

REVIEW



Immunotherapy for cow's milk allergy

Shoichiro Taniuchi ^{a,b}, Masaya Takahashi^b, Kazukiko Soejima^b, Yasuko Hatano^b, and Hiroataka Minami^a

^aDepartment of Pediatrics, Takatsuki General Hospital, Osaka, Japan; ^bDepartment of Pediatrics, Kansai Medical University, Osaka, Japan

ABSTRACT

Oral immunotherapy (OIT) is used regularly for young children with cow's milk (CM) allergy and has been shown to be effective in several studies. However, adverse events occur frequently during OIT. Furthermore, there are only 5 randomized controlled trial studies of CM-OIT and these are low-powered single center trials. Therefore, evidence levels are also low and sometimes frequent and severe allergic events occur during the OIT. Furthermore, there are no standardized protocols in pediatric allergy guidelines from several countries and studies with long-term follow-up observations and clinical tolerance defined as sustained unresponsiveness are rare. Additionally, clinical tolerance by OIT is generally not well defined and obscure. Thus, several problems remain to be resolved, however we hope OIT in combination with omalizumab and less allergenic heated CM products will resolve these problems in the future.

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Introduction

Allergy to cow's milk (CM) is the second most common immediate-type food hypersensitivity in Japanese children.¹ Regarding the worldwide incidence of CM allergy, the frequencies of self-reported adverse reactions to CM are much higher than the medically confirmed diagnoses, not only in children but also in adults.² A meta-analysis of relevant original studies since 1990 by Rona et al.³ demonstrated a large variation in self-reported prevalence of milk allergy between 1.2% and 17%, whereas the prevalence in studies using a double-blind placebo controlled food challenge or an open challenge varied between 0% and 3%. Moreover, in studies based on skin prick test (SPT) and immunoglobulin E (IgE) assessment CM allergy frequencies were between 2% and 9%.

Allergen avoidance is the basic approach for the management of food allergy until clinical tolerance is induced. Approximately 50% of children can tolerate CM by 5 y of age, increasing to 75% by their early teenage years.⁴ Nevertheless, some children experience persistent allergic reactions.^{5,6}

Oral immunotherapy (OIT) is used regularly for young children with CM allergy and has been shown to be effective by several studies.^{7–16} However, adverse effects occur frequently during OIT (especially during the escalation phase) and the use of parenteral epinephrine is frequent.

As many as 20–30% of patients with food allergy are refractory to desensitization, particularly those with higher initial food-specific IgE (sIgE) levels.^{15,16} The present review focuses on immunotherapy for CM IgE-mediated food allergies.

Allergenic epitopes of cow's milk proteins

Several protein components of CM have been well characterized. β -lactoglobulin occurs naturally as a 36 kDa dimer of 162

aminacid-residue polypeptides, each of which contains 2 disulfide bonds. In contrast, the 4 casein fractions of milk, α S1-casein, α S2-casein, β -casein and κ -casein, have minimal structural homology. α S-casein has chaperone-like properties that prevent the *in vitro* thermal aggregation of both itself and other proteins. Notably, patients with IgE antibodies against casein are reported to be less likely to outgrow CM allergy.

CM contains approximately 30–35 g of proteins per liter, which includes more than 25 different proteins, although only some of them are known to be allergenic. Through the acidification of raw skim milk to pH 4.6 at 20 °C 2 fractions can be obtained: the coagulum containing the casein proteins which accounts for 80% and the lactosermum (whey proteins) representing 20% of the total milk proteins.^{17–19} The casein fraction (Bos d 8, *Bos domesticus*) consists of 4 proteins which account for different percentages of the whole fraction: α S1-casein (Bos d 9, 32%), α S2-casein (Bos d 10, 10%), β -casein (Bos d 11, 28%) and κ -casein (Bos d 12, 10%) with α S1-casein being the most important allergen in the casein fraction.²⁰ Allergens found in the whey fraction are α -lactalbumin (Bos d 4), β -lactoglobulin (Bos d 5), immunoglobulins (Bos d 7), bovine serum albumin (BSA, Bos d 6) and traces of lactoferrin (Bos d lactoferrin). α -Lactalbumin and β -lactoglobulin are the most important allergens in the whey fraction, accounting for 5% and 10% of the total milk proteins.^{17,21} There are only a few reports describing allergies to minor whey proteins such as immunoglobulin, BSA or lactoferrin.²²

