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The Microbiome-Gut-Brain Axis in Multiple Sclerosis

Dušan Radojević, Svetlana Soković Bajić, Miroslav Dinić, Aleksandar Bisenić, Jelena Đokić, Nataša Golić*

Group for Probiotics and Microbiota-Host Interaction, Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 444a, 11042 Belgrade 152, Serbia

*Corresponding author: Nataša Golić, e-mail: natasag@imgge.bg.ac.rs

Abstract

The microbiome-gut-brain axis (MGBA) represents a close two-way relationship between the gut and the central nervous system (CNS) mediated by the immune system, the enteric nervous system (ENS), the vagus nerve, and the gut microbiome. Gut microbes, including bacteria, fungi, and viruses, can communicate with the CNS and modulate the physiology of the brain in health and disease, which marks them as an important MGBA factor. It is becoming increasingly evident that gut microbiome dysbiosis is implicated in the onset and severity of different neurodegenerative and psychiatric diseases including multiple sclerosis (MS). MS is a chronic disease of the CNS associated with different genetic and environmental risk factors. Neuroinflammation and demyelination in the brain and the spinal cord are hallmark features of MS. The accumulating evidence shows that the MGBA, although a relatively new concept, has an important role in MS. Therefore, the purpose of this article is to review recent research on the gut-brain connection in MS, and to highlight MS-associated gut microbiota constituents and the role of bacterial metabolites in MS.

Key words: gut microbiome, multiple sclerosis, gut-brain axis, bacterial metabolites, dysbiosis

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Microbiome in the gut-brain axis

There has been an epidemic of various neurodegenerative and autoimmune diseases, strongly associated with the modern lifestyle. Among them, neurodegenerative disorders are a huge burden on society, impairing the health and the quality of life of affected people and their families, as well as impacting society as a whole. Neurodegenerative diseases are a heterogeneous group of disorders characterized by the progressive degeneration of the structure and function of the central (CNS) or peripheral nervous system (PNS), with the most prevalent ones being dementia (more than 55 million people worldwide with Alzheimer disease [1] and Parkinson's Disease [2]), amyotrophic lateral sclerosis, synucleinopathies, Huntington disease and related polyglutamine diseases, prion disease, traumatic brain injury, chronic traumatic encephalopathy, stroke, spinal cord injury (3), and multiple sclerosis (MS; 2.8 million people globally [4]). Most of the neurodegenerative disorders in children and adults are considered multifactorial diseases prompted by environmental factors in genetically susceptible individuals (5). A number of preclinical and clinical studies indicate that patients affected by neurodegenerative diseases have gastrointestinal (GI) dysfunction, accompanied with alterations in the diversity and composition of gut microbiota and the microbiome-gut-brain axis (MGBA) as one of the common denominators (6, 7, 8, 9, 10). Gut microbiota is a term that refers to the bacteria, archea, fungi, viruses and protozoans residing in the gut, while gut microbiome includes microorganisms and their genetic material and metabolites (11). The MGBA is a term intended to describe the interactions between the host and gut microbiota, together with the effects within these interactions that have an impact on the CNS (Figure 1). Over the past decade, the MGBA has become appreciated as bidirectional communication between gut microbiome and the CNS, exerting a profound influence on neural development, neuroinflammation, activation of stress response, neurotransmission, and modulation of complex behaviours (12). Gut microbiota regulates host production of different molecules with known neuromodulatory properties, including endocannabinoids, neuropeptides and biogenic amines (13). Of these, the hormone and neurotransmitter serotonin (5-hydroxytryptamine (5-HT)) is expressed highly in the GI tract and regulated by gut microbiota, particularly the spore-forming bacteria dominated by families *Clostridiaceae* and *Turicibacteraceae* (14). Several bacterial taxa were found to be commonly disturbed in various neurodegenerative diseases, most of which are "anti-inflammatory" short-chain fatty acids (SCFA)-producing bacteria (15). In particular, *Firmicutes* (*Fecalibacterium*, *Anaerostipes*, and *Turicibacter*), *Bacteroidetes* (*Prevotella*, *Parabacteroides*), *Actinobacteria* (*Adlercreutzia* and *Collinsella*), *Lachnospiraceae* were found to be disturbed in the animal model of MS (9). Interestingly, besides changes in the gut microbiota composition, changes in metabolic pathways were observed as well, where SCFAs, major end-products of bacterial fermentation, decreased anxiety- and depressive-like behaviour in mice (16, 17). In addition, gut microbiota is an important regulator of γ-aminobutyric acid (GABA) and host tryptophan (TRP) metabolism along the kynurenine pathway, which both have implications for depressive disorder (18, 19). However, it is unclear how gut microbiota dysbiosis can trigger potential immunological changes in the

