REVIEW

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Intestinal microbiota in digestive diseases

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ABSTRACT – Background – In recent years, especially after the development of sophisticated metagenomic studies, research on the intestinal microbiota has increased, radically transforming our knowledge about the microbiome and its association with health maintenance and disease development in humans. Increasing evidence has shown that a permanent alteration in microbiota composition or function (dysbiosis) can alter immune responses, metabolism, intestinal permeability, and digestive motility, thereby promoting a proinflammatory state. Such alterations can mainly impair the host's immune and metabolic functions, thus favoring the onset of diseases such as diabetes, obesity, digestive, neurological, autoimmune, and neoplastic diseases. This comprehensive review is a compilation of the available literature on the formation of the complex intestinal ecosystem and its impact on the incidence of diseases such as obesity, non-alcoholic steatohepatitis, irritable bowel syndrome, inflammatory bowel disease, celiac disease, and digestive neoplasms. **Conclusion** – Alterations in the composition and function of the gastrointestinal microbiota (dysbiosis) have a direct impact on human health and seem to have an important role in the pathogenesis of several gastrointestinal diseases, whether inflammatory, metabolic, or neoplastic ones.

HEADINGS – Microbiota. Gastrointestinal microbiome. Dysbiosis. Gastrointestinal diseases.

INTRODUCTION

The human gastrointestinal microbiota ("microflora") consists in a group of microorganisms that live in the digestive tract. They comprise a metabolically active and complex ecosystem, consisting of hundreds of thousands of microorganisms (bacteria, viruses, and some eukaryotes) that colonize the digestive tract soon after birth[11,20]. The microbiota has established a dynamic association of mutual benefits (symbiosis) with the human organism, which results in the maintenance of normal immunological, metabolic, and motor functions, as well as correct nutrient digestion and absorption[11,20,30,53].

The microbiota acts as a true barrier to aggressive agents, as more than 10^{14} microorganisms cover the entire surface of the digestive tract, primarily in the intestine, competing with pathogens for nutrients and binding sites, producing inhibitory substances and preventing their penetration into the intestinal mucosa[30,42,59]. This population encodes 3 to 4 million genes, or approximately 150 times more than the human genome[20,42]. As it will be discussed below, the microbial genome allows the microbiota agents to perform several metabolic activities that are not encoded by the human genome and, therefore, can be beneficial to the host.

Humans have their own individual pattern of microbiota distribution and composition, which is in part determined by the host genotype and by the initial colonization that occurs immediately after birth[59]. Different factors such as the type of delivery and breastfeeding, lifestyle, diet, hygienic and environmental conditions, antibiotic use, and vaccination can determine definitive changes in the microbiota pattern[9,46,59].

The intestinal microbiota consists of more than one thousand and five hundred species, distributed in more than 50 different phyla[20,53], although most are represented by only two phyla: *Firmicutes* (more abundant) and *Bacteroidetes*. Other phyla also found in minor proportions are *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia*[53].

An important European study (the "MetaHit project") analyzed the intestinal microbiota of 700 healthy volunteers, showing that the participants' microbiota composition, regardless of age, gender and body mass index, belonged to one of the three major groups or types, called *enterotypes*[33]. Each enterotype was identified based on the abundance of bacteria in relation to three main genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3)[5,33]. However, it is not known what factors promote the aggregation of bacterial communities into enterotypes and it is believed that there may be a constant variation of these species in the digestive tract[5,42].

The distribution of the intestinal microbiota varies according to its location in the digestive tube[20,53]. In the stomach and duodenum, due to the presence of acidic gastric juice and pancreatic enzymes, the bacterial density is quite low. It gradually increases in the distal small intestine, reaching its highest concentration (10^{11}-10^{13} bacteria/g) in the colon, with the absolute predominance of anaerobes[11,20].

In recent years, especially after the development of complex metagenomic studies, research in this area has increased, radically transforming our knowledge on the intestinal microbiota and its association with human health maintenance.

Increasing evidence has shown that a permanent alteration in the microbiota composition or function (*dysbiosis*) can alter visceral sensitivity, intestinal motility, and permeability, as well as alter the immune response, thus promoting a proinflammatory state[4,53]. Such alterations, especially in the host's immune and metabolic

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functions, can originate or favor the onset of several diseases such as diabetes, obesity, as well as neurological and autoimmune diseases[42,54]. Recent studies have also demonstrated the participation of the microbiota in the etiopathogenesis of many gastroenterological diseases, such as irritable bowel syndrome, inflammatory bowel disease, celiac disease, non-alcoholic steatohepatitis and digestive neoplasms[22,34].

