



Contents lists available at ScienceDirect

Allergology International

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

Original Article

Effect of oral immunotherapy in children with milk allergy: The ORIMA study

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ARTICLE INFO

Article history:

Received 25 May 2020

Received in revised form

22 September 2020

Accepted 23 September 2020

Available online xxx

Keywords:

Cow's milk allergy

Efficacy

Immunotherapy

Pediatric

Safety

Abbreviations:

OFC oral food challenge test

DBPCFC double-blind placebo-controlled

food challenge

OIT oral immunotherapy

PBMC peripheral blood mononuclear

cell

ABSTRACT

Background: This study was aimed at evaluating the efficacy and safety of oral immunotherapy (OIT) in children with severe cow's milk allergy.

Methods: The subjects comprised 28 children (aged 3–12 years) with allergic symptoms that were induced by ≤ 10 mL of cow's milk in an oral food challenge test (OFC). The subjects were randomly allocated to the treatment group ($n = 14$) and control group ($n = 14$); the former received rush immunotherapy for 2 weeks, followed by a gradual increase of cow's milk volume to 100 mL for 1 year, and the latter completely eliminated cow's milk for 1 year. Both groups underwent an OFC with 100 mL of cow's milk after 1 year.

Results: The treatment group had significantly higher rates of a negative OFC [7/14 (50%) vs. 0/14 (0%), $p < 0.01$] compared with the control group. The cow's milk-specific IgE level significantly decreased in the treatment group ($p < 0.01$) but not in the control group ($p = 0.63$). During the study period, adrenaline was required in 6/14 patients (43%) of the treatment group and in 0/14 patients (0%) of the control group. Long follow-up data were available at the 2-year point after the study for 8 in the treatment group and 7 (87.5%) of these continued to ingest milk (>100 mL).

Conclusions: The effect of immunotherapy was 50%, but the incidence of adverse events was not low. Further studies focusing on safety is necessary to standardize OIT for cow's milk allergy.

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Introduction

Food allergy has increased in incidence among young children and may result in anaphylaxis and fatality. A survey in Japan revealed that the prevalence of food allergy was 10% in infancy, 5% in early childhood, and 1.3% at school age and thereafter.¹ The estimated rates of children having a history of anaphylaxis were 1.6% in the United States,² 0.3% in Europe,³ and 0.3%–0.6% in Japan.⁴ The basic treatment for food allergy is elimination of the causative food. Recently, oral immunotherapy (OIT) has been demonstrated

to be efficient and may be a new option for the management of food allergy. In patients with cow's milk allergy, the efficacy of OIT was reported to be 40%–90%, with a significant increase in the threshold intake.^{5–9} However, in the guidelines for major allergy, OIT is not recommended as a standard treatment, because it can induce serious adverse reactions such as anaphylaxis.¹⁰

Among the food allergies in Japan, cow's milk allergy is the second most frequent after hen's egg allergy, and among the causative food products accidentally ingested by young children, milk is the second highest following hen's egg.¹¹ Because cow's milk is provided in school lunches in the nursery, elementary, and junior high schools in Japan, cow's milk allergy has a large impact on the daily and social lives of the child and their parents. In previous reports on OIT for cow's milk allergy, important backgrounds such as threshold for symptom induction and severity, as well as the time of onset and severity of adverse events has often been

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Peer review under responsibility of Japanese Society of Allergy.

<https://doi.org/10.1016/j.alit.2020.09.011>

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unclear. Further, since there are few studies on the long-term outcomes of milk OIT, whether OIT induces not only desensitization but sustained unresponsiveness is not clear.¹²

Therefore, this study was performed to investigate the efficacy, adverse events and long-term outcomes in Japanese children with severe cow's milk allergy whom spontaneous acquisition of tolerance was unlikely.

