Immunomodulation properties of biotics and food allergy in pediatrics

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ABSTRACT
Preterm birth, C-section, antibiotic use, and limited breastfeeding are blamed in part for the increasing incidence of chronic non-communicable diseases among children, such as allergies, mainly cow's milk protein allergy (CMPA). Those who develop allergic diseases, against those who do not, show differences in the composition of their gut microbiota during the first months of life. Early interventions to modulate the intestinal microbiota and the immune system may be the key tools for the management of CMPA. Clinical judgment and the interdisciplinary work of allergists, gastroenterologists, immunologists, microbiologists, and nutritionists will allow pediatricians to achieve an adequate diagnosis and a timely treatment. In this setting, the use of biotics (prebiotics, probiotics, synbiotics, and postbiotics) as supplementary dietary tools is scientifically supported at present and seems to be very promising for the prevention and treatment of these conditions.

Key words: *immunomodulation, biotics, hypersensitivity, milk proteins.*

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INTRODUCTION

Translational medicine has made it possible to highlight the important role that intestinal microbiota plays in the maintenance of health and the occurrence of illness. The fact of observing a gut microbiota profile which differs between healthy populations and those with certain conditions has been the basis for determining a possible causal role of the microbiota in the development of metabolic disorders such as obesity and the associated metabolic syndrome.[1] This knowledge is being progressively applied to immunological diseases, functional digestive problems, emotional disorders, and neurodevelopment impairments, among others.[2]

In pediatrics, microbiota composition of exclusively breastfed infants is considered the gold standard for a healthy microbiota.[3] The predominance of bacterial genera such as *Bifidobacterium* and *Lactobacillus*, transferred from the maternal microbiota to the newborn through vaginal delivery and breastfeeding, and reinforced by natural prebiotics called human milk oligosaccharides (HMO), is the best example of an initial and successful gut "seeding" that sets the stage for a healthy childhood. With the recognition of the importance of dominant short-chain fatty acid-producing strict anaerobic bacteria present in the gut, the microbiota has emerged as the "missing link" in a more comprehensive understanding of the protective role of breastfeeding.

The concept that the microbiota can be in equilibrium stems from studies of its microbiological composition, in which ordinary microbiological methods such as standard counts in Petri dishes were used.[4] This approach provided a partial picture of the microbial complexity compared to what we know today by using culture-independent methods, namely those based on DNA sequencing. These modern techniques provide a powerful means to determine that day by day, the microbiota has great taxonomic variability rather than a state of equilibrium.[5] This dynamics, and the fact that the composition is highly variable among individuals,[6] renders it difficult to taxonomically define what microbiota is in eubiosis or dysbiosis in terms of the presence of microbial species and their relative abundance.[7] This has led to suggesting that a healthy microbiota is one with a high degree of microbial diversity and abundance of species,[8] without determining which specific species are associated with a state of eubiosis or dysbiosis. This issue in coming to terms regarding the taxonomic composition that would characterize microbiota in eubiosis implies that currently, studies on microbiota characterization lack validity and cannot be extrapolated to the clinical practice.

Dysbiosis is therefore a reduction in bacterial diversity and abundance. In the pediatric domain, possibly the only time when low diversity is not a sign of dysbiosis is along the first 6 months of life in exclusively breastfed children. In this period, there is a predominance of *bifidobacteria* and *lactobacilli*, considered as very healthy bacteria due to their ability to digest specific prebiotics, generating short-chain fatty acids, and modulating the metabolic and immunological programming of the child, hence resulting in a lower incidence of autoimmune and allergic diseases.[9] In case breastfeeding is not possible, it is recommended that prebiotics and probiotics are added to infant formulas to improve infants' functional capacity and nutritional status in order to foster a healthy microbiota by stimulating the growth of the above-mentioned microorganisms.