Microbiota and Immunity

The intestinal microbiota exerts an important effect on the immune response of humans, being crucial for the development and expansion of lymphoid tissues and for the maintenance and regulation of intestinal immunity[11,20,30,53].

Experimental studies on germ-free mice have shown that bacterial colonization is a fundamental condition for the development of gut-associated lymphoid tissue (GALT), as well as for the diversified formation of B-cell IgM^{+4} antibodies[46,59]. It was observed that the intestinal mucosa of these non-colonized animals had a lower number of B, T and dendritic cells, as well as immature mesenteric lymph nodes and Peyer's patches[59]. Additionally, their villi are narrower and longer and the crypts are deeper. It has been The authors emphasized that after being colonized by commensal microorganisms, the immune system of these mice develops and normalizes (Figure 1).

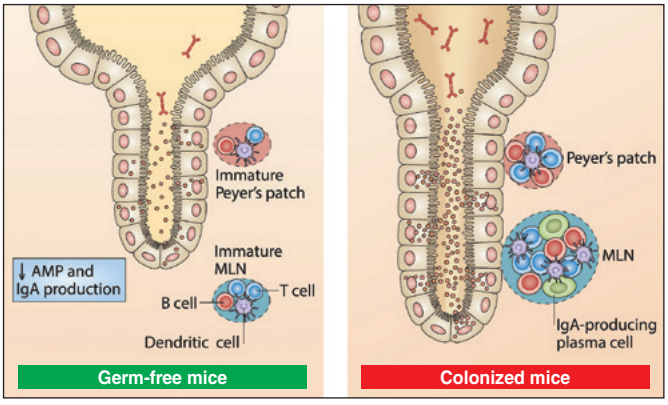

FIGURE 1. Immune system development after bacterial colonization in germ-free experimentation animals. Adapted from Sommer and Bäckhed, 2013[59].

The induction of the immunological tolerance in the intestine is essential and as such no undesirable inflammatory responses occur against food proteins or even against the intestinal microbiota[20,53]. T cells can generate subpopulations of which immune responses may be anti or proinflammatory[59].

Bacteroides fragilis is an example of bacteria present in the intestine, capable of inducing the differentiation of CD4+ T cells into regulatory T cells, which favors the production of anti-inflammatory cells and the transforming growth factor beta (TGF-β), neutralizing the pro-inflammatory response of Th17 (T helper)[62]. The capsule of this bacterium consists of polysaccharide A.

On the other hand, the group of filamentous bacteria, after contacting the antigen-regulating cells, seems capable of inducing pro-inflammatory cells such as Th17[44]. Obviously, inflammation induction, when well controlled, may even be beneficial and even necessary. Some authors suggest that the immunological responses triggered by the intestinal microbiota can strengthen the intestinal barrier[11,20,44]. In fact, mice that do not have filamentous bacteria have a more fragile immune response and, consequently, become more susceptible to infections[4,54,62].

All these findings from recent studies suggest that the intestinal microbiota and the immune system establish a constant interaction of mutualism with the host, in which both are benefited[11]. This interrelationship results in several immunological responses, such as immunoglobulin A secretion and the release of antimicrobial peptides, which allow the maintenance of a dynamic equilibrium with the commensal microorganisms.

Recent studies have confirmed the initial hypotheses that the decrease in the microbial population caused by the improvement of hygienic conditions may have contributed to the increase in the prevalence of autoimmune diseases in developed countries[61]. In fact, dietary and environmental alterations can lead to qualitative and quantitative changes in the intestinal microbiota and immune response and, therefore, can eventually contribute to the increased incidence of diseases with a significant inflammatory component such as asthma, obesity and diabetes[61,67]. It is concluded that a healthy diet contributes to the growth of bacteria that are beneficial to the host. An important characteristic in this case is the presence in the diet of substances that can be employed by bacteria, such as resistant starch fibers, found in fruits, vegetables, and garlic. These foods are more resistant to the high digestive enzymes found in the digestive tract[10].

Microbiota and metabolism

The intestinal microbiota contributes directly to the metabolization of nutrients and vitamins essential for host viability, collaborating to obtain energy from food[11,20,53]. This energy is acquired especially through the fermentation of non-absorbable carbohydrates, in a reaction that induces the production of short-chain fatty acids (SCFAs), hydrogen and carbon dioxide[44,62].