CNS in the presence of the blood–brain barrier (BBB), as well as how members of gut microbiota influence the MGBA. The gut microbiome can potentially influence these central processes through modulation of the immune system, production of neurotransmitters, through the regulation of gut barrier permeability, the increase of circulating lipopolysaccharide (LPS), alteration of neuroendocrine (hypothalamic-pituitary-adrenal [HPA] axis) and neural (e.g. vagus afferents, enteric nervous system) pathways (13, 20, 21). Decreased microbiota diversity seems to be one of the most consistent findings in gut microbiome dysbiosis, repeatedly associated with the modern lifestyle and autoimmune diseases, including gut microbiota from neurological patients (10). Recently, the gut microbiome has been shown to have a direct influence on the brain by modulating the immune system. Some evidence suggests that dysbiosis and increased gut permeability allow the translocation of bacteria or their metabolites from the lumen and induction or exacerbation of the immune response (e.g. production of pro-inflammatory cytokines tumor necrosis factor α [TNF-α], interleukin (IL)-6 and IL-1β). Peripheral inflammation, which may increase BBB permeability, is causatively implicated in the pathogenesis of neurological disorders (22). Furthermore, progress in MS treatment was achieved by interventional therapies on gut microbiota diversity and the metabolic traits of the microbiome, as well as by the use of probiotics in the treatment of experimental autoimmune encephalomyelitis (EAE), the animal model of MS (23–25). Currently, there is not enough evidence supporting the beneficial effects of gut microbiome manipulation in neurodegenerative and psychiatric diseases. Thus, further clinical and preclinical investigations are needed to specifically identify and counteract MGBA dysregulation.

