

ORIGINAL ARTICLE

Partially hydrolyzed cow's milk formula has a therapeutic effect on the infants with mild to moderate atopic dermatitis: a randomized, double-blind study

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Keywords

allergy; atopic dermatitis; infant; partially hydrolyzed formula; SCORing Atopic Dermatitis.

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Abstract

Objective: The allergy-preventing effect of partially hydrolyzed cow's milk formula (phCMF) in infants at high risk of atopic dermatitis (AD) has been demonstrated in many studies. However, the therapeutic potential of phCMF in treating the infants with AD has not been reported. We sought to assess such therapeutic efficacy of phCMF in infants with mild to moderate AD.

Methods: From 2006 to 2008, 113 infants < 6 months of age with AD were randomized to receive either partially hydrolyzed cow's milk formula (phCMF) or conventional cow's milk formula (CMF) in a double-blind clinical trial. Assessments were made at enrollment and at weeks 4, 8, and 12. The severity of AD was assessed using two scoring systems: Standard guideline for management (diagnosis, severity scoring, and therapy) of AD by the Japanese Dermatological Association Scoring System (JDASS) and the SCORing Atopic Dermatitis (SCORAD). Growth status of the infants was evaluated. Allergy profile was assessed by measuring total blood eosinophils (EOS), total/specific IgE, Th1/Th2 cytokine profiles, and the percentage of regulatory T cells.

Results: After follow-up for 12 wk, 27 infants (23.89%) dropped off study. Analysis was performed on 86 infants by the end of 12-wk observation. The AD severity scores were significantly reduced in the phCMF group ($n = 56$) compared with CMF group ($n = 30$) after 12 wk ($p < 0.05$). The severity scores of phCMF group were significantly reduced at weeks 4, 8, and 12 compared to enrollment ($p < 0.05$). In contrast, no significant improvement was observed for CMF group at any of those time points ($p > 0.05$). The number of AD flare-ups was significantly decreased in the phCMF group ($p = 0.002$). Th1/Th2 ratio in phCMF was significantly increased compared with CMF group ($p = 0.041$). The growth rates did not differ significantly between these feeding groups at any assessed time point ($p > 0.05$) and were in the normal range.

Conclusion: This study suggests a novel therapeutic effect of phCMF in treating infants with mild to moderate AD during the first 6 months of their life without affecting their nutritional status.

Abbreviations:

AD, atopic dermatitis; PhCMF, partially hydrolyzed cow's milk formula; CMF, cow's milk formula; JDASS, Japanese Dermatological Association Scoring System; SCORAD, SCORing Atopic Dermatitis; eHF, extensively hydrolyzed formulas; AAF, amino acid-based formula; CMPA, cow's milk protein allergy; Treg, regulatory T cells; CMP, cow's milk protein; CMA, cow's milk allergy.

Atopic dermatitis (AD) is a chronic, relapsing, and intensely pruritic skin disorder, which onsets during the first 6 months of life in 45% of children, the first year of life in 60% of affected individuals, and by 5 yr of age in at least 85% of affected individuals (1). However, the exact prevalence in China is not clear with incidence as high as 70% in the first year of life in some areas based on survey results (2, 3). AD affects the quality of life not only of infants but also of their families. The cost for seeking medical advice and treatment is quite high. Furthermore, AD is considered to be the initial step in atopic march as it plays a critical role in the development of allergic rhinitis and/or asthma (4). Therefore, to prevent AD and to alleviate the symptoms of AD have high social and clinical values.

Although the pathogenesis of AD remains obscure, it has been suggested that genetic background, environmental triggers, and immunologic dysfunctions all contribute to etiology of this disease (5). There is a strong association between food allergy and AD, as AD severity increases so does the prevalence of food allergy and the frequency of reported adverse reactions caused by food allergy (6). In addition, controlled studies have demonstrated that a low allergenic diet is associated with a significant reduction in the prevalence of AD in infancy (7, 8).