Current research demonstrates that SCFAs have much more refined functions than merely nourishing microorganisms and enterocytes, and are now considered important regulators of immunity, energy metabolism and adipose tissue expansion. Fatty acids can activate the G-protein coupled receptors – GPR41 and GPR43 – expressed in several cells (immune, endocrine, and adipose cells)[21]. This process is important for the YY peptide expression and the glucagon-like peptide 1 (GLP-1) production, directly contributing to lipolysis inhibition and adipocyte differentiation, with consequent increase in adipose tissue in animals. The SCFAs also allow the acidification of the colonic lumen, preventing the growth of bacterial pathogens[21,35].

It is important to emphasize that the intestinal microbiota has a direct participation in the metabolism of bile acids from dietary cholesterol. In the gut, primary bile acids bind to cell receptors, promote the absorption of fat and fat-soluble vitamins, and bind to cell receptors, such as TGR-5, which, when activated, trigger several protective metabolic effects such as resistance to weight gain and the development of hepatic steatosis[27].

The anaerobic metabolism of bacteria also includes proteolytic fermentation in the distal colon, originating nitrogenous derivatives such as amines and ammonia, some of which have carcinogenic effects[43].

Microbiota, obesity, and non-alcoholic steatohepatitis

Obesity arises mainly due to the consumption of high-calorie foods, carbohydrates, and saturated fats, although the simple

increase in caloric intake is not enough to explain the current obesity epidemic. Studies on germ-free mice showed that they did not increase their weight when exposed to high-calorie diets, from which one infers that diet alone is not enough to induce obesity[35]. On the other hand, obese mice have more genes encoding enzymes that break down nondigestible polysaccharides from the diet, in addition to having more fermentation products (SCFAs) and fewer calories in their feces, suggesting that in these animals the microbiota seems to help by extracting additional calories from the diet[65]. Other studies also found that germ-free mice, after being colonized, exhibited not only increased total body fat (despite a lower caloric intake), but many of them became insulin-resistant[14,65].

The intestinal microbiota also participates in the digestion of polysaccharides, increasing the amount of glucose in the liver and, therefore, increasing lipogenesis[14].

An "obese type" human microbiota associated with metabolic syndrome and overweight has been described, which shows an increase in the *Firmicutes*/*Bacteroidetes* ratio[29,42,65]. It has been shown that genetically obese mice have 50% fewer *Bacteroidetes* and more *Firmicutes* than lean animals[13,65]. It has been proven by several researchers that the microbiota of obese animals releases more calories during digestion than that of lean animals. It is also noteworthy that the administration of a typical high-calorie western diet in normal weight animals determines a marked reduction of *Bacteroidetes* and an increase of *Firmicutes*[22,30,42].

Recent studies suggest that *Bifidobacteria* and *Bacteroides ssp* seem to be able to protect against weight gain, giving rise to the "microbial hypothesis" for obesity, which may determine important therapeutic implications in the future[13,14,32].

In summary, the multiple metabolic mechanisms that associate the microbiota with obesity and hepatic steatosis are[13,14,29]:

1) Bacterial fermentation of polysaccharides that are not digested by man, which leads to the production of monosaccharides and short-chain fatty acids (SCFAs). These are substrates of colonocytes and precursors of cholesterol and fatty acids, which also participate in hepatic gluconeogenesis;

2) SCFAs bind to specific receptors in intestinal endocrine cells, increasing the YY peptide, capable of reducing appetite by slowing intestinal transit and increasing nutrient absorption and leptin levels;

3) Microbial regulation of some host genes that promote lipid deposition in adipocytes;

4) Reduction of the intestinal expression of the adipose factor induced by fasting, thus favoring the accumulation of fatty acids and adipose tissue expansion;

5) Increased hepatic capture of monosaccharides from the portal circulation with activation of substances involved in lipogenesis regulation;

6) Vascularization induced by inflammation and mucosal blood flow (stimulated by the intestinal microbiota), increasing nutrient absorption;

7) Promotion of a low-grade pro-inflammatory state, as well as increased insulin resistance and cardiovascular risk, through mechanisms involving exposure to bacterial products, especially lipopolysaccharides produced by gram-negative bacteria. This process is called "metabolic endotoxemia"[24].

In conclusion, the microbiota is potentially capable of affecting both sides of the energy balance, influencing energy acquisition from diet components and altering some host genes, which regulate energy consumption and storage.

Microbiota and Digestive Diseases

Irritable Bowel Syndrome

Although the etiopathogenesis of Irritable Bowel Syndrome (IBS) is not fully understood, several pathophysiological changes have been described (altered intestinal motility, visceral hypersensitivity, immune activation, dysregulation of the brain-bowel axis), thus constituting a multifactorial syndrome[39]. In recent years, important etiopathogenic contributions have been described, with the demonstration that some patients with IBS also have an alteration in the microbiota and changes in the intestinal mucosa (dysbiosis)[39,51,58].

