



SPECIAL ARTICLE

Microbiota and gastrointestinal diseases[☆]



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Abstract The bacterial colonisation is established immediately after birth, through direct contact with maternal microbiota, and may be influenced during lactation. There is emerging evidence indicating that quantitative and qualitative changes on gut microbiota contribute to alterations in the mucosal activation of the immune system, leading to intra- or extra-intestinal diseases. A balance between pathogenic and beneficial microbiota throughout childhood and adolescence is important to gastrointestinal health, including protection against pathogens, inhibition of pathogens, nutrient processing (synthesis of vitamin K), stimulation of angiogenesis, and regulation of host fat storage. Probiotics can promote an intentional modulation of intestinal microbiota favouring the health of the host. A review is presented on the modulation of intestinal microbiota on prevention, and adjuvant treatment of some paediatric gastrointestinal diseases.

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PALABRAS CLAVE

Microbiota;
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Microbiota y enfermedades gastrointestinales

Resumen La colonización bacteriana se establece inmediatamente después del nacimiento, por contacto directo con la microbiota materna, y puede modificarse durante la lactancia. Están apareciendo datos indicativos de que modificaciones cuantitativas y cualitativas de la microbiota intestinal son capaces de estimular cambios en la activación del sistema inmune que pueden conducir a la aparición de enfermedades gastrointestinales o extraintestinales. El equilibrio entre la microbiota patógena y beneficiosa durante la niñez y la adolescencia es importante para la salud gastrointestinal, incluyendo la protección frente a patógenos, la

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inhibición de patógenos, el procesamiento de nutrientes (síntesis de vitamina K), el estímulo de la angiogénesis y la regulación del almacenamiento de la grasa corporal. También los probióticos pueden modular la microbiota intestinal para favorecer la salud del huésped. Este artículo es una revisión sobre la acción moduladora de la microbiota intestinal en la prevención y el tratamiento coadyuvante de las enfermedades gastrointestinales pediátricas.

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Introduction

In recent years, extensive efforts have been made in research and clinical practice to gain a more thorough understanding of gut microbiota, its functions, its development, and its role in the pathophysiology of certain diseases.¹

The human gut is the natural habitat of a numerous, diverse and dynamic population of microorganisms, mainly bacteria that have adapted to live in the mucosal surfaces of the gastrointestinal lumen. The gut microbiota includes native species that colonise the gastrointestinal tract permanently, and a changing series of live microorganisms that pass through the digestive tract on a transient basis. The microbial population of the human gut includes about 100 billion bacteria of 500–1000 different species. The presence of certain bacteria as part of the intestinal flora is necessary for the adequate nutrition and development of the body, as well as for the appropriate maintenance of the immune system. Intestinal homeostasis is critical for the efficient extraction of energy from nutrients and for protection against microbial pathogens. Experiments conducted on mice² have demonstrated that germ-free animals are not only smaller than mice that have a normal microbiota from birth or that have been recolonised after being germ-free, but also have less body fat (are leaner); thus, it has been observed that normal-flora or recolonised mice store more fat while eating less. Furthermore, germ-free mice also consume less oxygen.

A thorough knowledge of the gut microbiota is key to determining the role of alterations at this level in the development of certain diseases. Many diseases result, to a great extent, from the presence of an altered intestinal microbiota,³ such as infectious diarrhoea and diarrhoea associated with antibiotic administration, complications of sepsis (multiple system organ failure, diverticulitis, appendicitis, etc.), functional gastrointestinal disorders (constipation, irritable bowel syndrome, etc.), obesity, diabetes type 2, metabolic syndrome, autoimmune diseases (including celiac disease), or colon cancer.

We proceed to review the main paediatric gastrointestinal diseases that are most strongly associated with the gastrointestinal microbiota. These include infection by *Helicobacter pylori*, necrotising enterocolitis, inflammatory bowel disease, celiac disease and acute diarrhoea.

