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REVIEW ARTICLE

Composition of gut microbiota and its correlations with neurological, intestinal, cardiovascular and metabolic diseases

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ABSTRACT

The intestinal microbiota is a microenvironment that has been the subject of studies for several decades. Over time, it has been reconsidered as a possible cofactor of multiple acute and chronic human diseases. In fact, alterations of the intestinal bacterial flora have been found in various neurological diseases. There are three modes of interaction between the intestinal microbiota and the gut-brain-axis: chemical signals, neural pathways and immune system. Even at the gastrointestinal level, the gut microbiota plays certainly an important role in the etiopathogenesis of chronic intestinal inflammatory diseases but also in irritable bowel syndrome. An important correlation has also been demonstrated with non-alcoholic fatty liver disease, as well as in other metabolic, cardiovascular and oncological diseases. Bacteria, viruses, fungi and various microorganisms that normally reside in our intestines can also be called into question as protective factors against these diseases. All this evidence leads researchers to consider the gut microbiota as a key element in the determination of aforementioned diseases. Therefore, it would be foreseeable in the future to associate the use of probiotics with the therapies used in the treatment of all these diseases. In this review we have condensed the main current knowledge regarding the link between the most frequent diseases and the gut microbiota.

KEYWORDS

gut microbiota, metabolic diseases, cardiovascular diseases, inflammatory bowel disease, dysbiosis, NAFLD

INTRODUCTION

In the human gut there is a set of different microbes (bacteria, fungi, viruses, and protozoa), known as the "gut microbiota" (GM). Normal human GM includes six major phyla: *Bacteroidetes, Firmicutes, Actinobacteria, Verrucomicrobia, Euryarchaeota, and Proteobacteria* (Fig. 1). A commensal relationship exists between the host and these microbes. In fact, one partner, that is the man, benefits from it while the other, that is the GM, does not seem to draw neither benefits nor disadvantages [1]. These microorganisms perform various actions which, under physiological conditions, turn out to be favorable for the human host. In fact, they are capable of producing metabolites, degrading potentially toxic substances, interacting with the host's immune system by stimulating its functionality. GM therefore influences both the physiological homeostasis of the gastrointestinal tract and the development of the disease [2, 3]. Furthermore, in order to survive inside the host, the GM has developed some essential characteristics. For example, the ability to evade bacteriophages and the host's immune system [4].

Human GM is classified into three enterotypes:

- Enterotype 1: *Bacteroides* predominate, which possesses saccharolytic and proteolytic activity; it is also involved in the synthesis of biotin, riboflavin, pantothenate and ascorbate [5].

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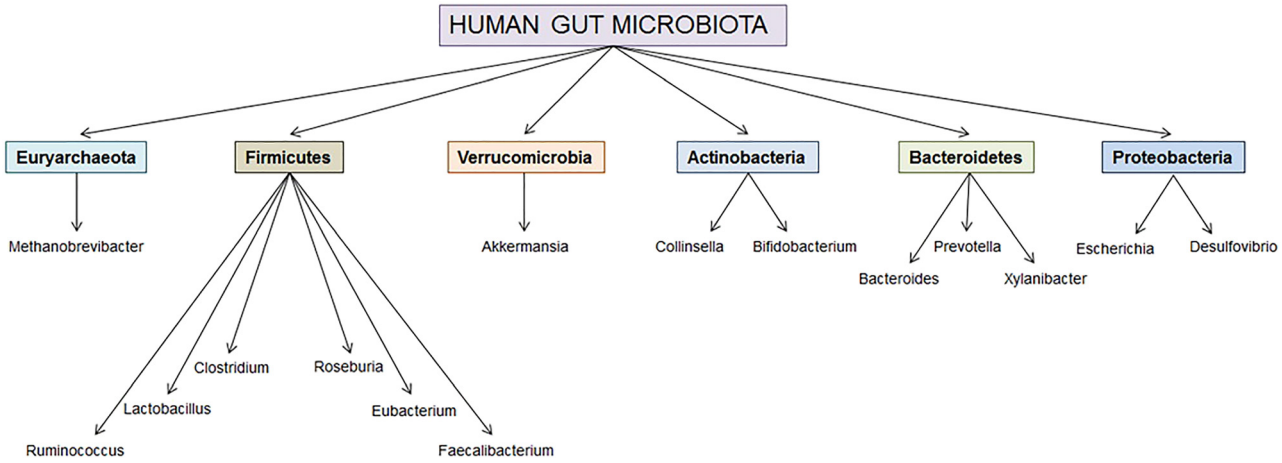

Fig. 1. The 6 main phyla of the human intestinal microbiota and the respective species most represented in the human intestine

- Enterotype 2: the prevailing genus is *Prevotella*, capable of degrading mucin glycoprotein and is also involved in the synthesis of thiamine and folate [6].
- Enterotype 3: *Ruminococcus* predominates, a group of bacteria capable of degrading mucin and carrying sugars on the membrane [6].