Allergy to mammalian milks other than cow's milk

Extensively hydrolyzed and soy-based formulas are the most commonly used substitutes for CM protein in children with CM allergy.^{23–24} Although their nutritional value is high, their

high cost and poor palatability by some children limit the use of extensively hydrolyzed formulas. For these reasons, there has been a continuous search for other nonbovine, mammalian milks as a replacement for CM. These trials included the milk of sheep, goat, ass or donkey, horse, and buffalo.²⁵⁻²⁸ Unfortunately, it has been demonstrated, by several studies, that children with CM allergy develop allergy to the milk proteins of other mammalian milks due to similarity in proteins between these milks and that of the CM.²⁹

Briefly, Bellioni-Businco's study of 26 children with CM allergy revealed that all children had a positive skin test, specific IgE titers to goat milk, and most of them (24 of 26 children) had positive challenge tests to goat milk, making goat milk an inappropriate substitute for children with CM allergy.²⁶ Infante Pina et al.³⁰ demonstrated through radioallergosorbent assay (RAST), sIgE, skin prick and challenge tests that only 25% of the patients showed adequate immediate and late oral tolerance and had negative results of immunological tests for adverse reactions, indicating a cross-reactivity between proteins *in vivo* and *in vitro*. Ehlayel et al. have recommended camel milk as an alternative to goat and cow milk.³¹ They reported that only 7 children (18.4%) tested positive to camel milk, but 24 (63.2%) were positive to goat milk. Six (15.8%) were positive to camel, goat, and cow milks. Patients with negative SPT tolerated both camel and goat milks well. They concluded that SPT indicates low cross-reactivity between camel milk and cow milk in CM allergy and that camel milk is a safer alternative than goat milk. Thus, mammalian milks including goat and sheep milk are not alternatives for most children with CM allergy, and only camel milk is applicable as a safer milk for some individuals with CM allergy.

Prevention of cow's milk allergy

Twenty years ago, IgE-mediated food allergy was considered to be triggered by exposure to food allergens in both the infant's and the maternal diet. Therefore, the American Academy of Pediatrics (AAP) recommended that families with an infant at increased risk of atopy based on family history should avoid common food allergens such as eggs, CM, and nuts and other highly allergenic food in the infant's diet during the first 3 y of life. Specifically, for common food allergens until the first (milk), second (egg), or third (tree nuts and fish) years of life.³² However, accidental ingestion often occurs in the complete elimination method and several studies reported that children with high sIgE levels and large wheal meter of SPT during infancy were not able to achieve natural tolerance³³⁻³⁶ Therefore, it is doubtful whether complete elimination of allergenic food is effective.

Lack³⁷ first advocated the dual allergen exposure hypothesis for the etiology of food allergy; i.e. sensitization to allergen occurs through environmental exposure to allergen via the skin and that consumption of food allergen induces oral tolerance. The mechanism of this hypothesis may be explained as follows; 1) low-dose exposure to foods in the environment (on tabletops, hands, and crumbs) penetrates the skin barrier and is taken up by Langerhans's cells and this leads to T_H2 responses and IgE production by B cells, 2) early high-dose oral consumption induces tolerance, and it is proposed that T_H1 and

regulatory T cell responses occur in the gut-associated lymphoid tissue, and 3) the timing and balance of cutaneous and oral exposure determine whether a child will have allergy or tolerance.

Two recent randomized clinical trials for the prevention of the development of food allergies support these hypotheses; the Learning Early About Peanut allergy (LEAP) study³⁸ and the Prevention of Egg allergy with Tiny amount InTake (PETIT) study, a 2-step introduction of egg for the prevention of egg allergy in high-risk infants with eczema which was a randomized, double-blind, placebo-controlled trial.³⁹ The LEAP study concluded that the early introduction of peanuts significantly decreased the frequency of the development of peanut allergy among children at high risk for this allergy and modulated immune responses to peanuts. The PETIT study investigated if the combination of stepwise introduction of egg with aggressive treatment of eczema reduces the risk of onset of hen's egg allergy at 12 months of age. The study found that introduction of heated egg in a stepwise manner along with aggressive eczema treatment is a safe and efficacious way to prevent hen's egg allergy in high-risk infants. In the other RCT, a high proportion (31%) of infants had allergic reactions to pasteurized raw egg powder. In the PETIT study, no participants had an allergic reaction to the heated egg powder. Another randomized clinical trial, the EAT study⁴⁰ of introduction of allergenic foods in breastfed infants, failed in the prevention of the development food allergies. The EAT study recruited from the general population 1,303 exclusively breast-fed infants who were 3 months of age and randomly assigned them to the early introduction of 6 allergenic foods (peanut, cooked egg, CM, sesame, whitefish, and wheat; early-introduction group) or to the current practice recommended in the United Kingdom of exclusive breast-feeding to approximately 6 months of age (standard-introduction group). The results demonstrated that the early introduction of all 6 foods was not easily achieved but was safe. The cause of failure to prevent the development of food allergy may be due to recruitment from the general population, not a high-risk group. Thus, to prevent food allergies, such as peanut and egg allergy, early oral exposure during infancy with aggressive eczema skin treatment is effective, although the effect was only observed in the high-risk group. Thus, it was demonstrated that Lack's hypotheses are true and complete elimination is incorrect for the prevention of these food allergies.

