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The potential for pre-, pro- and synbiotics in the management of infants at risk of cow's milk allergy or with cow's milk allergy: An exploration of the rationale, available evidence and remaining questions

Adam Fox [a,*], J. Andrew Bird [b], Alessandro Fiocchi [c], Jan Knol [d], Rosan Meyer [e], Seppo Salminen [f], Gong Sitang [g], Hania Szajewska [h], Nikolaos Papadopoulos [i]

[a] Dept of Paediatric Allergy, Guy's & St Thomas' Hospitals NHS Foundation Trust, Westminster Bridge, London SE1 7EH, United Kingdom
[b] University of Texas Southwestern Medical Center, Department of Pediatrics, Division of Allergy and Immunology, 5323 Harry Hines Blvd, Dallas, TX 75390-9063, USA
[c] Allergy Unit, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165 Rome, Italy
[d] Wageningen University, Stippeneng 4, 6708 WE Wageningen, the Netherlands
[e] Imperial College, Dept Paediatrics, London, UK
[f] Functional Foods Forum, Faculty of Medicine, Itäinen pitkäkatu 4A, 20014 Turku, Finland
[g] Guangzhou Women and Children's Medical Center, Tianhe, Guangzhou, 510620, China
[h] Department of Paediatrics, The Medical University of Warsaw, 02-091 Warsaw, Zwiriki i Wigury 63A, Poland
[i] Professor of Allergy & Paediatric Allergy Division of Infection, Immunity & Respiratory Medicine, University of Manchester, 5th Floor (Research), Royal Manchester Childrens Hospital, Manchester M13 9WL, UK

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ABSTRACT

Cow's milk allergy is one of the most commonly reported childhood food allergies, with increasing incidence, persistence and severity in many countries across the world. The World Allergy Organization Special Committee on Food Allergy has identified cow's milk allergy as an area in need of a rationale-based approach in order to make progress against what it considered an onerous problem, with worldwide public health impact. There is growing interest in the potential role of the gut microbiota in the early programming and development of immune responses and allergy. This discussion paper considers the rationale and available evidence for modulation of the gut microbiota and for the use of synbiotics in the management of infants at risk of, or living with cow's milk allergy and summarizes remaining research questions that need to be answered for the development of evidence-based recommendations.

Introduction

Cow's milk allergy (CMA) is now one of the most commonly reported childhood food allergies with studies showing that challenge-confirmed CMA affects between 2 and 5% of infants in some countries.[1–4] However, there is considerable variation between different countries. The EuroPrevall birth cohort of 12,049 children from nine European countries found an overall incidence of challenge-proven CMA of 0.54% in children up to the age of two, with national incidences ranging from 1.26% in the UK and 1.08% in the Netherlands to <0.3% in Germany, Greece and Lithuania.[1] Most of the affected children had serum IgE antibodies to cow's milk, although 23.6% of children with CMA had no cow's milk-specific IgE in serum.[1] Only the UK, the Netherlands, Poland and Italy identified children with non-IgE-associated CMA. The adjusted incidence ranged from 0.13% in Italy to 0.72% in the UK, where non-IgE-associated CMA was more prevalent than IgE-associated CMA (56.3% vs 43.7%).[1] However, it is thought that the prevalence of non-IgE mediated allergies is probably higher than documented in EuroPrevall.[5]

In addition to increasing incidence, CMA may be persisting longer, with a study suggesting slower rates of resolution and a higher

* Corresponding author. Dept of Paediatric Allergy, Guy's & St Thomas' Hospitals NHS Foundation Trust, Westminster Bridge, London SE1 7EH, United Kingdom.
E-mail addresses: adam.fox@gstt.nhs.uk (A. Fox), Drew.Bird@UTSouthwestern.edu (J.A. Bird), alessandro.fiocchi@allegriallergia.net (A. Fiocchi), Jan.Knol@wur.nl (J. Knol), r.meyer@imperial.ac.uk (R. Meyer), sepsal@utu.fi (S. Salminen), sitangg@126.com (G. Sitang), hania@ipgate.pl (H. Szajewska), nikolaos.papadopoulos@manchester.ac.uk (N. Papadopoulos).

