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REFERENCES

1. Powell WS, Rokach J. Biosynthesis, biological effects, and receptors of hydroxyeicosatetraenoic acids (HETEs) and oxoeicosatetraenoic acids (oxo-ETEs) derived from arachidonic acid. *Biochim Biophys Acta*. 2015;1851:340-355.
2. Konno Y, Ueki S, Takeda M, et al. Functional analysis of free fatty acid receptor GPR120 in human eosinophils: implications in metabolic homeostasis. *PLoS ONE*. 2015;10:e0120386.
3. Lingblom C, Wallander J, Ingelsten M, et al. Eosinophils from eosinophilic oesophagitis patients have T cell suppressive capacity and express FOXP3. *Clin Exp Immunol*. 2017;187:455-465.
4. Isobe Y, Kato T, Arita M. Emerging roles of eosinophils and eosinophil-derived lipid mediators in the resolution of inflammation. *Front Immunol*. 2012;3:270.
5. Arita M. Eosinophil polyunsaturated fatty acid metabolism and its potential control of inflammation and allergy. *Allergol Int*. 2016;65:S2-S5.
6. Miyata J, Fukunaga K, Iwamoto R, et al. Dysregulated synthesis of protectin D1 in eosinophils from patients with severe asthma. *J Allergy Clin Immunol*. 2013;131:353-360.
7. Feltenmark S, Gautam N, Brunnstrom A, et al. Eoxins are proinflammatory arachidonic acid metabolites produced via the 15-lipoxygenase-1 pathway in human eosinophils and mast cells. *Proc Natl Acad Sci USA*. 2008;105:680-685.
8. Jonsson K, Barman M, Moberg S, et al. Serum fatty acids in infants, reflecting family fish consumption, were inversely associated with allergy development but not related to farm residence. *Acta Paediatr*. 2016;105:1462-1471.
9. Barman M, Johansson S, Hesselmar B, Wold AE, Sandberg AS, Sandin A. High levels of both n-3 and n-6 long-chain polyunsaturated fatty acids in cord serum phospholipids predict allergy development. *PLoS ONE*. 2013;8(7):e67920.
10. Vahdaninia M, Mackenzie H, Dean T, Helps S. omega-3 LCPUFA supplementation during pregnancy and risk of allergic outcomes or sensitization in offspring: a systematic review and meta-analysis. *Ann Allergy Asthma Immunol*. 2019;122:302-313.
11. Stratakis N, Roumeliotaki T, Oken E, et al. Fish and seafood consumption during pregnancy and the risk of asthma and allergic rhinitis in childhood: a pooled analysis of 18 European and US birth cohorts. *Int J Epidemiol*. 2017;46:1465-1477.

SUPPORTING INFORMATION

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Oral immunotherapy with antigenicity-modified casein induces desensitization in cow's milk allergy

To the Editor

Cow's milk allergy (CMA), one of the most common types of food allergy, can cause life-threatening anaphylaxis.[1,2] Attempts to treat CMA using allergen-specific immunotherapy have yielded high rates of adverse events (45.4%-95% of treatment doses).[3] Although oral immunotherapy (OIT) is more effective than sublingual or epicutaneous immunotherapy, it can cause serious adverse reactions and thus has a safety disadvantage.[3] Therefore, it is relatively difficult to achieve clinical tolerance via OIT with cow's milk[4] (Figure 1).

Researchers have attempted to improve allergen immunotherapy using a variety of techniques involving antigenicity-modified allergens. Synthetic T-cell-tolerizing peptides corresponding to cat and ragweed could be used to suppress IgE-mediated allergic diseases, such as allergic rhinitis and asthma.[5] In food allergy, immunotherapy using T-cell-tolerizing peptides is not well established.

As casein is the dominant allergen component of cow's milk (rather than β-lactoglobulins),[6] we have developed a novel antigenicity-modified form of cow's milk casein digested to avoid cross-reaction with IgE and retain the T-cell reaction.[7] We used this casein hydrolysate in a pilot study of OIT in children with IgE-mediated CMA, with the objectives of determining whether this hydrolyzed casein could induce desensitization, and examining the safety of this system.

