Association of intestinal dysbiosis with susceptibility to multiple sclerosis: Evidence from different population studies (Review)

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Abstract. Understanding the relationship between microorganisms that live in our intestines and neuroinflammatory and neurodegenerative pathologies of the central nervous system (CNS) is essential, since they have been shown to have an immunomodulatory effect in neurological disorders, such as multiple sclerosis (MS). The gut microbiota can be affected by several environmental factors, including infections, physical and emotional stress and diet, the latter known as the main modulator of intestinal bacteria. An abrupt shift in the gut microbiota composition and function is known as dysbiosis, a state of local and systemic inflammation produced by pathogenic bacteria and its metabolites responsible for numerous neurological symptoms. It may also trigger neuronal damage in patients diagnosed with MS. Intestinal dysbiosis affects the permeability of the intestine, allowing chronic low-grade bacterial translocation from the intestine to the circulation, which may overstimulate immune cells and cells resident in the CNS, break immune tolerance and, in addition, alter the permeability of the blood-brain barrier (BBB). This way, toxins, inflammatory molecules and oxidative stress molecules can pass freely into the CNS and cause extensive damage to the brain. However, commensal bacteria, such as the *Lactobacillus* genus and *Bacteroides fragilis*, and their metabolites (with anti-inflammatory potential), produce neurotransmitters such as γ-aminobutyric acid, histamine, dopamine, norepinephrine, acetylcholine and serotonin, which are important for neurological regulation. In addition, reprogramming the gut microbiota of patients with MS with a healthy gut microbiota may help improve the integrity of the gut

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Abbreviations: AFB1, aflatoxin B1; AhR, aryl hydrocarbon receptor; BBB, blood-brain barrier; BDNF, brain-derived neurotrophic factor; CIS, clinically isolated syndrome; CNS, central nervous system; CVD, cardiovascular disease; EAE, experimental autoimmune encephalomyelitis; ENS, enteric nervous system; FMT, fecal microbiota transplantation; FoxP3, forkhead box protein 3; FXR, farnesoid receptor X; GA, glatiramer acetate; GABA, γ-aminobutyric acid; GALT, gut-associated lymphoid tissue; GPBAR1, G-protein-coupled bile acid receptor 1; GM, gut microbiota; IBS, irritable bowel syndrome; IFN-γ, interferon-γ; IL-2, interleukin 2; IL-10Rα, IL-10 receptor α; LDL-C, low-density lipoprotein cholesterol; LPS, lipopolysaccharides; miRNA, microRNA; MS, multiple sclerosis; NF-κB, nuclear factor κB; NMR, nuclear magnetic resonance; NLR, nucleotide-binding oligomerization domain-like receptors; NLRP6, pyrimidine domain of inflammasome 6; RCT, randomized controlled trial; ROS, reactive oxygen species; RRMS, relapsing-remitting multiple sclerosis; rRNA, ribosomal RNA; RXR, receptor X retinoide; SCFAs, short-chain fatty acids; SPMS, secondary progressive multiple sclerosis; T1DM, type 1 diabetes mellitus; Th, T-helper cell; TMAO, trimethylamine N-oxide; TNF-α, tumor necrosis factor-α; Treg, regulatory T cell; VDR, vitamin D receptor

Key words: multiple sclerosis, gut microbiota, intestinal dysbiosis, neuroinflammation, neurodegenerative disease

and BBB, by providing clinically protective anti-inflammatory effects and reducing the disease's degenerative progression. The present review provides valuable information about the relationship between gut microbiota and neuroinflammatory processes of the CNS. Most importantly, it highlights the importance of intestinal bacteria as an environmental factor that may mediate the clinical course of MS, or even predispose to the outbreak of this disease.

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1. Introduction

The gut microbiota (GM) comprises all of the bacteria, viruses, archaea and fungi that live in synergy in the body. If the set of genes of these microorganisms is included, the term is microbiome (1-3). Microbial diversity may be described as the number of different species in the same sample or in different samples, referred to as alpha and beta diversity, respectively (4). As for bacteria, the GM of a healthy person corresponds to three main phylae: *Firmicutes* (Gram positive), 60%; *Bacteroidetes* (Gram negative), 10%; and *Actinobacteria* (Gram positive), 10%. *Firmicutes* represent the largest proportion and include around 200 genera, of which the most important are *Mycoplasma*, *Bacillus* and *Clostridium*. The remaining 20% belong to the phylae *Proteobacteria*, *Verrucomicrobia* and *Euryarchaeota* (5,6). Certain studies report that microbial cells outnumber eukaryotic cells in the body, accounting for ~1 to 2 kg of the body weight, referring to the 10:1 ratio of microbial/eukaryotic cells (1,7,8). The large intestine is the part of the intestinal tract where there is a higher number of bacteria, as the neutral pH conditions, decrease in enzymes, decrease in secretions from other digestive organs, less oxygen and slow transit consequently give a greater possibility of survival and metabolic functions of microorganisms derived from the fermentation of non-digestible dietary products (1,8).

The functions of the GM are diverse and important and beneficial for the body, as it has a role both locally and systemically. At a local level, it participates in maintaining the health of the intestinal mucosa, prevents intestinal permeability and increases the absorption surface, among others. These microorganisms also have a metabolic function. They intervene in the digestion and absorption of dietary nutrients to obtain energy, in the absorption of minerals such as calcium, phosphorus, magnesium and iron, in the extraction of essential nutrients, and synthesize vitamins (such as K, B5, B8, B12 and folic acid), hormones and short-chain fatty acids (SCFAs). In addition, another important function is immunomodulation. Intestinal bacteria interact with the immune system, favor the maturation of the innate and adaptive immune system, and promote the health and maturity of the gut-associated lymphoid tissue (GALT) (1). Studies have demonstrated that the immune system depends significantly on GM diversity. An anti-inflammatory GM often demonstrates high taxonomic diversity and microbiome variation (9). All of this leads to an effective defense mechanism against pathogenic microorganisms which, in turn, may change the GM composition (1,10).

The GM is affected by several factors, such as consumption of xenobiotics, infections, individual genetic and immune response, diet and lifestyle. Diet is considered the main modulator. Research has indicated that GM is related to the maturation of cognitive and behavioral functions in the central nervous system (CNS) (11-13). The usual dynamics of the GM can be directed towards a state of dysbiosis when stress conditions quickly decrease microbial diversity and promote the spread of specific bacteria (6). The factors in intestinal dynamics, their natural variations and stress mediate cascades of destabilizing events of the intestinal microbiome. The underlying mechanisms of intestinal dysbiosis remain to be fully elucidated. However, in general, it has been observed that oxidative stress, the induction of viruses that specifically affect bacteria (bacteriophages) and the secretion of bacterial toxins can trigger rapid changes between intestinal microbial groups, thus producing dysbiosis (6). The human GM is composed of native and transient microorganisms (6). In this context, only a relatively small number of opportunistic (pathogenic) bacteria are considered members of the GM, which reside unaltered within the GM and only become a threat to the host's health when the ecosystem and homeostasis of the GM are altered (state of dysbiosis). Throughout an individual's life, the composition and function of the GM diversity are affected and lead to CNS alterations (14). In gastrointestinal disorders, the imbalance between pro-inflammatory and anti-inflammatory bacteria causes systemic inflammation, which generates alterations in the intestinal and blood-brain barrier (BBB) permeability. This, in turn, accelerates the neuro-inflammatory processes that can trigger neurological diseases, such as multiple sclerosis (MS) (14,15). Evidence has confirmed that GM is associated with the development of various disorders, including cardiovascular diseases (CVD), cancer, diabetes, brain disorders and chronic kidney disease. Therefore, its modulation has an important role in the prevention and treatment of multiple diseases, from microbial fecal transplantation to treat *Clostridium difficile* infection to the use of probiotics in inflammatory bowel diseases and diet-based modification for cancer. In October 2021, there were ~3,000 clinical trials in progress related to microbiota (16).

The Food and Agriculture Organization of the United Nations and the World Health Organization define probiotics as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'. Probiotic administration is used to improve GM homeostasis and maintain host health (17). As a result, the number of pathogenic bacteria that cannot survive in an acidic environment decreases and beneficial bacteria that grow well in an acidic state proliferate, thus balancing the GM, and prebiotics are defined as 'selectively fermented ingredients that produce specific changes in the composition and/or activity of the GM, thus conferring

benefits to the host's health'. Certain dietary fibers, such as carbohydrate polymers that are not digested or absorbed, are subject to bacterial fermentation in the gastrointestinal tract and, therefore, affect the composition of the bacterial community and metabolic activity. The combination of both is referred to as symbiotic (17).