Many studies have shown a reduced incidence of AD among infants who were exclusively breast-fed, indicating the protective effect of breast milk (9, 10). Although not all AD can be directly associated with specific allergens, the most common food allergen associated with AD is cow's milk protein (CMP). CMP constitutes the most common protein source for infants who are not exclusively breast-fed. Several formulas have been developed and used for infants who are not exclusively breast-fed in an effort to decrease the risk of allergy associated with CMP. Based on the degree of hydrolysis, the formulas are divided into partially hydrolyzed cow's milk formulas (phCMF) and extensively hydrolyzed formulas (eHF) and with respect to the protein source in whey and casein hydrolysates. PhCMF is widely used as the alternative formula for preventing AD owing to its low allergenicity. In contrast, the eHF or amino acid-based formula (AAF) is considered a treatment formula especially for infants with cow's milk protein allergy (CMPA) or severe AD. However, it is generally not recommended for the prevention and treatment of infants with mild to moderate AD (11).

We noticed previously that phCMF alleviated symptoms in infants with AD (12). It raised the question whether phCMF could have therapeutic effect on such patients with AD. If it is true, then phCMF could be a very convenient and economical treatment alternative for non-breast-fed infants. Moreover, this may give new insight into the role of dietary factors (CMP in particular) in the pathophysiology of AD, which is still a matter of debate. So we sought to investigate systematically the therapeutic efficacy of phCMF in treating infants with mild to moderate AD.

Methods

This study is a randomized, double-blind, and controlled trial to assess the therapeutic efficacy of phCMF (partially hydro-

lyzed whey and casein formula, Morinaga, 5-Chome, Higashihara, Zama-city, Japan) in treating Chinese infants with mild to moderate AD. Infants were recruited between April 2006 and August 2008 in Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, China. The protocol was approved by Institutional Review Board of Xinhua Hospital and consistent with ethical standards established by the Board.

Study population

Healthy infants below 6 months of age (birth weight > 2500 g) with mild to moderate AD were recruited in this study. Data collected during recruitment include duration of breastfeeding, time for introducing bottle feeding, family history of atopic diseases, and whether the subjects were receiving any hydrolyzed cow's or soy milk formula with or without other solid food. AD was defined by the criteria of Hanifin (13). Mild to moderate AD was defined as 10–50% of the whole body surface was involved. Written informed consent was obtained from their parents. Infants who tolerated exclusively CMF feeding or infants with breast-feeding for <10% were included in this study. Furthermore, all the infants included had no symptoms of cow's milk allergy (CMA). Exclusion criteria were as follows: severe acquired or congenital diseases (i.e., hematologic diseases, liver disorders, cardiac diseases, renal and gastrointestinal diseases), CMA, intake of any hydrolyzed cow's milk formula or soy milk at baseline, or incapability of the parents to comply with the study protocol.

Study design and protocol

The infants with mild to moderate AD were randomly assigned to receive either phCMF (Morinaga) or conventional cow's milk formula (CMF) (Morinaga) for 12 wk. The randomization was achieved by using SPSS software to generate a randomization list which study subjects were assigned based on. The parents of study subjects were advised to hold the introduction of other solid food throughout the study. The use of drugs including topical corticosteroid (mometasone furoate PRN) and oral sedating antihistamines remained unchanged. None of the infants required systemic steroids or antibiotics during the study. The infants were evaluated at the time of enrollment and at 4, 8, and 12 wk. The diagnosis and assessing the severity of AD were made by dermatologists and allergists. The detection of eosinophils, hemoglobin, and levels of serum total protein was carried out as routine well baby check. The nutrition and growth status including length, weight, head circumference, and chest circumference were evaluated by individual pediatricians. Infants were excluded from the study protocol if the diaries during the intervention period showed non-compliance with the specific feeding recommendations, diaries were missed for more than 1 wk, severe adverse reaction occurred, the infants suffered from other severe diseases required additional therapy, or the parents refused to continue the trial.

The appearance of the two types of formulas was identical to guarantee double blindness. The two formulas contain

similar calorie, fat, carbohydrate, minerals, vitamin, and protein contents to ensure adequate nutritional values. The only difference between the two formulas was that the proteins whey and casein in phCMF were partially hydrolyzed to about 3500 Da by molecular weight and the ratio of whey to casein was 60–40%, which was very close to human breast milk. Parents were asked to record in a diary details on formula intake for a random week. Infants were excluded from study if adjuvant food had been introduced.