Methods

Subjects

Of the patients seen at the Department of Pediatrics at the Daisan hospital of the Jikei University School of Medicine and the Department of Pediatrics at Showa University Hospital from August 2011 to July 2016 for cow's milk allergy, the children who met all of the following inclusion criteria were selected as candidate subjects: (1) were 3 to < 13 years old (children aged 3 to < 6 years were originally planned to be the subjects at study initiation, but the target age was changed to 3 to < 13 years in May 2013 because of the slow registration pace); (2) had a positive cow's milk-specific IgE level of ≥ 0.7 UA/mL; (3) had a medical history of development of symptoms after ingestion of cow's milk; (4) were able to make an emergency visit to the hospital within 30 min while performing the study; (5) parents were able to accompany their child during rush OIT; and (6) the child and/or their parents gave consent for participating in the study. The subjects were then excluded if they met any of the following criteria: (1) had history of life-threatening anaphylactic shock caused by ingestion of cow's milk; (2) had uncontrolled bronchial asthma; (3) had uncontrolled atopic dermatitis; (4) were allergic to other foods (soybeans, chocolate, and oats) used in the double-blind food challenge (DBPCFC) test; (5) had been judged by a physician as ineligible for the study because of complications; (6) were unable to obtain consent from the patient or his/her parents; and (7) had difficulty with the withdrawal of oral drugs in the food challenge test.

The DBPCFC test was judged as positive if a Sampson's classification¹³ of $\geq$ grade 2 (Sampson's Grade 2) symptoms appeared after cumulative ingestion of ≤ 10 mL of cow's milk. Of note, only children who developed symptoms after cumulative ingestion of ≤ 5 mL of cow's milk were included upon study initiation, but the criterion was changed in May 2013 because of the slow pace of registration. DBPCFC-positive patients were selected as subjects and randomly divided into the OIT (treatment) and observation (control) group. Randomization was managed by a data center independent of the rater and subjects. Dynamic allocation was performed using the minimization method with the hospital, sex, and cow's milk-specific IgE level ($<$ or ≥ 50 UA/mL) as allocation adjustment factors. OIT was continued for 1 year in the treatment group, whereas complete elimination of cow's milk was continued for 1 year in the control group. DBPCFC tests were performed after 1 year in both groups.

Oral immunotherapy

Patients in the treatment group were hospitalized for approximately two weeks for rush OIT. Ingestion of cow's milk started from 10^{-4} mL, and the volume was gradually increased daily. The protocol of rush OIT was prepared based on a study by Longo *et al.*⁵ (Supplementary Table 1). After this period, the patients were given cow's milk at home once daily, and the volume was increased by 10%–20% every 14 days (slow OIT) (Supplementary Table 2). When a volume of 100 mL was reached, it was ingested daily. The patients visited as outpatients once a month for 1 year. To suppress mild allergic symptoms, epinastine hydrochloride (0.5 mg/kg) was

taken once a day. If symptoms appeared during immunotherapy, the next doses were reduced depending on the severity of the symptoms (Supplementary Table 1, 2). Treatment was stopped if symptoms appeared three times after the same loading dose during rush immunotherapy. Therapy was discontinued and the patient was regarded as a dropout if the ingest volume of undiluted cow's milk was < 5 mL at this point. If the ingest volume was ≥ 5 mL, the patient was switched to slow OIT at home.

Patients who wished long-term follow-up continued to ingest milk depending on the individuals' threshold on a daily basis after the end of study.

The double-blind food challenge test

A DBPCFC test was performed using chocolate flakes prepared with cow's milk or soy milk (oats, chocolate syrup, vanilla essence, sugar, and salt). To eliminate the influence of drugs, different withdrawal periods were set for antihistamines, leukotriene antagonists, and oral steroids. Treatment with inhaled or topical steroids was regarded as continuable. The dose escalation method and dosing intervals are presented in Supplementary Table 3. The food challenge test was judged as positive if Sampson's Grade 2 or higher symptoms appeared. Treatment was performed for induced symptoms, as required.