Infection by *H. pylori*

More than 50% of the world's population is infected by *H. pylori*, with a prevalence of 30–40% in developed countries

and more than 80% in developing countries.⁴ In the population under 20 years, the prevalence is approximately 80% in developing countries, higher than in developed countries. The variations in prevalence are associated with sociodemographic factors, especially in individuals of low socioeconomic status.⁵

While the bacterial load in the stomach is low, *Helicobacter* species have received special attention due to their association with various gastric diseases. *H. pylori*, a gram-negative spirochete, is one of the most frequent agents of bacterial infection in humans,⁶ and can cause gastritis, gastric and duodenal ulcers, stomach cancer, adenocarcinoma and mucosa-associated lymphoid tissue lymphomas, such as MALT lymphoma.⁷ In the absence of ulcerative disease, most infections by *H. pylori* are asymptomatic, and when there are symptoms, they tend to be nonspecific. Recent studies in adults suggest that infection by *H. pylori* increases the risk of coronary heart disease.⁸

There is published evidence on the efficacy of lactic acid bacteria, bifidobacteria, fermented milks, *Lactobacillus salivarius* and *Lactobacillus acidophilus* for the mitigation of the adverse effects associated with treatment for *H. pylori* eradication. Several studies have demonstrated the efficacy of some probiotics (such as *Saccharomyces boulardii* alone or in combination with *Lactobacillus casei*) for the eradication of *H. pylori* in paediatric patients.⁹ There is an even greater volume of evidence in the adult population that supports the recommendation of probiotic therapy for increasing the rate of *H. pylori* eradication.^{9,10}

Necrotising enterocolitis

Necrotising enterocolitis is the most common gastrointestinal medical emergency that occurs in neonates. It represents a significant clinical problem in infants and affects up to 10% of infants that weigh less than 1500 g, especially neonates of extremely low birth weight (<1000 g) with less than 28 weeks of gestation.¹¹ Its mortality ranges between 20% and 30% of affected infants. Its morbidity is also high, mainly neurodevelopmental impairment in extremely low birth weight newborns.^{11,12} Despite advances in neonatal intensive care, NEC continues to be a potentially disastrous disease in preterm newborns, and there have been no significant changes in mortality and long-term morbidity "incidence".¹²

Its pathogenesis is poorly known. Several factors may contribute to its development, such as preterm birth, hypoxia, formula feeding, especially excess protein substrate in the intestinal lumen, sepsis, intestinal ischaemia,

and colonisation of the gut by pathogenic bacteria.^{11,12} A recently published study¹³ reported a higher proportion of *Proteobacteria* in newborns that had received a NEC diagnosis, consistent with other evidence. In this study, performed with nine neonates with NEC and nine in the control group, a lower proportion of these bacteria in patients with NEC was observed one week before diagnosis compared to the control group.¹⁴ The authors noted that preterm infants not sufficiently colonised by *Proteobacteria* in the first week of life may not be able to modulate an adaptive immune response against a subsequent bloom of *Proteobacteria*.¹⁴ Therefore, the immaturity of immune tolerance mechanisms, influenced by the quantity and quality of the microbiota, may be related to this disease.

Inflammatory bowel disease

In inflammatory bowel diseases (Crohn's disease and ulcerative colitis) there is an anomalous immune response to elements of the microbiota in the intestinal mucosa that causes intestinal damage.¹⁵ In the presence of commensal microorganisms, patients with inflammatory bowel disease show an increase in IgG antibodies and T cell activity in the mucosa, which suggests the suppression of local tolerance mechanisms.¹⁶ In fact, there are several factors that influence the stimulation and remission of inflammatory activity, such as derivation of the faecal stream, and the use of antibiotics to treat Crohn's disease and of broad-spectrum antibiotics in the intestinal lumen to treat ulcerative colitis.^{17,18} Considering that Crohn's disease in humans occurs in the colon and terminal ileum, where the bacterial concentration is highest,¹⁶ it is fair to assume that the intolerance generated by the microbiota combined with genetic susceptibility would contribute to the development of this inflammatory condition.¹⁵