We recruited children aged 4 years and older with immediate-type IgE-mediated CMA. After determining the threshold for casein (93% [w/w] of protein; Bean Stalk Snow, Tokyo, Japan) through open-label oral food challenges (OFC), slow OIT was performed (Figure S1). Detailed information about this study is available in this article's online repository. The casein or hydrolyzed casein dose was expressed as the equivalent amount of cow's milk, and the maximum casein hydrolysate dose was 1024 mg (equivalent to 40 mL of cow's milk).

The baseline characteristics of the participants and threshold of OFC during immunotherapy are summarized in Table 1. Fifteen participants with IgE-mediated CMA were voluntarily enrolled, and 13 participants were allocated to the OIT. The median age was 6 years (range: 4-14 years), and three participants were female. All had histories of acute reactions to cow's milk, including urticaria, vomiting, wheezing, and/or anaphylaxis, and 10 had a history of anaphylaxis (grade 3-4 according to the report by Sampson et al[8]). All exhibited elevated serum IgE (range: 387-8120 IU/mL), milk-specific IgE (range: 2.39 to ≥100 kU_A/L), and casein-specific IgE (range: 1.52 to ≥100 kU_A/L). Subject#2 was found to tolerate cow's milk, and Subject#7 developed anaphylaxis to the minimum casein and hydrolyzed casein doses; both withdrew from the study. Among the remaining 13 participants, 7 and 4 had atopic dermatitis and bronchial

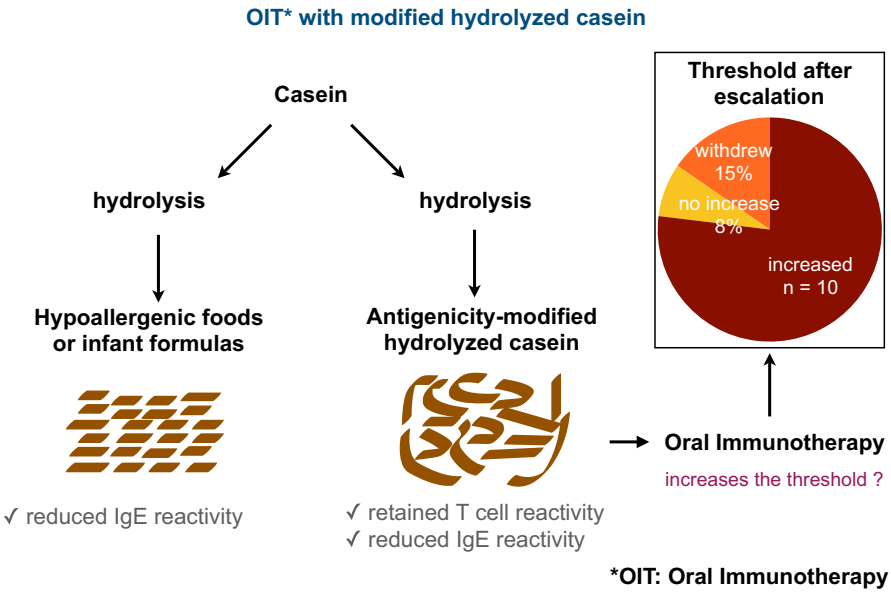

FIGURE 1 It is relatively difficult to achieve clinical tolerance via OIT with cow's milk. In food allergy, immunotherapy using T-cell-tolerizing peptides is not well established. A novel antigenicity-modified form of cow's milk casein can be digested to avoid cross-reaction with IgE and retain the T-cell reaction.

asthma, respectively. All enrolled participants had completely eliminated dairy foods from their diets at entry and showed allergic symptoms of OFC to unhydrolyzed casein.

Ten of 13 patients successfully increased their casein threshold (2-15 mL milk equivalent; Figure S2) during the escalation phase and entered the earlier maintenance phase. None of the 13 participants experienced anaphylaxis after consuming casein hydrolysate during the escalation phase. After the earlier maintenance phase, 6 patients achieved final casein thresholds of >40 mL milk equivalent and were desensitized to cow's milk (8 to >50 mL) after the later maintenance phase.

During the escalation phase, Subject#10 withdrew because of difficulty following the protocol due to school activity, and Subject#11 withdrew because of oral discomfort. During the earlier maintenance phase, Subject#9 withdrew because of oral discomfort, and Subject#12 withdrew because they developed anaphylaxis after performing extracurricular exercise 30 minutes following a daily dose of casein hydrolysate; this occurred despite our advice to maintain a longer resting period. During the earlier maintenance phase, Subject#15 experienced a protocol compliance issue involving oral discomfort and skipped to the next phase. Subject#14 had no increase in the casein threshold despite an increase during the escalation phase. During the later maintenance phase, Subject#5 developed anaphylaxis following the accidental consumption of cow's milk-containing sherbet during a bout of acute gastroenteritis, despite following our advice to avoid hydrolyzed casein on that day.

