCIT.⁵ Therefore, the development of an immunotherapy that is safe, effective and more convenient with a shorter regimen is needed.

Oral immunotherapy (OIT) is a promising method for modulating the immune response^{8–10} because many immune cells exist in the mucosa of the digestive tract. In 1980s, the treatment of pollinosis using OIT had been attempted.^{11–13} Although no severe systemic side effects were reported, oral immunotherapy induced many gastrointestinal adverse effects because the allergens were administered in their native form.¹³ In addition, the efficacy of OIT for pollinosis was controversial in the randomized control trials.¹⁴ Therefore, OIT for airway allergy including pollinosis is not currently recommended by World Health Organization.¹⁵ However, in recent years, the efficacy of OIT for subjects with food allergy including those allergic to cow's milk, peanuts, eggs, etc. has been confirmed.^{16–18} Thus, OIT for desensitization is in the spotlight again as an effective treatment.

OIT has some advantages compared with SCIT and SLIT. Subjects can ingest larger amounts of antigen each time and OIT is expected to induce immune tolerance in a short time because a large number of immune cells are present in the intestinal tract.¹⁹ However, it would be helpful if gastrointestinal adverse effects and structural failure of the antigen due to digestive enzymes in the stomach are avoided. Therefore, the development of a new agent and regimen to suppress adverse effects caused by the administered allergen in the gut is desirable.

Cry j1 is a major allergen of JCP.²⁰ In a previous study, we demonstrated that a Cry j1-galactomannan conjugate could mask the epitope sites of Cry j1, which completely inhibited the binding of patient serum IgE to these allergens,²¹ and was trafficked efficiently to dendritic cells in the gut lumen.²² These results suggest that galactomannan binding to Cry j1 is effective at reducing the risk of adverse effects and accelerate the uptake of antigen into gut dendritic cells compared with Cry j1 alone. We have recently reported that short-term OIT using the Cry j1-galactomannan conjugate for one month before the peak pollen season is effective and relatively safe for JCP.²³ However, the optimum regimen of OIT using the Cry j1-galactomannan conjugate has not been established and has been debated.

To enhance the therapeutic efficiency and safety of Cry j1-galactomannan conjugate for JCP, we studied efficacy (primary outcome), safety and immune responses (secondary outcomes) of the conjugate in a regimen that includes a build-up phase and extension of the maintenance phase to peak pollen season.

Methods

Participants

This study was conducted in Kyushu University Hospital, Fukuoka, Japan. Participants were recruited from Kyushu University Hospital. Of 52 individuals who expressed interest for this study, 48 came to the clinic for screening (Fig. 1). The authors recruited participants using the criteria described below. The study group fulfilled the inclusion criteria and consisted of 48 Japanese participants (27 men and 21 women), with an age range from 22 to 60 years, who were otherwise healthy but had moderate or severe

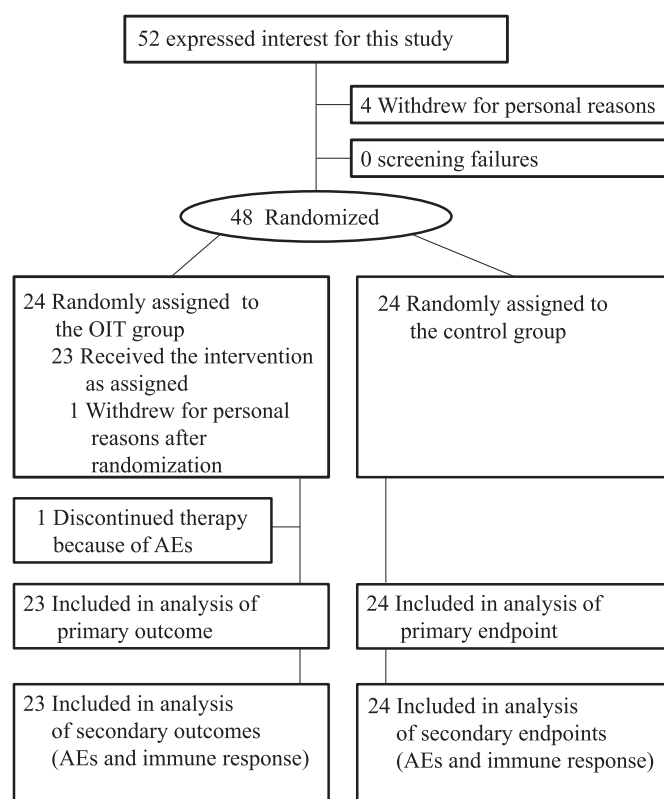


Fig. 1. Flow chart. Number of individuals assessed for the trial. Screening failures: Participants who did not fulfill the inclusion criteria or met the exclusion criteria. AE, Adverse events.

rhinoconjunctivitis due to JCP allergy. They had received pharmacological treatment for the last three consecutive cedar pollen seasons, and lived in or around the city of Fukuoka in Japan, where a similar amount of pollen spread was expected. The diagnosis of JCP allergy was based on clinical history and serum Cry j1-specific IgE levels associated with a score of 2 or greater using CAP-RAST (SRL Inc., Tokyo, Japan). The exclusion criteria were as follows: severe asthma, chronic sinusitis, previous immunotherapy or ongoing immunotherapy with other allergens, treatment with β -blockers or those on continuous corticosteroids, pregnancy or planned pregnancy, participation in another clinical trial, and other standard contraindications for immunotherapy.²⁴ Informed consent was obtained from all participants. The study was conducted according to the principles in the Declaration of Helsinki, and was approved by the Institutional Ethics Committee of Kyushu University Hospital (number 21082) and registered in UMIN-CTR (UMIN000013408).