Prebiotics are partially digested in the upper segments of the gastrointestinal tract. They then reach the colon to be fermented by beneficial bacteria (e.g., from the genus *Bifidobacterium*), achieving a state of synergy (symbiosis). This stimulates the selective growth and/or activity of intestinal bacteria potentially associated with health protection. Through their main mechanisms, such as the production of antimicrobial substances, competition for epithelial adhesion and nutrients, increased production of SCFAs, increased fecal mass, reduced colonic pH, reduced nitroso products and fecal enzymes, modulation of mucin production and improved immune system (by increased IgA secretion by GALT, which can stimulate the phagocytic function of inflammatory macrophages), prebiotics confer beneficial effects for human health. Some examples of prebiotics that are most commonly used in human nutrition are fructooligosaccharides, galactooligosaccharides, inulin, xylooligosaccharides and lactulose (18).

Lactobacillus, members of the GM, are producers of γ-aminobutyric acid (GABA) and serotonin, essential neurological regulators. Likewise, several neurotransmitters and neuromodulators, such as choline, tryptophan and SCFAs (acetate, propionate and butyrate), are also produced by the GM (14). In this sense, a change in the GM diversity or in certain bacteria may cause differential hormonal responses, which influence brain activity and instigate pathological processes (19). Probiotic supplementation has shown crucial benefits in stress and anxiety processes. Certain functions associated with the intake of probiotics are as follows: Reducing oxidative stress and inflammatory processes, facilitating modifications in hippocampal synapse by increasing the expression of brain-derived neurotrophic factor (BDNF) and increasing hypothalamic neuronal activity, which translates into better cognitive and learning processes (20). The present review aimed to highlight the role of the GM as an environmental risk factor in the development and progression of MS, and how the modulation of the GM may be part of the treatment for MS.

2. Methods

Articles published online in English from 2006 to August 2023 were searched in the electronic databases of PubMed of the National Library of Medicine (https://pubmed.ncbi.nlm.nih.gov). The search was conducted from September 2021 to August 2023. The following medical headers were used in various combinations: 'multiple sclerosis', 'lipopolysaccharides', 'gastrointestinal microbiome', 'intestinal mucosa', 'endotoxemia', 'intestinal dysbiosis', 'neurodegenerative diseases' and 'neuroinflammation'. More than 200 studies were assessed by title, abstract and study type regarding its potential inclusion in the current review.

The inclusion criteria were as follows: i) Articles written in English; ii) studies related to MS; iii) studies related to the GM; iv) the relationship between intestinal dysbiosis and impairment of CNS functions; v) studies on the existing evidence of the association of intestinal dysbiosis with MS in patients from different populations; vi) interventional studies including randomized controlled trials (RCTs) and experimental studies that evaluated the effect of probiotic administration on immune or inflammatory markers in patients with MS and the animal model of MS 'experimental autoimmune encephalomyelitis' (EAE); vii) studies that report the association between probiotic intake and immune and inflammatory response in MS. In addition, the full text of potentially eligible articles was analyzed independently.

Initially, a total of 1,764 articles were retrieved, as indicated in Fig. 1. After excluding duplicate studies (n=500), 1,264 citations remained. Subsequently, the titles and abstracts of the remaining articles were reviewed and 200 studies were considered potentially eligible after 1,064 were eliminated. The abstracts of the articles were read and 100 were excluded. After reviewing the full texts of the 100 remaining candidate articles, only 77 were included (23 were eliminated). Finally, 21 other studies were added due to being relevant for the present review, totaling 98 articles (60 reviews, 4 cases and controls, 4 systematic reviews, 10 *in vitro* studies, 3 RCTs, 13 animal studies, 3 case reports and 1 meta-analysis) (Fig. 1).

3. Multiple sclerosis

MS is a chronic, inflammatory, neurodegenerative disease of the CNS in which demyelination and axonal degeneration occur, making it highly disabling with a different disease phenotype for each individual patient who suffers from it (21). Out of every 100,000 individuals, 40 live with MS, meaning that there are almost 3 million cases of MS worldwide and numbers are increasing rapidly (22). MS occurs at the most formative stage of life, the productive stage of work life, with an average age at diagnosis of 32 years, and, as with other immunological diseases, women are affected twice as frequently as men (22). The progression and severity of the disease have a diverse pattern, where a small percentage of individuals present with a benign phenotype characterized by developing little or no disability over time, or an aggressive course with frequent relapses without reaching a full recovery between each, with rapid progression toward disability (23).

The vast majority of patients, ranging from 80 to 90%, present with relapsing-remitting variant MS (RRMS). They go through relapse episodes (active MS) followed by remission or partial or total recovery of symptoms (clinically silent periods). Approximately 50% of these patients progress to secondary progressive MS (SPMS), a form lacking clear periods of activity and remission, since patients deteriorate clinically more quickly and with serious sequelae that increase their disability. Another type is the primary progressive variant, where patients present with aggressive disease and constant progression, without the typical cycle of relapse-remission from the time-point of diagnosis. The prevalence of this type of MS is between 10 and 20% of patients (24,25).

Clinically isolated syndrome (CIS) is a clinical state where inflammation and demyelination may be observed in the CNS when only one attack or relapse has occurred, and the patient cannot yet be diagnosed as MS. MS begins as CIS in ~85% of patients and becomes clinically defined MS when they

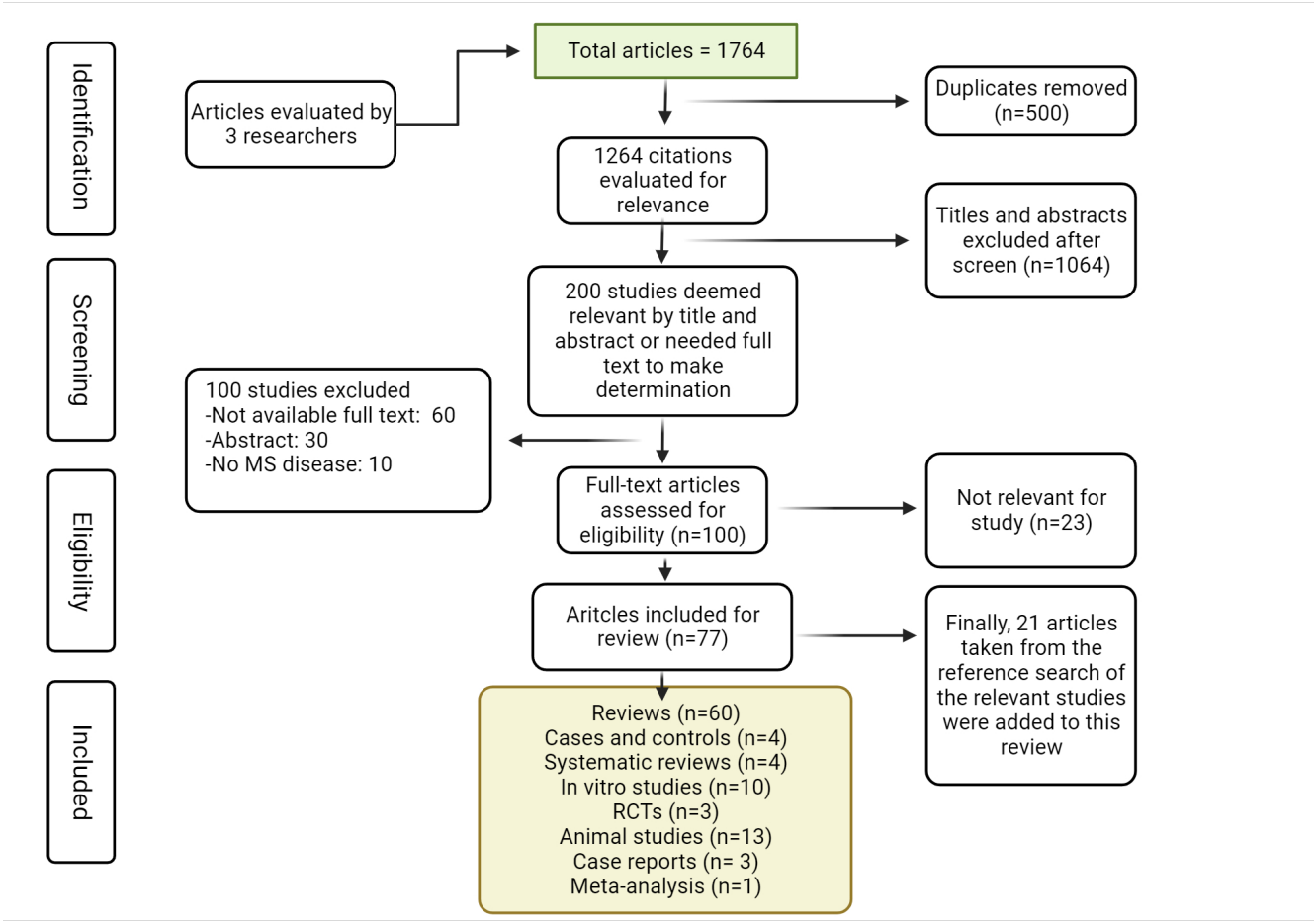

Figure 1. Flow chart of selection of studies for inclusion in the review. MS, multiple sclerosis; RCT, randomized controlled trial.

develop a second attack (26). In the most recent McDonald 2017 diagnostic criteria, CIS can be treated as MS when it is in accordance with the nuclear magnetic resonance (NMR) data for dissemination in space (involvement of different regions of the CNS) and time (involvement at different times in the disease course) (27), or oligoclonal bands are present in the cerebrospinal fluid and spread in space. Revisions to these criteria aim to diagnose most patients with less active disease with MS (28). The importance of these adjustments in the diagnostic criteria lies in increasing the identification of previously undiagnosed cases of MS and in not misdiagnosing individuals who do not have the disease (29).