However, there are no prospective randomized studies in the literature on the prevention of CM allergy. A recent study suggests that early exposure to CM protein as a supplement to breast-feeding might promote tolerance.⁴¹ Thus, it is speculated that early introduction of CM protein prevents development of CM allergy.

Protocol for cow's milk oral immunotherapy

In the pediatric allergy guidelines of several countries, including Japan, a standard protocol for oral immunotherapy for food allergies including eggs, CM and peanuts has not been established. Notably, there are various methods published, differing in the period of OIT, dosing and heating of food. The typical OIT protocol includes 3 steps⁴²; the rapid escalation phase, the build-up phase and the maintenance phase.

Escalation phase

Before the start of OIT, open food challenge (OFC) is needed and the protocol starts at a subthreshold dose in confirmation of OFC, which is rapidly increased every 30 min to 2 hours twice to 5 times a day until reaching the tolerated dose. The subject returns on a following day for a single administration of the highest tolerated dose during the initial rapid escalation day to confirm that this dose can be safely ingested every day at home.

Build-up phase

The daily dose is increased at typically weekly or biweekly increments until the target dose or highest tolerated dose (approximately 200 mL of CM) is reached. At the end of the build-up phase, the patient has achieved desensitization, in which hyporesponsiveness is maintained with regular ingestion of the food, but which may be lost with even brief dosing interruptions.

Maintenance phase

The dose achieved at the end of the buildup phase is continued daily during a maintenance phase of months to years, sometime after which a double-blind placebo controlled food challenge (DBPCFC) is performed to a regular serving of the food, referred to as a desensitization challenge as OIT has been continued until the day of the OFC. To assess the persistence of the desensitized state, daily dosing is then discontinued for a period of 4–12 weeks and reintroduced during a DBPCFC. If the food is ingested without any adverse reaction, this state is defined as sustained unresponsiveness, as the desensitized state has been maintained for a prolonged period of time in the absence of regular food ingestion.

We will now introduce our rush microwave-heated (MH)-CM protocol.¹⁶

Rush phase

CM was microwaved for 100 s at 550 W and cooled to room temperature. The starting dose was set approximately at a tenth of the threshold dose determined at OFC using CM or CM products 1.2–1.5-fold for each patient. The patients ingested MH-CM 2–4 times a day at 2-hour intervals. When 200 mL of MH-CH was reached, the ingestion was changed to once a day. When no further increases in dosage were possible because of repeated adverse events, the highest tolerated dose was continued for 3 consecutive days and if no allergic reactions occurred, the rush OIT was terminated.

Maintenance phase, microwave-heated cow's milk

The maintenance dose of 200 mL of MH-CM is ingested every day at home for maintenance. If the subject did not reach the target dose of 200 mL of MH-CM, the loading dose was gradually increased by 1 mL per day until the target dose of 200 mL was reached and the dose was continued. In cases where no adverse reactions were observed for 2 months with a daily

intake of 200 mL of MH-CM, the time spent heating the CM in the microwave oven was gradually shortened by 10 s every week. If there were no adverse events for 2 months with a daily intake of 200 mL of fresh CM, a CM-OFC was performed at 2 weeks after discontinuation of the daily ingestion of 200 mL of fresh CM.

Compared with the typical protocol, our protocol aims to avoid the lengthy build-up phase and rapidly reach tolerated doses. A downside of our protocol is the need for several days hospital admission as frequent adverse events occur. Although we did not compare the efficacy and safety of OIT for CM in patients using the 2 materials (fresh CM and MH-CM), according to the results of the OFC using the 2 materials, fewer adverse events and higher threshold doses were observed in the MH-CM OFC than in the fresh CM-OFC. Thus, adverse events were less frequent during MH-CM-OIT than during fresh CM-OIT.

Efficacy and safety of oral immunotherapy

Several studies of OIT for CM allergy have been reported since 2003.^{7–16,43} However, there are only 5 randomized control trials (RCT), which are judged as having a good evidence level.

In 2008, Long et al. first reported a RCT of OIT for severe CM allergy.⁷ Sixty subjects (aged 5–17 years) were randomized 1:1 to OIT or elimination groups. In the OIT group, the dose of CM was increased to 20 mL during the first 10 d after admission, followed by an increase to 150 mL of CM for 2–3 months. One hundred and 50 mL of CM was then maintained for 10 months. At one year after the start of the study, desensitization was compared in the 2 groups. Eleven of 31 participants in the OIT group were desensitized, 16 subjects tolerated ingestion of 50–150 mL of CM and 4 did not tolerate ingestion of CM at all. In the elimination group, no subjects tolerated the ingestion of CM.

