Cow's milk protein allergy: new knowledge from a multidisciplinary perspective

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ABSTRACT

In recent decades, a higher prevalence, persistence, and severity of cow's milk protein allergy (CMPA) have been observed. Different hypotheses have been proposed in relation to potential responsible mechanisms, with emphasis on the role of the microbiota in the induction and maintenance of immune tolerance as well as the importance of establishing a healthy microbiota in an early manner through the promotion of breastfeeding, vaginal delivery, rational use of antibiotics and proton pump inhibitors, along with an early introduction of varied foods. The use of probiotics and allergen-specific immunotherapy (AIT) come up as the treatment strategies with the greatest evidence in favor of tolerance acquisition.

The objective of this review was to describe the information currently available about the immune mechanisms involved in CMPA, the role of microbiota, and future treatment perspectives.

Key words: *food, hypersensitivity, tolerance, microbiota, treatment.*

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INTRODUCTION

Cow's milk protein allergy (CMPA) is one of the most common causes of food allergy in the early years of life, although it may also occur in school-aged children and adolescents.[1-6] CMPA is caused by a reproducible, immune-mediated response to subsequent exposure to milk proteins. Depending on the immune mechanism involved, signs and symptoms may develop immediately, within 2 hours after milk intake, or in a delayed manner, up to 3 weeks later.[7-9]

In recent decades, a higher CMPA prevalence has been observed, with more severe clinical manifestations and a higher risk for persistence, turning CMPA into a public health issue. The correct diagnosis and an adequate treatment are important to prevent the negative effects of this condition.[11-18]

The objective of this review is to describe the information currently available about immune processes, the role of microbiota, and future treatment perspectives. For this reason, a multidisciplinary team conducted a bibliographic search in Pubmed and selected the most relevant articles in this field.

Epidemiology

The estimate prevalence of CMPA ranges from 0.5% to 3% in industrialized countries,[19-23] and the lowest values are observed in breastfed infants.[9,24] Its incidence ranges from 2% to 7.5% in the first year of life.[24]

In Argentina, it is estimated that

the prevalence of medically diagnosed food allergies is 0.84%, and out of these, CMPA is the most common.[2] A retrospective, cohort study in newborns included in a health care program revealed that the prevalence of CMPA trebled in the past decade, from 0.4% in 2004 to 1.4% in 2014.[1]

Causes of food allergies

The development of food allergy depends on the individual predisposition to atopy and tolerance disruption due to an alteration in the immune response, which may not be established in the early stages or may be disrupted later on.[25,26]

There are several factors involved in the immune processes of tolerance, such as dendritic cells that process and present antigens to naive T lymphocytes and induce their differentiation into regulatory T cells (Tregs).[27] Tregs located on the intestinal lamina propria inhibit sensitization to food allergens.

Other important cells are CX3CR1+ macrophages and B cells secreting immunoglobulin (Ig) A in the intestinal lumen,[28] which, together with IgG4, may inhibit the allergic response and, therefore, contribute to tolerance.[29]

Mechanisms involved in tolerance disruption

Epithelial cells make up the first defense barrier. In the presence of an anatomical and/or functional alteration, epithelial cells become involved in mechanisms of allergic sensitization, a phenomenon that occurs both in the skin and the gut mucosa.[29-31]

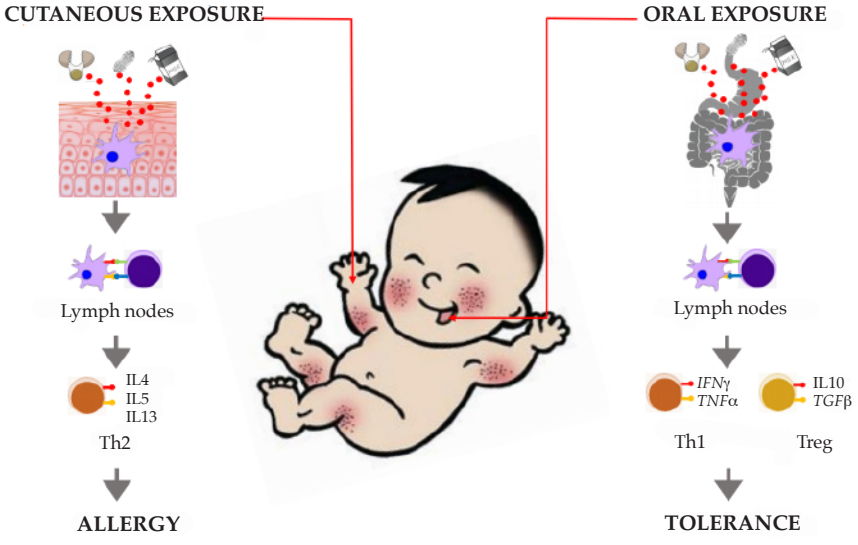
A defect in intestinal epithelial barrier promotes contact to the antigen and the production of proinflammatory cytokines, such as interleukin (IL)-33 and IL-25 and the thymic stromal lymphopoietin (TSLP),[32,33] that reprogram antigen-presenting cells to mediate in naive T cell differentiation from T helper 2 (Th2) cells which produce IL 4 and IL 13 to the detriment of Treg cells.[34-37]

