

IgE-Mediated Cow's Milk Allergy in Children

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Abstract The age at which the majority of children outgrow cow's milk allergy now appears to be later than previously reported. Recent studies have attempted to elucidate factors that may help prevent cow's milk allergy, assess markers of persistence, and evaluate the usefulness of new diagnostic methods. Strict avoidance of cow's milk has been the mainstay of treatment. However, given the potential nutritional, social, and immunologic ramifications of cow's milk elimination from a child's diet, there has been a focus on reevaluating this therapeutic approach.

Keywords Cow's milk · Cow's milk allergy · Food allergy · Immunotherapy · Baked milk · IgE mediated · Children · Pediatrics

Introduction

Cow's milk allergy (CMA) is the most common food allergy in children, affecting about 2 % of the pediatric population [1]. CMA represents a spectrum of immunologic presentations (e.g., eosinophilic enteropathies, proctocolitis, enterocolitis, etc.) [2–4]. However, for the purposes of this review, we will concentrate on IgE-mediated CMA. We will examine recent studies that have helped further our understanding of CMA in terms of epidemiology, pathophysiology, clinical manifestations, and natural history (Table 1). Furthermore, we will explore the potential utility of component-resolved diagnostics in the diagnosis of IgE-mediated CMA. Although the mainstay of management has been strict

avoidance of cow's milk, extensively heated or baked milk may be safely introduced into the diet of most children with CMA, representing a paradigm shift in food allergy management. Novel treatment modalities such as immunotherapy are also being explored.

Epidemiology

CMA is the most common IgE-mediated food allergy in children, and past literature has reported that about 2 % of children under the age of 4 years in the general population are affected [5–7]. In a population-based study of 2,848 one-year-old Australian infants published in 2011 [8•], an estimated 2.7 % (95 % CI: 2.1–3.4) had clinical symptoms consistent with IgE-mediated CMA (occurring within 1 h of ingestion), although oral challenges were not performed. The rate appears to be lower in older children, since only 0.3 % (1/301) of children >3 years of age from an unselected Danish cohort had challenge-proven CMA [9]. Not surprisingly, among children having food allergy, milk is also the most common allergen. In a large, university-based pediatric food allergy outpatient practice, CMA was reported in about 40 % of patients [10]. Cow's milk has also been reported as an allergen for food-dependent, exercise-induced anaphylaxis [11].

Pathophysiology/Immunologic Mechanisms

IgE-mediated CMA begins with a sensitization phase in which IgE antibodies to milk proteins attach themselves to the surface of mast cells and basophils. Exposure to milk proteins subsequently leads to binding of cell-associated IgE to allergenic epitopes on these milk proteins, triggering release of inflammatory mediators and, thus, resulting in clinical symptoms. Children with CMA may generate specific IgE antibodies against sequential and/or conformational epitopes.

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Table 1 Summary of 2011–2012 studies on IgE-mediated cow's milk allergy