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proportion of children with disease persisting into school age and older, and increased risk of developing other allergic conditions over time as part of the allergic march.[1,2,6–8] However, studies have shown widely varying results in the rate of resolution. The Milan Cow's Milk Allergy Cohort study found that just over half of infants with CMA (59 of 112) achieved tolerance at a mean age of 28 months.[7] Asthma and/or rhinitis at presentation was an independent predictor of persistence (hazard ratio 2.19). Multivariate analysis showed that a fresh milk wheal diameter of 1 mm and a positive skin prick test with soy were also predictors of persistence.[7] A US study of the natural history of milk allergy in an observational cohort of 293 children, of whom 244 were diagnosed with milk allergy at baseline, found that milk allergy had resolved in just over half (154, 52.6%) of participants at a median age of 63 months.[6] Baseline characteristics that were most predictive of resolution included milk-specific IgE level, milk skin prick test wheal size and severity of atopic dermatitis (all $p < 0.001$).[6]

The higher the baseline milk-specific IgE level (kU_A/L) the lower the milk allergy resolution. Baseline milk IgE level of <2 resolved milk allergy in 88 individuals (72.1%), ≥2.0–10 resolved milk allergy in 46 individuals (54.1%) and ≥10 resolved milk allergy in 19 individuals (23.1%). The higher the baseline milk skin prick test (SPT) response (wheal, mm) the lower the milk allergy resolution. Baseline milk SPT response of <5 resolved milk allergy in 62 individuals (72.1%), 5–10 resolved milk allergy in 55 individuals (52.4%) and >10 resolved allergy in 37 individuals (36.6%). The higher the baseline atopic dermatitus (AD) severity the lower the milk allergy resolution. No AD severity was recorded in 26 individuals (81.3%), mild AD severity was recorded in 22 individuals (64.7%), moderate AD severity was recorded in 69 individuals (47.3%) and severe AD severity was recorded in 37 individuals (45.7%).[6]

More recently, a retrospective cohort study of 131 children with a history of immediate reaction to cow's milk showed 32.6% were tolerant to cow's milk by the age of three years, 64.1% by five years and 84.8% by six years.[8] A history of anaphylaxis and high milk-specific IgE levels were associated with persistent CMA.[8]

Food allergy, of which CMA plays a major part in infants and children, is also becoming more serious with more adverse events reported and increasing rates of hospitalisation due to food-induced anaphylaxis (FIA). A study in Italy showed a rapid increase in hospital admissions for food-induced anaphylaxis between 2006 and 2011, with a 44% increase in admissions for children younger than four years over this five-year period and a 128% increase in children aged 5–14 years.[3] There was also an increasing trend in the number of hospital admissions for FIA in children older than 14 years, with a rise in all age groups. Cow's milk was the most frequent food responsible for anaphylaxis requiring hospitalisation, accounting for nearly half of cases in children aged four and younger.[3] These increases in FIA-related hospital admissions in Europe over recent years mirrored those previously reported in children in the US and Australia.[9,10]

Potential role of gut microbiota dysbiosis in food allergy and evidence for modulation in CMA

There is growing interest in the potential role of the gut microbiota. An estimated 3.8×10^{13} commensal bacteria inhabit the human colon and work together with the rest of the body to function as what's been termed a 'superorganism', on the programming and development of immune responses and allergy.[11–13] Studies suggest that the intestinal microbiota may modulate immunologic and inflammatory systemic responses and so influence the development of sensitisation and allergy.[14] These studies reflect the move towards more active management of food allergy, moving away from an approach based on avoiding allergens in the hope of prevention, to early active introduction to induce tolerance before allergy develops and active attempts to induce tolerance when already cow's milk allergic.[15]