Similarly, in 2008, Skripark et al. performed a double blinded placebo control RTC using soymilk as the placebo control in 20 patients (aged 6–21 years) with CM allergy.¹⁰ The primary outcome was the threshold dose of CM by DBPCFC at 21 weeks after study entry. In the OIT group, the threshold dose increased to 5,140 mg from 40 mg at baseline. In the placebo group, the threshold of 40 mg did not alter at the point. Among the 2,437 active OIT doses versus 1,193 placebo doses, there were 1107 (45.4%) vs. 134 (11.2%) total adverse reactions, with local symptoms being most common.

Martorell A et al. reported a RCT in 2-year-old children with CM allergy.⁸ A total of 60 children aged 24–36 months with IgE-mediated allergy to CM proteins (CMPs) were included in this multi-center study and were randomized into 2 groups. Thirty children (group A: treatment group) began oral desensitization immediately, whereas the remaining 30 (group B: control group) were kept on a milk-free diet and followed for one year. After the 1-year follow-up period, 90% of the children in group A had become completely tolerant vs 23% of the children in group B. In group A, CM skin reactivity and sIgE to milk and casein were decreased significantly from the initial assessment, whereas group B showed no significant change after 1 y of follow-up. Twenty-four patients (80%) developed some reaction during the treatment period: 14 children

developed moderate reaction (47%) and 10 mild reaction (33%). The most common manifestations were urticaria-angioedema, followed by cough. The authors concluded that oral desensitization appears to be efficacious as an alternative to elimination diet in the treatment of 2-year-old children with CM allergy.

In 2010, Pajano et al. reported a randomized single blind controlled oral immunotherapy study for CM allergy with a weekly up-dosing regimen.⁹ Briefly, 30 children with IgE-mediated CM allergy confirmed by DBPCFC were randomized equally to desensitization with CM or soymilk as control. The weekly up dosing lasted 18 weeks. Full tolerance to CM (200 mL) was achieved in 10 active patients and partial tolerance in one. Two active patients discontinued the desensitization after experiencing severe reactions, whereas no reactions occurred in controls, whose sensitivity to CM remained unchanged. They concluded that this weekly up-dosing desensitization protocol for CM allergy performed under medical supervision was effective and reasonably safe.

The Cochrane Database of Systematic Reviews⁴⁴ reported the efficacy and safety of CM-OIT in 5 RTCs, those were summarized in Table 1. Briefly, a total of 196 patients were studied (106 CM-OIT patients, 90 control patients) and all were children. Sixty-six patients (62%) in the CM-OIT group could tolerate a full serving of milk (approximately 200 mL) compared with 7 (8%) in the control group (RR 6.61, 95% CI 3.51 to 12.44). In addition, 27 (25%) in the CM-OIT group could tolerate ingestion of a partial serving of milk (10 to 184 mL) while none in the control group could (RR 9.34, 95% CI 2.72 to 32.09). None of the studies assessed the patients following a period off immunotherapy. Adverse reactions were common (97 of 106 CM-OIT patients had at least one symptom), although most were local and mild. For every 11 patients receiving CM-OIT one required intramuscular epinephrine. One patient required it on 2 occasions.

In these 5 RTCs, the change in CM-sIgE was evaluated before and after OIT in both groups.⁴⁵ In 3 of the articles,^{8,9,46} the authors

found no significant differences in the variations in sIgE before and after OIT. The results of Martorell et al.⁸ revealed a significant decrease in sIgE levels. Longo et al.⁷ observed a significant decrease in CM-sIgE levels in half of the patients. The results of our 1-step individual patient data meta-analysis show a difference of 8.1 kUa/L (95%CI, 7.8 to 24) in IgE levels between patients who were treated with OIT and those who were not, which was not statistically significant ($P = 0.318$). In the 2-step approach, the mean difference was 11.3 kUa/L (95%CI, -1.9 to 24.5; $P = 0.098$). Thus, a greater decrease was found in specific levels of serum CM-IgE in patients treated with OIT compared with placebo, although the difference was not statistically significant.

Thus, studies to date have involved small numbers of patients and the evidence is generally low quality. The current evidence shows that CM-OIT can lead to desensitization in the majority of individuals with IgE-related CM allergy although the development of long-term tolerance has not been established. A major drawback of CM-OIT is the frequency of adverse effects, although most are mild and self-limited. The use of parenteral epinephrine is frequent. The study concluded that guidelines would be required before incorporating desensitization into clinical practice because there are no standardized protocols.