EVOLUTION OF GM DURING GROWTH

GM is acquired from birth and develops parallel to the host, through a dynamic process [7]. The type of birth (eutocic or by caesarean section) influences the type of bacteria that will colonize the newborn. In fact, in subjects born by caesarean section, *Escherichia coli* and *Clostridium difficile* prevail, while *Bifidobacteria* are present to a lesser extent. Furthermore, GM colonization begins late in these individuals. On the other hand, in those born with vaginal birth, *Lactobacilli* and *Prevotella spp.* prevail [8, 9]. The method of feeding of the newborn also influences the composition of the GM. Breastfed individuals are mainly colonized by *E. coli*, *Streptococci* and *Bifidobacteria*. Instead, infant formula-fed infants are colonized primarily by *Enterobacteria*, *Clostridia*, and *Bacteroidetes* [10]. Then there are further differences also according to the geographical areas in which individuals grow. This is because in every country in the world there are different food cultures. In subjects who follow the western diet, which is richer in fats and carbohydrates, there is a high quantity of *Firmicutes* and *Proteobacteria* and a low quantity of *Bacteroidetes* and *Actinobacteria* [9]. In the same way, substantial differences in the composition of the microbiota were found between individuals living in cities compared to those living in rural suburbs, even though they are part of the same region [11]. For example, a study conducted in South Korea by Kim et al. found that the *Firmicutes* genus abounds in village elders, while *Bacteroidetes* tends to prevail in urban elders [12]. Therefore, throughout life, the GM undergoes various changes related to age, diet, environment, lifestyle, teething, and stress but also diseases and the use of drugs and substances of various kinds [13]. In particular, the aging process involves alterations of normal physiological processes which, in turn, alter the composition of the GM. In fact, during old age there is a reduction in gastrointestinal motility with consequent delay in gastric emptying; intestinal blood flow is reduced, alterations of the bowel habit and immune response occur. Furthermore, the elderly tend to follow a poor diet [14]. In addition, older people are more at risk of being hospitalized and developing infections that require the use of antibiotics [15].

Even some typically age-related diseases, such as Alzheimer's disease or osteoporosis, may not induce changes in GM [16].

Another important aspect to consider is that the composition of the GM is significantly related to the fragility and nutritional status of the individual. For example, the microbiota of the elderly housed in long-term care facilities seems to undergo greater diversification than that of peers residing in the community. This phenomenon seems to determine an increase in fragility [17]. Indeed, such changes in the microbiota promote inflammation, predispose to the development of opportunistic infections [18]. Several studies suggest that longevity can break the balance between pro-inflammatory and anti-inflammatory bacterial species [19].

GUT MICROBIOTA AND DISEASES

Neurological diseases

Several studies have shown that the gut microbiota can influence brain function through various neural, endocrine and immune pathways [20]. In particular, there are three main ways of interaction between the GM and the brain axis. The first is represented by chemical signals. Bacteria can affect the nervous system directly or indirectly through the production of metabolites and by regulating the release of various neurotransmitters. The second is the neural pathways. GM acts on the vagus nerve and intestinal nervous system, affecting the brain and behavior. The third is the

immune system, specifically microglia and systemic cytokines (Fig. 2). The latter pathway may be involved in the pathogenesis of neurodegenerative and other neuro-related diseases [21, 22].

Furthermore, other studies show how the intestinal microbiota is able to influence brain development, neurogenesis and interacts with the central and enteric nervous systems via the "gut-brain" axis. It is currently hypothesized, on the basis of studies conducted on animal models, that the intestinal microbiota is involved in the etiopathogenesis of diseases such as chronic headache, depression, anxiety, Parkinson's disease, Alzheimer's disease, multiple sclerosis [23].

Many bacteria are capable of producing neurotransmitters. These include dopamine, a precursor of the catecholamines epinephrine and noraepinephrine, produced by bacteria such as *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter cloacae, Shigella sonnei*, and *Staphylococcus aureus*, etc. [24, 25]. This is demonstrated by the fact that mice lacking intestinal flora possessed reduced levels of norepinephrine in the intestinal lumen. Not only that, in fact it also emerged that intestinal levels of norepinephrine could be restored through colonization with a mixture of 46 species of *Clostridia* [26].

Another neurotransmitter produced by GM is serotonin. This is involved in the regulation of various physiological processes, such as gastrointestinal secretion, neurological behavior and function, etc. [27]. However, despite the fact that various bacterial species have been shown to be able to produce serotonin, the relationships between its reduced production in the intestine and any cognitive alterations that may be caused are still unclear [23].

GABA is the main inhibitory neurotransmitter of the central nervous system. Several studies over the last few decades have supported the link between impaired GABAergic neurotransmission and numerous central and peripheral nervous system disorders [28].

It is also known that various GM bacteria, especially *Lactobacilli* and *Bifidobacteria* species, are capable of producing this neurotransmitter [29, 30]. GM appears to affect serum GABA levels, since guinea pigs lacking intestinal flora have reduced serum and intestinal levels of GABA [31]. In humans, too, it would seem that manipulation of the human microbiota can alter GABA levels [32].