Study design

We performed a prospective, randomized, open-label study. Participants were randomly divided into two groups: the OIT group and the control group without OIT (but who could receive other pharmacological treatment) using the envelope method. The OIT group consisted of 23 Japanese participants (14 men and 9 women) with an age range from 25 to 60 years. One woman in the OIT group withdrew from the study for personal reasons before commencement of OIT. The control group consisted of 24 Japanese participants (13 men and 11 women) with an age range from 22 to 58 years. The primary group for the analysis was the intention-to-treat (ITT) group, defined as all randomly assigned participants who received at least one dose of the study medication, who recorded

their weekly symptoms of rhinoconjunctivitis and usage medication in an e-mail sent to the data center at least once.

In the OIT group in the build-up phase, the dose was gradually increased to the maintenance dose over 18 days from the middle of January 2012, one month before the JCP season. First, one capsule (dose of Cry j1: 187.5 µg) of Cry j1-galactomannan conjugate was administered orally after breakfast for 6 days. Second, a total of two capsules daily (dose of Cry j1: 375 µg) of Cry j1-galactomannan conjugate was administered orally i.e., one capsule after breakfast and dinner, for 6 days. Third, three capsules daily (dose of Cry j1: 562.5 µg) of Cry j1-galactomannan conjugate were administered orally after breakfast (two capsules) and dinner (one capsule) for 6 days. Afterward, the maintenance phase of OIT was started. Four capsules (total dose of Cry j1: 750 µg) daily of Cry j1-galactomannan conjugate, divided into two capsules twice a day, were administered orally for 51 days from the beginning of February 2012 to the end of March 2012 during the JCP season. Participants in the control group did not undergo OIT and did not receive 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 groups in Kyushu University Hospital and checked the blood samples before the JCP season and OIT (visit 1), at the beginning of the JCP season (visit 2), and after the JCP season (visit 3) in both groups as shown in Fig. 2. The participants received pharmacological treatment for rhinoconjunctivitis due to JCP allergy throughout the JCP season according to the Japanese Guidelines for Allergic Rhinitis.²⁵ 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 electronic file diaries and relayed them by e-mail to the data center during the JCP season. Data were collected and analyzed after the JCP season.

Cry j1-galactomannan conjugates

Standardized JCP antigen-galactomannan conjugates were manufactured by Wako Filter Technology Co., Ltd. (Ibaraki, Japan) and were of Good Manufacturing Practice grade.²¹ A capsule of JCP

antigen–galactomannan conjugate contained 187.5 µg of Cry j1, which is the major allergen of JCP.

Pollen counts

The mean annual amount of cedar pollen in Fukuoka was measured using Durham pollen samplers in two different areas: Fukuoka City Medical Association Hospital and Fukuoka National Hospital.

Adverse events

Adverse events (AEs) were monitored throughout OIT and were graded according to the Common Terminology Criteria for Adverse Event (CTCAE) v4.0. Briefly, adverse events were graded as mild (grade 1), moderate (grade 2), severe (grade 3), life-threatening (grade 4), death (grade 5) according to the allergy/immunology category in the CTCAE v4.0 scoring system. Discontinuation criteria for OIT were grade ≥3 adverse events and even grade ≤2 adverse events if the participant wished to withdraw from the study. The occurrence of AEs with OIT was assessed as a secondary outcome.

Symptoms and medication use

During the cedar pollen season, 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 No 1).²⁶ The total symptom (TS) 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 the drug type and duration of usage, based on the Practical Guideline for the Management of Allergic Rhinitis in Japan^{25,26} as follows. Antihistamines, leukotriene antagonists and topical ocular antihistamines were listed as 1, topical nasal steroid sprays and ocular steroid drops as 2, and oral corticosteroids as 3.

First, we treated participants using antihistamines or leukotriene antagonists as rescue medications for the relief of symptoms. If symptoms were not improved and participants desired more drugs, we prescribed nasal steroid sprays and/or ocular antihistamine drops according to symptoms in addition to the initial rescue medication. Moreover, if symptoms were not improved and participants desired more drugs, we prescribed topical ocular steroid drops and/or oral corticosteroids. The total symptom medication (TSM) score reflected the average total symptom score (which comprised six types of scores, with 4 points for the maximum value plus the total medication score). In the present study, the primary outcome was the efficacy of OIT using the Cry j1-galactomannan conjugate for JCP that was assessed by examining the TSM score.