Severity scoring of atopic dermatitis

Two scoring systems were used to assess the severity of AD. Japanese Dermatological Association Scoring System (JDASS) was based on the Eczema Area and Severity Index (EASI) (14, 15). SCORing Atopic Dermatitis (SCORAD) was also employed in the current study (16). The former is a validated tool for objectively assessing the severity of eczema, taking into account both the severity of different morphological key signs of eczema and the surface area involvement (14, 15). The system divides AD into mild AD (with mild erythema, edema, oozing) and AD with severe infection according to the morphological key signs of eczema. It assesses erythema, edema, oozing, excoriation, and lichenification separately on a 6-point scale (0–5) on the head/neck, trunk, upper limbs, and lower limbs. The index assigns proportionate values (the head and neck = 1, trunk = 3, upper limbs = 2, lower limbs = 4) to each of these four body regions.

The SCORAD index includes the assessment of objective signs (extent and intensity) and subjective symptoms (pruritus and sleep loss). Extent is calculated using the 'rule of nine' and expresses the skin surface area involved. Intensity items are erythema, edema/papulation, oozing/crusts, excoriations, lichenification, and dryness of uninvolved skin (0–3 points for each item). The final score is calculated by using the following equation: $A/5 + 7B/2 + C$ (A extent; B intensity; C subjective symptoms). An objective SCORAD index range lies between 0 and 83. Based on the SCORAD index results, AD has been classified into mild (<25), moderate (25–50), and severe (≥ 50) forms (16).

Automated ImmunoCAP System analysis for serum-specific IgE and total IgE

Serum samples from most infants were collected at the time of the initial and the last visit. They were stored at -20°C until analyzed for the total IgE and antigen-specific IgE (milk, egg protein, soy, and fish) antibodies using the Pharmacia ImmunoCAP System (Pharmacia Diagnostics, Uppsala, Sweden) according to manufacturer's instruction. Briefly, 50 μl of samples including standards was absorbed with ImmunoCAP and incubated for 30 min. After washing, 50 μl of enzyme-labeled anti-IgE was added to each well and incubated for 2.5 h. After further washing, 50 μl of developing solution was added. Incubated for 10 min, the reaction was halted by adding stopping solution and the fluorescence intensity was measured and compared with standard curve generated from standards provided by manufacturer.

Flow cytometric analysis for Th1/Th2 balance

The expression of intracellular cytokines was assessed by flow cytometry as previously described (17). Briefly, 200 μl of whole-blood samples was diluted in equal volume of RPMI 1640. They were then stimulated with phorbol myristate acetate (50 ng/ml, Jingmei biotech), ionomycin (2 $\mu\text{g/ml}$, Sigma), and monensin (2 μM , Jingmei biotech), respectively, for 4 h at 37°C in 5% CO_2 . Stimulated cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% saponin. Cells were simultaneously stained with fluorescence-labelled monoclonal antibodies against the T-cell-surface markers (CD3, CD4, CD8) and antibodies against human IFN- γ and IL-4 (Becton Dickinson, San Jose, CA, USA). Fluorescence-labelled cells were analyzed on FACSCalibur (Becton Dickinson) by using Cellquest software. In all samples, 10,000 events were collected and analyzed. Results were presented as percentage of cytokine-producing cells in each cell subtype.

Flow cytometric analysis for regulatory T cells

The regulatory T cells were detected by a Foxp3 staining set (eBioscience, San Diego, CA, USA) following the manufacturer's instruction. Briefly, whole blood was incubated with PerCP-conjugated anti-CD4 and PE-conjugated anti-CD25 for 30 min at 4°C in the dark and then followed by fixation and permeabilization in Fix/Perm buffer (eBioscience) for 45 min. Subsequently, FITC-conjugated rat anti-human Foxp3 was added. After incubation for 45 min at 4°C , the cell suspension was washed and analyzed on a FACSCalibur (Becton Dickinson).