IL-4 induces the expansion of eosinophils and mast cells in the mucosa, as well as an isotype switch in local B cells to IgE production and the subsequent dissemination of Th2 cells.

On the other hand, the dual-allergen exposure hypothesis suggests that the contact of food with a defect in the skin barrier may overcome the normal tolerogenic response of the intestine.[38] Consequently, dendritic cells in the skin promote Th2 inflammation[39,40] (*Figure 1*).

The microbiota plays a relevant role in the

FIGURE 1. *Dual-allergen exposure hypothesis: it suggests that early life exposure to allergens through the skin causes T-cell skewing towards allergic type Th2 cells (IL-4, IL-5, IL-13) and subsequent food allergy, whereas early oral exposure causes T-cell skewing towards tolerance (subsets of Th1 and Treg IFNg, TNFa, IL-10, TGFb).*



Adapted from: Yu W, et al.[27]

maintenance of the barrier function and the induction and maintenance of tolerance.[41,42] Risk factors for developing food allergies, including C-section,[43,44] absence of breastfeeding,[45] early use of antibiotics[46] and proton pump inhibitors, vitamin D deficiency,[47] eating habits, and number of siblings,[48,49] among others, are capable of disrupting the acquisition and composition of a healthy microbiota.[50]

The role of microbiota

The gut microbiota appears to be a key player. A lower microbial diversity has been described in children with CMPA, which may lead to an imbalance of dysbiosis, the modification of which can be considered a treatment target.[51]

A healthy gut flora favors the immune balance of Th1 and Th2 cells, whereas an alteration in gut flora is associated with a Th2 response that will promote allergic manifestations.[52]

Biotics are nutritionally active components. When administered in sufficient amounts, they may be beneficial.[53] Biotics include probiotics, which are live microorganisms.[54]

The greater body of evidence refers to *Lactobacillus rhamnosus* GG (LGG).[55-58] The presence of LGG in baby formula, fermented milk products or dietary supplements has been assessed in several clinical studies carried out in pregnant women, newborn infants, adults, and elderly people, in whom the safety of LGG has been demonstrated.[59,60]

A comparative study conducted in 260 children aged 1-12 months with CMPA (42.7% were IgE-mediated) used different formulas with and without LGG to assess the acquisition of tolerance and reported that a formula with extensively hydrolyzed proteins with added LGG induced earlier tolerance rates (78.9%) than those without LGG (43.6%).[61] Another article described that extensively hydrolyzed formula (EHF) with added LGG reduced the incidence of other allergies and promoted the development of oral tolerance in patients with IgE-mediated CMPA.[62]

A prebiotic is "a substrate selectively used by the host microorganisms, which confers a health benefit".[63] Prebiotic intake may modulate the colon microbiota and increase the number of beneficial bacteria. Inulin, galactooligosaccharides (GOS), and fructooligosaccharides (FOS) are the most commonly used prebiotics, although raffinose and polydextrose are starting to emerge as new prebiotics.[64,65]

A synbiotic is defined as a "mixture of live microorganisms and substrates selectively utilized by the host organisms that confers a health benefit on the host".[66]

Studies that used EHF and amino acid-based formula with added synbiotics (a mixture of FOS, GOS, and *Bifidobacterium breve* M-16 V) demonstrated that their use is safe and that they achieve gut microbiota modulation, with an increase in beneficial microorganisms such as bifidobacteria and a lower percentage of bacteria of the genus *Clostridium*.[67-69]

Available treatment options

The recommended treatment for food allergies is to avoid exposure to the allergen involved. Human milk is adequate for most infants with CMPA. Therefore, it is important for mothers to continue breastfeeding while on an elimination diet, which should be supervised to prevent nutritional deficiencies.[23,70-73]

If breastfeeding is not exclusive or has been completely discontinued, it is necessary to use a nutritionally adequate substitute.