Analysis of allergen-specific serum antibodies

The levels of antigen-specific serum IgE and IgG4 were examined with Fluoro Enzyme Immunoassay (ImmunoCAP System, Phadia, Uppsala, Sweden) according to the manufacturer's instructions. The level of allergen-specific serum antibodies was assessed as a secondary outcome.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5. Comparisons between the control group and the OIT group at

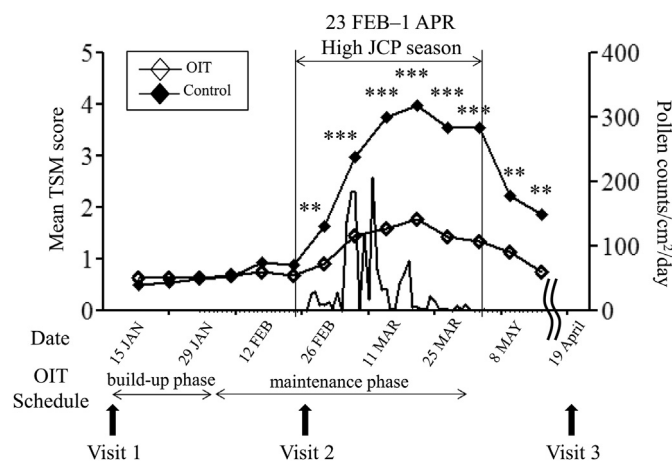


Fig. 2. Study design, daily Japanese cedar pollen counts and total symptom medication (TSM) scores. Visit 1: early January 2012, screening of potentially eligible subjects. Visit 2: mid-February 2012, the beginning of JCP season approximately 2 weeks after the beginning of the maintenance phase of OIT. Visit 3: middle of May 2012, the end of the JCP season. Duration of OIT: early January to late March 2012. JCP season: mid-February to late April 2012. Mean JCP counts in Fukuoka City, Japan, 2012, and TSM scores. Solid line: JCP counts. Open squares: TSM score in the OIT group ($n = 23$). Solid squares: TSM score in the control group ($n = 24$). ** $P < 0.01$; *** $P < 0.001$, between the OIT and control groups.

different time points were performed using the non-parametric Mann–Whitney *U*-test. Comparisons of paired samples from 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 $P < 0.05$.

Results

Patient characteristics

Fig. 1 describes the enrollment and characteristics of participants. Of 23 participants, 1 withdrew from OIT because of adverse effects. None of the 23 participants undergoing OIT and none of 24 participants who did not undergo OIT withdrew from the study during the pollen season. In the ITT analysis of primary outcome and AEs, the 23 participants in the OIT group and the 24 participants in the control group fully complied with the study protocol. The characteristics of each group are presented in Table 1. No differences in baseline characteristics were observed.

Adverse effects

Of the 23 participants, 9 suffered from AEs during OIT as shown in Table 2. During the build-up phase of OIT, 7 of 23 participants had grade 1 AEs as determined by CTCAE v4.0. Only one of the seven participants with AEs had diarrhea related to gastrointestinal disorders but the symptom resolved without any treatment. Three had pruritus on the body and one of them was treated with an antihistamine cream while the others had no treatment. Pruritus resolved in all patients either after 1 week or 1 month following the beginning of the build-up phase. Two of seven participants had rhinitis similar to pollinosis with or without sneezing and nasal congestion. One of them received an oral antihistamine for a few days while the others had no treatment. The symptoms including sneezing and nasal congestion resolved after about 1 week. In one of the seven participants, malaise and laryngopharyngeal dysphagia occurred immediately after immunotherapy but resolved within a week without any treatment. During the maintenance phase of OIT, 4 of 23 participants suffered from new AEs. Two of the four participants with AEs had grade 1 diarrhea and nausea/

Table 1
Participant demographics.

Group	OIT	Control	P value
Number	23	24	
Sex (M/F)	14/9	13/11	0.432
Mean age	38.6 ± 11.2	33.7 ± 9.2	0.107
Range	25–60	22–58	
Total IgE [IU/ml]	426 ± 1413	174 ± 191	0.685
Range	13–6870	11–850	
Cry j1-Specific IgE [UA/ml]	10.6 ± 10.6	22.6 ± 24.2	0.054
Range	1.43–46.1	2.11–92.2	
Other allergies (%)			
House dust mite	7 (30.4%)	12 (50%)	0.142
Asthma	1 (4.3%)	0 (10%)	0.489
Atopic dermatitis	4 (17.3%)	3 (12.5%)	0.475
Food allergy	3 (13.0%)	5 (20.8%)	0.375
Symptom score of pre-pollen season			
Total symptom score	2.1 ± 2.9	3.0 ± 2.4	0.134
Total nasal symptom score	1.6 ± 2.1	2.4 ± 1.9	0.095
Total ocular symptom score	0.5 ± 0.8	0.5 ± 1.0	0.746

Results of the intention-to-treat (ITT) group in percentages, ranges or means ± SDs. Total symptom (TS) 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 6 individual symptom scores, each assessed on a 5-point scale (from 0 = absent to 4 = very severe). Statistical analysis was performed using the Mann–Whitney *U*-test or Fisher's exact probability test. M, male; F, female.

Table 2

Adverse events (AEs) during oral immunotherapy (OIT) using the Cry j 1-galactomannan conjugate.