Statistical analysis

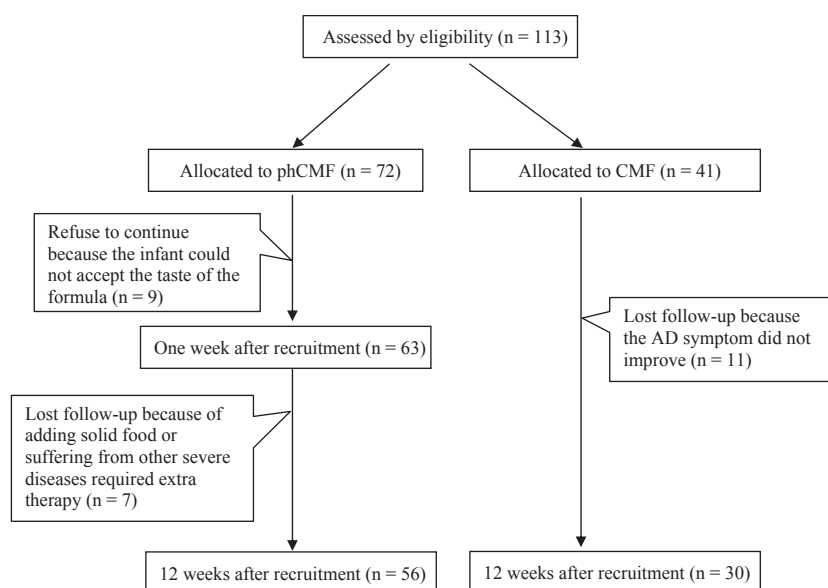
To fulfill the criterion that a therapeutic formula has to be tolerated by 90% of infants with a confidence of 95%, 30 subjects were required to complete the study in each group. Assuming a 20% dropout rate, a minimum of 75 subjects had to be recruited.

The randomized grouping and statistical analysis were performed with SPSS (Statistical Package for Social Science) version 14.0. Wilcoxon rank sum test was used to compare the scores at 4, 8, and 12 wk with the time of enrollment within each group. Mann–Whitney U non-parametric statistical test was used to compare the scores between groups. Chi-square test was used to compare the rate between groups. The growth data and immunologic data were assessed with t -test. A p value < 0.05 was considered significant.

Results

Patients' information

One hundred and thirteen infants with mild to moderate AD were enrolled. Sixteen (22.22%) dropped out during 12 wk' follow-up in phCMF group because of unacceptable taste of the formula ($n = 9$), introduction of solid food or suffering from other severe diseases that required additional therapy

**Figure 1** Trial profile.

($n = 7$). Eleven of the 41 infants (26.83%) in conventional CMF group lost follow-up because AD was not improving (Fig. 1). Analysis was performed on 86 infants. All 86 infants were term infants with normal weight and on either exclusively CMF feeding or mix feeding (breast milk amount: CMF amount < 1: 9). None had been exposed to hydrolyzed milk formula or introduced to solid foods before entering the study. Sixty-two were boys (72.09%) and 24 were girls (27.91%). Median age of the infants was 4.3 m, with range from 1 to 6 m. Fifteen (17.44%) of them were mild AD cases and 71 (82.56%) were moderate in nature according to the SCORAD scoring system. Fifty-six infants were enrolled in phCMF group and 30 cases in conventional CMF group. The details regarding age, sex distribution, severity of AD, and family history of atopy are shown in Table 1 and were not statistically significant between the two groups. Forty-nine infants had a family history of atopy with allergic rhinitis being dominant.

Clinical symptoms of atopic dermatitis

We evaluated the severity of the 86 infants with AD by JDASS and SCORAD scoring system. The median (range) scores of AD in conventional CMF group ($n = 30$) before enrollment were 4 (3–28) by JDASS and 37 (19–56) by SCORAD, while phCMF group ($n = 56$) scored 10 (2–40) and 37 (12–65), respectively. For each scoring system, there was no statistical difference between the two groups (JDASS: $Z = 1.054$, $p = 0.292$; SCORAD: $Z = 0.268$, $p = 0.789$). At week 12, phCMF group showed significantly lower AD scores than CMF group (JDASS: $Z = 2.332$, $p = 0.020$; SCORAD: $Z = 3.513$, $p = 0.001$). Within phCMF group, the scores at weeks 4, 8, and 12 were significantly low than that at enrollment time (JDASS: $p = 0.003$, 0.001 , 0.001 ; SCORAD: $p = 0.001$, 0.001 , 0.001) (Fig. 2). The effect did not differ between different age groups ($p = 0.090$, data not shown). In contrast, scores of CMF group showed no signifi-

Table 1 General characteristics of patients analyzed in the study protocol (Case)

Group	PhCMF $n = 56$ (%)	Conventional CMF $n = 30$ (%)
Age distribution (months)		
1–2	6 (10.71)	2 (6.67)
2–3	9 (16.07)	5 (16.67)
3–4	10 (17.86)	11 (36.67)
4–5	20 (35.71)	7 (23.33)
5–6	11 (19.64)	5 (16.67)
Sex distribution		
Male	39 (69.64)	23 (76.67)
Female	17 (30.36)	7 (23.33)
Severity (SCORAD)		
Mild (<25)	12 (21.43)	3 (10.00)
Moderate (25–50)	44 (78.57)	27 (90.00)
Family history for atopy		
Positive	33* (58.93)	16† (53.33)
Negative	23 (41.07)	14 (46.67)