The intestinal lamina propria is the site where immune cells first recognise bacterial antigens prior to their migration to distal lymphoid tissue to mount the inflammatory response.¹⁹ Toll-like receptors in epithelial cells and NOD2 receptors play an important role in the induction of the immune response. Once activated, these receptors can trigger an intracellular cascade leading to the production of proinflammatory cytokines.²⁰ This mechanism promotes the maturation of mucosal dendritic cells, which migrate to local lymphoid structures such as Peyer's patches and mesenteric draining lymph nodes following antigen recognition to initiate or maintain T- and B-cell immune responses. Dendritic cells initiate the immune response, control intestinal inflammation and maintain immune tolerance. In this context, it is believed that mucosal dendritic cells play a key role in the modulation of the immune response against the gastrointestinal antigenic environment, maintaining intestinal homeostasis and allowing the peaceful coexistence with the endogenous microflora.¹⁹ In normal individuals, the commensal flora cannot cross the epithelial barrier, and when some of these bacteria get through the intestinal barrier, they are quickly phagocytosed by the macrophages in the mucosa, preventing the activation of the intestinal immune response. On the other hand, when pathogenic microorganisms cross the barrier, the immune response is activated.²¹

Ulcerative colitis and Crohn's disease usually develop in areas with the highest concentrations of gut microbiota, which suggests that commensal bacteria, when associated with genetic susceptibility, may contribute to the pathogenesis of these diseases.²² Furthermore, various studies cited by Danese²³ suggest that the microbiota found in patients with inflammatory bowel disease is different from that of healthy individuals, and the authors noted that dysbiosis may lead to immune intolerance. These patients had a reduced microbial diversity with a predominance of *Clostridium*, *Bacteroides* and *Bifidobacteria* (commensal microbiota) and a concomitant increase in pathogenic bacteria such as *Escherichia coli*, which is rarely found in individuals without bowel inflammation.²³ The proportion of pathogenic bacteria may amount to 30–40% of the predominant bacteria, although it is well known that causality has not been properly established.²⁴ It is worth mentioning that reduced bacterial diversity has been associated with a lack of stability in the microbiota, making it more susceptible to changes in composition driven by environmental factors. Thus, changes in the microbiota may increase the risk of inflammation.

Celiac disease

Celiac disease (CD) is currently defined as an immune-mediated systemic disorder elicited by gluten ingestion in genetically susceptible individuals and characterised by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA DQ2 or DQ8 haplotypes and enteropathy.¹³ The exclusion of gluten from the diet is associated with the disappearance of the symptoms and the CD-specific antibodies and the normalisation of the intestinal mucosa in most of the patients.²⁵

Studies conducted in recent years have found a prevalence of CD of 1:100 (range, 0.5–2%). The disease affects children as well as adults, and the female to male ratio is 2:1.

In celiac patients, the immune response to gliadin fragments elicits an inflammatory response, mainly in the upper portion of the small intestine, characterised by infiltration of the lamina propria and the epithelium by inflammatory cells and villous atrophy. This response is mediated by innate and adaptive immunity. The adaptive response is mediated by T CD4+ lymphocytes in the lamina propria that recognise gliadin peptides, which bind class II HLA molecules (DQ2 or DQ8) expressed in antigen-presenting cells; T cells then produce proinflammatory cytokines, and interferon γ in particular. The enzyme tissue transglutaminase catalyses the deamidation of gliadin peptides in the intestine, enhancing their immunogenicity.²⁵

It is believed that besides immunologic and genetic factors, environmental factors such as type of feeding during infancy and the intestinal microbiota play a role in the aetiopathogenesis of CD.^{26,27}

The importance of the microbiota in CD remains to be determined.²⁸ Some genetic factors have been associated with colonisation by *Bacteroides* species.²⁹ Some studies conducted on celiac patients have shown that a strict gluten-free diet promotes a decrease in colonisation by beneficial bacteria, especially *Bifidobacterium* and