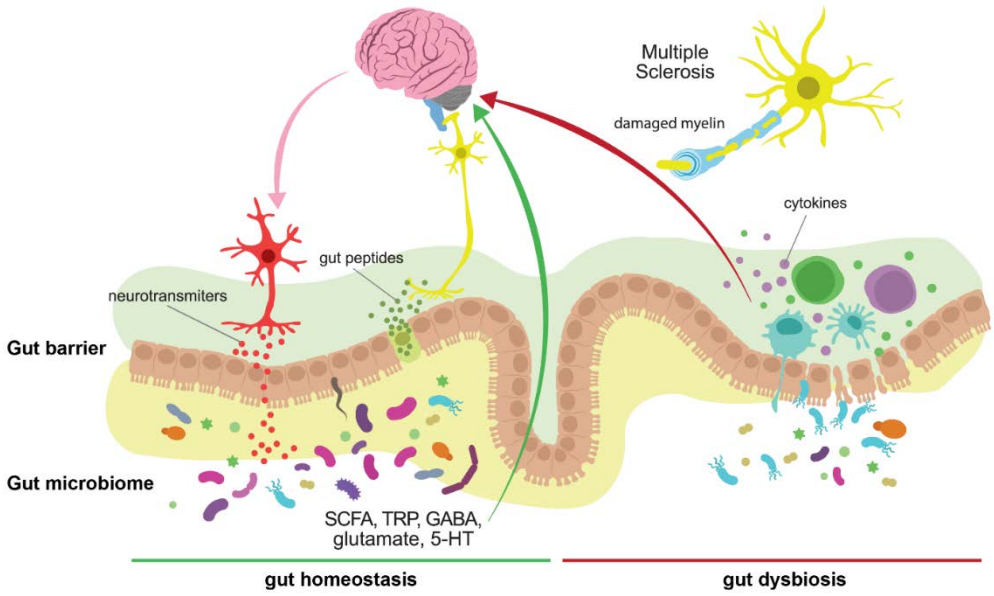

Figure 1. **The microbiome-gut-brain axis in the context of multiple sclerosis. SCFA, short chain fatty acid; TRP, tryptophan; 5-HT, 5-hydroxytryptamine.**

Slika 1. **Osovina crevo-mozak u kontekstu multiple skleroze. SCFA, masne kiseline kratkog lanca; TRP, triptofan; 5-HT, 5-hidroksitriptamin.**

The underlying pathology of multiple sclerosis

MS is a complex neurological disorder characterized by chronic autoimmune inflammation, demyelination, and neurodegeneration within the CNS (26). The exact etiology of MS remains elusive, but it is widely accepted that a combination of genetic predisposition, environmental factors, and dysregulated immune responses contributes to its development. It has been estimated that around 2.8 million people live with MS across the globe, with women being diagnosed more often than men (4). MS often occurs in early adulthood, between the ages of 20 and 40. MS can, however, arise in children and elderly individuals, though less frequently (27). Females are more likely than males to develop MS. The female-to-male ratio fluctuates but is considered to be around 3:1, indicating that the risk of developing MS is about three times higher for women compared to men (28). MS clinical manifestations might vary greatly from person to person. Fatigue is one of the most prevalent and debilitating symptoms of MS, and it can have a substantial impact on everyday living. Sensory symptoms include numbness, tingling, and burning sensations, which are most commonly felt in the limbs. Motor symptoms can include weakness, muscle spasms, and coordination issues. Optic neuritis, a common early sign of visual disruption in MS, may manifest as impaired vision, eye discomfort, or even temporary vision loss. Challenges in balance and coordination can restrict mobility and walking for individuals with MS. Cognitive irregularities, such as memory problems and reduced concentration, may arise in some cases, alongside mood fluctuations, depression, and anxiety, which are frequently observed in those affected by the condition (29).

MS comes in several subtypes, including the most common relapsing-remitting MS (RR-MS) and three other subtypes: secondary progressive MS (SP-MS), primary progressive MS (PP-MS), and progressive-relapsing MS (PR-MS) (30). RR-MS is characterized by instances of relapse or exacerbation, during which new symptoms or a worsening of existing ones occur, followed by periods of partial or total recovery (remission). Individuals frequently enjoy periods of stability in between relapses. Some people progress from RR-MS to SP-MS after a period of time. During the course of SP-MS, symptoms worsen gradually and steadily, with or without periodic relapses and remissions, and disabilities tend to accumulate more steadily. The PP-MS subtype is less prevalent than RR-MS, and it is distinguished by a progressive and constant escalation of disability from the outset of symptoms, with no discernible relapses and remissions. PR-MS is a less common subtype in which people have a continuous progression of disability from the start, but also have apparent relapses or exacerbations along the way (31). Our current understanding of MS has largely been shaped by research conducted on the experimental model for human inflammatory demyelinating diseases, such as EAE. EAE is a complex condition in which the interaction of several immunopathological and neuropathological pathways results in pathological hallmarks of MS: inflammation, demyelination, axonal loss, and gliosis (32).

Here we discuss recent findings about the MS underlying pathophysiology, with an accent on the role of the MGBA in this complex disabling neurological disease.