The development of the intestinal microbiota is a dynamic process in the first year, which is a time frame that is also critical for development and maturation of the immune system.[16,17] Immediately after birth, microbes derived from the maternal microbiota (vaginal, faecal, human milk, mouth and skin) and the environment colonise the infant's gut. Host genotype, gestational age, mode of delivery (vaginal vs caesarean section), medical practices (particularly use of antibiotics), geographic origin and cultural traditions, and early dietary exposure (human milk, complementary food), profoundly affect gut microbiota development.[16]

Several birth cohort studies have shown altered gut microbiota, or dysbiosis, in allergic infants compared to healthy infants, as well as specifically in CMA.[18,19] Although there are limited studies comparing gut microbiota in infants with CMA to healthy infants, from existing publications the gut microbiota of infants with allergic conditions typically has low levels of *Bifidobacteria* and *Lactobacilli* compared with healthy infants.[20]

Pre- and probiotics have been shown to influence the gut microbiota, either directly or indirectly, with the potential of influencing the onset of allergic conditions.[21] Synbiotics combine pre- and probiotics, with the aim of achieving a synergistic effect.[22]

Definitions: prebiotic, probiotic and synbiotic

- Prebiotic – a substrate that is selectively utilised by host microorganisms conferring a health benefit[23]
- Probiotic – live microorganisms, which when administered in adequate amounts confer a health benefit on the host[24,25]
- Synbiotic – a mixture of prebiotics and probiotics that affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, improving the health of the host[26]

Evidence for prebiotics, probiotics and synbiotics in the prevention and management of CMA

Prebiotics

Research studies have shown that prebiotic supplementation of infant formula with a specific mixture of short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) is a safe approach which results in a gut microbiota more similar to infants fed with human milk than babies fed with standard formula in a healthy population. The microbiota is enriched in *Bifidobacteria* and *Lactobacilli*.[27]

The worldwide, multicentre PATCH trial (Primary Allergy Prevention Through Cow's Milk Hydrolysates) studied 1047 infants at increased risk for allergy. Singleton infants born ≥36 weeks of gestational age and ≥2500 g were eligible for participation in the study if at least one of their parents had a documented history of allergic disease, confirmed by means of skin prick testing or a history of anaphylaxis. The study included infants with IgE and non-IgE medicated CMA. These infants were fed with cow's milk formula or a partially hydrolysed protein formula supplemented with non-digestible oligosaccharides for six months, comparing the rate of eczema with that seen in exclusively breastfed infants. Number, type and severity of adverse and serious adverse events were recorded. The study concluded that within specific categories of pregnancy, puerperium, perinatal conditions, infections and infestations the number of adverse events for the breast fed group were reduced compared to the formula fed group. For the category of surgical and medical circumstances the number of adverse events within the breast fed group were greater than the formula fed group. Active treatment was associated with increased length and head circumference at 4 and 12 weeks, but no differences in growth parameters were observed in active and control groups beyond the treatment period. Breastfed infants had increased stool frequency and increased watery stools, and were larger than formula fed infants from prior to the intervention period onwards.[28]

A sub-study in 138 of the infants showed that the faecal microbiota in infants receiving partially hydrolysed protein formula supplemented

with prebiotics was closer to that of breastfed infants than that of infants receiving standard cow's milk formula. The PATCH substudy did not report any adverse events.[29]

Probiotics

A randomised controlled open trial of extensively hydrolysed casein formula (EHCF) containing the probiotic *Lactobacillus rhamnosus* GG (LGG) in 55 infants with strongly suspected CMA showed accelerated development of tolerance to cow's milk protein compared to EHCF without supplementation with LGG. After both six and twelve months of an exclusion diet, the rate of acquisition of full clinical tolerance to cow's milk was higher in infants randomised to EHCF plus LGG than the EHCF only group. Subjects consumed regular doses of cow's milk without signs and symptoms related to CMA. Infants accepted the study formulas without problems, and no adverse events were observed.[30]