Follow-up studies and sustained unresponsiveness

More than 2 y of follow-up in studies from the start of the OIT is rare, and only 2 reports were found. In Italy, the desensitization rate is 86% a year after study entry, and it decreased to 70% at 4 y and 6 months after study entry.⁴⁷ In a study by Keet et al. with a median follow-up of 4 years,⁴⁸ 23% of the OIT group had no adverse events, 38% experienced, 19% had anaphylaxis, and 9% were injected with epinephrine. They conclude that the results of long-term follow-up study were not better because of the existence of frequent adverse event. In particular, high sIgE levels persisted in the patients who had adverse events in the long-term.

Table 1. Characteristics and results of the 5 RTCs.

| | Long et al [7] | Skipak et al [10] | Pajano et al [9] | Martorell et al [8] | Salmivesi et al [46] |
|------------------------------------|---|---|------------------------------------|---|---|
| Number of patients | 60 | 60 | 30 | 60 | 28 |
| Age range (years) | 5–17 | 6–17 | 4–10 | 2–3 | 6–14 |
| Study design | RCT, not blinded | RCT, double blinded | RCT, double blinded | RCT, not blinded | RCT, double blinded |
| Group of treatment (N) | OIT (30) Milk free Diet (30) | OIT (13) Placebo: placebo powder (7) | OIT (15) Placebo: soy milk (15) | OIT(30) Milk free Diet (30) | OIT (15) Placebo: oat, rice or soy milk (10) |
| Withdrawal (N) | 0 | 1 | 3 | 5 | 4 |
| Maximum tolerated Dose (mL) | 150ml | 500mg | 200ml | 200ml | 200ml |
| OIT duration (weeks) | 52 | 23 | 18 | 16 | 23 |
| Major outcome | Full desensitization | Full desensitization | Full desensitization | Full desensitization | Full desensitization |
| Measure major outcome | DBPCFC | DBPCFC | DBPCFC | DBPCFC | DBPCFC |
| Secondary outcome | Partial desensitization Safety Immunological change | Safety Immunological change | Safety Immunological change | Partial desensitization Safety Immunological change | Partial desensitization Safety Immunological change |
| Results of outcome | | | | | |
| Full desensitization RR (95%CI) | 23.00 (1.42–373.46) | 5.14 (0.32–83.70) | 21.0 (1.34–328.836) | 3.86 (1.99–7.46) | 13.74 (0.92–205.49) |
| Partial desensitization RR (95%CI) | 31.00 (1.94–495.61) | 9.71 (0.64–146.98) | 3.00 (0.13–68.26) | 3.00 (0.13–70.83) | 2.37 (0.13–44.40) |
| SigE to CM RR (95%CI) | 27.60 (15.10–40.10) | –16.73 (–61.61–28.15) | 0.66 (–2.81–4.13) | 17.50 (5.49–29.51) | 9.96 (–5.88–25.80) |

RCT, randomized control trial OIT, oral immunotherapy DBPCFC, double-blind placebo-controlled food challenge

In a 7 y follow-up study by Paassita et al., 14 of 24 subjects continued ingestion of 200 mL of CM, while 3 (21.4%) still reported symptoms associated with milk consumption.⁴⁹ Of the 10 remaining children, 2 children used milk products daily but consumed less due to symptoms and 8 (33.3%) had discontinued milk consumption. In our previous report using microwaved CM, the desensitization rate in the 31 enrolled children was 45%, 60%, 70% and 80% after 1, 2, 3 and 4 y of follow-up, respectively. The rate significantly increased in a time-dependent manner. The desensitization during CM-OIT was defined as the persistence for 2 months with no adverse event including oral swelling with daily ingestion of 200 ml of CM.¹⁶ The desensitization rate was 85% in our previous study, which was extremely high, compared with that of these 2 studies. Because the allergenicity of CM decreased after microwave heating, it resulted in fewer adverse events. Therefore, the dose of CM could be increased without allergic symptoms and so may induce earlier tolerance to CM, even if the mechanism is not clear. The effect of heating CM for 100 s at 600 W in a microwave may be considered similar to that of boiling for 30 minutes.

Generally, the definition of desensitization by clinical oral immunotherapy is difficult. In our previous study, desensitization by OIT was defined as the persistence of no adverse events during daily 200 mL CM ingestion for 2 months.

Clinical immunotolerance by OIT is also called sustained unresponsiveness (SU), which was defined as negativity of OFC 2 weeks after the discontinuation of 200 mL of daily milk ingestion. In our previous study the period following the discontinuation of OIT was relatively short (2 weeks) to assess sustained unresponsiveness. Notably, the timing was chosen to be in line with NIAID-FDA recommendations on food allergy clinical trial design at the time the study was designed and registered.⁵⁰ It is acknowledged that a longer period of at least 4 weeks after discontinuation of treatment would now be advised.