The most relevant evidence supporting a possible participation of the microbiota in the pathophysiology of IBS are[8,40]:

a) Frequently observed qualitative and quantitative alterations in the intestinal microbiota;
b) Subgroup of patients has a history of acute gastroenteritis preceding the chronic symptom onset, being considered in this case as having post-infection IBS;
c) Higher prevalence of intestinal bacterial overgrowth when compared to healthy controls;
d) Dietary restriction of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) often attenuates intestinal;
e) Possible modulation of the intestinal microbiota with antibiotics, probiotics and prebiotics and clinical improvement.

a) Qualitative and quantitative changes in intestinal microbiota in IBS

Initial studies of the intestinal microbiota in patients with IBS demonstrated a decrease in the proportion of *Bifidobacterium* and *Lactobacillus* and an increase in *Enterobacter*[67]. A greater reduction of *Lactobacillus* was detected in patients with IBS and diarrhea (IBS-D) than in those with IBS and constipation (IBS-C)[51]. It was also observed that a lower amount of *Bifidobacteria* is found in SII-D and a greater amount of *Veillonella* is found in IBS-C than in the control groups[57]. On the other hand, there is evidence of higher levels of *Enterobacteriaceae* and lower of *Faecalibacterium prausnitzii* in patients with IBS-D than in healthy controls[16].

Using DNA sequencing techniques, 24 fecal samples from patients with IBS and 23 controls were analyzed, confirming a decrease in the proportion of *Lactobacillus* and *Bifidobacteria*[36]. Another study observed a two-fold increase in the *Firmicutes*/*Bacteroidetes* ratio in patients with IBS (P<0.0002), in addition to an association between abdominal pain and low amounts of *Bifidobacteria* also being reported during a 7-week follow-up[52].

Research on experimental animals have demonstrated that behavioral changes, such as stress, can change the composition of the intestinal microbiota, making it more vulnerable to the inflammatory and immunological stimuli of the gastrointestinal tract[39,51]. It is worth mentioning that the great heterogeneity of the results is justified, at least in part, by the multiple methods used to determine the microbiota and the different inclusion criteria used for patients with IBS (Roma II/III).

b) Post-infection IBS and bacterial overgrowth of the small intestine

A strong indication of the importance of microbiota and low-grade inflammation in the etiology of IBS is the onset of chronic IBS-compatible symptoms after acute gastroenteritis [39]. Post-infection IBS has been associated with several microorganisms, including *Campylobacter jejuni*, *Yersinia*, *Salmonella* and *Shigella*[60].

More recent evidence indicates that, in addition to bacteria, viral infections (*rotavirus, adenovirus, calicivirus*) and parasitic infections (*Giardia lamblia*, *Blastocystis hominis*) may also be involved in the onset of this syndrome[58].

Epidemiological data published in recent years suggest that up to 20% of individuals with gastroenteritis will develop post-infection IBS and that there are factors considered to be of risk for its onset, such as: female gender, younger age, microorganism toxicity, prolonged diarrhea, and presence of stressful psychological events during the infection (e.g., anxiety and depression)[60]. The severity of the initial condition (based on the need to seek emergency care and hospitalization) is the main predictive factor for the subsequent development of a chronic IBS-compatible condition[40].

A meta-analysis showed the presence of IBS after acute gastroenteritis in 9.8% of the patients (4.0-13.3) when compared to 1.2% in the control groups (0.4-1, 8; P=0.01), showing that the relative risk of developing IBS was 7.3-fold higher after an episode of acute intestinal infection[31]. Another meta-analysis observed a nearly six-fold increase in the estimated risk of developing IBS after gastroenteritis and that this risk remains elevated up to 3 years after the acute infection[63].

In a well-conducted systematic review and meta-analysis including six studies, the presence of IBS after traveler's diarrhea was demonstrated in 5.4% of patients (2.35-6.49) compared to 1.4% in the control groups (P<0.00001). It was concluded that the relative risk of developing IBS after an episode of traveler's diarrhea (which can be caused by bacteria, mainly *Escherichia coli*, viruses, and parasites) is on average three to four-fold higher[55].

There is important evidence that the intestinal microbiota is altered in this subgroup of patients and that there is mucosal microinflammation characterized by cell infiltration, alteration in the number of mast cells and T lymphocytes, mRNA abnormalities of interleukin-1 (IL-1), reduction in the IL-10/IL-12 ratio, increased circulation of IL-6, IL-8 and tumor necrosis factor-alpha[8,52].