	No.	M/F	AEs	Severity (grade)	Days of treatment	Duration (days)
OIT group	1	M	Nausea	Grade 1	67	0.1
			Diarrhea	Grade 1	67	0.1
	2	M	Pruritus [†]	Grade 1	11	7
	3	M	Nausea	Grade 1	35	0.1
			Vomiting	Grade 1	35	0.1
			Cough	Grade 1	35	0.1
			Laryngopharyngeal dysphagia	Grade 1	35	0.1
			Urticaria	Grade 2	35	0.1
	4	M	Rhinitis	Grade 1	1	7
	5	M	Rhinitis	Grade 1	10	5
			Sneezing	Grade 1	10	5
			Nasal congestion	Grade 1	10	5
	6	M	Malaise	Grade 1	1	4
			Diarrhea	Grade 1	10	0.5
	7 [†]	M	Laryngopharyngeal dysphagia	Grade 1	1	7
			Pruritus [‡]	Grade 1	51	1
			Laryngopharyngeal dysphagia	Grade 1	51	1
			Edema face	Grade 1	51	1
			Urticaria	Grade 1	51	1
			Pruritus [‡]	Grade 1	53	3
			Urticaria	Grade 1	53	1
			Laryngopharyngeal dysphagia	Grade 1	53	3
			Pruritus [‡]	Grade 1	68	1
			Urticaria	Grade 1	68	1
			Laryngopharyngeal dysphagia	Grade 1	68	1
	8	M	Pruritus [‡]	Grade 1	11	28
	9	F	Pruritus [‡]	Grade 1	6	26
			Rash maculopapular	Grade 1	28	32

AEs were graded according to Common Terminology Criteria for Adverse Event (CTCAE) v4.0. Total AEs (%): 9/23 (39.1%); build-up phase: 7/23 (30.4%), maintenance phase: 4/23 (17.3%). M, male; F, female.

[†] Treatment-related withdrawal: 1/23 (4.3%).

[‡] Pruritus on any part of the body.

vomiting related to gastrointestinal disorders. One of them with grade 1 vomiting also had grade 1 cough, laryngopharyngeal dysphagia and grade 2 urticaria. The symptoms appeared about one hour after taking the oral capsules, and the participant visited the hospital immediately and was treated with intravenous antihistamines and steroids. The symptoms resolved a few hours after treatment. OIT was restarted at half the maintenance dose and the maintenance dose was reached in 12 days. After that, OIT could be administered without any further AEs. The other participant recovered without treatment a few hours after the appearance of symptoms. Furthermore, one of the four participants with AEs had a grade 1 maculopapular rash but it resolved within a month without any treatment. Only one of the four participants with AEs during the maintenance phase ceased OIT because of recurrent grade 1 urticaria on the face, pruritus and laryngopharyngeal dysphagia on days 51, 53, and 68 during OIT, although the symptoms resolved on the next day or after a few days without treatment. It was possible for this participant to continue OIT but he chose to discontinue OIT near the end of therapy because of recurrent grade 1 AEs.

Pollen counts

In 2012, the Japanese cedar pollen season started in late February and ended at the beginning of April in Fukuoka City, Japan (Fig. 2). The mean annual amount of cedar pollen was 1368/cm² in Fukuoka.

Clinical efficacy

Clinical efficacy was based on weekly symptom and medication scores. TSM score and pollen counts in the community during the JCP season are shown in Fig. 2. The temporal profiles of TSM scores were lower in the OIT group compared with the control group at all six time points from 26 February to 1 April, 2012, during the JCP season (P values for the time points in chronological order: P values of 0.006, <0.001, <0.001, <0.001, <0.001 and <0.001, respectively). The mean scores for the symptoms of sneezing, itchy nose, itchy eyes, the total nasal symptom score, the total ocular symptom score, TS score, medication score and TSM scores were also lower in the OIT group compared with the control group during the JCP season as shown in Fig. 3A–D (P values of 0.006, 0.004, 0.013, 0.015, 0.009, 0.010, <0.001 and <0.001, respectively).

Allergen-specific serum antibodies

To objectively determine the effects of OIT, we evaluated the levels of serum antibodies including allergen-specific serum antibodies in the OIT and control groups. Fig. 4A shows the change in

the serum levels of total IgE, total IgG, Cry j1-specific IgE and Cry j1-specific IgG4 in the OIT and control groups during the JCP season. The levels of total IgE and IgG in the OIT group remained the same through the JCP season from visit 1 before the JCP season to visit 3 after the JCP season. However, the levels of total IgE and total IgG decreased in the control group at visit 2 (the beginning of the JCP season) compared with those at visit 1 ($P = 0.028$ and 0.001 , respectively). Levels remained the same at visit 3. The levels of Cry j1-specific IgE and IgG4 increased in the OIT group at visit 2 (the beginning of the JCP season and the maintenance phase of OIT) compared with those at visit 1 ($P = 0.005$ and 0.006 , respectively). Furthermore, the level of Cry j1-specific IgE increased at visit 3 after the JCP season compared with that at visit 2 ($P < 0.001$). The level of Cry j1-specific IgG4 remained the same at visit 3. However, the levels of Cry j1-specific IgE and IgG4 at visit 2 remained the same compared with those at visit 1 and were increased at visit 3 in the control group ($P < 0.001$ and <0.001, respectively). Thus, the elevated levels of Cry j1-specific IgE and IgG4 during OIT at visit 2 of the JCP season compared with visit 1 before the JCP season show that an antigen-specific immune response was induced by the Cry j1-galactomannan conjugate. However, there was no significant