	Author, Year	Study summary	Findings
Epidemiology	Osbourne et al., 2011 [8•]	Population-based study of 2,848 one-year-old infants in Australia	Prevalence of IgE-mediated CMA was estimated to be 2.7 %
Pathophysiology	Jyonouchi et al., 2011 [20•]	- PBMCs from 15 children with cow's milk allergy, 12 children tolerant to cow's milk but with allergy to egg, and 13 healthy controls were incubated with α-galactosylceramide (αGal), cow's milk-sphingomyelin, or hen's egg-ceramide - iNKTs were quantified, and their cytokine production and proliferation were assessed	Milk sphingomyelin can induce invariant natural kill T-cell activation and T_H2 cytokine production that is more exuberant in children with IgE-mediated CMA
Natural history	Elizur et al., 2012 [24]	Prospective cohort study with 54 infants with CMA from a birth cohort of 13,019 children followed from birth for 48–60 months	57.4 % outgrew CMA during the study period - Infants reacting to less than 10 milliliters of milk or reacting in the first month of life, and those with a larger wheal size on SPT were found to be at increased risk for the persistence of CMA
	Ito et al., 2012 [46••]	83 children with suspected milk allergy (61 were diagnosed with CMA and 22 with no CMA based on an open milk challenge or convincing history)	- High levels of casein-sIgE antibodies are strongly associated with milk allergy in children - Casein IgE may be associated with prolonged allergy
	Savilahti et al., 2012 [96•]	95 infants with clinical eczema that was by history associated with the consumption of CM	Low β-lactoglobulin-specific serum IgG4 levels may differentiate eczematous infants with IgE-mediated CMA from infants who have eczema with only a suspected association with CM (and with a negative cow's milk challenge)
Clinical manifestations	Fleisher et al., 2012 [34••]	Five-site observational study of 512 infants 3–15 months of age with documented or likely allergy to milk or egg; the majority of allergic reactions were triggered by milk (495 [42.5 %]), egg (246 [21.0 %]), and peanuts (93 [7.9 %])	Severity of reactions was higher in peanuts than in milk (20.4 % vs. 11.4 % with severe reactions), but this difference was not statistically significant ($p=.06$)
Diagnostics	Ito et al., 2012 [46••]	83 children with suspected milk allergy were diagnosed as CMA ($n=61$) or non-CMA ($n=22$) on the basis of an open milk challenge or convincing clinical history - Serum concentrations of allergen-specific IgE and IgG4 antibodies were measured using ImmunoCAP®	- Casein IgE was deemed the best discriminating test (over milk and β-lactoglobulin sIgE) with a clinical decision point of 6.6 kU_A/L, which conferred 100 % specificity - At corresponding cutoff points with 100 % clinical specificity, milk and β-lactoglobulin IgE showed clinical sensitivity values of 33 % and 11 %, respectively
Prevention	Lowe et al., 2011 [51]	Single-blind, randomized controlled trial	Partially hydrolyzed whey-based formula or a soy at weaning in high-risk infants did not show differences in the outcomes for food reactions, sensitization, or eczema, as compared with a conventional milk-based formula
Management	Immunotherapy Martorell et al., 2011 [78]	Multicenter study of randomized 60 children 24–36 months of age with CMA into two groups: treatment group began oral desensitization immediately, while control group was kept on a milk-free diet	- After the 1-year follow-up period, 90 % of the children in the treatment group had become completely tolerant versus 23 % of the children in the control group. In the treatment group, cow's milk skin reactivity, and serum-specific IgE to milk and casein decreased significantly from the initial assessment, whereas the control group showed no significant change after 1 year of follow-up - Twenty-four patients (80 %) developed some reaction during the treatment period: 14 children developed moderate reactions (47 %) and 10 mild reactions (33 %)

Table 1 (continued)

Author, Year	Study summary	Findings
Keet et al., 2012 [82]	• 30 children 6–17 years of age were randomized to SLIT alone or SLIT followed by OIT	• After therapy, 1 of 10 subjects in the SLIT group, 6 of 10 subjects in the SLIT/OITB (1,000 mg of milk protein), and 8 of 10 subjects in the OITA group (2,000 mg of milk protein) passed the 8-g challenge • SLIT/OIT was more efficacious for desensitization to CM than SLIT alone but was accompanied by more systemic side effects
Nadeau et al., 2011 [85•]	Phase pilot I study	• Omalizumab treatment combined with oral milk desensitization in children with clinical reactions to cow's milk was found to permit rapid milk dose escalation in the majority of subjects
Baked Milk Kim et al., 2011 [87••]	• Prospective study reporting on the outcomes of children who incorporated baked milk products into their diets • 88 children underwent challenges at varying intervals (range, 6–54 months) over a median of 37 months	• Patients who were initially tolerant to baked milk were found to be 28 times more likely to become unheated milk tolerant, as compared with baked-milk-reactive patients. • Patients who incorporated dietary baked milk were 16 times more likely than a retrospectively assessed comparison group (that followed standard advice of strict avoidance of dietary milk) to become tolerant to unheated milk • Median casein IgG4 levels in the baked-milk-tolerant group increased significantly
Probiotics Berni Canali et al., 2012 [95•]	• Infants (1–12 months of age) consecutively referred for strongly suspected CMA were randomly allocated to one of the 2 groups of dietary interventions: group 1 received extensively hydrolyzed casein formula (EHCF), and group 2 received EHCF containing <i>Lactobacillus</i> GG (LGG)	• After 6 months of an exclusion diet, a DBPCFC was performed in 55 patients: 22 of 28 patients of group 1 and 11 of 27 patients of group 2 had positive results. At this time, the rate of full clinical tolerance acquisition was higher in group 2 than in group 1 • Infants with persisting CMA (22 in group 1 and 11 in group 2) were rechallenged at 12 months: 13 of 22 patients in group 1 and 5 of 11 patients in group 2 had positive DBPCFC results • Supplementation of EHCF with LGG accelerated the development of tolerance in infants to CMP

Unlike sequential epitopes, conformational epitopes are dependent on the food protein's tertiary structure.