Considering the abovementioned facts, researchers believe that post-infection IBS is often accompanied by bacterial overgrowth in the small intestine (BOSI)[60]. There is, in fact, a great overlap of symptoms in the two syndromes - diarrhea, constipation, abdominal pain, flatulence, and abdominal distension – and therefore, the possibility that IBS patients have underlying BOSI has been questioned.

A relevant study carried out by researchers from Los Angeles, USA, found a clear association between the presence of bacterial overgrowth and reduction of the interstitial cells of Cajal, which could explain the development of post-infectious IBS[48]. Additionally, it has been confirmed in different studies that the prevalence of overgrowth is significantly higher in patients with IBS than in healthy controls[47].

c) Efficacy of a diet poor in FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) and intestinal microbiota modulatory therapies in relieving IBS symptoms

It is believed that the products of bacterial fermentation may be implicated in the etiopathogenesis of IBS[39,58]. Increased fermentation can cause an increase in gas production, triggering the onset of typical symptoms of the syndrome, such as flatulence, abdominal pain, and distension. It has been reported that SCFA concentrations are increased in IBS-D and that they can stimulate serotonin release from the intestinal mucosa, with consequent increase in intestinal motility and transit[28].

FODMAPs are short-chained carbohydrates that are poorly absorbed in the small intestine and are used in the colon as a substrate for bacterial fermentation. These carbohydrates also have an osmotic effect, because they cause a displacement of water to the intestinal lumen. The effect of the FODMAP-rich diet on the onset of IBS symptoms seems to be directly associated with the intestinal microbiota composition, suggesting that its manipulation may constitute an effective therapeutic pathway in the treatment of IBS. In fact, several recent studies have shown that a diet poor in FODMAPs is effective in relieving IBS symptoms, especially flatulence and diarrhea, constituting an effective approach for patients with functional intestinal symptoms[28,39]. (Figure 2).

Category	High FODMAP content
Vegetables	Asparagus, artichoke, onion, garlic, legumes, peas, beets, corn, beans
Fruits	Apple, pear, mango, watermelon, plum
Milk/ Dairy products	Milk, yogurt, white cheese
Bread / Cereals	Made with wheat, rye; pasta, pizza
Cookies/Biscuits	Made with wheat, rye, barley
Seeds / Nuts	Pistachios, cashew nuts, walnuts

FIGURE 2. Examples of foods with high FODMAP content.

Evidence has suggested that therapies capable of modulating the intestinal microbiota (antibiotics, probiotics and prebiotics) may improve symptoms by reinforcing the role of dysbiosis in the syndrome pathogenesis[28,38,39].

Several clinical trials have shown that a significant portion of patients with IBS who have bacterial overgrowth improve with antibiotic therapy. A well-conducted double-blind, randomized study compared rifaximin and placebo in patients with IBS, showing an overall symptom improvement, especially flatulence in the group receiving the active drug[48]. Another study showed that a single treatment with rifaximin for 7 days may improve IBS symptoms in 46%-90% of patients and normalize the respiratory test in 20-75% of cases[49].

Rifaximin is not yet available in Brazil and the most commonly used antibiotics in our country for these cases are quinolones, metronidazole, amoxicillin-clavulanic acid, and tetracycline.

Significant improvement in abdominal distension and pain has been reported with the use of probiotics containing *Bifidobacterium infantis* 35624[38]. It has also been shown that *Lactobacillus* supplementation is associated with decreased gas-production related symptoms in patients with IBS[24].

d) Probiotics in IBS

Probiotics are living microorganisms that, when consumed in adequate amounts, can contribute to good health[45]. However, many questions regarding its use of probiotics in gastrointestinal disorders remain to be answered, such as most optimal doses, duration of treatment, physiological and immunological effects and safety in debilitated patients. Even so it is interesting to consider the crescent number of studies on the role of probiotics in different gastrointestinal disorders and the trend to consider them as an important adjuvant therapy.

The use of probiotics in the IBS is an example of this approach: a systematic review including 19 randomized controlled trials concluded that probiotics are superior to placebo in relieving the IBS symptoms[24].

The mechanism of action of probiotics seems to be broader than simply modulating the intestinal microbiota. It is possible that they also act to inhibit the colonization and adherence of pathogenic bacteria to the enterocytes, increasing the secretion of defensins and decreasing the synthesis of pro-inflammatory cytokines, such as IL-10 and IL-12[47]. The figure below is only demonstrative and may not include all the species/strains currently available on the market. (Figure 3).

Lactobacillus acidophilus NCFM
Lactobacillus acidophilus LA5
Lactobacillus rhamnosus R0011
Lactobacillus reuteri RC-14
Lactobacillus casei shirota
Saccharomyces cerevisae boulardi
Bacillus coagulans BC3
Bifidobacterium animalis lactis Bi-07
Bifidobacterium lactis Bb-12
Bifidobacterium bifidum CUL 20

FIGURE 3. Examples of probiotics.