Evaluation items

The primary endpoint was the rate of a negative cow's milk challenge test upon study completion, as defined by the absence of Sampson's Grade 1 or milder reactions after ingestion of 100 mL of cow's milk. The secondary endpoints were the percent change in the threshold of cow's milk intake, result of skin prick test with cow's milk, eosinophil count, antigen-specific IgE levels (cow's milk, casein, α -lactalbumin, and β -lactoglobulin), changes in antigen-specific IgG4 levels (cow's milk, casein, α -lactalbumin, and β -lactoglobulin), and comprehensive gene expression induction analysis after milk antigen stimulation.

The safety evaluation items were the incidence of adverse events, severity, and adrenaline use during the study period. Adverse events were divided into mild (minimal or no intervention required beyond reassurance and observation), moderate (neither mild nor severe, require intervention, and are usually managed on-site), or severe (extensive intervention with referral or specialist input). As for blood tests, the serum eosinophil count, antigen-specific IgE levels (Phadia, Thermo Fisher Scientific Diagnostics), and antigen-specific IgG4 levels were measured upon registration and 1 year after registration. In addition, a skin prick test was performed using a cow's milk antigen (Torii Pharmaceutical).

Transcriptome analysis of peripheral blood mononuclear cell (PBMC) after milk antigen stimulation was performed before the beginning of OIT to find molecules that can predict the outcome of OIT. In this analysis, the group in which the symptom induction threshold did not increase at all before and after OIT was defined as *the group that did not respond at all* (non-responder group). Contrarily, the group in which OIT progressed smoothly and 100 mL of milk could be taken without any problems after treatment was defined as *the group that progressed smoothly* (responder group). Each subject was selected and measured. The subjects' PBMCs before OIT were stimulated with 200 μ g/mL of cow's milk protein (kindly provided by Bean Stalk Snow, Tokyo, Japan) for five days and subsequently, total RNA in cultured-PBMCs was extracted using a RNeasy Mini Kit (Qiagen). The gene expression profiles were assessed using a microarray technology with Agilent Sure Print G3 Human GE 8 $\times$ 60 K Ver 3.0. The data was analyzed using the Subio Platform software (<http://www.subioplatform.com/>) and IPA

software (Qiagen). The strategy to identify the genes that are differentially expressed in non-responder and responder were described in [Supplementary Figure 1 and 2](#) respectively.

Post study data was collected by clinical follow-up including the daily dose of milk products, adrenalin use and cow's milk specific IgE level 2 years after the ORIMA study.

Statistical analysis

The statistical analysis was performed by a third party (Medical Statistics, present AC Medical). Patient background factors in the two groups were compared by Student's t-test or Wilcoxon test, with $p < 0.05$ regarded as significant.

Ethical considerations

The study was approved by the Ethics Committee of the Jikei University School of Medicine [approval number: 22–163 (6340)], the Medical Ethics Committee of Showa University School of Medicine (approval number: 1662), and the Ethics Committee of National Research Center for Child Health and Development (approved number: 476), and it was registered in UMIN-CTR (UMIN000005784). The study was explained to the parents using documents, and written informed consent was obtained. Written informed assent was also obtained if possible. If a patient who was randomly allocated to the observation group requested OIT after one year, OIT was performed after completion of the study period using the same protocol as that in the treatment group.

Results

Of 34 candidate patients, 28 who were positive in the DBPCFC test were selected as subjects. Of the 6 excluded patients, 4 did not have any reaction or had Sampson's Grade 2 or milder reaction to ≤ 10 mL of cow's milk; 1 had poor physical condition, and 1 could not ingest food in the challenge test. The 28 patients were randomly allocated to the treatment and control group ([Fig. 1](#)). The background factors for these two groups are presented in [Table 1](#). There were no differences between the treatment and control group in mean age ($\pm$ standard deviation) (5.5 ± 2.4 vs. 5.4 ± 2.3 years, $p = 0.55$); cow's milk-specific IgE level, which was high in both groups (51.7 ± 40.2 vs. 65.8 ± 39.1 UA/mL, $p = 0.38$); and the cow's milk intake threshold before registration (4.6 ± 5.0 vs. 3.6 ± 3.3 mL, $p = 0.54$), which was low as expected in severe cow's milk allergy.

Of the 14 patients in the treatment group, 2 dropped out during rush immunotherapy because of development of

Table 1

Demographic data of the treatment and control group.