The primary outcome was to determine whether this hydrolyzed casein can induce desensitization. Because there is a lack of information on whether this approach can be useful for the treatment of CMA, we performed this as a pilot study. No study has described OIT using hydrolyzed caseins. We previously developed β-lactoglobulin hydrolysate, which exhibited reduced antigenicity but retained the T-cell epitope[9]; however, this method was limited by the nature of β-lactoglobulin, a relatively minor allergen compared with casein.[6] The present study demonstrated that OIT with a specially designed hydrolyzed casein could increase the casein threshold within a few months of treatment, even in patients with a long history of CMA.

The secondary outcome was to determine the safety of this method. Although we included many participants with a history of anaphylaxis to cow's milk, no participants experienced anaphylaxis to hydrolyzed casein during the escalation phase. However, a patient experienced anaphylaxis to hydrolyzed casein at entry and another experienced it because they did not follow our advice to rest after each dose of hydrolyzed casein; this shows the need to educate participants and their guardians carefully especially for the patient of adolescence. Because the hydrolyzed casein was only supplemented with dextrin, flavoring techniques may also improve the adherence to OIT by reducing the bitterness.

The present study was limited by the lack of a randomized controlled design and relatively small number of recruited patients. Slow OIT is usually safer than rash OIT but it is difficult to induce desensitization in the patients who have a very low initial threshold. Our study included the patients who have a relatively low initial threshold and showed good response even in slow OIT. As this was the first study to administer specially designed casein peptides for OIT, we initially enrolled a limited number of patients with CMA. Then, we did not see the statistically significant decrease of specific IgEs (Figure S3). Although larger phase II randomized controlled trials are required to confirm clinical efficacy, our finding that 10 of 13 participants achieved an increased threshold for this form of casein is comparable to the desensitization rates achieved via OIT with ordinary cow's milk (71%-80%).[3] This indicated the possibility of using OIT with hydrolyzed casein before starting the OIT with normal milk.

In conclusion, we have demonstrated that slow OIT with an antigenicity-modified casein hydrolysate allowed dose escalation in the majority of pediatric patients with CMA with a considerable range of adverse reactions. Additionally, this therapy effectively increased the participants' thresholds to cow's milk.

TABLE 1 Patient summary and threshold during oral immunotherapy

Subject ID	Age at entry (y)	Sex	Symptom of CM allergy	Maximum grade of An	Comorbidity	Serum IgE (IU/mL)	Specifc IgE (kU_A/L/class) at entry			Threshold of casein OFC (mL milk eq.)			Threshold of cow's milk at 1 y from the entry (mL)	Disposition
							Cow's Milk	Casein	β-Lactoglobulin	$T_{initial}$	T_{Phase1}	T_{Phase2}		
1	6	F	An	G4	AD, BA	1520	31.50 / 4	35.60 / 4	2.79 / 2	0.75	>5	>45	>50	
3	4	M	Ur			685	45.20 / 4	22.70 / 4	0.53 / 1	0.75	>5	>45	>50	
4	5	M	Ur		AD	869	6.75 / 3	4.31 / 3	≦0.34 / 0	3.75	>11	>45	>50	
5	4	M	An	G2	AD, BA	8120	≧100.00 / 6	≧100.00 / 6	≧100.00 / 6	0.75	>7	>40	>20	
6	4	M	An	G4	AD	1420	34.40 / 4	41.20 / 4	2.13 / 2	11	11	-	-	
8	4	M	An	G3		387	4.68 / 3	4.35 / 3	0.53 / 1	1.75	2.0	>40	8	
9	6	M	An	G4		920	5.18 / 3	5.19 / 3	0.78 / 2	1.0	2.0	-	-	Abandoned due to oral discomfort at earlier maintenance phase
10	9	M	An	G4	AD, BA	2780	54.80 / 5	68.70 / 5	0.43 / 1	3.75	-	-	-	Withdrew because of difficulty with following the protocol
11	7	M	An	G4	AD, BA	668	59.00 / 5	55.90 / 5	2.61 / 2	3.75	-	-	-	Abandoned due to oral discomfort at escalation phase
12	14	M	An	G4		721	10.70 / 3	14.90 / 3	0.63 / 1	11	15	-	-	An(+; while extracurricular sports activities)
13	10	F	An	G4		563	6.15 / 3	3.98 / 3	1.14 / 2	0.25	>3.75	>45	34.5	
14	6	F	An	G4	AD	545	70.20 / 5	72.50 / 5	1.50 / 2	3.76	11	13	-	Still receiving hydrolyzed casein at 1 year after entry
15	10	M	An	G4		530	2.39 / 2	1.52 / 2	0.92 / 2	5.0	11	11	3.5	Skipped into later maintenance phase without increases of casein threshold