The etiology of MS remains largely elusive; however, it is known to occur in genetically susceptible individuals, but environmental factors are required to trigger the disease. The most studied environmental factors are a western diet (high in saturated fat, simple carbohydrates and sodium, and low in dietary fiber), vitamin D deficiency, childhood obesity, a sedentary lifestyle, tobacco smoking and certain infections, such as Epstein-Barr virus. In addition, other risk factors found in a systematic review, such as exposure to organic solvents, cytomegalovirus infection and vaccination against diphtheria and tetanus, are associated with the risk of MS (30). Environmental factors have become more relevant due to their relationship with autoimmune and neurodegenerative diseases, which had been previously explained only by genetic factors. Other environmental factors associated with autoimmune diseases are gastrointestinal disease and dysbiosis of the GM, as well as intestinal barrier permeability and consequent inflammation (31).

4. Intestinal dysbiosis and its relation with neuroinflammation and neurodegeneration in MS

The GM is a dynamic entity and there are numerous factors that predispose to its modification and, therefore, to its function. In fact, each individual has their own GM, similar to fingerprints. This is what prevents researchers from establishing a single GM as a model to follow. However, there are characteristics of these hosts that make them more or less desirable for the gastrointestinal tract. It is established that GM dysfunction depends on the type of bacterial strain and can trigger various pathologies (32). There are a number of studies on the relationship of dysbiosis with certain diseases, such as gastrointestinal diseases, inflammatory bowel disease and colon cancer, as well as obesity, diabetes, allergies, arthritis and CVD. However, for some years now, it has been suggested that the GM may have a role in CNS disorders (33). However, at present, only the tip of the iceberg is known, and the role of the GM in neuroinflammation and neurodegeneration has remained to be fully elucidated (32). For a long time, infections have been investigated as a possible trigger for MS, although without concrete evidence. Now, the concept of a pro-inflammatory GM as a trigger for autoimmunity has

emerged, with the possible implication of dysbiosis, as has been demonstrated in MS (34).

In the present review, it is argued that the link between the GM and MS is based on low-grade inflammation and that the outcome of this low-grade inflammation depends, to a large extent, on genetic factors, but above all on environmental factors, such as eating habits and their effects on the GM and the subsequent process of dysbiosis (32). It is thus possible that a trigger of intestinal inflammation, through dysbiosis, e.g. diet, may alter both the number and diversity of microbiota species. Studies have indicated that a high-salt diet may be associated with an increased risk of developing EAE and dementia, through the induction of T helper 17 (Th17) cell differentiation; in particular, mice fed a high-salt diet developed neurovascular and cognitive impairments through the expansion of Th17 cells in the small intestine, leading to an increase of interleukin 17 (IL-17) levels in plasma. This increase of Th17 cells possibly results from a change in the microbiome composition, is diet-dependent and may be induced by activation of serum glucocorticoid kinase 1 that regulates sodium intake (35). Studies in patients with MS have shown that a high-salt diet induced an increase of Th17 cells in the blood and a decrease in the intestinal strains of the *Lactobacillus* genus, and is associated with the worsening of clinical symptoms and increased activity on NMR (17,36,37). In addition, recent evidence suggests that the GM is one of the key environmental factors in the development of MS (38). Clinical patient studies have indicated the presence of a pro-inflammatory state originating in the intestine (17), specifically Th17 cells, which are involved in the pathogenesis of MS, and which are present in abundance in the peripheral blood, cerebrospinal fluid and brain lesions of patients with MS, and their counts and inflammatory mediators increase even further during relapses (39). Observations show that in MS, there is an expansion of Th17 cells in the intestine, which is associated with microbiota alterations and correlates with high disease activity (17).

Furthermore, multiple studies in which the GM was characterized by sequencing the 16S ribosomal RNA (rRNA) gene, have shown that patients with autoimmune diseases such as MS have alterations (they exhibit dysbiosis) in their microbiome (19). This dysbiosis is associated with various pathologies, including those that affect organs of the host that appear to have no connection to the intestine, e.g., the CNS (40). It has been observed that the modification of the GM composition, i.e., the state of dysbiosis, produces a wide range of amyloid proteins that serve for the crossing and propagation of pathological protein aggregates from the intestine to the CNS, favoring the neurodegenerative processes produced by the aberrant metabolism of these proteins (41). Clinical evidence shows that patients with neurodegenerative diseases present with symptoms of gastrointestinal disease, even several years before the diagnosis of their disease (42). Of note, a specific change in the GM of patients with RRMS has been observed: The presence of effector Th17 cells in the intestinal tissue was found to be associated with a decrease in the *Prevotella* genus and an increase in the *Streptococcus* genus (43). In another study, an association of certain bacterial species that are normally rare in the intestine of healthy humans was found. An increase in the *Acinetobacter* genus and a lower presence of *Parabacteroides distasonis* were found. It was also reported that exposing the lymphocytes of healthy individuals to the microbiota of patients with MS increased their differentiation into Th1 cells and reduced the proportion of $CD25^+$ forkhead box protein 3 $(FoxP3)^+$ regulatory T (Treg) cells. On the other hand, exposure of these lymphocytes to *Parabacteroides distasonis* led their T cells towards a regulatory phenotype (44). In another study on patients with MS who were given a mixture of the *Lactobacillus*, *Streptococcus* and *Bifidobacterium* genera, this probiotic mixture induced a change in the peripheral immune response to anti-inflammatory drugs and restored the GM composition of those patients (45). A similar probiotic mixture (*Lactobacillus* and *Bifidobacterium*) was evaluated in two randomized, double-blind, placebo-controlled trials of three and four months' duration. Their results suggest that daily supplementation with probiotics could improve the clinical symptoms of MS (46).

In addition to probiotics, other techniques are being used to modulate the composition and function of the dysbiotic GM in MS. Although studies are scarce, encouraging results have been obtained. To date, these studies have been conducted to treat severe gastrointestinal symptoms caused by the course of the disease, i.e., without the intention of observing clinical benefits for the disease. This was the case with the possible effect of fecal microbiota transplantation (FMT) in three patients with MS treated with an average of 8 FMT infusions for chronic severe constipation, where constipation resolution was demonstrated, but they also achieved progressive neurological improvement (47). Another longitudinal proof-of-concept study with 12 months of follow-up, also to evaluate the effect of FMT on RRMS, was carried out with a single subject who received FMT infusions from five healthy donors (48). Clinical progression, fecal microbiota composition, fecal SCFA concentrations and serum levels of inflammatory and neuroprotective biomarkers were evaluated. The results were a microbiota with greater bacterial diversity, with a higher relative abundance of butyrate-producing bacterial species and a higher butyrate concentration. The microbiota modification was associated with reduced levels of inflammatory cytokines and elevated serum levels of BDNF. Clinically, the patient showed a progressive improvement in gait and balance scores (disability status) (48). In this context, a case report indicated that following treatment with FMT for *Clostridium difficile* enterocolitis, disease stability was achieved in a patient with SPMS (34). All of these accidental findings and with limited studies provide evidence that the GM of patients with MS is in a state of dysbiosis and that, by restructuring it, remarkable benefits are obtained. Above all, they give us the lead to conduct research on FMT as a complementary approach to MS treatment.