Role of genetic and environmental factors

An important factor in the onset and development of MS is the interaction between environmental stimuli and genetic predisposition (33). Research by Brynedal and colleagues (34) has confirmed the significance of genetic variations in MS development, particularly in the Human Leukocyte Antigen (HLA) region, which influence immune responses and increase susceptibility to MS. The influence of the HLA complex is not uniform. The HLA-DRB115*:01 variant from the class II repertoire appears as a powerful factor, with a strong association with increased MS risk. With an odds ratio (OR) of roughly 3, the role of HLA-DRB115*:01 in enhancing MS susceptibility is clear. On the other hand, the class I variant HLA-A*02 plays the opposite role, with an OR of around 0.6 associated with MS protection, which emphasizes its potential to protect against the disease (35). In addition to MHC molecules, various non-MHC genes and genetic variants have been linked to susceptibility or protection against MS. Among these are IL2RA (Interleukin-2 receptor alpha), CD58 (Lymphocyte function-associated antigen 3, LFA-3), CD226 (DNAX accessory molecule-1, DNAM-1), CYP27B1 (Cytochrome P450 27B1), TNFRSF1A (Tumor necrosis factor receptor superfamily member 1A), IRF8 (Interferon regulatory factor 8), IL7R (Interleukin-7 receptor), and TYK2 (Tyrosine kinase 2). These genes play distinct roles in immune system regulation, activation, and response. Immune function can be affected by genetic differences within these genes, thereby increasing the likelihood of MS development (36). These genetic factors, however, do not solely determine disease susceptibility; environmental factors such as vitamin D deficiency, infections, and smoking have also been implicated. An early study by Munger and colleagues (37) underscores the importance of vitamin D in regulating immune responses and its potential role in modulating MS risk. Later research on supplementation and sun exposure has validated the function of vitamin D in lowering the risk of MS (38, 39). Vitamin D impact extends beyond risk reduction, as higher levels correlate with diminished axonal damage, highlighting its broader neuroprotective potential (40). Additionally, the work of Santiago and colleagues (41) emphasizes the intricate link between Epstein-Barr virus (EBV) infection and MS risk, suggesting a potential role for viral persistence in triggering autoimmune responses. Individuals with MS have been linked to increased antibodies targeting EBV nuclear antigen 1 (EBNA1) a specific section (amino acids 385-420) (42). Nested case-control research revealed that nearly all EBNA1-negative people had progressed to EBNA1 antibody positive prior to the beginning of MS (43). The pivotal studies, undertaken in recent years, have significantly advanced our understanding of the link between obesity and MS risk. Large-scale cohort investigations have decisively established a robust correlation between obesity during adolescence and an elevated risk of MS in the future, particularly among females (33). The association between smoking and MS risk was first proposed in small studies, with an OR of 1.5, and was later confirmed in a large case-control study (33). Importantly, smoking and MS risk have a dose-response connection, with cumulative smoking exposure corresponding with increased susceptibility, and even passive smoking enhanced the risk of MS (44). More recently, gut microbiome dysbiosis has been

recognized as a key environmental factor leading to the development of MS, which will be discussed further below.

Immunological Dysregulation and Inflammatory Responses

MS is an autoimmune disorder driven by dysregulated immune responses against self-antigens within the CNS (45). MS involves an autoimmune response against myelin components in the CNS. Some of the myelin proteins implicated in MS include myelin basic protein (MBP), a major component of the myelin sheath and one of the primary targets of the immune response in MS, proteolipid protein (PLP), another key myelin protein that can be targeted by the immune system in MS, and myelin oligodendrocyte glycoprotein (MOG), found on the surface of myelin sheaths (46). Our understanding of the underlying immunopathophysiology of MS has evolved, revealing the central involvement of various immune cell types in both the PNS and CNS. This complex network of interactions between immune cells, such as peripheral T cells, B cells, and myeloid cells, as well as resident CNS cells like microglia and astrocytes, is crucial to the pathophysiology of the disease. These immune responses lead to the secretion of inflammatory mediators that recruit inflammatory cells to the CNS across damaged BBB, resulting in neuronal demyelination and CNS inflammation (47).