By definition, hypoallergenic formulas are those tolerated by 90% of infants with CMPA, with a 95% confidence interval (CI). These are divided into EHFs, which contain short peptides (most below 1500 Da), and amino acid-based elemental formulas (AAFs).[71]

EHFs are the first line of treatment for mild or moderate CMPA. AAFs are the treatment of choice for severe cases. In addition, AAFs may be an option for patients who did not respond to an EHF treatment.[70-72] *Table 1* summarizes the indications of the different types of formulas based on the clinical presentation.

Hydrolyzed rice protein and soy formulas have demonstrated to be safe and well-tolerated among infants with CMPA and emerge as an alternative in countries where they are available. However, several documents do not recommend soy formulas for infants with CMPA during the first 6 months of life.[71,74-78]

Effect of early food introduction

In the 1990s, primary prevention consisted in delaying the introduction of potentially allergenic foods in all high-risk patients.[79-83]

Recent studies suggest that the early introduction (between 4 and 6 months old) of potential allergens may be effective in the prevention of food allergy.[60-88] This is based on the fact that the first year of life is a key period for the establishment of the gut microbiota and,

consequently, the development of oral tolerance.

When introducing supplementary feeding, family and cultural habits and the psychomotor development of children should be considered, and breastfeeding should be maintained for as long as possible, preferably up to 2 years of age.[89-91]

Another relevant factor is the variety of foods in the diet (vegetables, fruits, legumes, fish, etc.), which allows the development of a diverse microbiome favoring the intestinal barrier integrity and the immune system regulation.[92,93]

Baked goods

Different studies indicate that approximately 75% of patients with IgE-mediated CMPA tolerate baked goods (muffins, cake, cookies, and crackers, etc.).[94-98]

Epitopes recognized by the immune system may be present in the linear or conformational structure of food proteins. Proteins are denatured with heat and their 3-dimensional structure changes; thus, some epitopes are no longer recognized by the immune system and their allergenicity is modified.[94] Considering the main allergens, casein accounts for 80% of total protein content and is heat-stable, whereas whey proteins are affected by heat. Sensitization to casein is a risk factor for reaction regardless of baking.[99,100]

Protein allergenicity does not depend only on protein behavior during heating. There is evidence about the role played by the food matrix in relation to baked goods. Interactions with proteins, fats or sugars in a food matrix like wheat are as important as temperature and may reduce the exposure of specific epitopes to the immune system.[101-103]

Some articles describe that the consumption of baked milk goods would accelerate allergy resolution.[95,96,104,105] However, this fact has not been confirmed by other studies.[97,106] It is worth noting that the introduction of these goods into the diet has a positive effect on nutrition and quality of life. Such indication should be carefully assessed, together with the treating team.

Immunomodulation and immunotherapy

Food allergen specific immunotherapy (AIT) aims to restore immune tolerance through the administration of increasing doses of a specific food.

The initial immune switches caused by AIT result in a decrease in the activity and response capacity of effector cells, like mast cells and basophils,[107,108] and in an increase in specific IgG4, which binds to the allergen before allowing it to interact with IgE.

Then, there are changes in the modulation of T cell response, followed by a decrease in Th2 cells and their cytokines, and lastly, in oral tolerance.[109]

Specific immunotherapy for milk

The treatment for CMPA can be administered via various routes (*Table 2*).