THE BIOTICS FAMILY

Adequate maternal nutrition during pregnancy, term birth, vaginal delivery, breastfeeding, and the interaction with the environment and the family promote a diverse, abundant, and functional microbiota during the first 2 to 3 years of life.[10-12] It is in an unfavorable setting for the development of a functional microbiota (preterm birth, C-section, reduced breastfeeding, family history of allergies, or a combination of these factors) when the biotics family becomes relevant as a microbiological tool for prevention and treatment purposes. Biotics are microorganisms or substances that scientifically support the functioning of the gut microbiota and the gut associated immune system.[13]

A prebiotic is "a substrate selectively used by the host microorganisms, which confers a health benefit".[14] Examples of prebiotics with proven beneficial effects are inulin, galactooligosaccharides, and fructooligosaccharides, among others.[15] There are more than 200 oligosaccharides present in breast milk and are the first prebiotics an infant receives through breastfeeding.[16]

Probiotics were defined by the World Health Organization in 2002, included in the Argentine Food Code in 2011, and endorsed by the International Scientific Association of Probiotics and Prebiotics (ISAPP) in 2014.[17] A probiotic is "a live microorganism that, when administered in sufficient quantities, is capable of exerting a beneficial effect on the consumer's health".[17] Most food probiotics have been developed from specific strains of the genera *Lactobacillus* and *Bifidobacterium*. For instance, foods with undefined microbial species diversities, such as *kefir* or *kombucha*, should not be called probiotics because their microbiological composition is unknown *a priori*, and it also varies among products bearing the same name; hence, apart from knowing that it contains alcohol, it is not possible to determine their potential beneficial effects on health.[18] A comprehensive review of probiotics health benefits is beyond the scope of this article; however, in a nutshell, we can state that, always in a strain-dependent manner, probiotics can contribute to digestive health, promote food digestion, reinforce the epithelial barrier, and modulate intestinal inflammation to prevent intestinal infections (diarrheas) and airway infections (common respiratory infections), hasten the resolution of diarrheas, improve the response to vaccines (adjuvant effect), and prevent and treat certain allergies, among others. These benefits are achieved through different mechanisms (*Figure 1*).

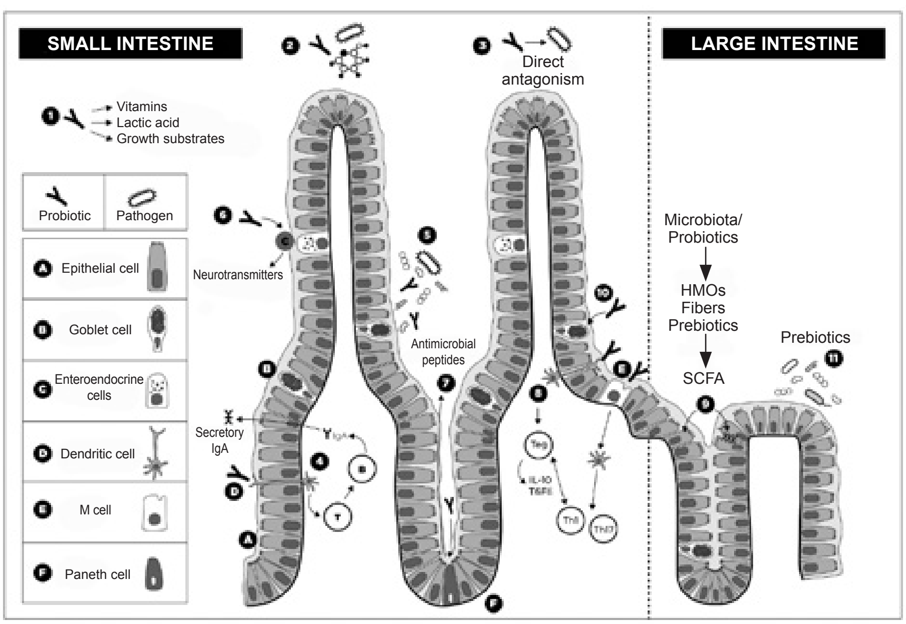
A synbiotic is defined as a "mixture of live microorganisms and substrates that are selectively utilized by the host organisms, which exerts a beneficial effect when administered in adequate amounts".[19] It is worth noting that the term "synbiotic", instead of symbiotic, is used. This neologism represents the true meaning of the term, since a prebiotic and a probiotic when administered together, can exert their beneficial effects independently, without interacting with each other, without necessarily establishing a symbiosis as raised by the ecological concept.