Cow's milk consists of two major proteins: casein and whey [12]. Casein proteins (Bos d 8) include alphaS1-, alphaS2-, beta-, and kappa-caseins and make up about 80 % of the total cow's milk protein [12, 13]. Whey proteins contain alpha-lactalbumin (Bos d 4), beta-lactoglobulin (Bos d 5), bovine lactoferrin, bovine serum albumin (Bos d 6), and bovine immunoglobulins (Bos d 7) [12, 13]. Casein, beta-lactoglobulin, and alpha-lactalbumin are the major milk allergens, and co-sensitization to these three allergens is common [12, 14, 15]. In a study using microarrays to assess IgE-mediated responses to milk-specific proteins in patients, bovine caseins were confirmed to be

the main allergens in milk. Specifically, alphaS1-casein was found to be more allergenic than alphaS2-, beta-, and kappa-caseins [16].

Unlike casein, which is relatively heat resistant, whey proteins, particularly beta-lactoglobulin, are heat labile. Exposure to heat diminishes allergenicity, presumably via denaturation of heat-labile proteins resulting in loss of conformational epitopes, which may explain why extensively heated milk is better tolerated by some patients [17–19].

T-cell-mediated pathways have been explored as well. Jyonouchi et al. [20•] suggested a possible role for invariant natural killer T-cells in the pathogenesis of IgE-mediated CMA. Milk sphingomyelin, a sphingolipid, was shown to act as a ligand for the T-cell receptor of invariant natural

killer cells, thereby promoting T_H2 cytokine production and facilitating IgE sensitization. This was found to be more significant in children with IgE-mediated CMA, as compared with those with egg allergy or healthy controls. Further investigation is needed to assess the role of this potential mechanism in allergy acquisition and tolerance development and confirm its specificity to cow's milk.

Natural History

The majority of children with IgE-mediated CMA naturally achieve tolerance [21], although the age at which this is reported to occur has differed among various studies. Reports from the 1990s suggested that a significant proportion (75–80 %) of children with IgE-mediated CMA become tolerant by 3–5 years of age [5–7, 22]. Skripak et al., in a 2007 retrospective chart review, suggested that CMA may be more persistent, with 79 % of children followed at a U.S. referral center outgrowing this allergy by 16 years [23]. However, this may not be applicable to the general population, due to the selection bias inherent in such a study. A 2012 study by Elizur et al. prospectively followed 54 Israeli infants with CMA identified from a birth cohort of 13,019 children; 65 % outgrew CMA by 4 years of age [24]. A major contributor to these differences is likely study design; country of origin may also be a factor.

Clinical Manifestations

Clinical manifestations of IgE-mediated CMA may appear during the first few months of life after the introduction of cow's-milk-based formula or via breast milk when the maternal diet is inclusive of cow's milk. Food-induced IgE-mediated reactions generally occur within minutes to 2 h after ingestion of the culprit food, and reactions vary from mild to life-threatening. Symptoms most commonly involve the skin and gastrointestinal tract (including the oropharynx) but may also affect the upper and lower respiratory tract and cardiovascular system [25, 26].

Although symptoms are often mild for many CMA-allergic patients, it is important to note that cow's milk can induce severe allergic reactions. Anaphylaxis is the most severe manifestation of CMA, occurring in up to 9 % of cases [6, 7, 27–29]. Cow's milk is the third most common food (after peanuts and tree nuts) to cause fatal or near-fatal food-induced anaphylaxis [30–32]. Three fatalities from milk exposure (in a hidden or nonobvious form) were reported in a study from Israel that characterized mortality due to food allergy over a 7-year period, 2004 to 2011 [33].