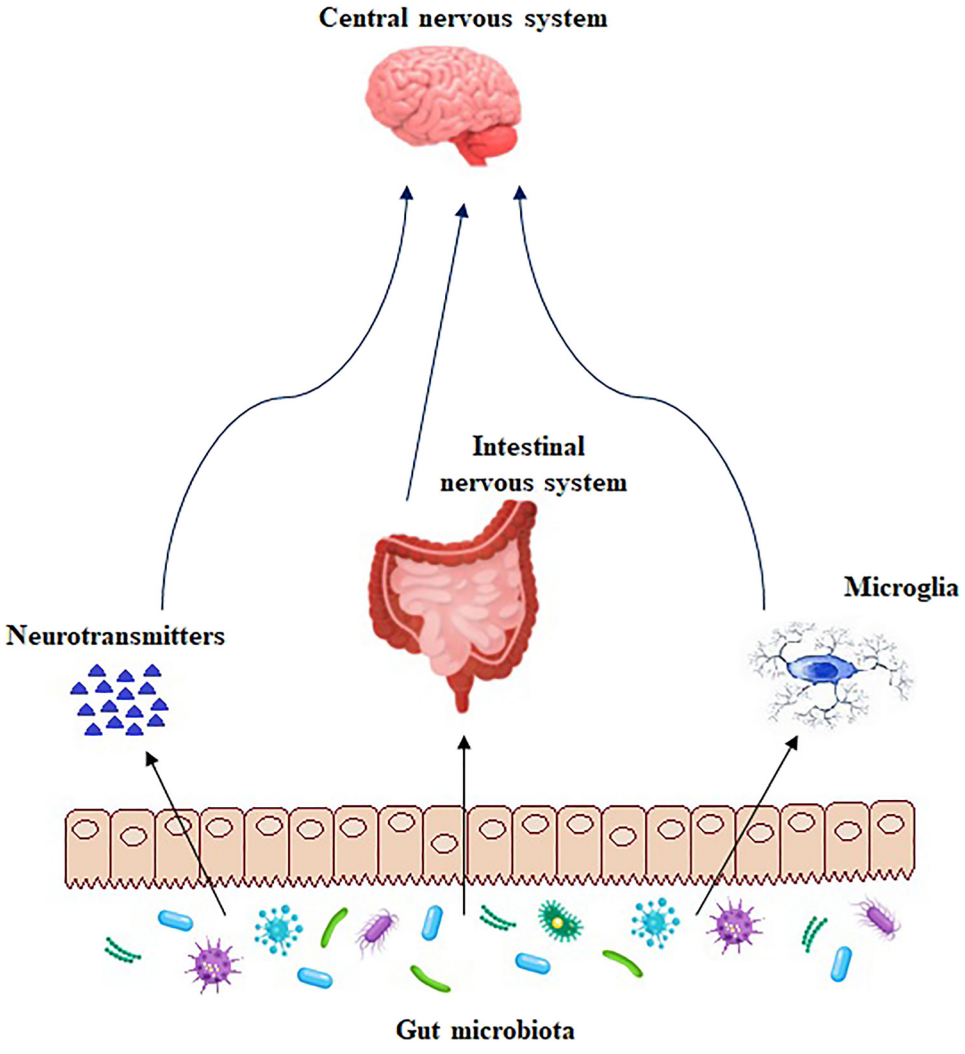

Fig. 2. The three ways of interaction between gut microbiota and central nervous system

Furthermore, GM normally stimulates the production of cytokines and chemokines aimed at regulating the function of the intestinal bacterial flora itself. These molecules under normal conditions do not spread to all systems, as the intestinal wall acts as a barrier and there is also an innate immune response capable of monitoring GM [33].

However, if for a given reason there is a dysregulation of the production of these molecules with consequent inflammation, there is a weakening of the intestinal barrier ("leaky gut"). From this derives the infiltration of bacteria and various toxic substances, which can also reach the bloodstream. Various studies have shown that GM can cause a neuro-inflammatory response and even affect the hypothalamus-pituitary-adrenal (HPA) axis via cytokines and chemokines that reach the brain [34, 35].

In some animal studies, it has been found that GM substantially influences the integrity of the blood-brain barrier also by regulating the production of proinflammatory cytokines and chemokines [36]. In addition, GM is also capable of producing other chemicals including bacteriocins and short-chain fatty acids (SCFA). Bacteriocins inhibit the growth of other bacteria, while SCFAs, such as butyrate, stimulate the synthesis of molecules including neurotransmitters [37, 38].

Another way of communication between GM and CNS is represented by the endocrine cells of the intestine. In particular, in the intestine there are enteroendocrine cells (EEC), capable of influencing the gastrointestinal functions in response to the various nutrients that reach the intestine [39].

GM can affect EEC in the release of neuropeptides and hormones, such as ghrelin, gastrin, cholecystokinin, and neuropeptide Y which can affect both peripheral and central neural communication by also interfering with behavior [37].

Another way of communication, perhaps less so-called, is represented by the vagus nerve. The latter is able to interact with the GM and transport this information to the CNS, resulting in an activation of neurons [40].

Even some bacterial species are able to influence host behavior using the vagus nerve, such as *Campylobacter jejuni*. This, in fact, in a study conducted on the animal model, would seem to have induced a more anxious behavior. This would have resulted from the activation of neurons in the nucleus of the solitary tract [41].

Another important association between GM and nervous pathologies concerns migraine and depression. Numerous studies have found a considerable alteration of GM in migraine patients compared to healthy subjects. These evidences are also supported by the finding of beneficial effects exerted by probiotics in the treatment of migraine. In fact it has been seen that these products have improved the quality of life in a group of individuals with migraine [42].

A recent study by Yong et al. found that *Tissierellia*, *Tissierellales*, and *Peptoniphilaceae* were more abundant in migraine patients than in controls. Furthermore, these authors also observed a quantitative association between the relative abundance of the different kinds of bacteria and the frequency of headache episodes. The frequency of headache is an important parameter for assessing its severity [43].

GM alterations have also been found in some psychiatric disorders such as schizophrenia, anorexia nervosa, bipolar disorder, etc. The genus *Eggerthella*, associated with intestinal inflammation, is increased in patients with these disorders, while the genera *Faecalibacterium* (with known anti-inflammatory properties) and *Coprococcus* are reduced [44].

As far as depressive disorders are concerned, it is interesting to underline that antidepressants not only affect brain biochemistry, but also GM. Similarly, some antibiotics, such as β-lactams and tetracyclines, also have potential antidepressant properties. Conversely, several studies have suggested that other classes of antibiotics, such as fluoroquinolones, are associated with the development of depression and anxiety [45].

This can lead us to conclude that a certain composition of the intestinal flora can facilitate or hinder the development of depressive-type disorders.

Parkinson's disease (PD) is the second neurodegenerative disease by annual incidence, with a global prevalence of over 6 million affected subjects. The disease is characterized by the presence of neural inclusions in the form of Lewy bodies, with degeneration of neurons in the *substantia nigra* and other brain areas [46].