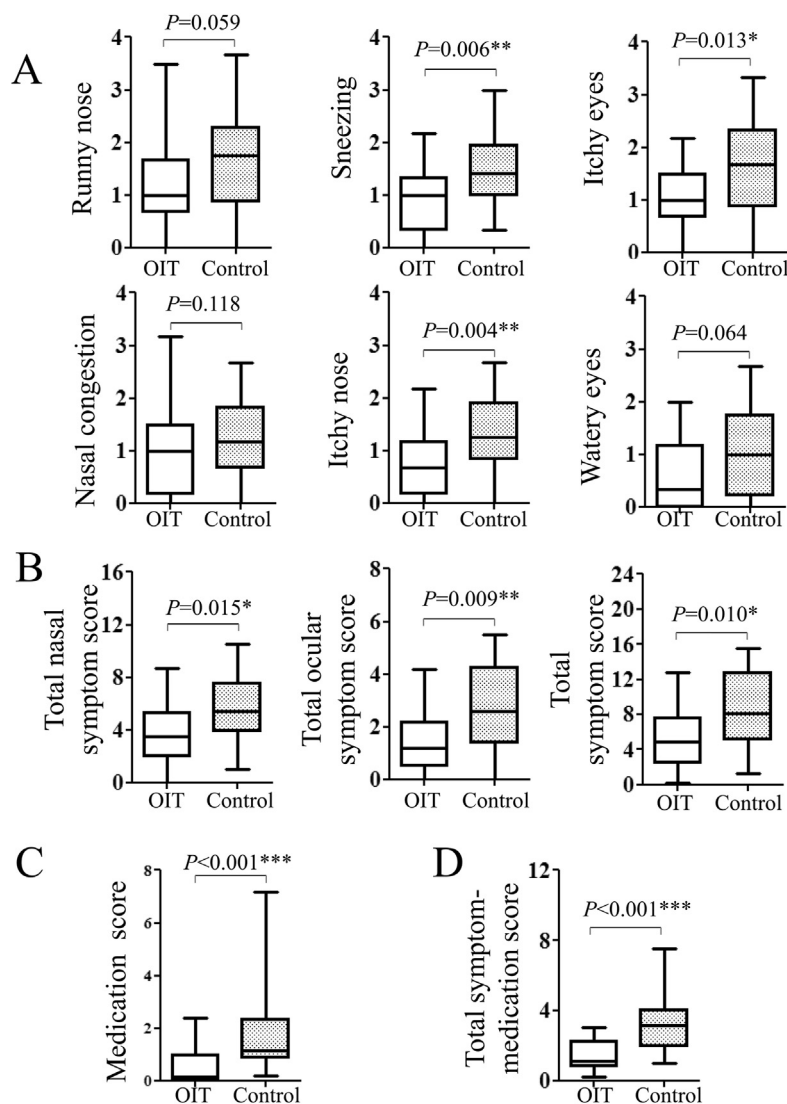


Fig. 3. Symptoms and medication use. A, Symptom scores. B, Total nasal and ocular symptom scores and total symptom scores. C, Medication score. D, TSM scores. Open squares: OIT group ($n = 23$); solid square: control group ($n = 24$). Each score represents the mean weekly score during peak pollen season (23 February to 1 April, 2012). Data are depicted as box-and-whisker plots. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, between the OIT and control groups.