Finally, a postbiotic is "a preparation of inanimate microorganisms and/or their components that confers a health benefit to the host".[20] To fulfill this definition, the product should have non-viable microorganisms, accompanied (or not) by metabolites and/or fermentation products. There are products on the market, such as infant formulas containing prebiotics and postbiotics.[13] Since these non-viable microorganisms or their microbial fractions are unable to grow and occasionally cause an infection in immunosuppressed populations, or in subjects whose intestinal barrier is not strong enough, they would offer possibilities for nutritional intervention in cases where translocation is an issue of concern. Additionally, since these products contain non-viable microorganisms, their shelf-life might be longer since refrigeration is not required, and thus be supplied to geographical regions without adequate cold chain infrastructures.

FUNCTIONAL DISORDERS: DIFFERENTIAL DIAGNOSIS BETWEEN GASTROESOPHAGEAL REFLUX DISEASE AND COW'S MILK PROTEIN ALLERGY

In 2016, the modified Rome IV criteria for functional gastrointestinal disorders,[21] very frequent among infants and children, were published. Crying in spells at this stage of life is interpreted as a digestive discomfort or an abdominal pain, and parental concern may lead to inappropriate behaviors (overfeeding, termination of breastfeeding, multiple changes of infant formulas, medical consultations

Figure 1. *Mechanisms of action exerted by probiotics and prebiotics on the gut. 1) Synthesis of vitamins, lactic acid, and growth substrates for microorganisms residing in the microbiota (intestinal trophic chain), 2) competition with pathogens for nutrients, 3) direct antagonism through the production of antimicrobial substances, 4) stimulation of the immune system for the production of polyvalent secretory IgA, 5) barrier effect against pathogens by temporarily integrating into the resident microbiota, 6) stimulation of the synthesis of neurotransmitters from enteroendocrine cells, 7) stimulation of antimicrobial peptide synthesis in Paneth cells, 8) stimulation of the immune system for the induction of oral tolerance and regulation of innate immunity, 9) production of short chain fatty acids with anti-inflammatory activity (butyric acid) and ability to reinforce the tight junctions of the intestinal epithelial barrier, 10) stimulation of Goblet cells for the production and reinforcement of the mucus layer, and 11) selective stimulation of the beneficial bacteria residing in the colon with prebiotics.*



Source: Developed by the authors.

and use of unnecessary medications, such as antacids, for example).[22] Symptoms that should raise the suspicion of an organic problem are frequent regurgitations, vomiting and cough episodes, Sandifer's syndrome (abnormal posturing of the head and neck), family history of atopy, respiratory or skin symptoms of allergy, gastrointestinal bleeding or clinical signs of anemia, failure to thrive, abdominal distension, perianal dermatitis, and poor general condition.[23] The most relevant organic causes to rule out in children with inconsolable crying are CMPA and, less frequently, gastroesophageal reflux disease (GERD).[24] Acid inhibitors are often empirically and incorrectly prescribed, and can cause dysbiosis.[25]

Attempting to unveil the clinical enigma

Colics and regurgitation mainly occur during the first 3 to 4 months of life, and most regurgitation events resolve naturally by 6 months.[26] When the onset of regurgitation occurs in the first 2 weeks of life, the vomiting is bilious, and when projectile vomiting is the predominant symptom, it is most likely related to anatomical malformations or conditions, such as CMPA.

In the ESPGHAN guidelines, the involvement of symptoms in different organ systems in association with regurgitation increases the likelihood of CMPA. Both regurgitation and atopic dermatitis are common disorders in the first months of life and the nature of this relationship remains to be clarified, especially in infants with severe eczema.[27]

Withdrawal of cow's milk for 4 weeks is the recommended initial diagnostic approach,[28] with a subsequent challenge test for diagnostic confirmation. In a systematic review of controlled trials, the administration of probiotics was associated with a higher rate of acquisition of tolerance to cow's milk protein by 3 years compared to placebo (n = 493, $p = 0.0009$), but was not associated with an earlier resolution of hematochezia.[29] Given their immunomodulatory properties, biotics have been proposed as a strategy for the management of CMPA.