Increasing evidence demonstrates a significant difference in the GM composition of subjects with PD versus healthy patients [47, 48].

It was observed that bacteria such as *Bifidobacterium*, *Pasteurella* and *Enterococcus* are significantly increased in PD patients, while strains of *Brautella*, *Prevotella* and *Faecococcus* are reduced [49].

Despite this, it has also been seen that, as the disease progresses, in certain patients there is a subsequent reduction of this microorganism in parallel with a worsening of the disease state. This means that maintaining elevated levels of *Bifidobacterium* in PD could represent a protective factor against neurodegenerative aggravation. Thus the administration of probiotics containing *Bifidobacterium* could prevent or slow down the progression of PD to more severe stages [50]. Many experimental studies have also confirmed the gut-brain axis in PD. In particular, GM alterations in PD patients may promote α-synuclein accumulation and excessive microglial activation. In an experiment on an animal model it was shown that more α-synuclein aggregations were present in the brains of GM mice than in mice lacking gut bacteria. In this study, transplantation of fecal microbiota from PD patients to gut *germ-free* mice aggravated α-synuclein-induced motor symptoms to a greater extent than fecal transplantation from healthy people [51].

Alzheimer's disease (AD) is a neurodegenerative disease in which there is a progressive decline in cognitive function. In the disease there is the formation of amyloid plaques and neurofibrillary tangles. The former are extracellular accumulations of abnormally folded amyloid-beta (Aβ) proteins. Neurofibrillary tangles, on the other hand, are composed primarily of hyperphosphorylated *tau* protein [52, 53]. Recently, it has been speculated that there may be a connection between AD and intestinal dysbiosis. This is due to the fact that not only is GM connected to the nervous

system via the gut-brain axis, but it is also somehow involved in the inflammatory processes that underlie AD [54]. The brain, in fact, triggers an immune reaction in response to pathogens or any other harmful event. This immune response is initiated by microglia and shuts down as soon as the trigger is removed. However, in pathological conditions, a persistent immune response can be established with the consequent chronicization of the inflammatory process. This event is clearly harmful to neurons and is referred to as "neuroinflammation" [55]. AD patients have been shown to have elevated serum levels of proinflammatory cytokines (i.e. interleukin 1 – IL-1 -, IL-6, tumor necrosis factor α, etc.) that also play a role in neuroinflammation. The production of these cytokines in AD patients appears to be due to the continuous deposition of the Aβ peptide [56].

In a meta-analysis conducted by Hung et al., studies conducted in patients with AD aimed at detecting any differences between the GM of these patients and that of healthy subjects were compared. There were many differences found. In fact, AD patients first showed reduced GM diversity. Then the most represented species in AD were *Proteobacteria*, *Bifidobacterium* and *Phascolarctobacterium*, while *Firmicutes*, *Clostridiaceae*, *Lachnospiraceae* and *Rikenellaceae* were less abundant in the AD spectrum compared to healthy subjects [57].

Not surprisingly that neurotoxins produced by the *Proteobacteria* member *E. coli* correlate with AD pathogenesis and, in general, increase the release of pro-inflammatory cytokines [58]. In fact, an increase in the level of *Proteobacteria* has also been associated with greater memory dysfunction [59].

The phylum *Firmicutes* is also somehow connected with inflammatory states. For example, various evidences show that the decrease in *Firmicutes* was associated with the development of obesity and type 2 diabetes, pathologies closely interconnected with systemic inflammation and beyond [60].

The insulin resistance that underlies the pathogenesis of DM2 could cause cerebral hypometabolism of glucose and increased accumulation of Aβ, which results in an increased risk of AD [61]. Conversely, the abundance of *Firmicutes* was positively associated with executive brain function [62].

Clostridiaceae play a crucial role in the production of SCFAs, which among others exert protective effects on the permeability of the blood brain barrier [63]. Furthermore, these bacteria also produce indole-3-propionic acid, which could prevent Aβ-induced oxidative injury on neurons [57].

Similarly, *Lachnospiraceae* produce butyrate which has anti-inflammatory actions and facilitates the barrier functions of the intestinal wall [64]. Numerous studies have shown that a lower proportion of *Lachnospiraceae* is associated with insulin resistance, alterations of the homeostasis of the central nervous system and an increase in the rate of progression of AD. The genus *Bifidobacterium* has a neuroprotective action through the production of acetate and γ-aminobutyric acid. Animal model studies confirmed it because these substances reduced the development of AD-like pathologies. Furthermore, it was found that *Bifidobacterium*-enriched probiotics improved cognitive impairments in AD patients [65].

All of this current knowledge has several implications for AD therapy. In fact, in addition to the drugs used for the treatment of the pathology and which aim, for example, at increasing the quantity of neurotransmitters at the level of the synaptic terminal, it would be useful to administer probiotics containing strains considered protective against AD.

Irritable bowel syndrome

Irritable bowel syndrome (IBS) is one of the most common diseases in the world. It is a functional disorder of the digestive system which greatly affects the quality of life and is often associated with disorders such as anxiety and depression [66]. A possible predisposing condition to the development of IBS is represented by intestinal dysbiosis. In fact, a different microbial flora is detected in patients with IBS compared to healthy subjects [67]. The most common dysbiosis seen in IBS is an increase in *Streptococcus* spp. and a reduction of *Lactobacillus* spp. and *Bacteroidetes*. There is a reduction in beneficial bacteria and an increase in pathogenic species [68]. Patients with IBS present a scarcity of *Erysipelotrichaceae* and *Ruminococcaceae* compared to healthy subjects. These species are capable of producing butyric acid, which is an excellent nutrient for colonocytes. On the other hand, in constipation-prevalent IBS, *Methanobacterialles*, producers of methane, are more abundant, but they are more deficient in diarrhea-prevalent IBS [69]. In fact, methane is able to inhibit the contractility of the smooth muscle cells of the proximal colon by activating the voltage-dependent potassium channel and increasing the voltage-dependent potassium current [70]. This could also suggest a possible role in determining the diarrheal or constipated habit of these bacterial species.