Characteristics	Treatment group (n = 14)		Control group (n = 14)		P value
Age (year)	5.5	(± 2.4)	5.4	(± 2.3)	0.55
Male, sex	7	(50)	7	(50)	0.65*
Complication of bronchial asthma	6	(43)	5	(36)	0.50*
Complication of atopic dermatitis	7	(50)	11	(79)	0.12*
Complication of allergic rhinitis	2	(14)	6	(43)	0.10*
Complication of hives	2	(14)	2	(14)	0.70*
Food allergies other than milk	12	(86)	9	(64)	0.19*
Cow's milk-specific IgE level at the time of registration (UA/mL)	51.7	(± 40.2)	65.8	(± 39.1)	0.38
DBPCFC at registration (threshold of reactivity) (mL)	4.6	(± 5.0)	3.6	(± 3.3)	0.54
Severity of reaction at DBPCFC [†]					
Grade 2	3	(21)	1	(7)	
Grade 3	6	(43)	8	(57)	
Grade 4	5	(36)	5	(36)	

Values are presented as mean ($\pm$ standard deviation) or number (%). Statistics were performed using Chi-squared test (*) or Mann–Whitney U test.

DBPCFC, double blind placebo control food challenge.

[†] Severity at DBPCFC according to Sampson's grading.

symptoms three or more times after ingesting the same volume. The remaining 12 patients completed the 1-year treatment. The course of the treatment group is shown in [Supplementary Table 4](#). None of the 14 patients in the control group dropped out. The primary endpoint of the rate of a negative cow's milk challenge test upon study completion was significantly higher in the treatment group than in the control group [50% (7/14) vs. 0% (0/14), $p < 0.01$] ([Fig. 2](#)). Moreover, the treatment group had a significantly higher cow's milk intake threshold (77.1 ± 35.1 vs. 8.6 ± 10.5 mL, $p < 0.01$) and percent change in the cow's milk intake threshold ($3090\% \pm 2808\%$ vs. $443\% \pm 1114\%$, $p < 0.01$) compared to those in the control group upon study completion ([Table 2](#)).

The cow's milk-specific IgE level significantly decreased during the study period in the treatment group ($p < 0.01$), but there was no change in the control group ($p = 0.63$). In addition, the IgE levels specific to casein and β -lactoglobulin significantly decreased in the treatment group ($p < 0.01$). The two groups had no significant differences as of changes in the results of the skin prick test with cow's milk nor eosinophil count ([Table 3A, B](#)). The antigen-specific IgG4 level was measured, but evaluation was not possible because many samples were missing.

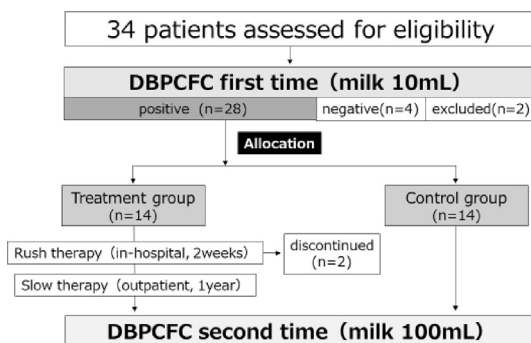


Fig. 1. Enrollment and randomization of the population for analysis of the primary outcome. DBPCFC, double blind placebo control food challenge.

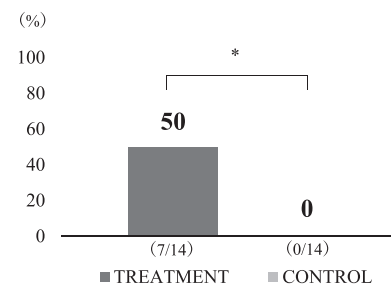


Fig. 2. Rate of a negative cow's milk challenge test. Negative cow's milk challenge test defined by the absence of reactions, or Grade 1 (Sampson's classification) or milder reactions after ingestion of 100 mL of cow's milk. The rate of a negative cow's milk challenge test was significantly higher in the treatment group ($p = 0.001$). *A p -value < 0.05 was considered statistically significant; Chi-squared test.