Native bacteria from fermented foods, such as curds, sourdough and fermented milk, are another technique that may be used to benefit various pathologies (49). For instance, *Lactobacillus fermentum* from fermented milk was studied by Kumara *et al* (50). The study evaluated the ability of *Lactobacillus fermentum* to detoxify aflatoxin B1 (AFB1) *in vivo* in albino mice. *Lactobacillus fermentum* was administered orally to mice 24 h before AFB1 administration. The results showed that *Lactobacillus fermentum* significantly reduced AFB1 levels in the liver and kidneys of

mice. In addition, *Lactobacillus fermentum* also modulated the production of pro-inflammatory cytokines, suggesting that it has immunomodulatory activity. In summary, the study found that *Lactobacillus fermentum* has the potential to detoxify AFB1 and modulate the immune response, which may be beneficial for human health (50) and it would be worth using it in patients with MS, taking into account that this pathology has significant inflammatory potential. The same bacteria were also studied in humans, but this time in the form of an encapsulated probiotic (51). Researchers evaluated the effects of supplementation with various types of *Lactobacillus*, including *Lactobacillus fermentum*, on gene expression related to inflammation, insulin and lipids in patients with MS. The study was a randomized, double-blind and placebo-controlled trial. The intervention group (n=20) received a daily probiotic capsule containing a blend of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum* and *Lactobacillus fermentum* for 12 weeks. Each capsule contained 2±109 colony-forming units/g. Patients were assigned to receive 100 g of probiotics or placebo per day for 12 weeks. The results showed that patients who received probiotics had a significant decrease in the expression of genes related to inflammation [IL-8 and tumor necrosis factor α], but did not affect the expression of genes related to insulin PPAR-γ or lipids/low-density lipoprotein (LDL) receptor. In addition, patients who received probiotics also exhibited an improvement in MS symptoms. In summary, the study found that supplementation with *Lactobacillus* may be a potential therapy for MS (51). In addition, another study focused on the molecular characterization of *Bacillus* sp. bacteria that hydrolyze gluten and in their potential as a probiotic. The bacteria were isolated from sourdough and curd samples and characterized by their biochemical and molecular properties. The results showed that the bacteria were able to hydrolyze gluten and that they had antibacterial, anti-adhesive and pathogen exclusion properties. Furthermore, the bacteria were also able to modulate gene expression in Caco-2 cells (used in research as a model of the intestinal epithelial barrier) (50).

In addition to probiotics, SCFA supplementation has also shown good results as an adjuvant in the inflammatory response in MS, as propionate has been indicated to be reduced in the serum and stool of patients with MS at the same time that they have an altered GM (34). In another study, supplementation for 3 years achieved clinical improvement in patients; these SCFAs were associated with a rebalancing of the Th17/Treg cell ratio towards a more regulatory profile, in addition to positive regulation of genes related to the induction of Treg cells in the intestine (52). Therefore, it appears that inflammation in the intestine may result in the activation of encephalogenic T cells that may travel to the CNS, where they can induce inflammatory damage with subsequent demyelination and axonal loss. In addition, inflammation in the intestine can also influence the diversity of the microbiota and lead to dysbiosis, which in turn may enhance intestinal inflammation (34). Chen *et al* (38) also presented results consistent with the hypothesis that patients with MS have GM dysbiosis. They designed a study with patients with RRMS to investigate whether their GM was altered. They compared their fecal microbiota with that of healthy controls, revealing that patients with MS had a distinct microbial profile compared to controls, with the genera *Pseudomonas*, *Mycoplana*, *Haemophilus*, *Blautia* and *Dorea* found in patients with MS, while the control group had a higher abundance of the genera *Parabacteroides*, *Adlercreutzia* and *Prevotella*. Therefore, they made two important observations: First, that certain gut microbes show decreased or increased abundance in patients with MS compared to controls, and second, the importance of the microbiota in this disease (38).

Possible mechanisms of the influence of the GM in MS. It is important to clarify the mechanism by which intestinal bacteria are related to MS. According to Wekerle (53), the pathogenic mechanism of MS requires at least 3 factors: A genetic predisposition, a pro-inflammatory intestinal bacterial profile and the accumulation of self-reactive T cells in GALT. In this bidirectional communication between the gut and the brain, the mild inflammation originating in the gut passes to the brain. In this path to the brain, there are two barriers, the intestinal barrier and the BBB, and it is easier for intestinal inflammation to cause a disruption of the BBB than *vice versa* (34). However, to obtain an answer that will lead to further clarification of the scope of intestinal dysbiosis in MS, it would be necessary to clearly define the sequence of events that lead to the dysfunction of both barriers and 'trace' the moment at which they occur. This is easier to demonstrate in EAE, but in MS, it is practically impossible. However, research is underway that may lead to a good understanding of this intricate relationship (34). It is thus that a large amount of current evidence indicates that the gut-microbiota-brain axis probably has a crucial role in the pathogenesis of neurological diseases, such as MS. Both in MS and in its murine model, gastrointestinal symptoms, altered GM and increased intestinal permeability have been reported (34).

The gut-microbiota-brain axis acts as a link between the external environment and the CNS. Its main components are the microbiota, for its role in gastrointestinal homeostasis; the intestinal barrier, which regulates the entry of food and microbial metabolites into the body; and the sympathetic and parasympathetic arms of the autonomic nervous system, specifically the enteric nervous system (ENS) and the vagus nerve, which transmits signals to the brain (34). In fact, the possible implication of intestinal dysbiosis in MS was first studied in EAE using germ-free mice and it was shown that the microbiota has a crucial role in directing both pro-inflammatory and anti-inflammatory immune responses in the CNS, possibly increasing Treg- and Th2-cell responses (53,54). EAE experiments with mice treated with specific antibiotics or GF mice monocolonized with particular bacteria have clearly shown that signals from an altered GM can induce inflammation in extraintestinal tissues (54). This concept has become even stronger through experiments in which microbiota from patients with MS was administered to modulate EAE (34). However, the dilemma of the initial trigger for the dysfunction of the gut-microbiota-brain axis in MS is still unknown within a chicken or egg causality conundrum. Thus, innate immune activation in the brain could be triggered by signals from the gut. On the one hand, metabolites from the GM can affect the innate immune response in the CNS. This was demonstrated in a study of mice fed high amounts of fiber, where the diet modulated the GM and led to increased production of SCFAs,

particularly butyrate, and there was a decrease in the expression of the inflammatory microglia gene (34).

The BBB is composed of tightly joined endothelial cells with tight junctions along the blood vessels that vascularize the brain. This barrier allows the CNS to be isolated from the circulation and precisely regulates the passage of molecules, ions and cells between the brain and the periphery (55). It also protects the brain from pathogenic microorganisms and uncontrolled inflammatory reactions that could damage brain neurons. Unfortunately, in response to a state of dysbiosis, i.e., a high number of bacteria with pro-inflammatory function, the intestinal barrier can become permeable, consequently contributing to the chronic low-grade bacterial translocation from the intestine to the circulation. This overstimulates immune cells and cells of the CNS, leading to alterations in the permeability of the BBB. This is how the GM plays a fundamental role in the susceptibility to the development of autoimmune diseases (55).

A study of mice with BBB permeability observed an increase in the passage of molecules from the periphery, such as lipopolysaccharides (LPS) and oxidative stress. Both molecules are known to induce systemic inflammation and neuroinflammation. This permeability was due to a decrease in bacterial species, which in turn caused a 75% decrease in tight junction proteins, including occludin and claudin-5 in the BBB (56,57). Another study reported that this effect could be reversed with SCFA supplementation, such as propionate and butyrate, which demonstrates that SCFA production by the GM also has a beneficial effect on the BBB (41). In addition, bacterial products and metabolites may also cross the permeable BBB (40) and contribute to the development and progression of autoimmune diseases (57,58), since they cause inflammation, demyelination of neuronal axons and scarring (gliosis), which triggers abnormal neuronal signaling function. In addition, studies in aged mice have indicated that endotoxemia, i.e., the passage of LPS to the circulation (LPS >200 pmol/ml), may alter the brain vasculature and promote a neuroinflammatory phenotype, leading to memory problems and cerebral amyloid angiopathy (59). Thus, when MS patients do not have a known exogenous risk factor, the question arises of whether MS originates in the periphery or in the CNS. Therefore, there is an extrinsic CNS theory where self-reactive T cells that are activated in the periphery, possibly by molecular mimicry, activation and co-expression of T-cell receptors with double specificity (60), travel to the CNS along with activated B cells and monocytes. This theory is analogous to the method used to induce EAE: These mice are administered emulsioned CNS antigen along with immunostimulants, resulting in the generation of pathogenic $CD4^+$ Th1 and Th17 cells in the draining lymph nodes; subsequently, these cells pass to the circulation, cross the BBB and exert their effector functions within the CNS (60).

In a healthy state, there is a regulatory center in the thymus where numerous self-reactive T cells are eliminated, maintaining central tolerance in order. However, despite this regulation, certain self-reactive T cells escape to the periphery, possibly because peripheral tolerance mechanisms are compromised by reduced function of Treg cells or by increased resistance of effector B cells and T cells to CNS-directed suppressor mechanisms. Subsequently, self-reactive B cells and T cells are activated in the periphery and become highly aggressive effector cells. This activation of lymphocytes may be due to the recognition of CNS-derived antigens sequestered in the periphery. Once lymphocytes are activated and have differentiated into Th1 and Th17, they infiltrate the CNS, along with B cells and innate immune cells, causing inflammation and tissue damage there (61) (Fig. 2). In this sense, the health of the microbiota is a crucial factor in maintaining the integrity of the intestinal barrier, as the latter is also part of the correct maturation and response of T and B cells, and of course, is involved in the tolerant communication between the host and the microbial environment, i.e., that there is an adequate tolerant response during exposure in the intestinal mucosa and an attack in the systemic circulation (61). In addition, for triggering an adequate immune response, it is necessary that there is adequate colonization of the microbiota, since, in demyelinating lesions of the CNS, it may be observed that the recruitment of self-reactive B and T cells depends on the availability of target autoantigens and commensal bacteria. In a study with germ-free EAE model mice, a reduction in inflammation was observed, as the immune system cannot mount an effective Th17-mediated response (61).