Several studies have also highlighted an abundance of *Lachnospira* and *Clostridium* in patients with IBS and especially *C. difficile* infection has been shown to increase the risk of post-infectious IBS [71].

In IBS, although it is a functional pathology, a non-specific infiltrate of inflammatory cells can be detected in association with a state of visceral hyperalgesia. This inflammation can cause epithelial but also neuromuscular dysfunction with consequent alteration of peristalsis [72].

Another mechanism by which intestinal bacteria can determine the establishment of an altered functioning of the intestine is the alteration of the epithelial barrier. Its integrity is essential for intestinal homeostasis since it prevents the translocation of pathogens from the intestinal lumen to the mucosa, thus avoiding the development of mucosal inflammation (Fig. 3) [73]. In particular, some intestinal bacteria, such as *Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii* and *Ruminococcus* spp., influence the composition of the intestinal wall [4].

Furthermore, some bacteria that abound in IBS patients, such as *Clostridium* and *Streptococcus* spp., are capable of producing polyamines (putrescine, spermidine and spermine),

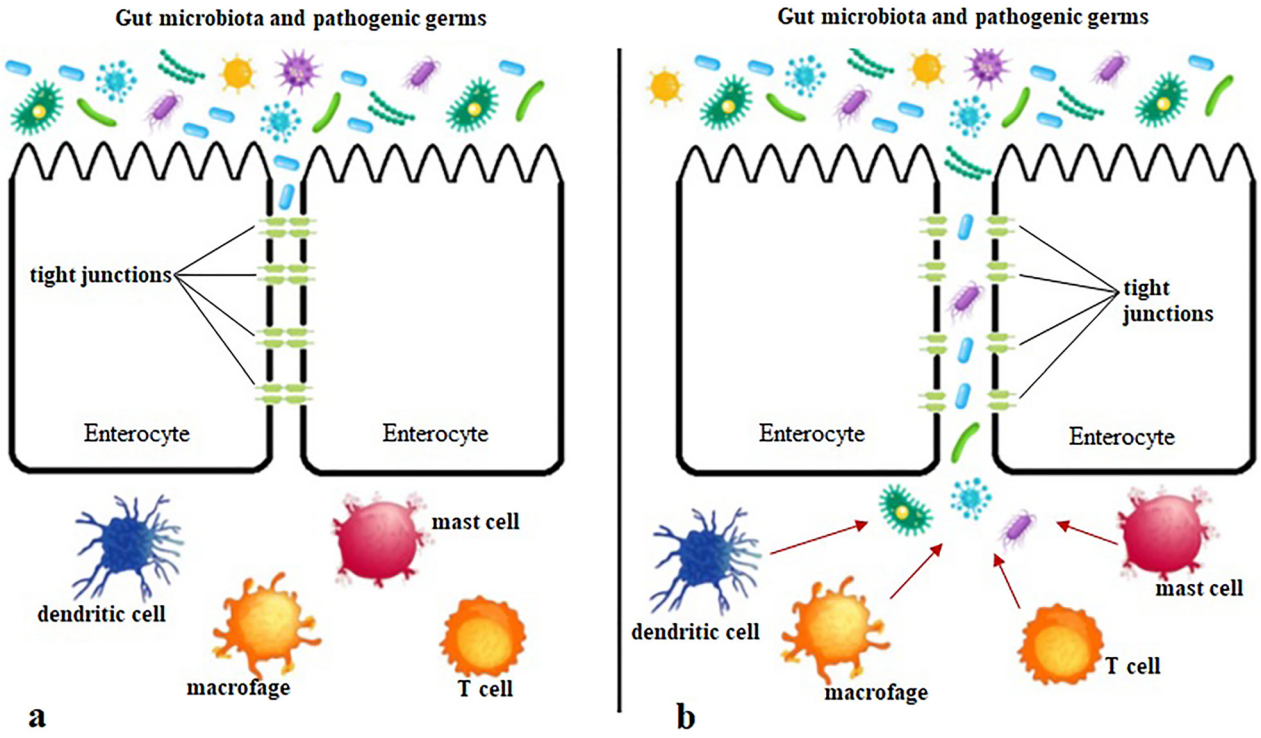

Fig. 3. **a: healthy gut**. In this condition the integrity of the tight junctions prevents the passage of microbes. **b: pathological condition**. In this case, the rupture of the tight junctions allows the passage of microbes which thus trigger an inflammatory response (red arrows) by the cells of the immune system

capable of inducing alterations in the molecular composition of the intestinal barrier [74].

The consumption of prebiotics has therefore been suggested as a possible treatment for IBS. This option is being considered not only for the modulation of the microbiome, but also for the anti-inflammatory and antioxidant effects of the prebiotics themselves [75].

The intestinal virome can also influence the development of IBS symptoms. In particular, the most abundant viral clusters belong to the families *Siphoviridae*, *Myoviridae* and *Podoviridae* [76]. Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) is also related to IBS. In this case, patients with diarrhea tend to have a higher proportion of viral RNA and the virus can be detected in the faecal samples of these patients. Furthermore, compared to controls, the levels of faecal IL-8 and IL-23 are higher, while the levels of IL-10 are lower. These evidences suggest that the gastrointestinal tract is immunologically active during this type of infection [77].