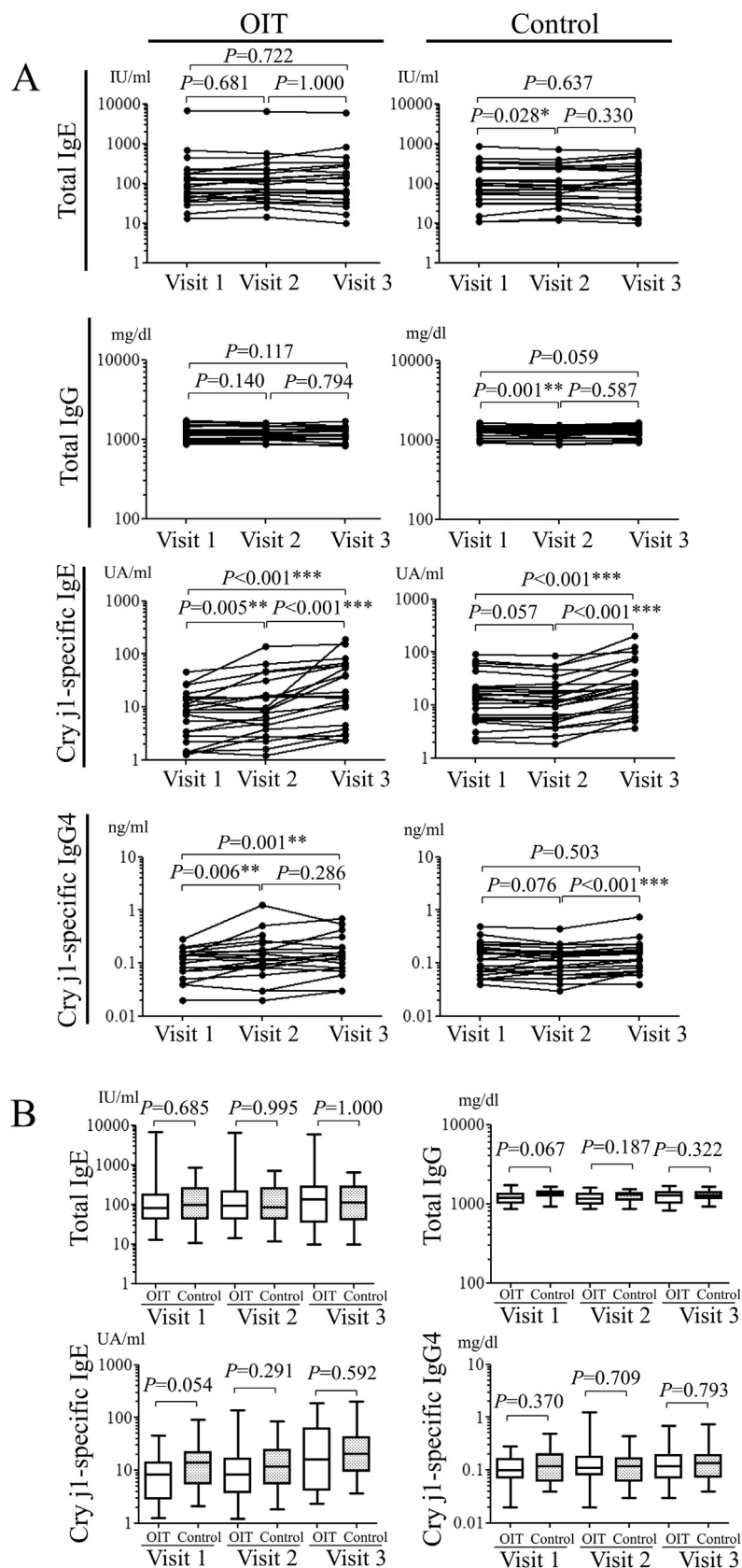


Fig. 4. Measurement of serum total IgE and IgG, and Cry j1-specific IgE and IgG4. A, Changes in individual serum total IgE and IgG, and Cry j1-specific IgE and IgG4. Open squares: OIT group; solid squares: control group. V1: OIT group ($n = 23$); control group ($n = 24$), V2: OIT group ($n = 23$); control group ($n = 24$), V3: OIT group ($n = 21$); control group ($n = 24$). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, between each visit in individuals. B, Group comparisons of serum immunoglobulins. Open squares: OIT group; solid squares: control group. Data are depicted as box-and-whisker plots. V1: OIT group ($n = 23$); control group ($n = 24$), V2: OIT group ($n = 23$); control group ($n = 24$), V3: OIT group ($n = 21$); control group ($n = 24$).

differences in the levels of total IgE, total IgG, Cry j1-specific IgE, and Cry j1-specific IgG4 in serum samples collected before JCP season and OIT (visit 1), at the beginning of JCP season (visit 2), and after JCP season (visit 3) between the OIT and control groups as shown in Fig. 4B.

Discussion

OIT is in the spotlight again as an effective treatment for food allergy including cow's milk, peanuts, eggs, etc.^{16–18} In addition, we recently reported for the first time that short-term OIT for only one month before the peak pollen season using the Cry j1-galactomannan conjugate for JCP is effective and relatively safe.²³ However, the optimum regimen of OIT using the Cry j1-galactomannan conjugate has not been established. In this study, we show that a new regimen of short-term OIT using the Cry j1-galactomannan conjugate with a build-up phase and a maintenance phase that extends to the peak pollen season is effective, relatively safe, and can induce immune tolerance in participants who are sensitized to the JCP allergen.

In our recent study, we showed that the TS score and the TSM score were significantly improved in the OIT group during the peak pollen season.²³ However, the medication score was not improved because it was lower in the OIT group than in the control group at the beginning of the pollen season, and it was the same in the late period of the pollen season. Therefore, the TSM score was not improved significantly in the OIT group compared with the control group in the late period of the pollen season. These results raise the possibility that tolerance induced by OIT cannot be maintained throughout the pollen season. We thus hypothesized that OIT through the peak pollen season in addition to OIT before the pollen season, such as the regimens of SCIT and SLIT, might enhance the therapeutic effect of OIT. In this study, the medication score was significantly improved in the OIT group throughout the peak pollen season, and the medication score was also significantly improved even in the late period of the pollen season in the OIT group. In addition, compared with the control group, the OIT group showed significant improvements in the TS score and TSM score, which represent the primary endpoint, during the peak pollen season. On the other hand, a significant difference in each symptom score including runny nose, nasal congestion and watery eyes was not observed. Generally, the severity of symptoms including nasal discharge and nasal obstruction increases with greater amounts of cedar pollen. The annual amount of cedar pollen in 2012 around Fukuoka city was less than the annual average of 1888/cm² in the last decade.²⁷ Thus, each symptom score in the control group in 2012 was relatively small compared with those in our previous study of the 2011 season that had a large annual amount of cedar pollen (2621/cm²),²³ which was not a direct comparison. The small annual amount of cedar pollen in 2012 may contribute to the small difference of each symptom score between the control and OIT groups and make it more difficult to detect a difference in each symptom score.

Furthermore, although the maintenance phase of OIT was extended to the late period of the pollen season, it did not affect compliance because all participants found the regimen acceptable and completed the OIT, except for one person who withdrew because of AEs. In the standard regimen of both SLIT and SCID for cedar pollinosis, it is necessary to start the immunotherapy more than six months before the pollen season and continue the immunotherapy for a few years before the effectiveness of the treatment can be determined.⁶ The period of immunotherapy is long and this has been a major disadvantage for patients, especially those who do not benefit from immunotherapy. In contrast, OIT in this study started from one month before the pollen season and continued for about two months until the late period of the pollen

season, which is very short compared with the standard regimen of both SLIT and SCID. This length of treatment is the same as that of conventional drug therapy for JCP. Thus, good compliance is expected for this OIT regimen. Moreover, patients can choose whether to continue the immunotherapy next year because its efficacy is known after one course of treatment in a single pollen season. It also benefits patients who do not experience the therapeutic effect of immunotherapy.