Besides, the microbiota and its metabolites also participate in the differentiation fate of T cells in the intestine, thus modulating the immune response. For instance, *Bacteroides fragilis* participates in the differentiation of $FoxP3^+$ Treg cells (62). In addition, SCFAs increase the expression of Treg cells, particularly butyrate, suppress the activation of Th17 cells, and reduce the symptoms of EAE and axonal damage (63). Neurodegenerative diseases have shown a compositional and functional modification of the GM with resulting neuroinflammation. A diet high in simple sugars, high in saturated fats and low in fiber (64), fructose and the heme component, are the few factors that have also been reported to alter the GM composition and the permeability of the intestinal epithelial barrier in mouse models. In this way, GM dysbiosis in mice fed a high-fructose diet was the main cause of neuroinflammation (65), as a healthy microbiota can modulate the neurological function of the mouse brain by being part of the maturation and function of microglia (66). The pyrimidine domain of inflammasome 6 (NLRP6), part of the family of nucleotide-binding oligomerization domain-like receptors (NLR), mediates innate cellular immunity to defend the intestinal mucosa against bacterial pathogens. As mentioned above, commensal bacteria and their metabolites are essential for epithelial barrier integrity, perhaps by regulating NLRP6 inflammasome signaling to maintain intestinal microenvironment homeostasis (67). As mentioned, commensal bacteria-derived metabolites are critical in neurodegenerative diseases. They have shown a notable role in immune-mediated neurodegeneration, as Treg cells promote the differentiation and remyelination of oligodendrocytes (68). Certain commensal bacteria, such as *Streptococcus* sp., and the most representative species of the genus *Lactobacillus*, such as *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri* and *Lactobacillus delbrueckii* (subspecies *bulgaricus*), are able to increase levels of anti-inflammatory cytokines, such as IL-10 (69).

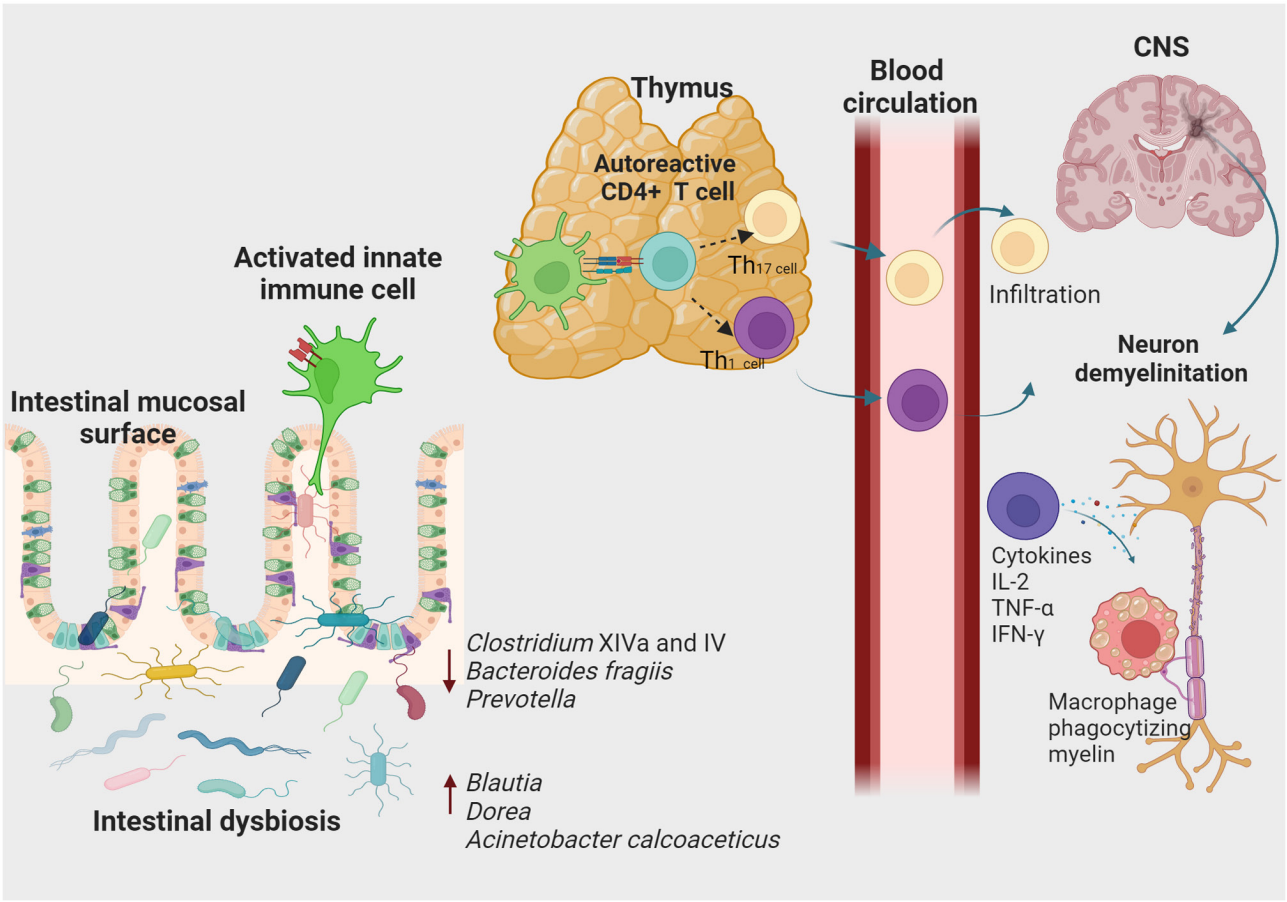

Figure 2. Association of intestinal dysbiosis with susceptibility to the development of MS. According to an extrinsic model of the CNS, autoreactive T cells in the periphery are activated through molecular mimicry, some circulating cells or the co-expression of T-cell receptors with different specificity. Different genetic and environmental factors, such as modification of gut microbiota composition, are thought to contribute to these events. When activated, $CD8^+$ T-lymphocytes, $CD4^+$ T-lymphocytes differentiated into $CD4^+$ Th1 and Th17 cells, B cells and cells of the innate immune system can infiltrate the CNS, causing inflammation and tissue damage. In addition, the Th1-type response produces pro-inflammatory cytokines such as IL-2, TNF-α and IFN-γ that activate antigen-presenting cells. In turn, pro-inflammatory cytokines activate macrophages responsible for phagocytizing myelin. CNS, central nervous system; MS, multiple sclerosis; Th1, type 1 T-helper cell.

In a mouse study, direct binding of *Streptococcus* sp. and *Lactobacillus* ligands initiates a signaling cascade that induces increased expression of IL-10 by C-type lectin-positive macrophages and macrophage galactose-type lectin-1/CD301a, and downstream mediators such as spleen tyrosine kinase, caspase recruitment domain-containing protein 9 and ERK are also involved in the induction of IL-10 mRNA (69). The immunoregulatory effects of this cytokine are critical for brain health, as indicated by another study of oxygen and glucose deprivation-induced cerebral ischemia, as IL-10 has an immunoregulatory role that attenuates neuronal death and promotes neurite growth and synapse formation through the JAK1/STAT3 pathway (70). Sun *et al* (71) have demonstrated the influence of the *Bifidobacterium* genus on Treg cells, which increased the intracellular level of IL-10 and IL-10 receptor α (IL-10Rα), suggesting that *Bifidobacterium* promotes the IL-10/IL-10Rα autostimulatory circuit in intestinal Treg cells. On the other hand, the metabolites of segmented filamentous bacteria in the ileum activate C-X-C motif chemokine receptor 1^+ macrophages that act as antigen-presenting cells that present microbial antigens to naive T cells, which consequently differentiate into RAR-related orphan receptor-γ^+ T cells and, ultimately, into Th17 cells. Therefore, these intestinal bacteria modulate the $CD4^+$ T-cell compartment in the GALT and promote the differentiation and expansion of Th17 cells, which, in turn, can promote neuroinflammation in the circulation. As observed in a rat study using the EAE model, pro-inflammatory Th17 cells promote neurodegeneration directly in neuronal cells; IL-17 inhibits the maturation of oligodendrocyte cells *in vitro* and reduces their survival. Oligodendrocyte lineage cells are susceptible to IL-17-mediated toxicity, suggesting a direct connection between intestinal bacteria, inflammation, neuroinflammation and the pathogenesis of MS (72).