PhCMF, partially hydrolyzed cow's milk formula; Conventional CMF, conventional cow's milk formula. *16 allergic rhinitis, 3 drug allergy, 6 food allergy, 1 asthma; 2 urticaria, 5 multiple. †7 allergic rhinitis, 2 drug allergy, 4 food allergy, 2 asthma; 1 urticaria.

cant difference between week 12 and enrollment time. (JDASS: $p = 0.431$; SCORAD: $p = 0.132$, Fig. 2).

During the 12-wk intervention, in phCMF group, there was a significant shift of patients from moderate AD to mild AD as the number of moderate AD cases decreased, while the number of mild AD cases increased. The change was statistically significant ($\chi^2 = 35.654$, $p = 0.001$). Again, in

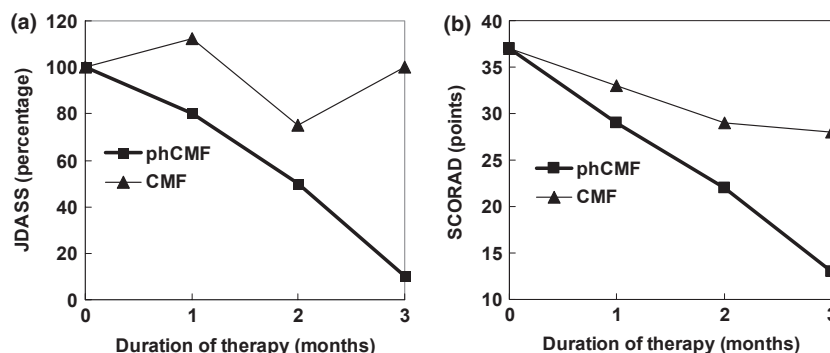


Figure 2 Severity of AD during treatment. Data presented as median (range). PhCMF, partially hydrolyzed formula; CMF, conventional cow's milk formula. (a) JDASS index between phCMF and CMF groups. Statistical analysis: 1 m, $p = 0.292$; 2 m, $p = 0.389$; 3 m, $p = 0.020$. Data expressed as percentage of enrollment which was normalized to 100%. The median (range) scores for phCMF were 1 m, 8.00 (1–40); 2 m, 5.00 (0–29); and 3 m, 1.00 (0–39) and 1 m, 4.00 (3–28); 2 m, 3.00 (0–24); and 3 m, 4.00 (0–27) for CMF. The difference between the scores at weeks 4, 8, and 12 and enroll-

ment for phCMF: $p = 0.003$, 0.001, 0.001 and CMF: 0.430, 0.035, 0.431. (b) SCORAD index in phCMF and CMF treatment patients. Statistical analysis: 1 m, $p = 0.251$; 2 m, $p = 0.070$; 3 m, $p = 0.001$. The median (range) scores for phCMF were 1 m, 29.00 (7–58); 2 m, 22.00 (0–73); and 3 m, 13.00 (0–84) and for CMF, 1 m, 33.00 (6–61); 2 m, 29.00 (0–61); and 3 m, 28.00 (0–64). The difference between the scores at weeks 4, 8, and 12 and enrollment for phCMF: $p = 0.001$, 0.001, 0.001 and CMF: $p = 0.001$, 0.002, 0.132.

conventional CMF group, the ratio between mild and moderate AD cases did not differ significantly ($\chi^2 = 2.917$, $p = 0.405$, Table 2).

To evaluate the effect of phCMF on the distribution of skin lesions of patients with AD, we compared skin lesions in four areas of the body between week 12 and at enrollment time (upper limb, lower limb, trunk, and pate). Except for the lower limb ($p = 0.263$), there was a significant decrease in percentage involvement in all other three body locations in phCMF group ($p = 0.032$, 0.016, 0.019). On the other hand, such change was not noticed in conventional CMF group ($p = 0.138$, 0.256, 0.117, 0.213).

Next, we compared the number of acute flare-ups of AD in phCMF and conventional CMF groups. Such episodes were observed and recorded by their parents. As shown in Table 3, phCMF treatment significantly reduced the number of flare-ups ($p = 0.003$), while conventional CMF failed to show such benefit ($p = 0.348$).