The new regimen of prolonged OIT for about two months in this study showed therapeutic efficacy throughout the pollen season and there was good compliance. Thus, we think it is a reasonable and convenient method but the cost of therapy is increased. Further study of the efficacy and method of administration is required to reduce costs (for example, administration every other day).

The safety of OIT for pollen allergy has been confirmed in many trials, and no severe systemic side effects were reported. However, many minor side effects (not life-threatening) were observed because the native form of the allergen was administered for OIT.¹² These effects tended to increase with an increased dosage of allergen.¹³ It is therefore desirable to develop a new agent to suppress gastrointestinal AEs associated with OIT. In a recent study, we reported that short-term OIT using the Cry j1-galactomannan conjugate for JCP was relatively safe.²³ Although the OIT regimen did not have a build-up phase, only 7 of 23 participants who underwent OIT had mild (grade 1) or moderate (grade 2) AEs (5 had mild and 2 had moderate AEs), and only 2 of 7 participants with adverse events developed mild gastrointestinal disorders. No severe AEs were occurred.

In previous studies of OIT for food allergy, AEs were more frequent with initial day dose escalation and in the build-up phase. The rates of AEs were the highest during the early period of oral immunotherapy.^{17,18} In addition, the risk of anaphylaxis was highest with rush protocols and overdose.²⁸ These observations suggest that a build-up phase in the OIT regimen using the Cry j1-galactomannan conjugate is necessary to enhance therapeutic safety.

In this study, 7 of 23 OIT participants had mild (grade 1) AEs during the build-up phase. During the maintenance phase, the rates of AEs were decreased compared with the build-up phase. Only 4 of 23 participants had new adverse effects. Three of four participants with AEs had mild (grade 1) AEs and one had moderate (grade 2) AEs. No severe AEs occurred in both the build-up phase and the maintenance phase. Furthermore, the rate of AEs in the maintenance phase decreased relative to that in the build-up phase, which was similar to the phenomenon in previous studies of OIT^{17,18} and this shows that the new regimen of OIT with a build-up phase is safe compared with the regimen without a build-up phase. With respect to the duration and dose escalation in the build-up phase, further research is needed to improve the safety of this regimen.

To demonstrate objective evidence for the *in vivo* effect of OIT using the Cry j1-galactomannan conjugate, we investigated the levels of serum Cry j1-specific antibodies. The levels of serum Cry j1-specific IgE and IgG4 during OIT were significantly increased in the OIT group but not in the control group at the beginning of the cedar pollen season (Visit 2). This result shows that the antigen-specific immune response for cedar pollen antigen arose *in vivo* during OIT using the Cry j1-galactomannan conjugate. The change in serum Cry j1-specific IgG4 and IgE levels during the pollen season is very similar to previous studies of OIT for birch pollen allergy¹¹ and peanut allergy.¹⁷ Moreover, the increased level of serum Cry j1-specific IgG4 in the OIT group throughout the cedar pollen season suggest that OIT with the Cry j1-galactomannan conjugate is also an effective treatment that is comparable to SLIT and SCIT.²⁹

There are some limitations in our study: the study cohort was small; the study did not use a placebo arm; and the trial was an open-

label study. Further research is required to determine whether a new regimen of OIT using the antigen–galactomannan conjugate is a universally effective method for the treatment of airway allergy.

In summary, we reported that the new regimen of short-term OIT using the Cry j 1–galactomannan conjugate is effective, relatively safe, and can induce antigen-specific immune responses. Our findings suggest that OIT using allergen–galactomannan conjugates permits a shorter, effective, and thus convenient immunotherapy regimen for cedar pollinosis compared with SLIT or SCIT that takes a few years before the therapeutic effect is apparent.

Acknowledgments

This work was supported by MEXT/JSPS KAKENHI (23791905).

Wako Filter Technology Co. Ltd. provided Cry j1–galactomannan conjugates for this study free of charge and supported the measurement of allergen-specific serum antibodies, but was not involved in this study financially.

We thank Dr. R. Kishikawa (National Fukuoka Hospital, Fukuoka, Japan) and E. Koto (The Kyushu Chapter of Japanese Allergy Foundation, Fukuoka, Japan) for their permission to use the information on the annual amount of cedar pollen in 2012 in Fukuoka.

Conflict of interest

AS is an employee of Wako Filter Technology Co., Ltd. The rest of the authors have no conflict of interest.

Authors' contributions

DM and MS designed and performed clinical experiments and analyzed data; DM wrote the main paper; SK and ME performed clinical experiments; AS and AK made allergen–galactomannan conjugates; AK supervised the study as a designer of the fundamental theory; ShK managed all experiments. All authors except for AS discussed the results and implications and commented on the manuscript at all stages. AS had no role in the study design, conduct of the study, data collection, data interpretation or preparation of the paper.