In a prospective, five-site observational study of 512 infants 3–15 months of age with documented or likely

allergy to milk or egg, allergic reactions were most frequently triggered by milk (42.3 %) over a median follow-up period of 36 months [34••]. Reaction severity was not statistically different between those reactions induced by peanuts and those induced by milk ($p=.06$). In addition, when the first reaction to milk was compared with the fourth from the same subject ($n=48$), an increase in severity was found ($p=.002$) [34••]; additional research is needed to substantiate this finding.

Diagnosis

Diagnosis of IgE-mediated CMA is based firstly upon clinical history. Testing can be used to confirm clinical suspicions [35, 36]. Although the gold standard for diagnosis is a clinician-supervised double-blind, placebo-controlled oral food challenge, this is neither practical nor necessary to perform in the vast majority of cases. A convincing clinical history, along with a demonstration of the presence of cow's milk IgE antibody (via serologic testing and/or skin prick test), may be sufficient to establish a diagnosis. Skin testing using commercially available cow's milk extract is 95 % predictive of a clinical reaction for a wheal diameter of ≥ 8 mm in children over 2 years of age and ≥ 6 mm in children 2 years and under [37, 38]. A cow's-milk-specific IgE level of ≥ 15 kU_A/L using the ImmunoCAP® assay (Thermo Fisher Scientific, Waltham, MA) is ≥ 95 % predictive of a clinical reaction to ingested cow's milk [39], and a level of ≥ 5 kU_A/L in children less than 2 years of age is similarly predictive of a reaction [40]. Milk-specific IgE levels determined by other assays may be correlated but are not interchangeable [41, 42].

Component-resolved diagnostics is being examined in the field of food allergy as a potential diagnostic tool. Thus far, a handful of studies have examined the role of cow's milk allergen-specific IgE measurement with microarrays [35]. Three studies used a self-engineered allergen chip, and one study used a commercially available allergen microarray that was custom modified for the study (ImmunoCAP ISAC™, Thermo Fisher Scientific, Waltham, MA) [16, 43–45]. In children with suspected IgE-mediated CMA, the estimated sensitivity of the ISAC chip was 0.6 (95 % CI: 0.43–0.74) with a specificity of 0.84 (95 % CI: 0.69–0.93).

Recently, casein IgE was deemed the best discriminating test in a 2012 study of 61 children with CMA (based on open milk challenges or convincing history) [46••]. A clinical decision point of 6.6 kU_A/L for casein IgE conferred 100 % specificity and 72 % sensitivity within this Japanese cohort (median age, 3.5 years; range, 0.8–15.8 years), while a corresponding cutoff point for milk IgE with 100 % specificity corresponded with a sensitivity of only 33 % [46••]. In a prospectively followed cohort of 66 children with CMA in Spain (2004), the cutoff points for casein IgE for ≥ 95 %

predicted clinical reactivity varied by age: 2, 4.2, and 9 kU_A/L for casein IgE for age ranges 13–18 months, 19–24 months, and 3 years, respectively [47]. On the basis of these small studies, it is difficult to assess the performance characteristics of component testing for milk. Age should be taken into account, although further research is required to confirm these data in larger patient populations [45].

Cross Reactivity

The greatest homology exists among milk proteins of cows, sheep, and goats. Proteins that have less structural similarity include milks from pigs, camels, horses, and donkeys [35]. However, from a practical standpoint, cow's-milk-allergic patients are generally instructed to avoid all mammalian milks (aside from human milk, assuming maternal dietary elimination). In addition, beef allergy implies CMA, while the reverse is generally not true [48, 49].

Management and Treatment

Prevention

Breast feeding is recommended for all infants when possible. However, its role in the prevention of IgE-mediated CMA has not been well established. With regard to formula choice, a Cochrane review and meta-analysis of six studies of this topic [50] suggested a benefit of partially hydrolyzed whey formula (pHWF) for the prevention of allergy, as compared with conventional cow's milk formula (CMF). However, analysis of the two largest studies in this area (GINI and Melbourne Atopy Cohort Study) failed to show any benefit of pHWF, as compared with conventional CMF [51–54]. More recently, the utility of pHWF and soy formula to conventional CMF at weaning in high-risk infants was evaluated in a single-blind, randomized controlled trial; there was no evidence that infants allocated to pHWF or soy were at a lower risk for food reactions or eczema [51].