Only 2 studies including our report, evaluated SU during OIT. Sato et al.⁵¹ described the rate of SU in CM-OIT in which 75% of patients were successfully desensitized. However, patients who passed the OFC 2 weeks after ceasing the OIT one year after the start of OIT included 27.1% of patients on CM, and those patients might have achieved SU. The published rates for SU are similar to those obtained in our previous study, but the rates of achieving desensitization were higher than in our study. Also in our study the rate of SU at 1, 2, 3, and 4 y after the start of OIT was 21%, 47%, 53%, and 70%, respectively. Thus, children enrolled in CM-OIT could achieve clinical tolerance as defined by SU when OIT was maintained for as long as possible.

Thus, there are few studies on long-term follow-up and SU in CM-OIT. However, current results suggest that children with CM allergy can achieve clinical immunotolerance, which is characterized as desensitization followed by SU, for as long as OIT is continued.

Mechanism of oral immunotherapy

The mechanism of CM-OIT has not been elucidated in detail. It is speculated that dynamic immunological change, as characterized by the basophil activation test *in vitro* and the skin prick

test *in vivo*, correlates with the desensitization of mast cells and basophils by allergic stimulation in the early stages of OIT.⁵² Frequent allergic stimulation induces mast cell desensitization, resulting in an increased tolerated dose of allergen. Furthermore, this event may induce allergen-specific FoxP3⁺ T regs, producing the cytokines interleukin (IL)-10, transforming growth factor (TGF)- β , and interferon γ .^{53,54}

Collins and Jackson⁵⁵ suggest that early in the germinal center reaction, IgM⁺ B cells first switch to both IgE and IgG3, then to IgG1 cells, followed by IgG2-committed cells and finally, upon continued exposure to the antigen, to IgG4-producing cells, which coincides with the arrangement of the immunoglobulin heavy gene locus. As the patients repeatedly received high doses of the allergen during OIT, the fold changes in the levels of IgG subclasses observed in this study can be explained by class switching pathways ($\mu \rightarrow \gamma 3 \rightarrow \gamma 1 \rightarrow \gamma 2 \rightarrow \gamma 4$). IgG3 and IgG1, which are encoded by adjacent gene segments of immunoglobulin heavy-chain C-region genes in the switching pathways, show similar inverted V-shape response patterns. IgG2 and IgG4, which are also encoded by adjacent gene segments, showed a similar increase.

In addition, high-affinity IgE is also generated through sequential class switching ($\mu \rightarrow \gamma 3 \rightarrow \gamma 1 \rightarrow \epsilon$).⁵⁶ During OIT, repeated high-dose allergen stimulation may cause class IgG subclass switching ($\mu \rightarrow \gamma 3 \rightarrow \gamma 1 \rightarrow \gamma 2 \rightarrow \gamma 4$), instead of sequential class switching ($\mu \rightarrow \gamma 3 \rightarrow \gamma 1 \rightarrow \epsilon$), by producing cytokines such as IL-10 and TGF- β .

It is acknowledged that the affinity of antibody for allergen is weak in IgM antibodies, gradually increasing through IgG class switching.⁵⁵ Allergen-specific IgG4 antibodies act as blocking antibodies, that is, they are able to compete for allergen binding. Thus, specific IgG4 antibodies inhibit the release of mediators from mast cells and basophils. Therefore, specific IgG4 antibodies can produce a decrease in the processing and presenting activity of APCs (dendritic cells and B cells) thereby inhibiting the binding of allergen-IgE complexes to CD23⁵⁷ completely blocking allergen to bind IgE. Furthermore, IgG antibodies induced during OIT could act through the inhibitory receptor Fc γ IIb to decrease IgE-mediate hypersensitivity.⁵⁸ Dendritic cells in the early stages of OIT produce IL-10, interferon γ and decreased IL-6, resulting in suppression of IgE mediated-hypersensitivity.⁵⁹

Oral immunotherapy with omalizumab

In general, patients with severe asthma can be treated with the anti-IgE monoclonal antibody omalizumab (OMB).⁶⁰⁻⁶² Nadou et al. first reported CM-OIT with omalizumab (OMB) in 2011.⁶³ After 9 weeks of the OMB pretreatment, the OIT protocol was started in 11 children with severe CM allergy. Nine of ten milk-allergic patients (age 7–17 years) reached the dose of 1 g of milk protein during the rapid phase. Nine of the 10 patients could tolerate 2 g of milk protein over 5 weeks of the build-up phase. Eight weeks after discontinuation of OMB, these 9 patients tolerated 7.25 g of milk protein in the DBPCFC. The mean frequencies of adverse reactions during the study were very low (1.6%), especially in the escalation phase where epinephrine was required for only 2 patients. The study confirmed the efficacy and safety of the combination of OMB and

OIT. However, after the discontinuation of OMB, adverse allergic reaction may occur during the home maintenance phase, although these children were previously tolerant.