References

- Bousquet J, Van Cauwenberge P, Khaltaev N, Aria Workshop G, World Health O. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;**108**: S147–334.
- Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* 1998;**351**: 1225–32.
- Sakashita M, Hirota T, Harada M, Nakamichi R, Tsunoda T, Osawa Y, et al. Prevalence of allergic rhinitis and sensitization to common aeroallergens in a Japanese population. *Int Arch Allergy Immunol* 2010;**151**:255–61.
- Yamada T, Saito H, Fujieda S. Present state of Japanese cedar pollinosis: the national affliction. *J Allergy Clin Immunol* 2014;**133**:632–9.
- Di Bona D, Plaia A, Leto-Barone MS, La Piana S, Di Lorenzo G. Efficacy of subcutaneous and sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a meta-analysis-based comparison. *J Allergy Clin Immunol* 2012;**130**:1097–107.
- Dretzke J, Meadows A, Novielli N, Huissoon A, Fry-Smith A, Meads C. Subcutaneous and sublingual immunotherapy for seasonal allergic rhinitis: a systematic review and indirect comparison. *J Allergy Clin Immunol* 2013;**131**: 1361–6.
- Okamoto Y, Ohta N, Okano M, Kamijo A, Gotoh M, Suzuki M, et al. Guiding principles of subcutaneous immunotherapy for allergic rhinitis in Japan. *Auris Nasus Larynx* 2014;**41**:1–5.
- Weiner HL. Oral tolerance. *Proc Natl Acad Sci U S A* 1994;**91**:10762–5.
- Suko M, Mori A, Ito K, Okudaira H. Oral immunotherapy may induce T cell anergy. *International archives of allergy and immunology. Int Arch Allergy Immunol* 1995;**107**:278–81.
- Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. *J Allergy Clin Immunol* 2005;**115**:3–12.
- Moller C, Dreborg S, Lanner A, Björkstén B. Oral immunotherapy of children with rhinoconjunctivitis due to birch pollen allergy. A double blind study. *Allergy* 1986;**41**:271–9.
- Björkstén B, Möller C, Broberger U, Ahlstedt S, Dreborg S, Johansson SG, et al. Clinical and immunological effects of oral immunotherapy with a standardized birch pollen extract. *Allergy* 1986;**41**:290–5.
- Taudorf E, Laursen LC, Lanner A, Björkstén B, Dreborg S, Søborg M, et al. Oral immunotherapy in birch pollen hay fever. *J Allergy Clin Immunol* 1987;**80**: 153–61.
- Canonica GW, Passalacqua G. Noninjection routes for immunotherapy. *J Allergy Clin Immunol* 2003;**111**:437–48.
- Bousquet J, Lockett R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998;**102**:558–62.
- Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* 2008;**122**:1154–60.
- Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* 2009;**124**:292–300.
- Burks AW, Jones SM, Wood RA, Fleischer DM, Sicherer SH, Lindblad RW, et al. Oral immunotherapy for treatment of egg allergy in children. *N Engl J Med* 2012;**367**:233–43.
- Vickery BP, Scurlock AM, Jones SM, Burks AW. Mechanisms of immune tolerance relevant to food allergy. *J Allergy Clin Immunol* 2011;**127**:576–86.
- Yasueda H, Yui Y, Shimizu T, Shida T. Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *J Allergy Clin Immunol* 1983;**71**:77–86.
- Usui M, Saito A, Taniguchi N, Nishijima N, Azakami H, Kato A. Reduction of antigenicity of Cry j 1, major allergen of Japanese cedar pollen, by the attachment of polysaccharides. *Biosci Biotechnol Biochem* 2003;**67**:2425–30.
- Aoki R, Saito A, Azakami H, Kato A. Effects of various saccharides on the masking of epitope sites and uptake in the gut of cedar allergen Cry j 1-saccharide conjugates by a naturally occurring Maillard reaction. *J Agric Food Chem* 2010;**58**:7986–90.
- Murakami D, Kubo K, Sawatsubashi M, Kikkawa S, Ejima M, Saito A, et al. Phase I/II study of oral immunotherapy with Cry j1–galactomannan conjugate for Japanese cedar pollinosis. *Auris Nasus Larynx* 2014;**41**:350–8.
- Malling HJ, Weeke B. Position paper: immunotherapy. *Allergy* 1993;**48**:9–35.
- Fujieda S, Kurono Y, Okubo K, Ichimura K, Enomoto T, Kawauchi H, et al. Examination, diagnosis and classification for Japanese allergic rhinitis: Japanese guideline. *Auris Nasus Larynx* 2012;**39**:553–6.
- Okubo K, Kurono Y, Fujieda S, Ogino S, Uchio E, Odajima H, et al. Japanese guideline for allergic rhinitis. *Allergol Int* 2011;**60**:171–89.
- Oshikawa C, Kishikawa R, Koto E, So N, Kamori C, Shimoda T, et al. [Trends of the *Cryptomeria japonica* and Cupressaceae pollen counts and the number of patients with Japanese Cedar pollinosis in Fukuoka]. *[Otol Fukuoka]* 2012;**58**: 268–77 (in Japanese).
- Calderón MA, Simons FE, Malling HJ, Lockett RF, Moingeon P, Demoly P. Sublingual allergen immunotherapy: mode of action and its relationship with the safety profile allergy. *Allergy* 2012;**67**:302–11.
- Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van Overtvelt L. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* 2006;**61**: 151–65.