$CD4^+$ T cells, specifically T helper cells (Th)1 and Th17 cells, are key players in the MS development (48). The pro-inflammatory milieu in MS involves the production of cytokines such as TNF-α, IL-12, IL-6, IL-23, and IL-1, all of which influence Th1 and Th17 cell development (49). Th1 cells play a central role in the disease's progression, orchestrating a cascade of immune responses that contribute to the characteristic demyelination and neuroinflammation seen in MS (50). Th1 cells are characterized by their secretion of pro-inflammatory cytokines, most notably interferon-gamma (IFNγ). Within the context of MS, these cells are implicated in driving the immune response towards a pro-inflammatory profile. IFN-γ in particular has been linked to the activation of immune cells such as astrocytes and microglia in the CNS, which exacerbates the inflammatory environment (51). The aberrant activation of Th1 cells in MS is intricately linked to antigen presentation by antigen-presenting cells (APC)s, including B cells and myeloid cells such as macrophages and dendritic cells. These APCs present CNS-specific antigens to Th1 cells, fueling their activation and the subsequent immune response targeted at CNS tissue (52).

Th17 cells, which produce IL-17, and $CD8^+$ T cells are implicated in direct injury of astrocytes, oligodendrocytes, and neurons. These immune cells can also indirectly cause tissue damage by activating other immune cells, such as macrophages (53). Th17 cells collaborate with Th1 cells to induce a pro-inflammatory environment in the CNS. IL-17, best known for its function in extracellular bacterial and fungal defense, has a strong effect on astrocytes. By synergizing with other cytokines, Th17 cells amplify the secretion of proinflammatory cytokines (such as IL-6, Granulocyte Macrophage Colony-Stimulating Factor [GM-CSF], and TNF-α), chemokines, and effector proteins, contributing to immune pathology and neuroinflammation (48). This synergistic effect

enhances neuroinflammation and tissue damage, contributing to the clinical manifestations of MS.

$CD8^+$ T cells are notably more abundant in both white and grey matter demyelinating lesions and closely correlate with axonal damage. Their activation and response, as well as epitope spreading, play a significant role in MS pathogenesis (54). Immune cells in MS lesions lead to myelin loss, oligodendrocyte damage, and axon damage, all of which contribute to neurological disability. $CD8^+$ T cells carry cytolytic granules containing perforin and granzyme molecules that are polarized toward demyelinated axons and will release them to kill oligodendrocytes and neurons (48). When these lesions are inflamed, the body activates immune-modulating systems to suppress the immune response and initiate repair processes, which can result in partial remyelination and clinical improvement (55). However, in the relapsing form of the disease, despite these repair attempts, over 80% of patients experience disease progression (48).

A potential cause of aberrant effector T cell activation in MS is the inadequacy in the function of regulatory T (Treg) cells, coupled with the resistance of CNS-specific effector T cells to Treg cell-mediated regulation. Abnormalities in circulating Treg cells, including decreased expression of FOXP3, have been observed and are implicated in MS. These regulatory cells are crucial for maintaining immune homeostasis (56).

Building upon our understanding of MS pathogenesis, B cells have emerged as significant contributors, and therapies directed at B cells have displayed potential. Notably, pro-inflammatory B cells, particularly $CD27^+$ GM-CSF-expressing memory B cells, are more prevalent in the bloodstream of MS patients. These B cells play a crucial role in driving abnormal Th1 and Th17 cell responses by secreting cytokines such as TNF-α and IL-6, ultimately provoking pro-inflammatory responses in myeloid cells, predominantly through GM-CSF (57).