Inflammatory Bowel Diseases (IBD)

Environmental factors, genetic factors and immune responses have been considered as the major etiology of IBD (Crohn's Disease and Ulcerative Colitis) which have a diversified pathogenesis. However, in recent years researchers have been very interested in evaluating the possible involvement of the intestinal microbiota in the complex etiopathogenesis of IBD [10,30,42]. The main evidence that supports a possible participation of the microbiota in the pathophysiology of IBD are shown in Figure 4[7,15].

Qualitative and quantitative alterations in intestinal microbiota
Occurrence of lesions in intestinal segments with higher concentrations of bacteria
Polymorphisms of genes encoding bacterial receptors
Experimental "germ free" animals do not develop ulcerative colitis
Increased intestinal permeability in Crohn's disease

FIGURE 4. Participation of the microbiota in the pathophysiology of IBD – main evidence.

Initial studies, based on stool cultures, observed a significant decrease in the intestinal microbiota biodiversity in patients with IBD[7]. Obviously, only alterations in diversity would not be the cause of the inflammatory disease, with a susceptible genotype being indispensable, which occurs in the presence of specific mutations[15,42]. A reduction in the *Bacteroidetes* and *Firmicutes* phyla and the concomitant increase in *Proteobacteria* and *Actinobacteria* have been described[7]. There is also a tendency for the excess of pro-inflammatory organisms with concomitant depletion of organisms with anti-inflammatory properties, such as *Faecalibacterium prausnitzii*[30,42,53] – (Figure 4).

Studies have shown an increase in Enterobacteriaceae (*Escherichia coli* and *Shigella*) in IBD[18,19]. Colonization by *E. coli* with adherent and invasive properties is more frequent in the ileal mucosa of patients with Crohn's disease than in healthy controls[19]. This group of bacteria has been identified more frequently in the stool of patients with active IBD[7,18,19].

Studies have shown that there is a higher concentration of bacteria of the genus *Fusobacterium* in the colonic mucosa of patients with ulcerative colitis[7,42]. Additionally, it has been emphasized in experimental studies that the *Fusobacterium varium* species can cause erosions in the colon similar to the lesions seen in patients with rectocolitis[37].

Mutations in the nucleotide-binding oligomerization domain containing 2 (NOD2) gene that may be associated with the onset of Crohn's disease and variations of the interleukin receptor gene (IL-23) have been reported both in Crohn's disease and ulcerative rectocolitis[12,41].

It has been shown that NOD2 dysfunction causes the translocation of enteric bacteria into the lamina propria, with alterations in cytokine expression. Mice with NOD-2 depletion have a significant increase in *Bacteroides*, *Firmicutes* and *Bacillus* in the terminal ileum and reduced capacity to eliminate more pathogenic bacterial species such as *Helicobacter hepaticus*[41].

Studies comparing "germ-free" and colonized animals have shown that animals raised in sterile environments are less susceptible to experimental IBD and that the presence of commensal bacteria can trigger and/or exacerbate inflammatory bowel diseases[7,15]. It can be concluded that, in susceptible individuals, the inadequate immune response of the mucosa in relation to the gastrointestinal microbiota may result in Crohn's disease or ulcerative rectocolitis.

Similar to NOD2, TLRs (toll-like receptors) are also active participants in luminal interactions and studies have suggested that IBD may be associated with alterations in the selective expression of these receptors. Recent data have shown that levels of TLR-3 and 4 are altered in active IBD[23].

Defects in autophagy may also lead to some inability to maintain the stability of the intestinal microbiota. Studies have implicated the gene ATG1666L1, which codifies a protein involved in autophagosome formation, in the etiology of Crohn's disease[50]. Additionally, changes in intestinal permeability resulting in altered epithelial barrier function have been described[7].

The concentration of bacteria in the colonic mucosa is substantially higher in patients with IBD than in healthy volunteers and this concentration also increases according to disease severity[42]. On the other hand, an experiment carried out on mice with suppressed IL-10 gene indicated that these animals developed ulcerative rectocolitis when they were colonized, but not when they were raised in a germ-free environment[15].

It is known that the mucus has a protective function in the intestinal epithelium, forming an actual barrier and selectively preventing bacterial colonization. It has been shown that the mice with the thinnest and least sulphated intestinal mucus layer have a BOSI and are also more susceptible to developing rectocolitis[37].