Table 2
Change of cow's milk intake threshold.

	Treatment group (n = 12)		Control group (n = 14)		P value
Threshold of reactivity at registration (mL)	4.6	(±5.0)	3.6	(±3.3)	0.54
Threshold of reactivity at the end of study (mL)	77.1	(±35.1)	8.6	(±10.5)	<0.01
The change of milk intake threshold [†] (%)	3090	(±2808)	442	(±1114)	<0.01

Values are presented as mean (±standard deviation). Statistics were performed using Mann–Whitney U test. = (Threshold of reactivity at the end of study - Threshold of reactivity at registration)/Threshold of reactivity at registration*100.

[†] Change of milk intake threshold (%).

Transcriptome analysis was performed using total RNAs from two subjects in the non-responder group and two subjects in the responder group. The >1.8-fold up/down-regulated genes (1180 up-regulated, 704 down-regulated) in milk-stimulated PBMCs than vehicle-stimulated PBMCs in responder group, and > 2-fold up/down-regulated genes (41 up-regulated and 21 down-regulated) in milk-stimulated PBMCs than vehicle-stimulated PBMCs in the non-responder group were compared (See detail in [Supplementary Fig. 1, 2](#)). As a result, milk-specifically up/down-regulated genes were identified in each group ([Supplementary Table 5, 6](#)). Gene ontology analysis showed that genes whose expression was differentially expressed only in non-responder group were functionally associated with cellular movement significantly ([Supplementary Table 6A](#)).

Adverse events were reported in 12/14 patients in the treatment group during the study period and were mild in 2, moderate in 8, and severe in 2 cases. All adverse events were treatment-related adverse reactions. By contrast, only mild adverse events were reported in 3/14 patients in the control group ([Table 4](#)). Adrenaline was used during the study period in 1/14 patients (7%)

Table 3
Changes in laboratory test values between pre and post of the study.
A. Changes in laboratory test values in treatment group: "Pre" indicates the test values at the time of registration, and "Post" indicates the test values at the end of the study.

Treatment group	Pre	Post	P value
milk-skin prick test (mm)	9.9 (±7.3)	8.8 (±5.1)	0.55
Eosinophil count (/μL)	526 (±251)	454 (±279)	0.68
Cow's milk-specific IgE (U _A /mL)	51.7 (±40.2)	27.1 (±32.0)	<0.01
CN-specific IgE (U _A /mL)	52.4 (±41.5)	24.8 (±30.7)	<0.01
αLA-specific IgE (U _A /mL)	14.4 (±16.5)	7.9 (±16.0)	0.11
βLG-specific IgE (U _A /mL)	10.1 (±12.9)	4.1 (±7.6)	<0.01

Values are presented as mean (±standard deviation). Statistics were performed using Wilcoxon signed rank test.

CN, casein; αLA, α-lactalbumin; βLG, β-lactoglobulin.

B. Changes in laboratory test values in the control group: "Pre" indicates the test values at the time of registration, and "Post" indicates the test values at the end of the study.

Control group	Pre	Post	P value
milk-skin prick test (mm)	9.7 (±5.5)	9.4 (±4.5)	0.30
Eosinophil count (/μL)	538 (±241)	489 (±168)	1.00
Cow's milk-specific IgE (U _A /mL)	65.8 (±39.1)	63.7 (±37.2)	0.63
CN-specific IgE (U _A /mL)	65.4 (±39.7)	67.5 (±38.9)	0.92
αLA-specific IgE (U _A /mL)	15.9 (±18.1)	16.1 (±18.5)	0.73
βLG-specific IgE (U _A /mL)	10.1 (±11.6)	7.4 (±7.3)	0.95

Values are presented as mean (±standard deviation). Statistics were performed using Wilcoxon signed rank test.

CN, casein; αLA, α-lactalbumin; βLG, β-lactoglobulin.

Table 4
Adverse event incidences.