Avoidance

Currently, there is no effective treatment for IgE-mediated CMA in children aside from avoidance of cow's milk, the standard of care. Yet elimination diets have the potential to affect nutritional status, particularly of young children. Studies in which growth measurements were evaluated against diet records suggest that children with food allergy are at risk for inadequate nutritional intake [55, 56], and severe cases of malnutrition have been reported in children

treated with milk elimination for various reasons [57–59]. Thus, the NIAID-sponsored expert panel recommends nutritional counseling and regular growth monitoring for all children with food allergy [36].

Before the age of 2 years, a substitute with adequate nutritional value should be used. For breast-fed infants, strict milk avoidance (maternal) during breast feeding has been suggested by expert panels [35, 60–62], since some case reports and series have reported that allergic reactions, including anaphylaxis, may occur in infants from transfer of maternally ingested milk via breast milk [63, 64]. However, there are no controlled trials upon which to base these recommendations. Thus, we recommend taking into consideration the clinical history, as well as having a discussion with the mother, reviewing options that may include strict maternal avoidance of milk, reduced ingestion (avoiding large boluses), ingestion of only extensively heated milk, or no avoidance.

For infants not breast fed, current guidelines define a therapeutic formula as one that is tolerated by at least 90 % of cow's-milk-allergic infants. These criteria are met by some extensively hydrolyzed cow's milk whey and/or casein formulas, soy formulas, and amino-acid-based formulas [65].

Elimination of milk from the diet is a difficult and complex task. Thus, educating and providing families with resources is of utmost importance. Education for parents includes examples of unacceptable foods and suitable substitutes, as well as online resources, such as the Food Allergy Education Program developed by the Consortium of Food Allergy Research (<https://web.emmes.com/study/cofar/EducationProgram.htm>). In the United States, the Food Allergen Labeling and Consumer Protection Act states that all milk products require an ingredient statement written in plain English. Similar laws are currently in place in the U.K. and Australia and are being implemented in Canada as of August 2012. In general, we recommend that children be assessed annually to review avoidance strategies and emergency plans (including demonstration of autoinjectable epinephrine) and to ascertain whether tolerance has been achieved naturally [35].

Immunotherapy

Since the 1980s, immunotherapy has been regarded as a possible approach for the treatment of food allergy. It has been shown in aeroallergen and venom allergy to modify the IgE-mediated immune response and induce long-term protection [66–73]. However, early studies for food allergy utilized subcutaneous peanut extracts, which resulted in an unacceptable rate of severe events [74, 75]. Immunotherapy delivered orally, sublingually, or epicutaneously may be more promising routes of administration.

Desensitization Versus Tolerance

Throughout our discussion, we will be using the terms *desensitization* and *tolerance* as two distinct states. The goal of treatment is permanent oral tolerance, which is established when the food can be ingested without the appearance of allergic symptoms, despite periods when the food is not being ingested regularly. The term *desensitization* is used to describe a state in which the patient must regularly ingest the food in order to maintain the status of being able to do so without having a reaction; when the ingestion is stopped or interrupted, the protective effect may be lost or decreased.

Oral Immunotherapy

The first randomized, double-blind, placebo-controlled trial of oral immunotherapy (OIT) was published in 2008 by Skripak et al. [76]. This study involved 19 subjects 6–17 years of age who were assigned 2:1 to milk or placebo OIT. In the active group, the median threshold dose for reaction during oral challenge increased from 40 mg pre-treatment to 5,140 mg post-treatment, as compared to no change in the placebo group.

Additional milk-specific OIT studies were reviewed by Brozek et al. in a 2012 systemic review and meta-analysis. Among 1,034 articles screened, 5 randomized controlled trials (RCTs), including that of Skripak et al., and 5 observational studies [76–80] fulfilled criteria. OIT, as compared with strict avoidance alone, was shown in 218 patients to increase the likelihood of achieving full tolerance of cow's milk (relative risk: 10.0 [95 % CI: 4.1–24.2]). Adverse effects of immunotherapy included local symptoms (16 % of doses), mild laryngospasm (relative risk: 12.9 [1.7–98.6]), mild asthma (rate ratio: 3.8 [2.9–5.0]), reactions requiring oral glucocorticoids (relative risk: 11.3 [2.7–46.5]), and epinephrine injection (rate ratio: 5.8 [1.6–21.9]). The results of observational studies were found to be consistent with the RCTs. However, the number of studies is small and the quality rather low. Thus, rigorously designed, well-executed, larger RCTs are still needed to determine whether tolerance can be achieved and whether benefits are outweighed by the risks of adverse events [76, 81].