Inflammatory bowel disease

Different studies have found differences in GM composition between inflammatory bowel disease (IBD) patients and healthy individuals. These differences mainly concern microbial diversity and the predominance of certain bacterial strains [78]. Among them, the phylum *Firmicutes* is often reduced in the stool of patients with Crohn's disease, while bacteria from *Proteobacteria* phylum are more abundant [79].

Furthermore, differences were also found in the GM composition of members of the same family, some with IBD and others healthy, thus suggesting that in these cases dysbiosis is mainly associated with the disease state rather than with environmental or genetic factors [80]. Some pediatric studies have focused on the temporal relationship between dysbiosis and inflammation in sick and healthy subjects, with substantial differences found between both [81]. Dysbiosis has been described in the feces and mucosa of children with newly diagnosed and treatment-naïve Crohn's disease. This suggests that dysbiosis could precede clinical disease and develop independently of longstanding inflammation and/or medical therapy [82].

Another study of pediatric patients with Crohn's disease concluded that dysbiosis reflects not only the presence, but also the severity of inflammation [83].

Therefore, in the early stage of IBD there may already be changes in the composition of the gut microbiota contributing to the onset of the disease. But over time, environmental factors and inflammation itself likely contribute to dysbiosis [78].

Not only bacteria are implicated in the etiopathogenesis of IBD. In fact, even bacteriophages, viruses that are part of GM, can be involved in the development of these pathologies. In patients with IBD there is an increase of bacteriophage compared to healthy subjects [84]. In particular, a significant increase in *Picornaviridae* and *Enterovirus B* was found in the colon of IBD patients compared to healthy subjects. *Enterovirus B* include *Coxsackie viruses B1–B6* and

A9 and over 30 *Echovirus* serotypes and more than 20 EV-B serotypes. Among these, the levels of *Echovirus B75, Echovirus B5* and *Echovirus 26* were found to be particularly high in colon resection samples from patients with IBD. Therefore, a potential role of these viruses in UC and CD has been suggested [85].

Phages, in particular, are related to changes in intestinal bacteria. These in fact integrate into the bacterial hosts as prophages. In case of environmental stress they can induce the lytic cycle, replicate and determine the destruction of host cells. This demonstrates that the intestinal virome is also closely related to bacterial microbial species [86].

This transition to the lytic phase appears to be particularly related to the development of IBD. One of the first studies that investigated the correlation between intestinal virome dysbiosis and the pathogenesis of IBD highlighted an increase in phages infecting the bacterial orders *Alteromonadales* and *Clostridiales* [87].

A possible pathogenic action of viruses, as in the case of *Caudovirales* bacteriophages, can be represented by their ability to cause intestinal dysbiosis [84].

And there were observed specific viral species alterations as well as reduced intestinal virome diversity in patients with IBD, revealing that the IBD-associated virome was mainly represented by members of the *Caudoviridae* family [84]. These results were also confirmed by a study of *Fernandes* et al. conducted in a pediatric population. These authors found an increase in *Caudovirales* and a reduced proportion of strains in the *Microviridae* family in patients with CD compared to healthy controls [88].

Hepadnaviridae is also very abundant in UC patients. For this family it has been hypothesized that it may have an indirect impact on the host transcriptional activity so as to influence the host immune response, leading to the genesis of chronic intestinal inflammation [89]. In contrast, *Polydnaviridae*, *Tymoviridae*, and *Virgaviridae* are less abundant in IBD patients. So they could be somewhat considered protective for humans [90].

As far as eukaryotic viruses are concerned, a greater abundance of *Pneumoviridae* emerged in patients with UC compared to healthy ones, while the opposite was found for *Anelloviruses* [91]. Another study found an increase in the *Herpesviridae* family in patients with IBD [92].

Furthermore, fungal dysbiosis may also be involved in the development of IBD. In fact, it has been seen that prolonged treatment of mice with antifungal drugs causes colitis to worsen [93]. One study found that overall fungal diversity was higher in IBD patients than in healthy individuals, with some species only detected in IBD samples [94]. A predominance of *Ascomycota* and *Basidiomycota* has been observed in various studies both in healthy patients and in patients with IBD [95].

What is different is the *Basidiomycota*/*Ascomycota* ratio which varies not only between IBD patients and healthy subjects, but differences are also found between patients in the acute phase and those in remission. In particular, the ratio is generally higher in patients with IBD in exacerbation while it is lower in subjects in remission and in healthy subjects. This suggests that this relationship may be influenced by inflammation or even involved in the pathogenesis of the inflammatory process [96].

Saccaromyces cerevisiae is normally part of the fungal microbiota. Its levels are reduced in patients with IBD. Furthermore, this microbe has been shown to reduce colitis induced by adherent-invasive *E. coli*, which is associated with ileal CD [97]. It also exerts beneficial effects in the prevention of various forms of diarrhea, from that associated with antibiotics, to that from *C. difficile* infection, to that related to enteral nutrition. Thus, anti-inflammatory potential can be hypothesized for *S. cerevisiae* [98].

Malassezia is a fungal genus that can be found on the skin of mammals and is associated with numerous diseases, such as dandruff, atopic eczema or pityriasis. Some species of *Malassezia* have also been identified in human GM [99].

In the study by *Qiu* et al., a high proportion of *Candida* spp. was also found in IBD patients compared to controls. This genus has, in fact, been implicated in the pathogenesis of IBD. In particular, *C. tropicalis* and *Candida albicans* have been found to trigger intestinal inflammation in IBD [100].

As for *Aspergillus*, this too has been found in GM, especially in immunocompromised individuals. Certain *Aspergillus* species (i.e., *Aspergillus fumigatus*) may exacerbate colitis [93].