	Treatment group (n = 14)		Control group (n = 14)	
Number of patients experiencing adverse events	12	(86)	3	(21)
Grade of event [†]				
Mild [‡]	2	(14)	3	(21)
Moderate [§]	8	(57)	0	(0)
Severe [¶]	2	(14)	0	(0)

Values are presented as number (%).

[†] Adverse events have been classified into three categories of severity: mild, moderate, and severe.

[‡] Mild classification indicates minimal or no intervention required beyond reassurance and observation.

[§] Moderate classification relates to adverse events that are neither mild nor severe, require intervention, and are usually managed on-site.

[¶] Severe classification requires extensive intervention with referral or specialist input.

during rush immunotherapy and in 6/12 (50%) during slow immunotherapy. The ratio of episodes of adrenaline use to frequency of treatment was 2/822 (0.2%) during rush immunotherapy, 19/4109 (0.5%) during slow immunotherapy, and 21/4931 (0.4%) in total ([Table 5](#)). Some patients with treatment-related induction of symptoms also used adrenaline in the dose escalation and maintenance periods during rush and slow immunotherapy ([Supplementary Fig. 3A, B](#)).

Data were available at the 2-year point for 8 children and 7 (87.5%) of these continued to ingest milk (>100 mL) for 2 years. The cow's milk threshold increased 1 year after the study [78.8 ± 11.9 vs. 123.8 ± 16.7 mL, $p = 0.046$] ([Fig. 3A](#)). The cow's milk-specific IgE level decreased 1 year after the study [14.0 ± 4.0 vs. 8.6 ± 3.1 U_A/mL, $p = 0.02$] and further decreased was recognized 2 years after the study [8.6 ± 3.1 vs. 4.6 ± 1.4 U_A/mL, $p = 0.03$] ([Fig. 3B](#)). Neither adrenalin use nor severe adverse events were reported for 2 years after the study period.

Discussion

In this randomized controlled study, 50% of patients treated with OIT in whom remarkable immediate-type allergic symptoms had been induced by ingestion of ≤10 mL of cow's milk were able to ingest the target volume of 100 mL of cow's milk. The percent change in the threshold was 3090%, with a significant increase, compared with that in the control group. All IgE levels specific to cow's milk, casein, and β-lactoglobulin decreased after 1 year of immunotherapy, similarly to what has been reported in previous studies.^{14,15}

To our knowledge, six randomized controlled studies on OIT with cow's milk have been reported,^{5,9,16–19} and the efficacy for desensitization was 37%–67%. However, the patients' background such as age, severity, threshold for symptom induction, and the criteria for efficacy varied among the studies. In our study, the DBPCFC test with cow's milk which was performed on registration confirmed the appearance of Sampson's Grade 2 or higher symptoms after ingestion of ≤10 mL of milk. In addition, children aged ≤2 years were not included in our study, suggesting that spontaneous tolerance to milk was unlikely during the intervention period.^{20,21} In comparing the characteristics of this study with those of previous studies,^{5,9,16–19} most of them had some limitations. In the studies by Martorell *et al.*¹⁶ and Morisset *et al.*,¹⁹ the target age was 2–3 years and 1.1–6.5 years, respectively; these include the age group in which spontaneous acquisition of tolerance can be expected. In the studies by Martorell *et al.*¹⁶ and Sal-mivesi *et al.*,¹⁷ the threshold prior to initiation of intervention was not described, making evaluation of severity difficult. In the studies

Table 5

Frequency of adrenaline use, intervention, adverse events during oral immunotherapy.