Hydrolyzed formulas can vary significantly in terms of protein source, method, and degree of hydrolysis, macro- and micronutrient content, additional components such as prebiotics, probiotics, and postbiotics, and proof of clinical benefit. Therefore, the results of a specific formula cannot be extrapolated to a "new" or "similar" formula.

COW'S MILK PROTEIN ALLERGY AND FOOD ALLERGIES. REVIEW OF ORIGIN AND SUSPICION

Food allergy has a significant socioeconomic impact and, in recent decades, there has been concern about its increase. It is defined as an adverse reaction resulting from an immune response triggered by the ingestion of a specific food. The immune reaction may be IgE-mediated, non-IgE-mediated or mixed. The maximum prevalence is reached in infancy and the highest incidence during the first year of life.[30] A prevalence of up to 10% has been reported in preschool children.[31] While 90% of food allergies are caused by milk, egg, wheat, soy, peanuts, tree nuts, fish, and shellfish, the food most frequently involved is milk.[28]

The importance of a correct diagnosis minimizes the burden on patients and their families. Without it, which includes the evaluation by a specialist and testing, there is a high risk of over- and under-diagnosis and, consequently, suboptimal treatment. The correct diagnosis, through the gold standard, which is an elimination and reintroduction diet, allows the prescription of an adequate diet that facilitates the child´s normal growth and development. On the contrary, an elimination diet that is not necessary or that is maintained even when the child has already developed tolerance can harm the child's development, their quality of life, and cause unnecessary medical expenses.[32]

The ingestion of cow's milk can lead to acute skin symptoms (hives, erythema, rash, swelling around the lip and eyelids), gastrointestinal symptoms (vomiting, gastroesophageal reflux, diarrhea, enterorrhea, proctocolitis), respiratory symptoms (asthma and rhinitis), and severe symptoms, such as anaphylaxis.[33] The treatment of choice is the complete elimination of milk and dairy products from the diet, both for the baby and the breastfeeding mother. For infants who cannot be breastfed, other nutritional solutions (infant formulas) are available. Currently there are different formulas based on cow's milk proteins, ranging from those formulated with partially or extensively hydrolyzed proteins to amino acid-based formulas, which in turn may contain prebiotics, probiotics and/or postbiotics.

The introduction of baked milk into the child´s diet may speed up the resolution of cow's milk allergy. The introduction of such products in these children's diet appears to markedly speed up the development of tolerance to milk compared with strict avoidance. Encouraging diet diversification, healthy nutrition, and participation in social activities can also improve their quality of life.[33]

The measurement of allergen-specific IgE or the skin prick test (SPT) are useful markers for the diagnosis and prediction of food tolerance. A cow's milk-specific IgE level ≥ 15 KUA /L and a SPT ≥ 8 mm in children ≤ 2 years are highly predictive of a positive reaction to an oral milk challenge.[34]

THE INTERACTION OF MICROBIOTA, BIOTICS, AND THE IMMUNE SYSTEM

Immunomodulation by microbiota has been extensively studied. Different immune cells such as T and B cells, intraepithelial lymphocytes, dendritic cells (DCs), and macrophages can be found in the gut-associated lymphoid tissue. Microbiota microorganisms, together with probiotics, interact with the mucosal immune system through numerous pathways. This leads to Toll-like and Nod-like receptor expression modulation in immune cells, DC activation, cytokine synthesis, and leukocyte phagocytic activity. These reactions are predominantly innate immune responses that result in the activation of Th1 and Th17 lymphocyte populations. The intestinal microbiota also exerts an appropriate modulation for the control of mucosal immune responses with the development of T helper and T regulatory (Treg) cells associated with the induction of tolerance through IL-10 and TGF-b production (*Figure 1*).[35]