GM-derived neurotransmitters, such as histamine, dopamine, norepinephrine, acetylcholine and serotonin, as well as GABA produced by *Lactobacillus brevis*, have direct activity in the CNS (73,74). Studies have shown a decrease in the functionality of the serotonergic system in MS. Furthermore, pro-inflammatory cytokines that are elevated in dysbiosis can activate indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase, enzymes that activate the kynurenine pathway and deplete the availability of tryptophan for serotonin synthesis. Serotonin regulates the function of T cells and other immune components (75). Approximately 90% of serotonin is synthesized by gut enterochromaffin cells and is mediated by the microbiome. In addition, the

GM not only independently generates serotonin, but it can also stimulate the release of these molecules (76). For instance, microbial metabolites such as SCFAs promote the transcription of an enzyme that synthesizes serotonin from tryptophan. In addition, the GM in animal models has been shown to have an influence on the synthesis of serotonin receptors outside the gut. Therefore, serotonin may be a critical mediator of the gut-brain axis in MS. Similarly, a decrease in GABA brain transporters is expressed in MS patients. GABA is the main inhibitory mediator in the CNS and downregulates pro-inflammatory T-cell mediators. The production of this neurotransmitter is regulated by the GM, with *Lactobacillus brevis* being the most effective producer. Furthermore, a decrease in dopamine levels is linked to MS relapse. Dopamine controls locomotion, cognition, endocrine regulation and immune regulation. It also suppresses IL-17, suggesting an anti-inflammatory effect. *Staphylococcus aureus*, *Escherichia coli* 157:H7 and other bacteria have shown improved growth or mobility *in vitro* in the presence of dopamine. However, there is no clinical evidence to confirm that bacteria produce dopamine (77).

The mechanism behind this gut-microbiota-brain axis remains to be fully elucidated. This axis may be regulated through different pathways, and among them, neural pathways are most important, mainly due to the stimulation of the vagus nerve, the alteration of the activity of the hypothalamic-pituitary-adrenal axis and the secretion of SCFAs that can activate microglial cells and the systemic circulation (41), which explains how the brain and the gut are bidirectionally linked. The neurotransmitters produced by the GM bind to sensory neurons in the ENS, sending afferent impulses through the vagus nerve and sympathetic/parasympathetic pathways (77). On the other hand, intestinal inflammation induced by bacterial pathogens causes activation of the vagal sensory ganglia and the solitary tract nucleus in the brainstem, providing an early warning pathway to the brain during infections. In addition, the interaction between the GM and brain also occurs indirectly, as the afferent vagal fibers do not cross the epithelial layer of the intestine, so the passage of metabolites produced by intestinal bacteria or their compounds is the main way to activate the afferent fibers (78). Enteroendocrine cells present in the intestinal epithelium are responsible for communicating with the afferent fibers of the vagus nerve and release certain substances, such as serotonin, ghrelin, cholecystokinin and peptide YY that modulate motility, secretion and nutrient intake within the gastrointestinal tract (79). As mentioned above, SCFAs are the main metabolites produced by bacteria and their production is affected during dysbiosis, leading to an inflammatory phenotype (80). SCFAs can induce transcription factors and modulate the expression of critical enzymes through histone deacetylation. In addition, SCFAs have vital roles in structural, metabolic and neurohormonal regulation. In particular, the functions of SCFAs in MS, in addition to those already described, are as follows: Promoting immune cell tolerance, inducing immunoglobulin secretion, inhibiting pathogens, stimulating mucus secretion and contributing to intestinal barrier integrity (81).

In one study, a decrease in the *Clostridium* genus was associated with the production of Treg cells and an increase in the anti-inflammatory cytokine IL-10, and administration of *Clostridium tyrobutyricum* or sodium butyrate restored the BBB permeability in pathogen-free mice (81). A fiber-rich diet and oral butyrate supplements reduced symptoms and increased $CD4^+FoxP3^+$ T cells in mouse EAE models (82). A high-fat diet (such as the Western diet) reduces butyrate production by the *Prevotellaceae* family and *Bacteroides* genus in mouse EAE models (82). On the other hand, SCFA treatment mitigates the high-fat diet-induced change in the GM and intestinal epithelial barrier integrity in C57BL/6 mice, possibly by inhibiting systemic inflammation (83). SCFAs can modulate the maturation and function of microglia in the brain, suggesting the potential benefits of GM-derived SCFAs in protecting against the neuroinflammatory process (65). In a recent study, intestinal dysbiosis induced by a high-fat and choline-deficient diet in C57BL/6N male mice occasionally increased the *Proteobacteria* phylum and decreased the *Firmicutes* phylum, and as a consequence, SCFA production deteriorated, combined with a sudden increase in neurogenesis, possibly depleting the pool of neural stem cells available for long-term neurogenesis, neuroinflammation, oxidative stress, synaptic loss and cell death in different brain regions (83). Other proposed mechanisms linking dysbiosis with neuroinflammation and neurodegeneration in MS, as the relationship between certain elevated bacteria in MS and mucin degradation, bacteria metabolize bile acids with anti-inflammatory and pro-inflammatory effects in patients with MS, and certain vitamin and other compound deficiencies related to intestinal barrier disruption and inflammation are presented in Table I. In any case, whether in the EAE model or in MS, it is clear that the gut-microbiota-brain interaction is crucial in the progression of the disease and therapeutic approaches targeting intestinal dysbiosis and intestinal barrier dysfunction should begin to be considered within the MS drug regimen (34). Therefore, in view of the great impact that dysbiosis may have on MS, the use of probiotics has been proposed as a potential therapy (34).

5. Evidence of the association of intestinal dysbiosis with MS in different human populations

Certain pathologies, such as MS, are related to modifications of the GM composition, showing intestinal dysbiosis compared with healthy controls. The relationship between MS and intestinal dysbiosis has been scrutinized by different studies, from *in vitro* analyses using mouse models to clinical trials comparing MS patients with healthy subjects. Even though different reviews regarding MS and the microbiome were found, only a few studies (cohorts from the US, UK, Japan, Germany and Italy) describe the cohorts in detail. Most were predominantly Caucasian, although a few included Hispanic subjects from the US. In addition, an increased intestinal permeability prevalence in MS patients is related to this disease. However, it has not been related to brain NMR lesion load, which is also true for EAE. The mice also exhibited an increase of inflammatory T-cell migration to the brain, linking intestinal permeability with BBB permeability (88). Furthermore, an increase of the LPS endotoxin in EAE compared with controls may imply

Table I. Other proposed mechanisms link dysbiosis to neuroinflammation and neurodegeneration in MS.

Molecule or biological compound	Definition or function	Related bacteria	Effect of bacteria and their relationship with intestinal dysbiosis	(Refs.)
Mucin	A family of glycosylated proteins secreted in the gut, forming the mucus barrier gel that protects the epithelium	*Akkermansia muciniphila, Ruminococcus, Bifidobacterium* and *Dorea*	They are capable of degrading mucin and they were found to increase in patients with MS	(8)
Bile acids	Primary and secondary bile acids modulate the immune response by increasing FXR and the GPBAR1 expression. FXR, IL-1β and TNF-α mediate colon inflammation by increasing LPS in dysbiosis	*i) Lactobacillus, Clostridium, Erysipelotrichaceae* and *Parabacteroides* *ii) Acinetobacter, Bifidobacterium, Pseudomonas* and *Bilophila*	i) Bile acids metabolizing bacteria with anti-inflammatory effects decreased in patients with RRMS ii) Bile acids metabolizing bacteria with pro-inflammatory effects increased in patients with MS	(84)
Vitamin D	Vitamin D has an anti-inflammatory function by inhibiting the expression of NF-κB. It does this by binding to VDR and RXR receptors, creating a heterodimer that modulates gene expression	*Faecalibacterium*	Vitamin D deficiency has been linked to intestinal barrier disruption, decreased butyrate-producing bacteria and dysbiosis	(32)
Phytoestrogens	There are four main types of phytoestrogens (isoflavones, prenyl flavonoids, coumestans and lignans), and some bacteria are responsible for metabolizing isoflavones and lignans into equol and enterolactone, respectively. In intestinal epithelial cells, isoflavones suppress pro-inflammatory cytokines through estrogen receptors. They also inhibit nitric oxide production induced by LPS (leading to inflammation and neurodegenerative processes) and decrease the expression of NF-κB and other pro-inflammatory cytokines	*i) Prevotella, Parabacteroides, Adlercreutzia, Slackia* and *Lactobacillus* *ii) Lactobacillus plantarum* and *Lactobacillus paracasei*	i) These bacterial genera are related to the conversion of phytoestrogens to their metabolites; therefore, they possess an anti-inflammatory potential and are decreased in patients with MS ii) Their administration was effective in preventing EAE development in mice	(11,10,85,86)
Tryptophan	Tryptophan is an essential amino acid metabolized from food to indole and indole derivates by microbiota. These metabolites regulate immune cell responses in the gut mucosa and increase mucin production. They also activate the pregnane X receptor, essential in gut barrier integrity	*Lactobacillus*	Bacteria that metabolize tryptophan to indole, which activates AhR with anti-inflammatory functions	(8,82)

Table I. Continued.