A larger prospective study allocated 260 children with CMA to five groups based on the formula used for their clinical management: EHCF; EHCF plus LGG; hydrolysed rice formula; soy formula; and amino acid based formula (AAF). As in the previous study, result showed that EHCF accelerated tolerance acquisition compared with other types of formula and this effect was even greater with LGG. No adverse events were observed during the study.[31]

A recent study that randomly allocated 220 children (median age 5.0 months) with suspected IgE-mediated CMA to either EHCF or EHCF + LGG showed reduced incidence of other allergic manifestations, including eczema and asthma, and hastened the development of oral tolerance with the probiotic-supplemented formula. The study authors suggested that further studies were needed to assess whether EHCF + LGG can prevent a single allergic manifestation, which was suggested but not proven by this randomised trial, and to better elucidate the mechanisms of the beneficial effect they observed. No child was intolerant to the study formulas. No case of placebo refusal was observed during the double-blind, placebo-controlled food challenges. No adverse events were attributed to the consumption of the formulas, and no difference was detected in their daily intake. Moreover, the time-related changes in weight, length, and height were comparable between the EHCF + LGG and EHCF groups.[32]

A study analysing faecal samples from healthy controls (n = 20) and from CMA infants (n = 19) before and after treatment with EHCF with (n = 12) and without (n = 7) LGG supplementation suggested that EHCF + LGG promoted tolerance of cow's milk, in part by influencing the strain-level bacterial community structure of the infant gut. Study did not report any adverse events.[33]

Synbiotics

A trial randomising 90 exclusively formula-fed infants younger than seven months with atopic dermatitis (AD) to extensively hydrolysed formula (EHF) plus synbiotics (a mixture of scGOS and lcFOS and *Bifidobacterium breve* M-16 V) or the same formula without synbiotics for 12 weeks showed no difference in the primary outcome of severity of atopic dermatitis (SCORAD), although infants with IgE-associated AD (defined as patients with AD and associated with elevated total and/or specific serum IgE levels at baseline) in the synbiotic group showed significantly greater improvement in SCORAD score than those in the placebo group ($p = 0.04$).[34] There was significant modulation of intestinal microbiota in infants given EHF with synbiotics, with higher percentages of *Bifidobacteria* and lower percentages of *Clostridium liuseburense/Clostridium histolyticum* and *Eubacterium rectale/Clostridium coccoides* than without synbiotics. The synbiotic effect remained at one-year follow-up, with significantly reduced risk of asthma-like symptoms and use of asthma medication. The percentage of patients experiencing any adverse event was similar in the synbiotic and the placebo group (91.1% vs. 84.1%, Chi-square test: P = 0.35). Two serious adverse events were reported (hospitalization because of respiratory syncytial virus bronchiolitis and because of severe cow's milk allergy) in the synbiotic and none in the placebo group. None of the reported adverse events were considered to be treatment-related.[34]

The multicentre ASSIGN study randomised 71 infants with suspected non-IgE mediated CMA to an AAF including synbiotics ([scFOS]/lcFOS/*Bifidobacterium breve* M-16 V) or an AAF without synbiotics. Results at eight weeks showed that infants receiving the AAF plus synbiotics had a faecal microbiota pattern of increased *Bifidobacteria* and decreased *Eubacterium rectale/Clostridium coccoides* (ER/CC) bacteria approximating that seen in age-matched healthy breastfed infants. In contrast, infants receiving the AAF without synbiotics had lower levels of *Bifidobacteria* and higher levels of adult-like ER/CC bacteria. The synbiotic-supplemented AAF in this trial was shown to be safe in terms of adverse events, use of concomitant medications, and achievement of growth targets.[35]