Fungal dysbiosis may also be related to bacterial dysbiosis involved in the pathogenesis of these diseases [100]. A study found increased fecal levels of *Candida Tropicalis* in patients with CD, which correlated with fecal concentrations of *E. coli* and *Serratia marcescens*, as well as serum anti-*S. cerevisiae* titers. In practice, the two bacteria and the fungus, in vitro, acted in synergy to form a sort of enhanced biofilm [101]. The latter may underlie the combined pathogenic capacity of these microbes.

Changes in GM composition also lead to alterations in metabolites that may play a role in the pathogenesis of these diseases. Specifically, there is a reduction in the biosynthesis of amino acids and carbohydrate metabolism, while increasing the absorption and secretion pathways of nutrients [102]. For example, bile acid signaling via the nuclear farnesoid-activated X receptor (FXR, also known as the bile acid receptor) has been shown to be protective in patients with colitis. Since bile salt hydrolases (BSH) produced by gut bacteria play a key role in bile acid modification, dysbiosis could affect FXR signaling. Indeed, the relative abundance of BSH in the gut microbiota was found to be markedly reduced in IBD patients compared to healthy individuals [103]. Furthermore, it has also been shown that bile acid levels are reduced in patients with IBD, especially at the time of exacerbations [104]. Thus it can be inferred that microbial enzymatic activity is impaired in IBD resulting in impaired bile salt metabolism and loss of anti-inflammatory signaling through FXR.

Another bacterial metabolic pathway involved in the pathogenesis of IBD is the production of short-chain fatty acids (SCFA) by specific strains of *Clostridia* spp. SCFAs have been shown to enhance regulatory T cell function in the intestinal mucosa via the activation of G protein-coupled

receptors resulting in epigenetic effects with subsequent inhibition of histone deacetylase. In the animal model this process promotes the restoration of immune tolerance and reduces inflammation [105]. Furthermore, SCFAs together with L-arginine helps to maintain the integrity of the intestinal wall, which is in fact altered in inflammatory pathologies [106].

Cardiovascular diseases

Hypertension, heart failure and atherosclerosis are the most frequent cardiovascular diseases and are now known to be related to GM as well. In hypertensive patients, the GM diversity is reduced, while the *Firmicutes*/*Bacteroidetes* ratio is increased [107]. The intestinal flora is closely connected to pressure variations as it produces molecules capable of modifying this vital parameter. For example, the imbalance of SCFA that occurs during dysbiosis stimulates intestinal enterochromaffin cells to produce 5-hydroxytryptamine which, in the blood, causes vasoconstriction [108]. Conversely, in the course of dysbiosis there may be a reduction of hydrogen sulphide, a molecule capable of causing vasodilation and hypotension. Therefore, its deficiency contributes to the onset of arterial hypertension [109].

GM may also be involved in atherosclerosis and also in this case this involvement may depend on the production of various molecules. Among these should be mentioned the Trimethylamine-N-Oxide (TMAO). It is able to increase the production of proinflammatory cytokines and reduce the production of anti-inflammatory molecules [110]. Furthermore, it is capable of increasing platelet reactivity, facilitating thrombotic processes. This set of events contributes to atherosclerosis [111]. Therefore it can be deduced that an excess of TMAO and, in particular, of bacteria that produce this molecule can increase the risk of developing atherosclerotic disease.

Metabolic diseases

GM alterations have also been detected in patients with type 1 (T1DM) and type 2 (T2DM) diabetes mellitus. In particular, a reduction in the diversity of bacterial species was observed in patients with T1DM, as well as a reduction in *Clostridium* and *Prevotella* [112]. In patients with T2DM, compared to healthy ones, a reduced amount of *Bifidobacteria* and *Akkermansia* and a high amount of *Dallella* were found [113].

GM alterations have also been found in gestational diabetes. In particular, there were increases in *Rumenococcus*, *Desulfovibrio*, *Prevotella*, *Bacteroides*, and *Enterobacter*, with a reduction instead of *Bifidobacterium* and *Fischeri* [114]. In these cases, dysbiosis increases the risk of developing inflammation, impaired glucose tolerance, and obesity [115].

Inflammation can also be caused by bacterial components such as lipopolysaccharide, peptidoglycans, flagellins which lead to the destruction of tight junctions [116].

Intestinal dysbiosis is also found in obese individuals, in whom *Bacteroides*, *Akkermansia* and *Faecalibacterium* are deficient and *Firmicutes* species abound [117].

Bacterial metabolites such as SCFAs and succinate are also involved in the pathogenesis of obesity. SCFAs regulate energy balance and reduce appetite [118]. Succinate, on the other hand, is produced by *Bacteroides* and *Prevotella*. It facilitates gluconeogenesis and the consumption of glucose in the brain [119]. This molecule therefore tends to counteract obesity.

Non-alcoholic fatty liver disease (NAFLD) is a widespread disease in the world, especially in Western countries, which can evolve into more serious pathological conditions, such as cirrhosis of the liver [120]. A meta-analysis found that intestinal permeability was often increased in patients with NAFLD compared to healthy controls and was also associated with the degree of fatty liver disease [121]. This then promotes the passage of microbes inside the intestinal wall. But not only that, it has been seen that in many cases in patients with this liver disease there is also a certain degree of intestinal dysbiosis [122]. In particular, a reduction of *Bacteroides* and an increase of *Firmicutes* and *Proteobacteria* are reported, compared to healthy subjects [123].