Immunotherapy category	Number of cases	Percentage (number of cases/all patients)	Number of events	Percentage (number of events/all intakes)
Events requiring adrenaline treatment				
Rush (n = 14)	1	7%	2	0.2%
Slow (n = 12)	6	50%	19	0.5%
Rush + Slow (n = 14)	6	43%	21	0.4%
Events requiring intervention				
Rush (n = 14)	7	50%	14	2.2%
Slow (n = 12)	10	83%	117	2.8%
Rush + Slow (n = 14)	11	79%	131	2.7%
All adverse events				
Rush (n = 14)	9	64%	51	8.1%
Slow (n = 12)	10	83%	180	4.3%
Rush + Slow (n = 14)	12	86%	231	4.8%

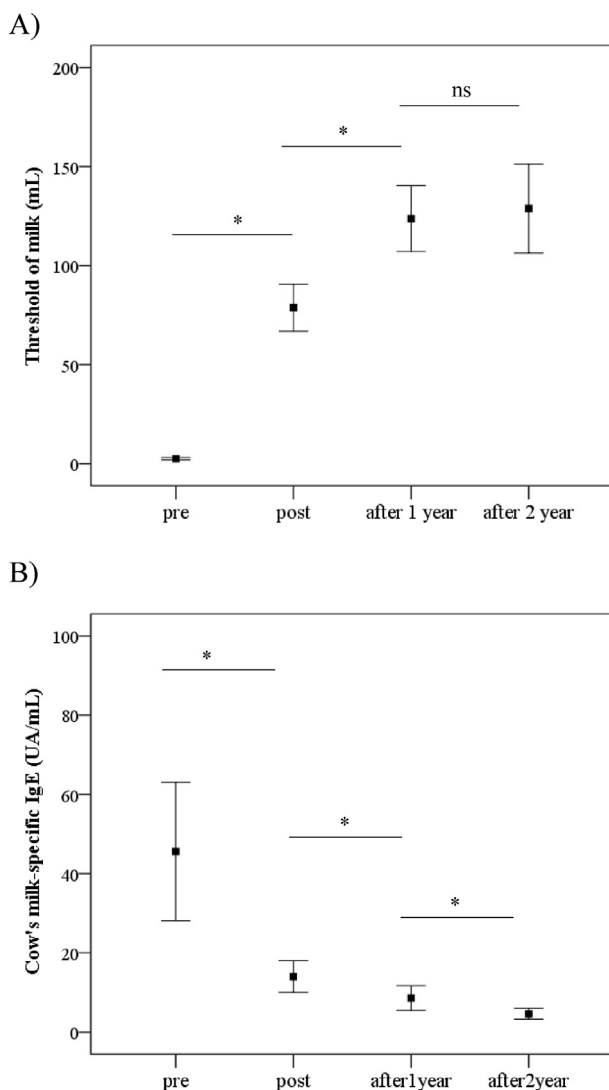


Fig. 3. Course of threshold of cow's milk ingestion and cow's milk-specific IgE after 2 years of follow-up. Evolution of milk ingestion threshold (A) cow's milk-specific IgE (B) at baseline (pre), end of the study (post), after 1 year from the end of the study (after 1 year) and after 2 years from end of the study (after 2 year). Means and SD are shown. *p-value <0.05, ns, not significant; Wilcoxon's test.

by Skripak *et al.*,⁹ Pajno *et al.*,¹⁸ and Morisset *et al.*,¹⁹ the threshold before initiation of intervention was set at a high level, resulting in inclusion of many mild cases, compared with those in our study. In

the study by Longo *et al.*,⁵ the symptoms induced in the challenge test prior to initiation of intervention were unclear, but the target age, threshold prior to initiation of intervention, and the intervention period were mostly the same as those in our study.

Regarding safety, 6/14 patients (43%) needed adrenaline throughout the entire period, and this can be considered a high rate. The frequency of adrenaline use was similar during rush and slow immunotherapy (frequency of adrenaline self-injection/frequency of treatment of 0.2%–0.5%). The annual rate of anaphylactic symptoms was about one per patient, indicating a major problem with safety.

Since the study focused on subjects who had severe allergy, 80% of whom had Sampson's Grade 3 symptoms or worse (and 5 out of 14 patients with Sampson's Grade 4 symptoms or worse) with 5 mL of consumption, we directed physicians and parents not to hesitate the use of adrenaline shots. Including these factors, the rate of using adrenaline shots for each case in the treatments group was 0.4% per dose, which is relatively low. However, this does not significantly prove the overall safety of OIT, rather emphasizing the need of safer procedures. Although serious adverse events such as anaphylaxis are inevitable in OIT, the risk should be reduced as much as possible. To minimize this risk, use of a low target volume,²² sublingual immunotherapy,²³ percutaneous immunotherapy,²⁴ and a method to standardize the dose using a formulated antigen²⁵ have been tried. Concomitant use of omalizumab for severe cases has also been reported as an approach to reducing adverse events.²⁶ All these methods have potential, but none have reached the level of standard treatment.