Sublingual Immunotherapy

Delivering cow's milk allergen to the mucosa under the tongue has also been studied. In sublingual immunotherapy (SLIT), dosing is limited by volume considerations but has less potential to induce adverse effects. In a randomized controlled trial [82], 30 milk-allergic children were randomized to SLIT alone or SLIT followed by OIT. OIT/SLIT was found to be more efficacious for desensitization to cow's milk than was SLIT alone. Although the overall reaction rate

was similar, OIT was accompanied by more systemic side effects. Additionally, in some cases, clinical desensitization was lost within 1 week of therapy.

Epicutaneous Immunotherapy

Another route of allergen delivery is through the skin. In a 3-month pilot study [83], children with challenge-confirmed CMA received three skin patches weekly, each patch applied for 48 h. Children treated with epicutaneous immunotherapy (EPIT) trended toward having an increased threshold dose of reactivity during the follow-up oral milk challenge, compared with no change in the placebo group. Common side effects included local pruritus and eczema at the sites of patch application.

Readiness for Clinical Practice

At this point in time, it is not clear whether or not cow's milk immunotherapy is a step toward tolerance or whether it induces only desensitization [84]. Although immunotherapy appears to be promising, it is not considered to be ready for use in clinical practice, due to uncertainty as to whether tolerance can be achieved as well as the significant adverse effects potentially induced, including anaphylaxis and need for epinephrine injections. Adjuncts, such as omalizumab, a humanized antibody that is FDA-approved for patients with moderate to severe asthma, may help minimize risks. Nadeau et al. [85•] in a phase I pilot study performed oral desensitization with omalizumab in 11 subjects. Nine were dosed up to 2,000 mg of milk within 7–11 weeks of initiating desensitization. The rate of severe (grade 3) reactions was 0.1 %. Aside from the small sample size, limitations of this study include the lack of a placebo group and lack of a baseline oral food challenge. Thus, further studies are required to establish the role of omalizumab in OIT. Controlled trials are ongoing [86].

Baked Milk

For most children with CMA, a more “natural” form of immunotherapy may be to incorporate baked milk into the diet. Preliminary findings suggest that children with CMA ingesting baked products may experience accelerated tolerance development [87••]. The rationale behind these studies builds on the concept that high heat—specifically, baking—reduces the allergenicity of milk proteins, resulting in the loss of conformational epitopes [17, 18, 88, 89]. Similar studies in egg-allergic subjects have shown that children with transient egg allergy produce IgE antibodies directed primarily against conformational epitopes that are destroyed during extensive heating or processing [90, 91]. Sequential IgE-binding epitopes have been shown to be recognized by the majority of patients with persistent CMA but not by

those with transient CMA [92]. Children with persistent CMA also demonstrated increased epitope diversity and higher affinity of IgE binding compared to those who outgrew CMA. Binding to greater numbers of IgE peptides in a peptide microarray assay was also associated with more severe allergic reactions [93].

Nowak et al. [19] challenged children 2–17 years of age with CMA to baked milk products (i.e., muffin and waffle). Sixty-eight of 91 (75 %) children with milk allergy were able to tolerate baked milk but not unheated milk. Children who reacted to baked milk had significantly larger skin-prick wheal sizes and higher milk and casein IgE levels, as compared with baked-milk -tolerant or unheated-milk-tolerant groups. At 3 months, children regularly ingesting baked milk products had significantly smaller skin-prick wheals and higher casein-sIgG₄, as compared with baseline.