Ongoing and future studies

The ongoing, prospective double-blind PRESTO study is randomly allocating infants with confirmed IgE-mediated CMA to AAF with or without synbiotics (scFOS/lcFOS/*B breve* M-16 V) for 12 months, assessing acquisition of tolerance to cow's milk at 12, 24 and 36 months.[36] The large-scale TEMPO study is investigating the use of partially hydrolysed whey protein infant and follow-on formula supplemented with scGOS/lcFOS/*B. breve* M-16 V compared to standard cow's milk formula without synbiotics in healthy, high-risk, not-exclusively breastfed infants, compared to a reference group of breastfed infants. The primary outcome is faecal levels of *Bifidobacteria* at 17 weeks of age. Secondary outcomes are levels of *Bifidobacteria* and adult-like ER/CC group bacterial cluster and IgE-mediated allergic manifestations up to 52 weeks of age.[37] A second large study with the exact same design as the TEMPO study will be initiated. This study will assess any allergic manifestation at 12 months as the primary outcome.[38]

Current guideline recommendations

Currently available evidence does not indicate that probiotic supplementation reduces the risk of any allergic manifestation in children. However, considering all critical outcomes including efficacy, safety and tolerability data in this context, the World Allergy Organization (WAO) guideline panel stated 'that there is a likely net benefit from using probiotics resulting primarily from prevention of eczema.'[14]

In terms of preventing food allergy, WAO guidelines suggest using probiotics in:

- Pregnant women at high risk for having an allergic child
- Women who breastfeed infants at high risk of developing allergy
- Infants at high risk of developing allergy[14]

However, the guideline group acknowledged that the evidence supporting these recommendations was of low quality and it is not currently clear which probiotic to use and when to start and stop use of probiotics.[14] The ESPGHAN group noted that decisions on the use of a particular probiotic should be based on the data for that probiotic.[39,40]

The European Academy of Allergy and Clinical Immunology (EAACI) guidelines on the primary prevention of food allergy found conflicting results with the studies reviewed when the guidelines were published in 2014, and considered the evidence available at the time did not support use of prebiotics or probiotics for food allergy prevention.[41] However, the guideline authors noted that different microorganisms had been used in different studies and suggested that different microbial strains may have different effects, which may have explained the inconsistent results regarding a possible preventive effect of specific strains of probiotics.[41] This highlighted the need for research studies using very precisely specified strains of probiotics.

The EAACI panel suggested using prebiotic supplementation for the prevention of allergy in not-exclusively breastfed infants regardless of

their allergic risk.[14,42] The guideline group acknowledged that this recommendation is conditional and based on very low certainty of the evidence.[43]

There are numerous guidelines for the treatment of cow's milk allergy, which recommend and actively support continued breast feeding as the ideal nutrition for allergic infants but where this is not possible, typically advise EHF first for allergy management, moving to AAF if EHF fails.[44] They also advise using AAF first for children at high risk of anaphylactic reactions and more complex presentation of non-IgE CMA (i.e. eosinophilic esophagitis) if continued breast feeding with maternal milk exclusion is not possible.[4,44] In China, the new expert consensus on food allergy-related gastrointestinal diseases recommends using AAF for infants diagnosed with CMA.[45] However, guidelines do not make specific recommendations on the use of probiotics and prebiotics in the treatment of CMA because of a lack of evidence at the time they were developed.[46] The WAO guidelines considered modulation of the immune system using functional foods offers a promising research hypothesis as part of efforts to induce a tolerogenic immune environment in the context of CMA. However, in the guideline authors considered that more evidence from randomised controlled trials is needed. They identified further research on probiotic supplementation in CMA treatment as an important area for the development of a stronger evidence base in CMA.[4]

Further research questions

Improving the recognition and clarifying the diagnosis of CMA

Despite the growing incidence of CMA in most countries, there is an ongoing problem of poor recognition by general clinicians, particularly those working in primary care. This is particularly the case for non-IgE-mediated CMA. This failure to recognise possible CMA results in delays in investigations, diagnosis and management.[47,48] There is also confusion about the terminology used for CMA, which is used to describe several conditions, including intolerance. There is also frequent failure to correctly classify CMA, which can lead to over diagnosis and incorrect treatment. The problem differs between healthcare systems, with poor recognition and identification of CMA in some systems and misclassification and over diagnosis in others. Research needs to be carried out with the aim of facilitating the early recognition and correct classification and treatment of CMA.