Molecule or biological compound	Definition or function	Related bacteria	Effect of bacteria and their relationship with intestinal dysbiosis	(Refs.)
Cysteine	Cysteine is a non-essential amino acid required in a rate-limiting step for glutathione production. Glutathione is an antioxidant that protects cells from ROS and peroxide damage, which plays a crucial role in recruiting immune cells that start the myelin phagocytosis at CNS. Superoxide increases disrupt the cell junction at the BBB	*Desulfovibrionaceae*	They may induce a deficiency of bioactive sulfur-containing antioxidant glutathione by sequestering cysteine and are increased in patients with MS and EAE	(82)
Choline	A water-soluble nutrient grouped in complex B vitamins	*Acinetobacter baumannii*	Metabolizes choline to trimethylamine, which is metabolized in the liver to TMAO. An increase in these bacteria has been observed in patients with MS	(8,32)
MicroRNAs	In MS and EAE, miRNAs mediate the upregulation and downregulation of different miRNAs in cells like $CD4^+$ T, Th17, Th1, Th2 and Treg. miRNAs produced by host cells regulate GM	Lower levels of butyrate-producing bacteria such as *Clostridium*	The GM may regulate miRNA production in host cells. For example, butyrate triggers the expression of miR-375, a biomarker related to MS progression. On the other hand, certain exome miRNAs, such as let-7i, prevent Th1 and Th17 cell differentiation during MS onset	(87)
Steroid hormones	Microbiota dysbiosis may also relate to an increase in glucocorticoids, mineralocorticoids or catecholamines in the CNS by the enteric nervous system	Interaction between the CNS and the enteric nervous system	This release may increase gut permeability and immune responses	(87)

MS, multiple sclerosis; FXR, farnesoid receptor X; GPBAR1, G-protein-coupled bile acid receptor 1; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor α; LPS, lipopolysaccharide; RRMS, relapsing-remitting MS; NF-κB, nuclear factor κB; VDR, vitamin D receptor; RXR, receptor X retinoide; EAE, experimental autoimmune encephalomyelitis; AhR, aryl hydrocarbon receptor; ROS, reactive oxygen species; CNS, central nervous system; BBB, blood-brain barrier; TMAO, trimethylamine N-oxide; miRNA/miR, microRNA; Th, T helper cell; Treg, regulatory T cell; GM, gut microbiota; let7i, exome miRNA (a lethal weapon against angiotensin II).

bacterial translocation due to an altered intestinal barrier. LPS can stimulate immune microglial responses (88).

Although intestinal dysbiosis is a continuum seen in different studies that include individuals with MS, there are differences and similarities in the changes observed in each phylum. In general, the GM in MS compared with healthy controls shows a depletion of the phylum *Bacteroidetes*, particularly the genera *Prevotella* and *Parabacteroidetes* (8), which is related to RRMS, expansion of Th17 cells and disease activity (8). *Prevotella* depletion in MS has been consistent in different geographical areas (8). By contrast, in a predominantly adult caucasian cohort from the USA, a *Prevotella* increase was observed after pharmacological treatment or disease remission in fecal samples of patients with RRMS in comparison with untreated patients with RRMS (89).

Changes around the *Firmicutes* phylum are contradictory among studies, with both a decrease or increase of the genera *Clostridium* and *Faecalibacterium* reported (38). On the other hand, Chen *et al* (38) found an increase of the genera *Dorea* and *Blautia* (also of the *Firmicutes* phylum) in fecal samples of an RRMS cohort from the US (n=62). High levels of *Dorea*

are also related to other inflammatory diseases such as Crohn's disease. However, the *Dorea* genus also shows an anti-inflammatory action, possibly due to different species of this same genus or their proportion with respect to another genus, since for example, gasses produced by *Dorea* are utilized by *Blautia* (which can promote mucin degradation) (38).

An increase of the *Akkermansia* genus (*Verrucomicrobia* phylum) has been reported by different studies comparing MS patients with healthy controls (90). The *Akkermansia* genus is related to mucin degradation and increases of pro-inflammatory cytokines. It has been shown that *Akkermansia muciniphila* induces a pro-inflammatory T lymphocyte response *in vitro*; however, *in vivo* studies using mouse models of MS have so far failed to induce a similar response (90). Nevertheless, *Akkermansia muciniphila* also may improve the intestinal barrier in metabolic diseases, highlighting the entanglement of the microbiome function (90).

Another genus consistently increased in patients with MS compared with healthy controls is *Methanobrevibacter* (*Euryarchaeota* phylum) (90). *Methanobrevibacter* and *Ak kermansia muciniphila* are methanogen bacteria associated with constipation in patients with MS, a common condition in this disease (90). Methane acts as a neurotransmitter that slows bowel movement and may contribute to microbiota changes and the pro-inflammatory local environment (90). *In vitro* analyses and mouse models linked the increase of both *Methanobrevibacter* and *Akkermansia* genus, in addition to the decrease of *Butyricimonas* genus, with the regulation of dendritic cell maturation, interferon (IFN) signaling and nuclear factor κB signaling (90). After medical treatment with IFN-β and glatiramer acetate (GA), the genera *Prevotella* and *Sutterella* increased in a predominantly caucasian US cohort (91). This study comparing patients with MS with healthy controls showed that GA treatment also decreased the abundance of *Bacteroidaceae and Lactobacillaceae* families, *Ruminococcus and Clostridium* genera and other clostridial microbes (91).

Regarding the *Proteobacteria* phylum, human cohorts from different countries observed enrichment of the *Mycoplana*, *Haemophilus*, *Sutterella*, *Pseudomonas*, *Bilophila* and *Acinetobacter* genera (86). It should be noted that, although they do not contradict each other, each research group reports an enrichment in different *Proteobacteria*. A Japanese cohort study comparing healthy controls with patients with RRMS also showed a decrease of *Clostridia* XIVa and IV clusters (both of the *Clostridium* genus), constituting up to 40% of healthy gut bacteria (92).

The contradictions around the *Firmicutes* phylum and the differences between genera of the *Proteobacteria* phylum may be derived from the absence of standardized sequencing methodologies and the bias represented by using primers for the specific identification of the 16S rRNA gene (8), which is a region of bacterial DNA used to classify the sample, identifying the abundance and variability of species. Although it is one of the most conserved regions of bacterial DNA, it is formed by regions with high variation, making it challenging to generate an accurate microbiome profile. Other factors that limit GM studies are individual variability and environmental influences. Genetics, diet, geographical area and therapies may modify the microbiome (8).

Despite the discrepancy, the GM profile in patients with MS is usually enriched with pro-inflammatory bacteria and depleted from anti-inflammatory bacteria, which may influence the severity of the disease. These changes in bacterial distribution are related to the digestion/metabolism of several compounds, resulting in either fewer nutrients reaching the host or an increase of metabolites with detrimental effects. Thus, diet may be a determining factor in GM.

6. Beneficial effects of GM on various diseases

Most microbiota research focuses on the relationship between changes in GM composition and various pathological states. Environmental (external) factors affect the balance of the microbial community, leading to the deregulation of the organism's vital functions and diseases. Thus, there is increasing evidence that the GM is associated with the development of pathologies such as allergies, gastrointestinal disorders, obesity, CVD, cancer and diabetes, among others (16). Also, in the scientific literature, it has been indicated that the modulation of the GM by means of probiotic administration brings important health benefits (93).

Allergies. The relationship between microbiota and diseases is complex. For instance, infants born through cesarean section, who acquire bacteria from the maternal epidermis, face an elevated risk of developing allergies and asthma compared to those born through the maternal vaginal canal. Although this distinction diminishes as the infant develops, the microbiota has an important role in immune system development. Certain pathogenic bacteria, such as *Haemophilus influenzae*, have the potential to trigger allergic reactions, whereas others like the Proteobacterium *Moraxella catarrhalis* can exacerbate the allergic airway reaction. Meanwhile, SCFAs may stimulate the generation of Treg cells and contribute to reducing inflammation in allergic models (16).

Possible mechanisms by which probiotics could modulate the allergic response are as follows: Probiotics can regulate the immune response, decreasing the production of IgE, Th2 cells and pro-inflammatory cytokines, restore the GM, which has been shown to be altered in allergic patients and increase the production of SCFAs, which have anti-inflammatory properties (93). The results of clinical trials evaluating the efficacy of probiotics for allergies are mixed. Certain studies have found that probiotics are effective in reducing allergic symptoms, such as allergic rhinitis and asthma. However, other studies have found no benefit from probiotics. More studies are needed to confirm the efficacy of probiotics for allergies. Future studies should evaluate different types of probiotics, doses and formulations, as well as different types of allergies (93).