Such findings in studies in experimental animals and in patients with IBD suggest that the intestinal microbiota may play an important role in the pathogenesis of Crohn's disease and ulcerative rectocolitisA[50].

Celiac disease

In addition to the known immunological and genetic mechanisms, it is believed that environmental factors and the intestinal microbiota may have an effective participation in the pathophysiology of celiac disease[30,42]. Recent studies have shown the presence of intestinal dysbiosis in these patients (both in the untreated group and in those treated with the gluten-free diet) when compared to healthy individuals. In fact, some altered genes and/or their expression in celiac disease seem to play an active role in bacterial colonization and sensitization. On the other hand, dysbiosis can provoke the abnormal response to gluten (or other environmental factors that promote the disease) in genetically predisposed individuals[66].

The gluten-free diet, alone, can influence the composition of the intestinal microbiota and thus constitutes a complicating factor in studies of celiac patients. Some researchers have shown that the gluten-free diet favors the reduction of beneficial bacteria, such as *Bifidobacterium* and *Lactobacilli* and the increase of gram negative bacteria, such as *E. coli* and *Bacteroidetes*[17,66]. To understand whether intestinal dysbiosis is the cause or the consequence of the disease, studies are required with healthy newborns from families at increased risk of the disease. These investigations might reveal which genes and genotypes are involved, as well as identify environmental factors capable of influencing the development of oral tolerance to gluten[17]. It is worth considering that it is possible that new probiotics and prebiotics will show to be of value in the treatment and even in the prevention of celiac disease.

Gastrointestinal neoplasms

There are a multiplicity of factors that influence the initiation of neoplasia and its subsequent course. Factors that may not be considered strictly carcinogenic may, nevertheless, affect the ultimate result in neoplasms. As such, several microbial species participate directly or indirectly in the genesis of many malignant neoplasms; according to conservative estimates, at least 15% of all cancer cases are attributable to infectious agents[10,42,53]. Very little is known about the contribution of the intestinal microbiota to the development of digestive neoplasms. Enteric microorganisms that can promote carcinogenesis through different mechanisms[1,42,68] are: 1) Inflammation induction; 2) Increased cell proliferation; 3) Alteration in stem cell dynamics; 4) Production of some substances such as butyrate, capable of affecting DNA integrity and immune regulation.

Studies in experimental animals and in humans have identified effector species and/or interrelations between members of the microbial community of the stomach and colon, which increases the risk of developing malignant lesions in these organs. The several manipulation strategies of the microbiota or the host's immune response to these microorganisms may in the future prevent or even treat certain types of gastrointestinal neoplasms[1,68].

Esophageal cancer

Although there is still little research on the effects of the microbiome on the development of esophageal cancer, it is possible to consider that alterations in the gastric microbiota may contribute to the increased incidence of esophageal adenocarcinoma – particularly those arising near the gastroesophageal junction[1]. Some studies have suggested that the esophageal endogenous microbiota differs between individuals with healthy esophageal mucosa and those with Barrett's esophagus[26,68]. It is worth mentioning that the studies in this area are still very incipient, but it is possible that the differentiation between the esophageal and gastric microbioma will constitute an important factor for future investigations.

Gastric cancer

Helicobacter pylori (*H. pylori*) is considered a type 1A carcinogen by the WHO[3]. It initiates the inflammatory cascade that progresses through chronic gastritis to intestinal metaplasia, gastric atrophy, and gastric cancer. Only a minority of infected patients, however, will develop cancer, and the bacterium is absent or minimally present in neoplastic lesions. Experimental studies in animals demonstrate that the interrelationships between *H. pylori* and *non-H. pylori* gastric microbiota represent a new challenge to the dogma of gastric carcinogenesis[3,68].

In contrast to the current model of gastric carcinogenesis, which is focused on the interaction between different strains of *H. pylori* and the host's genetic factors, research has suggested a new hypothesis, where early interactions between this microorganism and the gastric mucosa can determine alterations in the gastric microbiota structure, which could result in gastric atrophy[1,56]. It is possible that the new microbiota that develops in the stomach in response to low acidity conditions effectively participates in gastric carcinogenesis[3,56].

Colorectal cancer

The culmination of multiple genetic and epigenetic events directly contributes to the pathogenesis of colon and rectal cancer (CRC)[1]. The neoplasia is initiated by mutations in some tumor suppressor genes (APC, CTNNB1, p53) and oncogenes (KRAS) that transform healthy intestinal mucosa into adenoma and neoplasia[25]. Although the mutations in these genes already have defined roles in the development of CRC, the events that lead to the acquisition of these mutations and epigenetic modifications are still unclear[68]. Recent studies have identified the intestinal microbiota and some environmental factors, such as diet and lifestyle, as potential promoters of CRC development[2].