Oral immunotherapy

Oral immunotherapy (OIT) with cow's milk

TABLE 1. *Indications for formulas in terms of clinical presentation*

Clinical presentation	First option	Second option	Third option
Anaphylaxis	AAF	EHF	SF
Immediate gastrointestinal allergy	EHF	AAF/SF	
FPIES	AAF	EHF	
Asthma and rhinitis	EHF	AAF/SF	
Acute urticaria/angioedema	EHF	AAF/SF	
Atopic dermatitis	EHF	AAF/SF	
Gastroesophageal reflux	EHF	AAF	
Allergic eosinophilic esophagitis	AAF		
Milk protein-induced intestinal disease	EHF	AAF	
Constipation	EHF	AAF	
Severe irritability (colics)	EHF	AAF	
Gastroenteritis and proctocolitis	EHF	AAF	
Heiner syndrome (milk protein-induced chronic pulmonary disease)			

FPIES: food protein-induced enterocolitis syndrome; EHF: extensively hydrolyzed formula; AAF: amino acid-based elemental formula; SF: soy formula.
Adapted from: Hill C, et al. [54]

was associated with better tolerance and reduction in symptoms.[110] However, approximately 90% of participants developed adverse reactions, with a significant number of severe side effects,[111] including anaphylaxis and eosinophilic esophagitis.[112] Two systematic reviews suggested that OIT should not be recommended as standard treatment.[113,114] In the light of its potential benefit in carefully selected patients, OIT should only be administered in specialized health centers, by experienced personnel, using adequate equipment and in accordance with the clinical protocols approved by local ethics committees.

Sublingual immunotherapy

Sublingual immunotherapy (SLIT) uses less-concentrated allergen extracts than OIT.[115] Current data support a model in which antigens administered by SLIT are uptaken by a population of myeloid dendritic cells in the oral mucosa, the oral Langerhans cells. This leads to IL-10 release promoting the T cell production of tolerogenic cytokines, like IL-10 and TGF-β.[116,117]

Epicutaneous immunotherapy

Epicutaneous immunotherapy (EPIT) has recently emerged as an alternative method for allergen administration using a delivery system applied to intact skin.[118] Since the epidermis is not vascularized, EPIT prevents systemic reactions caused by allergen circulation. It is believed that its preventive effects are modulated via epidermal Langerhans cells.[119]

Treatments with biologics

These are drugs produced by living organisms that target different molecular pathways involved in inflammatory processes. Biologics could be used as monotherapy or as adjuvant therapy in addition to AIT to reduce the risk for adverse reactions.[120]

They have begun to be evaluated for treating food allergies in the last few years. Omalizumab—an anti-IgE antibody—used together with AIT for milk, egg, or peanut allergy, shows increased safety and efficacy compared to placebo.[121,122] Using omalizumab plus OIT with cow's milk, higher doses could be reached over shorter periods of time, with greater safety and efficacy.[123]

Dupilumab is an antibody that targets the IL-4 and IL-13 receptor alpha chain.[124] It is currently under investigation for this kind of conditions.

Other treatment targets are being studied for other foods.[125]

CONCLUSIONS

The prevalence of CMPA is increasing and has become a reason for global concern. New knowledge about the pathophysiology of CMPA highlights the role of barrier mechanisms and the microbiota. Therefore, all measures aimed at the development of the normal microbiota play an important role since birth.

Studies with probiotics targeted at correcting dysbiosis during the first years of life have

TABLE 2. *Routes of administration of immunotherapy: Advantages, limitations, and potential adverse events*

Route of administration	Immune effects	Adverse reactions	Limitations
Oral (OIT)	• Reduces response with patch testing • Increases IgG4 • Reduces specific IgE • Activates Treg	• Common • Occasional systemic reaction requiring epinephrine	• Safety • Variable results in long-term tolerance
Sublingual (SLIT)	• Reduces response with patch testing • Increases IgG4 • Reduces basophil activation • Skewing of Th2 cytokines towards Th1	• Less common • Usually local • Develop at the time of treatment initiation	• Low effectiveness compared to OIT • Lack of long-term tolerance studies
Epicutaneous (EPIT)	• Evidence of Treg induction • Switch in cytokine level towards Th1	• Even less common • Only local skin reaction	• Unclear mechanism • Lack of randomized controlled studies

promising results, but some are contradictory, probably due to their heterogeneity, the different strains, the duration of treatment, the doses and the time at which treatment should be initiated, among other factors.

AIT has the potential to balance the immune response; however, it has some disadvantages related to its adverse effects and its effectiveness over time that require further studies for it to be considered a systematic treatment plan.

The use of biologics has emerged as an alternative, but additional studies and consensuses on their use in CMPA and other food allergies are still required, in addition to the fact that they are expensive.

Current data are encouraging, but further studies are required to find new and improved therapeutic tools that will result in the benefit of our patients and their families in the immediate future. ■

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