The importance of the gut microbiota in the pathogenesis of NAFLD has also been demonstrated in mouse models. In particular, the effects of a high-fat diet were tested on mice with GM and germ-free mice. The first group of mice experienced weight gain, glycemia, and pro-inflammatory cytokines, while the second group experienced only weight gain, but without changes in glycemia and cytokines. Furthermore, the group of mice with GM developed fatty liver disease, whereas the mice lacking gut microbes did not. These results suggest an evident role of GM in the development of hepatic steatosis and metabolic pathologies [124].

Lactobacillus rhamnosus supplementation in NAFLD mice strongly reduced fat accumulation in the liver. In particular, it resulted in an increase in *Firmicutes* and *Bacteroidetes*, attenuated the expression of proinflammatory cytokines such as TNF-α and IL-1β in the liver [125]. Even the administration of the *Lactobacillus casei* strain seems to have positive effects on the regression of NAFLD. In fact, this microbe can contribute to the restoration of tight junctions and the reduction of the inflammatory state [126].

Chronic alcohol intake not only causes direct liver damage, it can also compromise the integrity of the intestinal barrier and contribute to intestinal dysbiosis. This is demonstrated by the fact that the cessation of alcohol abuse restores intestinal eubiosis and reduces intestinal permeability. In a condition of increased intestinal permeability, some microbial components, such as LPS, can translocate through the portal venous circulation to the liver. Here LPS is recognized by specific receptors, such as toll-like receptors (TLRs), with consequent activation of the immune system which sets up liver inflammation, with subsequent hepatocyte damage and liver fibrosis [127].

Boursier et al. demonstrated a correlation between significant liver fibrosis (F≥2) and some species of *Ruminococcus* abundance [128] which are also capable of producing alcohol, that could have harmful effects on both intestinal permeability and liver inflammation [129].

Hyperammonemia has long been considered a marker of the severity of chronic liver disease, particularly cirrhosis [130]. In particular, it has been hypothesized that ammonia may exert direct effects on hepatic stellate cells by activating them. Which would suggest that hyperammonaemia itself may promote fibrosis. And an excess of ammonia is produced by certain bacteria in the intestine [131].

Cancer

Numerous human cancers have been associated with GM dysbiosis. Among them should be mentioned gastric cancer. The main risk factor for gastric cancer is *Helicobacter pylori*. This microbe may in fact be responsible for chronic gastric inflammation, which represents the ideal substrate for the development of a tumor [132]. Conversely, a reduced incidence of esophageal cancer was observed in subjects with gastric *H. pylori* infection. In fact, it inhibits the function of the parietal cells and/or induces the development of atrophic gastritis, preventing the production of hydrochloric acid. The latter is mainly responsible for Barrett's esophagus in patients with gastroesophageal reflux disease, a precancerous condition [132, 133].

H. pylori can also reach the liver by blood. *H. pylori* lipopolysaccharide (LPS) was found to promote liver cancer growth by increasing the levels of IL-8 and transforming growth factor β1 (TGF-β1) [134]. *H. pylori* LPS is also involved in the genesis of pancreatic cancer as it induces the production of large amounts of inflammatory cytokines that damage the pancreas [135]. Furthermore, at the pancreatic level, LPS can also facilitate mutations of proto-oncogene K-Ras, which is mutated in over 90% of cases of pancreatic adenocarcinoma [136].

Colorectal cancer (CRC) is the third most common cancer in the world and is currently burdened with high morbidity and mortality. Several risk factors are known including age >50, smoking, obesity, etc. [137]. Nutrition may or may not influence the development of CRC. In fact, a diet rich in processed foods, sausages and red meats and low in fiber and fruit can favor the development of this neoplasm [138]. In addition to these exogenous factors, endogenous factors have also been identified, such as, for example, intestinal dysbiosis [2]. In particular, in the case of neoplastic pathologies, dysbiosis seems to be involved in the development of cellular mutations and in the uncontrolled proliferation of abnormal cells [139].

The bacteria which, on the basis of current knowledge, seem to be most involved in tumorigenesis are *Fusobacterium nucleatum, Bacteroides fragilis, Streptococcus bovis, E. coli*, etc.

Several studies have been carried out on these bacterial species over the years which have allowed us to strengthen the hypotheses on an existing link between the microbes and the increased neoplastic risk. These bacteria have in fact demonstrated the ability to induce an inflammatory state in the intestine, to inhibit the immune response against tumor cells, to promote the progression of preneoplastic lesions [140]. While other bacterial species (e.g. *Lachnospiraceae* spp., *Bifidobacterium animalis*, etc.) have often been found to be deficient in CRC patients. Therefore, a protective effect against CRC was hypothesized for these [141].

Similarly, some fungi, such as *C. albicans*, are able to promote tumorigenesis through inflammation and the production of molecules, such as reactive oxygen species, which can cause chromosomal damage and mutations [142]. Others such as *Malassezia* and *Trichosporon*, on the other hand, have proven capable of promoting the progression of neoplastic lesions [143].

As far as viruses are concerned, especially Human Papilloma Virus and John Cunningham virus (JC virus) have been associated with colon carcinogenesis because they are capable, among other actions, of inhibiting the transcription of immunosuppressants (e.g. p53 and pRb) through the production of various proteins [144, 145].

CONCLUSIONS

From what has been illustrated, it can be deduced that GM is actually involved in the development of certain pathological disorders. This can occur directly, for example through its action on the integrity of the intestinal barrier, or through the production of molecules capable of interfering with normal cellular functioning. However, not all microbes are harmful to the body; some have obvious anti-inflammatory properties, others are capable of preventing the colonization by pathogenic germs. Therefore, the use of probiotics could support the treatment of pathological disorders that are also associated with intestinal dysbiosis. Future studies that will be conducted in this direction will certainly be useful and decisive.

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