Although limited in number, 8 patients were followed up 2 years after the completion of the study (ie, 3 years after the start of OIT) to assess the prognosis of treatment. Of the 8 patients assessed 7 were able to continually ingest more than 100 mL of cow's milk, and no adrenaline use nor severe adverse events were reported. In addition, the amount of cow's milk that patients were able to ingest increased and the cow's milk-specific IgE level decreased in long-term. This suggests that the effect of OIT is persistent and also that OIT is more effective if it is continued for years.

In this study, we performed a comprehensive expression analysis after specific antigen stimulation, to search for antigen-specific markers that predict the results of OIT in advance. As a result, certain genes with characteristic induced or attenuated expression were found in the responder and non-responder groups. These genes can be candidates for antigen-specific markers that predict the outcome of OIT in advance. However, because the number of subjects is limited, it is necessary to consider increasing the number of cases in the future.

The strong point of this study includes its focus on patients aged 3–12 years with severe cow's milk allergy and the performance of

OIT in a randomized controlled design. Although having severe allergy, 50% of the children were able to ingest 100 mL of cow's milk after a 1-year intervention, and the percent threshold increase was 3090%. A latest systematic review²⁷ points out that OIT of milk needs to accumulate high quality RCTs in order to be established as a safe standard of therapy. Because there was no ongoing RCTs for OIT of milk except for the ORIMA study, the results of this study may be valuable additional data. There are certain limitations for this study. End point DBPCFC without an elimination period, lacks significance as an evaluation of sustained unresponsiveness. Additionally, the 1-year intervention period may be insufficient for acquisition of tolerance. Therefore, this study shows additional data concerning continuous observation of the long-term effect of OIT with cow's milk for participants who requested further follow-up. As a result, we found that the effect of OIT had a fairly long persistency with correlation between length of time and effectiveness. Since the appropriate time length of OIT is still unclear, we emphasize the importance of further studies in this field.

In conclusion, the ORIMA study was performed in children with severe cow's milk allergy. The treatment efficacy for desensitization was 50%, but the incidence of adverse events was high. These results suggest that standardization of OIT with cow's milk requires further investigation, with priority placed on safety.

Acknowledgements

We thank the children who participated in this study and their parents. We thank Ms. Kazue Takeda, Ms. Nao Aida, Ms. Michiko Yamada, Ms. Noriko Hashimoto, Dr. Kyoko Futamura, Dr. Kanami Orihara, Dr. Akio Matsuda and Mr. Motohiro Kuriyama of the National Research Institute for Child Health and Development for their excellent assistance in transcriptome and post-transcriptome analyses.

This study was funded in part by Kawano Masanori Memorial Public Interest Incorporated Foundation for Promotion of Pediatrics, Grant Number: 22-13 and 23-12 (to K.A.) and by the Ministry of Health, Labor and Welfare, Japan, Grant/Award Number: 201126005B (to K.M.). All sponsors had no roles in study design; collection, analysis, and interpretation of data; or writing of the report.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2020.09.011>.

Conflict of interest

TKat received lecture fees from Kyorin Pharmaceutical, Maruho, and Torii Pharmaceutical. The rest of the authors have no conflict of interest.

Authors' contributions

MM collected, analyzed and interpreted the data and wrote the draft. KA conceived and designed the study, collected, edited the manuscript. TN and RI designed the study and collected data. TKam and AK collected data. SF revised the manuscript. HT performed data analysis. HM and KM performed transcriptome analysis and critically revised the manuscript. TI designed the study and collected the data, critically revised the manuscript. TKat provides overall supervision, and critically revised the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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