Certain strains of *Bifidobacterium*, *Lactobacillus*, and *Clostridium*, among others, promote the development of Treg cells and have been shown to inhibit transcription and secretion factors of proinflammatory chemokines. The interaction of an altered gut microbiota with the immune system can damage the intestinal barrier, increasing bacterial translocation and triggering systemic proinflammatory effects. In contrast, *Lactobacillus* strains have been shown to induce tolerogenic DCs, and those of the genus *Bacteroidetes*, abundantly represented in the microbiota, activate cell-mediated immunity and lymphoid organogenesis. Peptidoglycans and lipopolysaccharides from commensal gram-negative bacteria of the microbiota also stimulate mucosal IgA, which is key not only in bacterial recognition, but also in mechanisms of tolerance induction and regulation of inflammation.[36]

RELATIONSHIP BETWEEN MICROBIOTA, DIET, AND PREDISPOSITION TO DISEASES

The gut microbiota plays major roles that is essential in the maintenance of health. This is partly due to the production of short-chain fatty acids (SCFA) produced by the fermentation of HMOs and dietary fibers, which act as local and systemic signaling molecules. SCFAs are also actively involved in the induction of an anti-inflammatory phenotype and mucosal IgA production. The main examples are acetate, propionate, and butyrate. Butyrate has an immunomodulatory function by suppressing macrophage proinflammatory cytokines in the lamina propria as well as long-chain fatty acids, omega-3, and conjugated linoleic acid (CLA), which decrease eicosanoid production and increase IL-10 release, thereby further inhibiting inflammation. Dietary supplementation with CLA increases IgA, promotes the decrease of serum IgE levels and the synthesis of antimicrobial peptides. The intestinal microbiota is involved in the metabolism of amino acids such as tryptophan (a precursor to serotonin); it is also essential for the synthesis of vitamin K and of several vitamin B complexes, the degradation of dietary polyphenols, the elimination of xenobiotics, and also the metabolism of bile acids. There is a bidirectional interaction in the gut microbiota; it produces and controls different elements that, in turn, modulate it.[37] The quantitative and qualitative loss of the composition of the intestinal microbiota is called dysbiosis, and this characterizes conditions, including infections and chronic non-communicable diseases (NCDs), such as type 2 diabetes mellitus and obesity. In children who will have a higher overweight at 7 years of age, a reduced early bacterial diversity is observed, with a lower number of bifidobacteria.[38]

To help prevent NCDs, as well as asthma or allergies, breastfeeding is essential, although certain nutritional strategies may be helpful, such as the use of prebiotics (capable of stimulating indigenous populations of intestinal bifidobacteria) or HMOs in infant formula feeding,[39] as well as providing fiber and omega-3 fatty acids to the mother during pregnancy. In turn, polyphenols (flavonoids in fruits and vegetables) promote the growth of beneficial microorganisms such as *Lactobacillus*. All these nutrients will favor epigenetic mechanisms that will have a health effect later in life as the maintenance of eubiosis in the gut microbiota, the integrity of the intestinal barrier, and the development of oral tolerance.[40]

CONCLUSIONS

The prevalence of allergies has been steadily increasing in recent decades. While this is likely to be multicausal, a limited early microbial exposure is one of the possible factors interfering with postnatal immune maturation. In support of this hypothesis, children who later develop allergic diseases show differences in the composition (diversity and abundance) of their gut microbiota during the first months of life compared to those who do not. Consequently, early interventions to modulate the gut microbiota and the immune system may be key to developing strategies for the treatment and prevention of CMPA. Clinical judgment and interdisciplinary work with specialists (allergists, gastroenterologists, immunologists, microbiologists and nutritionists) will allow the primary care pediatrician to determine the timely diagnosis and treatment, since they know the child and their family best, and can add a biopsychosocial approach to the situation, in addition to the appropriate clinical treatments tailored to each particular case, combined with the use of biotics (prebiotics, probiotics, synbiotics, and postbiotics) as complementary nutritional tools. ■

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