Table 2 Number of patients in phCMF and conventional CMF groups with different severity (SCORAD) with time prolonged (%)

	PhCMF (n = 56)		Conventional CMF (n = 30)	
	Mild <25	Moderate 25–50	Mild <25	Moderate 25–50
0 month	12 (21.43)	44 (78.57)	3 (10.00)	27 (90.00)
1 month	22 (39.29)	34 (60.71)	7 (23.33)	23 (76.67)
2 month	36 (64.29)	20 (35.71)	8 (26.67)	22 (73.33)
3 month	40 (71.43)	16 (28.57)	6 (20.00)	24 (80.00)
χ^2	35.654		2.917	
p	0.001		0.405	

PhCMF, partially hydrolyzed cow's milk formula; Conventional CMF, conventional cow's milk formula.

Growth data

To evaluate the impact of phCMF feeding on the growth of infants, we compared growth parameters of selective patients at pre- and post-treatment. Data were obtained from 21 patients at enrollment time (age, 3–4 months, phCMF, $n = 10$, CMF, $n = 11$) and at the end of treatment (age, 4–5 months, phCMF, $n = 20$, CMF, $n = 7$). There was no difference between phCMF and conventional CMF groups regarding their length, weight, head circumference, and chest circumference (all $p > 0.05$, data not shown). The mean level of hemoglobin and total serum protein in phCMF was 118.65 ± 12.71 and 57.39 ± 5.46 g/l for pre-treatment ($p = 0.324$ and 0.068) and 115.88 ± 8.98 and 60.69 ± 3.45 g/l for post-treatment ($p = 0.279$ and 0.059) with no difference comparing to conventional CMF group.

Eosinophil counts and the level of total IgE and serum-specific IgE

To evaluate the allergy status of infants enrolled in the study, we measured blood eosinophil numbers and the levels of total

Table 3 The effect of phCMF and conventional CMF on disease flare-ups ($\chi \pm$ s.d.)

Group	PhCMF (n = 56)	Conventional CMF (n = 30)
1 month	9.17 ± 6.38	6.31 ± 6.40
2 month	5.06 ± 4.45	9.38 ± 5.27
3 month	3.44 ± 3.57	8.00 ± 5.99
F	6.415	1.081
p	0.003	0.348

PhCMF, partially hydrolyzed cow's milk formula; Conventional CMF, conventional cow's milk formula.

IgE and serum-specific IgE at enrollment and by the end of the follow-up. The median (range) of eosinophil counts was 649 (44–2900)/ μ l (pre-treatment) and 573 (66–2860)/ μ l (post-treatment) in phCMF group ($n = 56$) and was 561 (132–3600)/ μ l (pre-treatment) and 430 (44–2794)/ μ l (post-treatment) in conventional CMF group ($n = 30$). The median (range) of serum total IgE concentration in phCMF group ($n = 36$) was 8.43 (3.54–105) IU/ml (pre-treatment) and 21.15 (4.60–122) IU/ml (post-treatment). There was no significant difference in eosinophil counts and the level of total IgE between phCMF and conventional CMF groups at both pre- and post-treatment time ($p > 0.05$). For all the infants enrolled in the study, serum-specific IgE to milk, egg protein, soy, and fish was measured at baseline (pre-treatment) and the values were all under detectable level (< 0.35 IU/ml). After 12 wk' follow-up, 2 (3.57%) infants in phCMF group showed positive cow's milk-specific IgE and 4 (7.14%) patients had egg albumin-specific IgE in their sera. In conventional CMF group, 4 (13.33%) were cow's milk-specific IgE-positive and six patients (20.00%) had egg albumin-specific IgE in their sera. The level of cow's milk-specific IgE varied from 0.53 IU/ml to 2.19 IU/ml, while egg albumin-specific IgE between 0.59 IU/ml and 2.67 IU/ml. There was no significant difference in various specific IgE between phCMF and conventional CMF groups ($p = 0.177$ and 0.090, respectively).