Only one randomized controlled study on the use of OMB in CM-OIT using a placebo control without OMB has been reported in 2016.⁶⁴ Fifty-seven milk-allergic patients (7–32 years) were randomized 1:1 (for 4 months before the treatment and continued dosing for 24 months of into OIT) of placebo. At month 28, 24 (88.9%) OMB-treated subjects and 20 (71.4%) placebo-treated subjects passed the 10 g “desensitization” OFC ($P = 0.18$). At month 32, SU was demonstrated in 48.1% in the OMB group and 35.7% in the placebo group ($P = 0.42$). Adverse reactions were markedly reduced during OIT escalation in OMB-treated subjects for percentages of doses per subject provoking symptoms (2.1% vs 16.1%, $P = 0.0005$), dose-related reactions requiring treatment (0.0% vs 3.8%, $P = 0.0008$), and doses required to achieve maintenance (198 vs 225, $P = 0.008$). Thus, the study found a significant improvement of safety but not in efficacy. However, the study control was CM-OIT, not complete elimination of CM protein. Previous studies did not demonstrate the efficacy of CM-OIT compared with elimination of CM in combination with OMB. Therefore, RTCs of CM-OIT with OMB with elimination of CM protein as a control will need to be performed in the future.

We also reported successful desensitization in a boy with severe CM allergy by a combination therapy using OMB and rush oral immunotherapy and long-term follow-up observations after OMB discontinuation.⁶⁵ A 5-year-old boy presented with a history of 2 severe episodes of anaphylaxis (at the age of 2 and 3 years) after consuming small amounts of CM. Before the OIT, sIgE levels for CM were 77.0 kUA/L. The SPT for CM showed a wheal (diameter, 20 mm). In the open food challenge, he reacted to the ingestion of 0.2 mL of CM and presented with dyspnea and laryngospasms, and he was then administered 150 mg OMB every 2 weeks for 8 weeks. In the ninth week, he was admitted to hospital for the rush phase of the OIT. Once he was able ingest a dose of 200 mL CM without having an adverse reaction, he was discharged and allowed to continue a daily dose of 200 mL CM at home. During this phase, his sIgE levels were elevated, but the end-point titration values from the SPT gradually decreased, and the SPT was negative after one year of OMB treatment. Five months after discontinuation of OMB, the daily CM ingestion was ceased for 2 weeks period, followed by an oral food challenge (OFC) that was negative. The patient experienced only 5 mild adverse events during the course of the rush OIT, even after the discontinuation of OMB and his quality of life improved dramatically afterwards. This case report describes how the combination of OIT with OMB enables children with very severe life threatening milk allergy to achieve complete desensitization to CM without any allergic reactions. Furthermore, it indicates SPT is useful to find the timing of discontinuation of OMB.

Collectively, the combination of OIT with OMB may accelerate desensitization and could be useful to inhibit frequent adverse events during OIT, especially during the escalation phase. However, whether this combination of OIT and OMB will be effective with long-term follow-up remains to be elucidated.

Baked and heated milk oral immunotherapy

Heating destroys many conformational epitopes and reduces allergenicity of some foods. In 2015, Goldberg et al. investigated the efficacy of baked milk OIT in baked milk-reactive allergic patients.⁶⁶ Briefly, 15 patients (>4 y of age) who previously failed to complete our milk OIT program were enrolled into the baked milk (BM) OIT protocol. A dose of BM (180°C for 30 minutes), which was less than the eliciting dose, was increased 50% monthly until the primary outcome dose of 1.3 g/d BM protein was achieved. In terms of the primary outcome, only 3 (21%) of 14 patients tolerated the 1.3 g/d BM dose. Although some patients initially progressed with BM OIT, 8 of 11 failed because of IgE-mediated reactions. Three did not complete the program because of non-IgE-mediated factors. An increase in challenge threshold to BM was noted in patients continuing until 12 months ($P = 0.003$), including those among whom reactions precluded continuation in the program. The authors concluded that the use of hypoallergenic BM in OIT is a promising therapy, but care must be taken before its administration in BM-reactive patients because of the risk of anaphylaxis and only limited increase in challenge threshold attained.