Irritable bowel syndrome (IBS). A meta-analysis found an association of probiotics with improvement of symptoms, specifically decreased pain in IBS compared to placebo (94). Another randomized, double-blind study of a probiotic containing *Lactobacillus plantarum* and *Bifidobacterium breve* also found improvement of pain in 38% of patients after 14 days and 52% after 28 days of consumption (95). Another clinical trial found that a product containing 8 bacterial species

significantly improved IBS symptoms. In general, evidence suggests that probiotics may be a useful therapy for certain patients with IBS. However, more studies are needed to determine the efficacy of probiotics in the treatment of different types of IBS and to identify the most effective types and doses of probiotics (96).

Obesity. The idea that the GM may have a role in obesity has been around for several years, based on studies that confirmed a higher proportion of *Firmicutes* to *Bacteroidetes* in obese mice compared to lean mice. There was also a higher representation of genes involved in the extraction of energy from food in the GM of the obese host compared to the microbes of the lean host (97). In humans, one of the first studies to link the GM with obesity compared the GM of lean and obese individuals and revealed that obese subjects had a reduced proportion of *Bacteroidetes* and higher levels of *Firmicutes*. When a dietary intervention was given to these individuals, the relative abundance of *Bacteroidetes* increased, while that of *Firmicutes* decreased (97). It is possible that the GM has a role in obesity, but more research is needed to better understand this association (97).

In different studies where symbiotic supplementation (mixture of probiotics and prebiotics) was administered to obese subjects, different results were observed depending on the type of bacteria supplemented. With *Lactobacillus gasseri* SBT2055, a reduction in body mass index, waist circumference, abdominal visceral fat area and hip circumference was observed. With strains of *Enterococcus faecium* and *Streptococcus thermophilus*, a reduction in body weight, systolic blood pressure and LDL cholesterol (LDL-C), and an increase in fibrinogen levels were obtained. With *Bifidobacterium*, *Lactobacillus* and *Streptococcus thermophilus*, there was an improvement in the lipid profile and insulin sensitivity, and a decrease in C-reactive protein. With *Lactobacillus acidophilus* La5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 (*Bifidobacterium lactis*), there was a reduction in the fasting glucose concentration and an increase in the homeostasis model assessment of insulin resistance (18).

CVD. There is growing evidence that the GM has a role in maintaining cardiovascular health and its dysregulation can contribute to CVD. The gut and oral microbiota have an important role in cardiovascular health. GM dysbiosis, or an imbalance in the composition of gut bacteria, has been associated with an increased risk of CVD, such as atherosclerosis, hypertension and heart failure (16). The mechanisms by which the GM can contribute to CVD include the following: i) Inflammation-gut bacteria can release inflammatory mediators that can damage endothelial cells, which line the blood vessels; ii) oxidative stress-gut bacteria can produce free radicals that can damage cells and tissues in the body; Iii) blood pressure modulation: Metabolites produced by gut bacteria can affect blood pressure (16).

Cancer. The microbiota, which is the collection of microorganisms that live in the human body, may have a role in the development of cancer (16). Researchers have found that microbiota dysbiosis, or an imbalance in the composition of the microorganisms, is associated with an increased risk of cancer in several organs, including the colon, lung and mouth (16). The mechanisms by which the microbiota may contribute to cancer include the following: i) Inflammation-chronic inflammation caused by microbiota dysbiosis can damage DNA and promote the growth of cancer cells; ii) invasion and metastasis-certain bacteria can invade body tissues and help cancer cells to spread; iii) metabolic alteration-metabolites produced by bacteria can affect cellular metabolism, which can increase the risk of cancer (16).

In particular, studies have found that the bacteria *Fusobacterium nucleatum*, *Escherichia coli*, *Streptococcus bovis*, *Bacteroides fragilis* and *Helicobacter pylori* are associated with an increased risk of colon cancer. The genera *Streptococcus*, *Prevotella* and *Veillonella* are associated with lung cancer, while *Porphyromonas gingivalis* and *Fusobacterium nucleatum* are associated with oral cancer (16). More research is needed to better understand the mechanisms by which the microbiota may contribute to cancer. However, emerging evidence suggests that the microbiota is an important target for cancer prevention and treatment (16). In several studies, beneficial effects have been identified with supplementation with *Lacticaseibacillus rhamnosus* GG, *Bifidobacterium lactis* and inulin. These include an increase in *Lacticaseibacillus rhamnosus* and *Bifidobacterium lactis* in the feces, reduction of *Clostridium perfringens*, prevention of increased IL-2 secretion in polypectomized patients and increased production of IFN-γ in cancer patients (18).

Diabetes. The intestinal and oral microbiota may have a role in the development of type 1 diabetes mellitus (T1DM) and T2DM. In T1DM, the GM is characterized by a decrease in bacteria producing butyrate, a fatty acid with anti-inflammatory properties. In addition, an increase in pathogenic bacteria, such as the genus *Salmonella*, has been observed. The mechanisms by which the GM may contribute to T1DM are not fully elucidated, but it is thought that GM dysbiosis may lead to chronic inflammation, which may damage insulin-producing cells in the pancreas. In T2DM, the GM is also characterized by a decrease in butyrate-producing bacteria and an increase in pathogenic bacteria. In addition, an increase in bacteria that produce primary bile acids, which can negatively affect glucose metabolism, has been observed. It is thought that GM dysbiosis may lead to chronic inflammation, abnormal intestinal permeability and altered glucose metabolism, acting as a possible mechanism that can contribute to T2DM (16).

The oral microbiota may also have a role in T2DM. Oral bacteria can travel to the intestine, changing the GM composition and potentially mediating the immune response (16). In general, research on the intestinal and oral microbiota in T1DM and T2DM is ongoing. However, emerging evidence suggests that the intestinal and oral microbiota may be an important target for the development of new therapies for these diseases (16). Various combinations with probiotic strains are associated with a reduction in fasting blood glucose, antioxidant status, hemoglobin A1c, total cholesterol and LDL-C (*Lactobacillus acidophilus* La5 and *Bifidobacterium lactis*) (18).

7. Concluding remarks

The present review deals with the relationship between dysbiosis of the GM and neuroinflammatory processes in MS. Although research is still ongoing, gut bacteria appear to be a predisposing factor to the development of neurodegenerative diseases such as MS. Studies carried out in different populations around the world reported that patients with MS exhibit marked changes in their GM composition, described by the decrease or increase of determined bacterial phylae and species compared to control subjects. Despite the controversy existing in the results of microbiota studies in different populations, the difference in composition and function between study groups remains clear. The contradictions regarding the differences between species may be due to the proportion between genera or to the fact that the products of certain bacteria are used in the metabolic processes of other bacteria. In addition, the medical treatment is different in each patient; the clinical course of the disease and disease-modifying treatment may also influence the bacterial response in the host.

In this light, it appears that the factors that interfere with the GM composition are multiple and diverse for each population, from genetics to eating habits and lifestyle, the complex and entangled relationship between the microbiota and the host, and the relationship that exists between the same microorganisms; therefore, further work is required on the implementation of standardized techniques for the sequencing of the 16S rRNA gene and the protocols for studying the GM. It may be argued that MS is triggered in the periphery, i.e., in the intestine, where the inflammatory molecules produced by a dysbiotic microbiota can enter the circulation as a result of an intestinal barrier impaired by the same inflammatory process and reach the CNS and initiate an immunological cascade that sensitizes T-lymphocytes to myelin, also weakening the BBB and causing inflammation at the local level and demyelination of neurons, and consequently the nervous tissue. In its eagerness to repair the damage, it produces remyelination by oligodendrocyte precursor cells causing scarring, so axons with partial or complete loss of myelin do not transmit full nerve impulses and classic MS symptoms begin to appear.

In conclusion, ongoing research should be performed to answer the question of whether intestinal dysbiosis triggers MS, or whether MS causes dysbiosis in patients. Studies indicate that intestinal dysbiosis may be the most critical environmental factor for developing the disease. Animal and *in vitro* studies suggest that probiotic administration modifies the GM composition towards an inflammation-mediating phenotype, which positively affects CNS disorders and the demyelination process in EAE. However, more research in humans is needed. For this reason, future studies using large-scale RCTs are required to review effect-doses in MS. Future therapies may target inflammation control via the gut-CNS axis.

For instance, the oral administration of myelin antigens suppressing the immune response has proven effective in EAE animal models. A number of case reports have suggested a potential benefit of FMT on patients with MS. Despite FMT, antibiotic treatment is more widely used; however, it significantly alters the GM composition. Meanwhile, probiotic, prebiotic and diet-based microbiota treatments may be a promising approach for MS, since accessibility is wider, enabling GM modification, competitive adherence to the mucosa and epithelium, strengthening the gut epithelium and BBB, preventing bacterial translocation and modulating immune response (98). Likewise, molecular characterization of bacteria indigenous in fermented foods, such as sourdough or curd, may have a broader biotherapeutic potential (17).

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The authors declare that they have no competing interests.

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