Th1/Th2 cytokine ratio and regulatory T cells (Treg)

Th1/Th2 cytokine ratio and the percentage of $CD4^+CD25^+Foxp3^+$ Treg were evaluated to explore the effect of phCMF and conventional CMF on infants' immune function. The results showed that there were no significant differences between phCMF and conventional CMF groups at either enrollment or week 12 time point. In phCMF group, both Th1/Th2 cytokine ratios and the percentage of $CD4^+CD25^+Foxp3^+$ Treg cells showed a trend of increase (from 4.82 ± 3.26 to 7.07 ± 2.89 , $n = 34$, $t = 2.131$, $p = 0.041$; and from $0.57 \pm 0.28\%$ to $0.72 \pm 0.34\%$, $n = 18$, $t = 1.390$, $p = 0.174$, respectively). Conventional CMF had no effect on Th1/Th2 cytokine ratios and the percentage of $CD4^+CD25^+Foxp3^+$ Treg cells ($p = 0.113$ and 0.071, respectively).

Discussion

To our knowledge, this is the first study in evaluating the therapeutic effect of phCMF on the infants with AD. We found that partially hydrolyzed whey and casein formula has a therapeutic effect on the infants with mild to moderate AD. Such a formula not only alleviated patients' symptoms but also reduced the flare-ups of AD. The result suggests that this formula can be applied as one of the 'maintenance' treatments for infants with mild to moderate AD. And the role of phCMF in the treatment of AD is less likely due to the elimination of CMA as infants with clear CMA were excluded in our study. In addition, all infants enrolled in our study tolerated CMF as the exclusive diet intake or constituted $> 90\%$ of infants' energy intake. The levels of total IgE and serum

milk-specific IgE were all negative at enrollment. The levels of milk-specific IgE after 12 wk' follow-up were all below the critical decision-making levels established by Sampson's group (18) (milk-specific IgE, 32 kU/l; egg albumin-specific IgE, 6 kU/l). Although we cannot completely exclude the possibility of elimination of CMA as part of the mechanism, other explanations may include the introduction of immune tolerance owing to lower molecular weight. Many studies in rodents showed that oral tolerance to whey allergens can be induced by partially hydrolyzed formulas but not by eHF (19). Development of oral tolerance to intact proteins has been explained by several mechanisms, which include Th anergy, Ts activation, and bystander suppression. Using phCMF as a treatment alternative for infants with mild to moderate AD avoids allergen contact as well as serving as diet substitution. The complete mechanism remains to be elucidated.

A number of experimental and clinical data indicate that the partially hydrolyzed formula has preventive effect against allergic diseases in high-risk infants (20, 21). Such effect may last until age 6 (22). However, whether this type of formula has the therapeutic effect in infants with mild to moderate AD is unknown. Our study confirmed such speculation for the first time.

Besides the therapeutic effect, the nutritional value is one of the clinically important pre-request in choosing an alternative formula. AAF and eHF are sometimes associated with growth impairments (23). In contrast, our studies and many others' have shown that the partially hydrolyzed CMF does not affect the nutritional status of patients (24).

Th1 and Th2 cells are two distinct T-cell subsets, defined by cytokine profiles and effector function. $IFN-\gamma$ is known to be the signature cytokine of Th1 cells and IL-4 for Th2 cells. In allergy diseases, the patient's Th1/Th2 cytokine ratio often shifts to the enhancement of Th2 function. In this study, we have shown that phCMF remarkably increased Th1/Th2 cytokine ratios. The phCMF used in our study has an average molecular weight of 3500 Da, which corresponds to a size of about 30 amino acids. These fragments are less immunogenic than intact CMP with regard to binding to immunoglobulins but will very likely to be processed by antigen-presenting cells and recognized by CMP-specific T cells. Whether Th1 cells preferentially bind to such antigen is unclear.

Early manifestation of AD has been associated with an increased risk of respiratory diseases such as bronchial asthma, hay fever, or perennial allergic rhinitis at later age, suggesting that AD can be a starting point for the so-called atopic march or allergy march (25). Effective management of patient at AD stage may prevent or slow down the development of subsequent allergic diseases. Early intervention appears to be crucial for infants with AD and those high-risk infants. The most suitable time for intervention is before the addition of solid food (26).

In conclusion, we demonstrated the therapeutic efficacy of partially hydrolyzed CMF in Chinese infants with mild to moderate AD in the first 6 months of life. We further illustrated that phCMF provided sufficient nutrition for patients'

growth. Therefore, phCMF could be considered as a beneficial alternative for the infants with mild to moderate AD. Limitations of our study include high dropout rate, relative small sample size, and non-assessment of the use of eczema topical treatments. Future study is necessary to further elucidate the underlying mechanism of phCMF on immune system.

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