We also reported that MH-CM OIT was useful to achieve desensitization and 2-weeks SU for the children with CM allergy.¹⁶ Briefly, 48 children were enrolled in this study. Thirty-one children received rush OIT using MH-CM (the OIT group) and the other 17 children formed the untreated group. No children in the untreated group failed an open food challenge to CM. Of the 31 children in the OIT group, 14 ($P = 0.002$) achieved desensitization, and 8 ($P = 0.036$) achieved 2-weeks-SU to CM at 1 y from the start of OIT. Two years after the start of OIT, both the rate of desensitization and the rate of 2-weeks-SU in the OIT group significantly increased compared with the rates at 1 y ($P = 0.025$ and $P = 0.008$ respectively). We concluded that the rush OIT protocol using MH-CM was effective at inducing 2-weeks-SU to CM and had a good safety profile in children with CM allergy.

Recently, Bloom reported on the effect of heat treatment on the allergenicity of milk and egg proteins.⁶⁷ Interestingly, gel electrophoresis showed strongly staining casein bands that withstood up to 60 min of heating. In contrast, β -lactoglobulin and α -lactalbumin bands became progressively weaker with increasing heating times, with no detectable β -lactoglobulin after 15–20 min of heating. The presence of wheat during heating resulted in decreased IgE antibody binding to milk proteins.

Thus, it is likely that OIT using heated CM may accelerate desensitization and SU for milk-allergic children. However, why there is less allergenicity by this process remains to be elucidated.

Other immunotherapies

There is only one study on each of sublingual immunotherapy (SLIT) and epicutaneous IT (EPIT) in CM allergy. In 2009, Dupont et al. reported on the efficacy and safety of epicutaneous immunotherapy (EPIT) for CM allergic children.⁶⁸ They

concluded that the effect of EPIT was not significant in open food challenge but was safe. The study suggested that significance of EPIT has not been demonstrated because of only a 3 months study period. In 2010, Keet reported the safety and efficacy of combined sublingual and oral immunotherapy for milk allergy.⁶⁹ They compared the safety and efficacy of 2 protocols; sublingual immunotherapy followed by OIT and SLIT only. They concluded that OIT was more effective for desensitization to CM than SLIT alone but was accompanied by more systemic side effects.

Thus, these 2 protocols (SLIT, EPIT) were less effective than the OIT protocol, but OIT combination of this protocol may be safer than an OIT only protocol. Further study is needed to elucidate the efficacy and safety of the 2 protocols.

Expert opinion on future protocols

In summary, it may be effective and mostly safe to perform the conventional 3 steps procedure: rush, build up and maintenance phase or the 2 steps procedure: rush and maintenance phase using MH-CM for the mild allergic CM patient. However, alternative protocols should be available for complete desensitization to CM for patients with severe adverse signs such as high levels sIgE or reaction with only a very small amount of CM in the food challenge. The best procedure for these severe cases could be considered to first start SLIT, or EPIT, followed by conventional OIT with MH-CM. To safely complete desensitization or immunotolerance to CM allergy may require a 2-steps theory: low dose tolerance followed by high dose tolerance.⁷⁰ Low dose tolerance is mediated by regulatory T cells and cytokines, such as IL-10 and TGF- β . These tolerogenic factors may be induced by SLIT or EPIT using a small amount of CM. High dose tolerance is mediated by lymphocyte anergy or clonal deletion, which may be induced by conventional OIT. In general, in conventional OIT severe adverse event occasionally occur in the rush phase or in the early maintenance phase. Therefore, if SLIT or EPIT induced regulatory T cells and immunosuppressive cytokine before rapid dosing, it is possible to rapidly dose and maintain high doses of CM safely, resulting in inducing and maintaining high-dose CM ingestion quickly. The patient could thus achieve complete desensitization or immunotolerance. The period of use of SLIT and EPIT may be evaluated in future studies.

Conclusions

There are only 5 RTC studies of CM-OIT and these are low powered single center trials. Therefore, evidence levels were also low and sometimes frequent and severe allergic events occurs during OIT. Furthermore, studies containing long-term follow up observations and SU are rare. Additionally, clinical tolerance is not well defined and remain obscure. Thus, several problems remain to be resolved, but hopefully OIT in combination with OMB and using less allergenic heated CM products will resolve these problems in the future.

Abbreviations

| | |
|--------|--|
| CM | cow's milk |
| DBPCFC | double-blind placebo controlled food challenge |
| EPIT | epicutaneous immunotherapy |
| MH | microwave heated |
| OFC | open food challenge |
| OIT | oral immunotherapy |
| OMB | omalizumab |
| SPT | skin prick test |
| SLIT | sublingual immunotherapy |

Disclosure of potential conflicts of interest

All authors declare no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 y and no other relationships or activities that could appear to have influenced the submitted work.

Authors' contributions

ST is guarantor for this work. Drafting of the manuscript, ST and MT; critical revision, SK and YH; and supervision, ST and HM.

ORCID

Shoichiro Taniuchi  <http://orcid.org/0000-0002-1185-9705>

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