It is still unclear whether there are specific microorganisms that are particularly pathogenic and can directly participate in carcinogenesis or whether the process requires specific interactions between host tissues and the colonic microbiota and, probably, several mechanisms must be involved[1,25]. A future challenge will be to identify which microorganisms are effectively associated with adenocarcinoma development.

In vitro and *in vivo* studies indicate that high fiber intake can bring benefits to intestinal health and decrease the incidence of colorectal cancer. This is due to the fact that fibers are fermented by the colonic bacteria, later forming short-chain amino acids, and among them butyrate, which after being captured by the enterocytes, is used as a local source of energy[2]. Butyrate seems to be capable of inducing apoptosis and inhibiting the proliferation of neoplastic colon cells. The formation of a higher percentage of this substance is associated mainly with the action of anaerobic bacteria, such as *Clostridium*, *Eubacterium* and *Fusobacterium*[3,42,68].

Analysis of the fecal microbiome of patients with CRC shows the increase of the *Bacteroides* species, decrease of butyrate-producing bacteria and increase of potentially pathogenic bacteria, such as the *Fusobacterium* species[25]. It has been demonstrated that the microbiota present in the neoplastic lesion differs from that found in the surrounding colonic mucosa (normal), with greater abundance of *Coriobacteriaceae*. It is probable that the alterations in the microbiota next to the neoplasms are associated to nutrient availability and other conditions created by the neoplastic cells themselves[1]. Future studies will investigate whether a state of dysbiosis precedes the development of adenomas and CRC.

Experimental studies suggest that probiotics may inhibit CRC by interfering with the immune system and apoptosis, being able to modulate intestinal bacteria and their metabolism[2,25]. It has been observed that *Lactobacillus johnsonii* reduced the concentrations of *Enterobacteria*, modulating the immune response in patients with CRC. This effect was not described with strains of *Bifidobacterium longum*[6]. A clinical trial showed that strains of *Lactobacillus casei* were able to reduce tumor growth after 2 to 4 years of treatment[64]. Clinical trials in this area, however, are still quite limited by the small number of enrolled patients and a short follow-up period.

CONCLUSION

Alterations in the composition and function of the gastrointestinal microbiota (dysbiosis) have a direct impact on human health and seem to have an important role in the etiopathogenesis of several gastrointestinal diseases, whether inflammatory, metabolic, or neoplastic ones.

New studies on the interrelationship of the intestinal microbiota with the host will be essential to recognize the possible strategies of how to favorably handle the thousands of microorganisms that inhabit the digestive tract, promoting eubiosis and fighting the associated diseases.

Authors' contributions

Passos MCF designed the study, collected and analyzed data, drafted the manuscript, criticaly revised the article for important medical content and approved the final manuscript draft. Moraes-Filho JP collected and analyzed data, critically revised the article for important medical content and approved the final manuscript draft.

Passos MCF, Moraes-Filho JP. Microbiota intestinal nas doenças digestivas. Arq Gastroenterol. 2017;54(3):255-62.

RESUMO – **Contexto** – Nos últimos anos, especialmente a partir do desenvolvimento de sofisticados estudos metagenômicos, as pesquisas acerca da microbiota intestinal se intensificaram, transformando de forma radical os nossos conhecimentos sobre o microbioma e sua relação com a manutenção da saúde e o desenvolvimento de doenças no ser humano. Evidências crescentes demonstram que uma alteração permanente da composição ou da função da microbiota (*disbiose*) pode alterar as respostas imunológicas, o metabolismo, a permeabilidade intestinal e a motilidade digestiva, promovendo, dessa maneira, um estado pró-inflamatório. Tais alterações podem comprometer, sobretudo, as funções imunes e metabólicas do hospedeiro, favorecendo o aparecimento de doenças como diabetes, obesidade, doenças digestivas, neurológicas, autoimunes e neoplásicas. Este artigo de revisão é uma compilação da literatura disponível sobre a formação do complexo ecossistema intestinal e seu impacto na incidência de doenças como obesidade, esteatohepatite não alcoólica, síndrome do intestino irritável, doença inflamatória intestinal, doença celíaca e neoplasias digestivas. **Conclusão** – Alterações na composição e função da microbiota gastrointestinal (disbiose) têm um impacto direto sobre a saúde humana e parecem ter um papel importante na patogênese de várias doenças gastrointestinais, sejam elas inflamatórias, metabólicas ou neoplásicas.

DESCRITORES – Microbiota. Microbioma gastrointestinal. Disbiose. Gastroenteropatias.

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