Lactobacillus, compared to colonisation by gram-negative bacteria (*Bacteroides* and *E. coli*).³⁰ This change in the microbiota following gluten exclusion may be due to the elimination of important sources of carbohydrates, the main source of energy for the commensal microbiota.²⁹ Dietary treatment in these patients does not promote intestinal homeostasis; however, the immunosuppressive effects of such a microbiota may be beneficial for patients with CD.³¹

Ongoing research on the roles of specific components of the microbiota in the pathogenesis of CD may allow the future development of nutritional intervention strategies (probiotics and prebiotics) for preventive or therapeutic purposes.

Acute diarrhoea

Acute gastroenteritis (AGE) is defined as the passage of loose or watery stools three or more times a day and with a duration of less than 14 days, which distinguishes it from chronic diarrhoea, usually of an infectious origin, with the aetiological agent being a microbial pathogen: viruses, bacteria or parasites. It may be accompanied by nausea, vomiting, fever, abdominal pain or dehydration, and it is usually self-limiting.^{32–34} In Europe, the incidence of AGE in children less than 3 years of age ranges between 0.5 and 1.9 episodes per year.³²

The most common causative agents are *Rotavirus* and, among bacteria, *Campylobacter* followed by *Salmonella*. Parasites such as *Giardia lamblia* and *Cryptosporidium* are a rare cause of diarrhoea in healthy children. The predominant causative agents may change with the age of the child: in children younger than one year, *Rotavirus*, *Norovirus*, *Adenovirus* and *Salmonella* are most common. Between 1 and 4 years, the same pathogens predominate along with *Campylobacter* and *Yersinia*. In children older than 5 years, they are *Campylobacter*, *Salmonella* and *Rotavirus*.³² Diarrhoea associated with antibiotic administration is also common, with an incidence of up to 30%.³⁵

In the management of patients with AGE, the initial treatment must include rehydration by means of oral solutions and a rapid replenishment of nutrients. Rehydration with small volumes of oral solution can compensate the loss of water and electrolytes caused by AGE, but it does not have a favourable impact on the duration of diarrhoea nor on the quality and intensity of the bowel movements; it also does not restore the intestinal microbiota that was altered by the infection.

The rationale for the use of probiotics to treat and prevent AGE is based on the modification of the composition of the gut microbiota to prevent the growth of pathogenic enteric strains. It is also believed that probiotics secrete antibacterial substances, competing with pathogens and preventing their adhesion to the intestinal epithelium, and that they have an anti-toxin effect and reverse some of the consequences of infection of the intestinal epithelium, such as secretory changes and neutrophil migration. Used in combination with rehydration therapy, certain probiotics seem to be safe and have clear beneficial effects in the reduction of the duration and frequency of acute infectious diarrhoea.

The effect of probiotics depends on the species and strain used, and the effects of one microorganism cannot

be extrapolated to other species or strains.³² The probiotics for which the efficacy has been clearly demonstrated are basically *Lactobacillus rhamnosis* GG and *S. boulardii*.^{36–38} The effect seems to be dose-dependent, with higher doses being more efficacious. Probiotics are also more useful when they are administered early in the course of diarrhoea.

Final considerations

The microbiota of the human gut constitutes a universe in itself, alive within our bodies, and it has a significant impact in our physiology and pathophysiology.

The main functions carried out by the human gut microbiota, such as metabolic functions (fermentation of nondigestible dietary substrates and endogenous mucus), protective functions (protection against pathogens in a barrier effect) and trophic functions (control of the proliferation and differentiation of epithelial cells), and its role in the development and homeostasis of the immune system, allow us to say that the gut microbiota is a metabolic organ.

A good understanding of the intestinal microbiota is essential to elucidate the role of changes at this level associated with the development of certain intestinal and extraintestinal diseases.

Conflicts of interest

The authors have no conflicts of interest to declare.

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