Note: Casein OFC was performed with unhydrolyzed casein; Milk threshold was either the threshold of OFC of cow's milk or the maintenance dose of cow's milk at 1 year from entry; The OFC threshold is indicated by the symbol ">" if the patient did not develop the symptoms during OFC, as OFC during the maintenance phase involved doses that were 3 stepwise doses higher than the previous threshold to ensure the participant's safety.

Abbreviations: AD, atopic dermatitis; An, anaphylaxis; Ur, urticaria; BA, bronchial asthma; OFC, oral food challenge; $T_{initial}$, threshold of initial eliciting dose at study enrollment; T_{Phase1}, threshold at the end of the escalation phase with casein hydrolysate; T_{Phase2}, threshold at the end of earlier maintenance phase (maintain with maximum doses of casein hydrolysate).

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CONFLICT OF INTEREST

HU and TN are employees of Bean Stalk Snow Co., Ltd. The other authors declare no conflicts of interest.

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REFERENCES

1. Ebisawa M, Ito K, Fujisawa T,Committee for Japanese Pediatric Guideline for Food Allergy, The Japanese Society of Pediatric Allergy Clinical Immunology, The Japanese Society of Allergology. Japanese guidelines for food allergy 2017. *Allergol Int*. 2017;6:248-264.
2. Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol*. 2010;125:S116-125.
3. Kobernick AK, Burks AW. Active treatment for food allergy. *Allergol Int*. 2016;65:388-395.
4. Sato S, Yanagida N, Ogura K, et al. Clinical studies in oral allergen-specific immunotherapy: differences among allergens. *Int Arch Allergy Immunol*. 2014;164:1-9.
5. Hoffmann HJ, Valovirta E, Pfaar O, et al. Novel approaches and perspectives in allergen immunotherapy. *Allergy*. 2017;72:1022-1034.
6. Shek LP, Bardina L, Castro R, Sampson HA, Beyer K. Humoral and cellular responses to cow milk proteins in patients with milk-induced IgE-mediated and non-IgE-mediated disorders. *Allergy*. 2005;60:912-919.
7. Ueno HM, Kato T, Ohnishi H, et al. Hypoallergenic casein hydrolysate for peptide-based oral immunotherapy in cow's milk allergy. *J Allergy Clin Immunol*. 2018;142:330-333.
8. Sampson HA. Anaphylaxis and emergency treatment. *Pediatrics*. 2003;111:1601-1608.
9. Ueno HM, Kato T, Ohnishi H, et al. T-cell epitope-containing hypoallergenic beta-lactoglobulin for oral immunotherapy in milk allergy. *Pediatr Allergy Immunol*. 2016;27:818-824.

SUPPORTING INFORMATION

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Barrier disruptive effects of mucus isolated from chronic rhinosinusitis patients

To the Editor,

Chronic rhinosinusitis (CRS) is a heterogeneous disease involving a complex interplay of host, microbial, and environmental factors. Phenotypically, CRS is divided into two subtypes: CRS with nasal polyps (CRSwNP) or without nasal polyps (CRSsNP). Patients with these subtypes have different inflammatory profiles, suggesting a possible underlying difference in pathophysiology.[1] Common to both, however, is the ultimate disruption of the normal mucosal barrier, considered the first line of defence against airborne pathogens.[2,3] The immune barrier hypothesis proposes that chronic inflammation with barrier dysfunction triggers the development and ongoing symptoms of CRS.[1] This theory is supported from the findings in patients with cystic fibrosis who demonstrate a higher incidence of CRS and show mucociliary dysfunction, diminished tight junction protein expression and increased epithelial permeability.[4-6] The aim of this study was to investigate the effect of nasal mucus