Characterising healthy and allergic microbiome patterns

The tools are not yet in place to assess the microbiome or to describe the allergic microbiome with enough specificity to use it clinically. Further work is needed on gut microbiota analysis as part of managing and treating allergy. There are currently no normative data or reference values, and there is a lack of clarity on what can be diagnosed specifically from the analysis. Better understanding is needed of the dynamic nature of the microbiome in early life, including the succession of species over time, comparing allergic infants with healthy breastfed infants.

Clearer definitions and signatures of healthy and allergy microbiomes are needed, taking into account that these will vary in different parts of the world, across different categories of risk, and in different social classes. Tools are needed to assess the microbiome and to ascribe an allergic signature with enough specificity to discuss with patients as part of informing specific recommendations for them.

Diagnosing and intervening on dysbiosis

Accurate and fast bedside diagnostics are needed to diagnose dysbiosis and research the impact of interventions. More studies are required to assess whether correcting dysbiosis can help prevent and/or treat allergic disease.

Further research in this area must be very specific on the prebiotics, probiotics and synbiotics being investigated. The dose and timing should also be carefully considered. Critical for this is viable, affordable targeted probiotics that are available to treat dysbiosis.

Assessing allergic outcomes with gut microbiota modification

Studies have shown the potential to influence the gut microbiota in infants, however more work is needed on patient outcome benefits, particularly in terms of allergic outcomes.[35]

Identifying the optimal timing for intervention

The window of opportunity for preventing and treating CMA in early life is important but currently unclear. This is a potential focus for future research to explore the optimal timing for interventions. Ultimately this will help confirm the optimal timing for use of synbiotics and explore whether early intervention has the potential to halt the allergic march, redirect it or even stop it completely.

Understanding the multiple risk factors for allergy development.

Understanding complex diseases such as allergies, including CMA, is challenging with multiple risk factors and mechanisms occurring and interacting at the same time.[49] Research methods, such as bioinformatics approaches, are being developed to assess this multi-causality and complexity but more work is needed.

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List of abbreviations

AAF	Amino acid based formula
AD	Atopic dermatitis
ASSIGN	An Amino Acid based Formula with synbiotics: Effects on gut microbiota diversity and clinical effectiveness in suspected gastrointestinal non-IgE mediated Cow's Milk Allergy
CMA	Cow's milk allergy
EAACI	The European Academy of Allergy and Clinical Immunology
EHCF	Extensively hydrolyzed casein formula
EHF	Extensively hydrolysed formula
ER/CC	*Eubacterium rectale/Clostridium coccoides*
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FIA	Food-induced anaphylaxis
lcFOS	Long-chain fructo-oligosaccharides
LGG	*Lactobacillus rhamnosus* GG
PATCH	The effect of early nutrition in high-risk infants on allergy prevention during the first 12 months of life.
PRESTO	A prospective double blind randomised controlled study to evaluate the immunological benefits and clinical effects of an elimination diet using an amino acid formula (AAF) with an added pre-probiotic blend in infants with Cow's Milk Allergy (CMA)
scGOS	Short-chain galacto-oligosaccharides
SCORAD	Scoring atopic dermatitis/Severity of atopic dermatitis
SPT	Skin prick test
TEMPO	A Clinical Study to Investigate the Effect of a Partially Hydrolysed Infant Formula With Added Synbiotics on Gut Microbiota Composition and Clinical Effectiveness in Infants at High Risk of Developing Allergy
WAO	World Allergy Organization

Declarations

Ethics approval and consent to participate

Not applicable (paper includes no primary data).

Consent for publication

Not applicable (paper includes no primary data).

Availability of data and material

Not applicable (paper includes no primary data).

Competing interests

The authors declare that they have no competing interests.

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