A follow-up study [87•] demonstrated that the addition of baked milk to the diet of children who tolerated such foods appeared to accelerate the development of unheated milk tolerance, as compared with those who avoided milk strictly. Children underwent challenges at varying intervals (range, 6–54 months) over a median of 37 months. Unheated milk tolerance was achieved by 60 % in the baked-milk-tolerant group ($n=65$) but only 9 % in the baked-milk-reactive group ($n=23$). Patients who were initially tolerant to baked milk were found to be 28 times more likely than baked-milk-reactive subjects to become unheated milk tolerant ($p<.001$). Patients who incorporated dietary baked milk were 16 times more likely than a retrospectively gathered comparison group (following standard advice of strict avoidance) to become tolerant to unheated milk ($p<.001$) [87•]. The median casein IgG₄ levels in the baked-milk-tolerant group increased significantly although there was no significant change in median milk IgE levels. Chronic conditions such as asthma, atopic dermatitis, and allergic rhinitis were not worsened, growth parameters were unaffected, and eosinophilic esophagitis was not more common in those ingesting baked milk products, as compared with those adhering to strict avoidance [19, 87•]. In addition to the immunomodulatory benefits, the inclusion of extensively heated milk to the child's diet may greatly improve quality of life, facilitate the fulfillment of nutritional requirements, and reduce anxiety. However, 35 % of baked-milk-reactive subjects were treated with epinephrine for systemic reactions during baked milk challenges. Due to such risks, the introduction of baked milk should be performed carefully under physician supervision.

Probiotics

There has been a surge of interest in the role of probiotics in different disease processes in the past decade. In the field of allergy, the bulk of the research has involved probiotics' relation to atopy—specifically, atopic dermatitis [86, 94].

Currently, there are few studies on the effects of probiotics on CMA. One randomized trial on the effect of *Lactobacillus* GG (LGG) on tolerance acquisition in 55 infants (mean age, 3 months) with CMA showed that supplementation of extensively hydrolyzed casein formula with LGG accelerated the development of tolerance in infants to cow's milk protein [95•]. In this study, all patients consumed regular doses of cow's milk daily without showing any signs or symptoms related to CMA for 6 months after the negative DBPCFC result, suggesting the persistence of tolerance [95•]. The possible effect of probiotics on tolerance acquisition in patients with CMA requires further investigation.

Markers of Persistence

As was discussed previously, baked milk reactivity is a likely marker of persistent CMA. Additional clinical markers that may help identify children with persistent IgE-mediated CMA were identified by an Israeli birth cohort study [24]. Using multivariate analysis, an increased risk of persistent CMA was significantly associated with a reaction threshold of <10 milliliters of milk (demonstrated by an oral food challenge or estimated by a guardian). Of borderline significance was having a history of reaction within the first month of life (i.e., early introduction) [24].

While the utility of components in diagnosis is debatable, they may help predict the course of the disease. Higher levels of casein IgE may be associated with persistent allergy (>5 years of age) [46•]. Another study suggests that lower IgG₄ levels to β -lactoglobulin are associated with IgE-mediated CMA in eczematous infants than in infants having a negative cow's milk challenge [96•].

Other immunologic parameters, such as T regulatory cells, may also be helpful indicators. A study on T cell responses in children who outgrew CMA showed that these patients had higher frequencies of circulating CD4+CD25+ regulatory T-cells and decreased in vitro proliferative responses to bovine β -lactoglobulin in peripheral blood mononuclear cells (PBMCs) when compared to children who retained clinically active allergy. Depletion of CD25+ cells from PBMCs of tolerant children led to a fivefold increase in in vitro proliferation against β -lactoglobulin. These findings suggest that tolerance is associated with the presence of CD4+CD25+ regulatory T-cells that are capable of suppressing the effector T-cells generated after reintroduction of cow's milk [97].

Conclusions

New research has focused on many aspects on CMA that have helped us gain further insights into identifying

strategies that have the potential to modify the natural history of this disease, although the mechanisms of tolerance induction are still largely unknown. Markers of persistence include reactivity to baked forms of milk and having a low threshold of reactivity. Since CMA is the most common IgE-mediated food allergy, research on prevention as well as management, can greatly impact the lives of many patients. Studies on immunotherapy (oral, sublingual, and epicutaneous), as well as baked milk have begun to yield valuable information for possible future treatments for CMA. Well-controlled randomized trials are still needed to demonstrate safety and efficacy.

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- Of importance
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