Neurodegeneration and Remyelination Impairment

While inflammation and demyelination are hallmark features of MS, the disease also encompasses neurodegenerative processes that contribute to irreversible neurological deficits (58). Lesions occur in both white matter and grey matter and are typically found throughout the CNS, including the brain, optic nerve, and spinal cord (26). In the early stages of MS such as Clinically Isolated Syndrome (CIS) and RR-MS, characterized by active demyelinating lesions, there are active areas in the brain with a lot of antigen-specific immune cells like $CD8^+$ T cells and $CD20^+$ B cells (59). These areas also have activated microglia, macrophages, containing myelin debris, and large, reactive astrocytes (60). However, as MS progresses to SP-MS and PP-MS, these active areas become less common. Instead, there are areas with fewer active cells and clear signs of damage, but they are not actively getting worse (61, 62). There are also other types of damaged areas, like chronic active plaques, which are more common in people with longer-lasting MS. These have a different pattern of cell activity. Slow expanding lesions are also seen in people with SP-MS, and they show very slow damage to the brain's

protective covering, with fewer cells involved, but still causing damage over time (26). Gray matter damage in MS patients begins early in the disease and can be more extensive in those with PP-MS and SP-MS, involving more than 60% of the cortex of the brain in severe cases (63). Grey matter lesions can also appear in deep brain structures and the grey matter of the spinal cord, where they are more widespread than in the white matter (64). These grey matter lesions often form in the cortical sulci and in deep invaginations of the brain and are linked to inflammation in the brain's meninges (65). Interestingly, these grey matter lesions are different from the more common white matter lesions seen in MS, tend to have less disruption of the BBB, less swelling, and fewer infiltrating activated microglia and macrophages (66). Additionally, they can lead to the loss of nerve connections, brain cells, and synapses (67). Remyelination, a critical repair mechanism, is impaired in MS due to factors such as the presence of inhibitory molecules and insufficient oligodendrocyte precursor cell recruitment (68). Understanding these complex issues is essential for creating ways to enhance remyelination and slow disease progression.

Intestinal microbiota biomarkers of multiple sclerosis

Although genetic predisposition is important and may play a significant role in the MS onset, growing evidence suggests that interactions between gut microbiome and immune system are crucial for the development of MS (7, 69).

Among microbiome members, researchers are mostly focused on the gut bacteria, with little attention given to the contribution of fungi, parasites or viruses to MS development and severity. Although fungal components make up a smaller proportion of the gut microbiome, fungi have a significant impact on human health (70). Probably the first report on this subject was made by Truss in 1981, where he reported amelioration of symptoms in several MS patients following nystatin treatments (71). A recent case-control observational study showed that people with MS have higher fungal alpha diversity and increased relative abundances of *Saccharomyces* and *Aspergillus* genera when compared to healthy subjects (72). Another study reported the presence of antibodies against *Candida* in the cerebrospinal fluid of MS patients (73). Interestingly, one study reported increased fungal to bacterial ratio in RR-MS patients (74). Further research is also needed in order to better understand the role of gut mycobiome in MS, which should not be underestimated.

The role of viruses in MS development is proposed based on the findings of viral genetic material and antiviral antibodies in the cerebrospinal fluid and blood of patients with MS (75). Besides EBV, human herpesvirus 6 (HHV-6) has been linked to MS and is notably more prevalent within MS plaques when compared to EBV in both MS and non-MS brain white matter. Intriguingly, reactivation of HHV-6 has been observed during clinical relapses in MS (76).

In addition to microbiome, emerging evidence suggests that members of microbiome such as certain parasites, particularly helminths, and protozoa, may confer protective effects in the context of MS. Multiple studies have lent support to the notion

that parasitic infections, such as those caused by *Toxoplasma gondii* and *Schistosoma mansoni*, demonstrate a protective effects in humans (77) and in the C57BL/6J mice model of MS (78). Notably, a study involving *Trichinella spiralis* reveals that infection with L1 stage muscle larvae (TSL1) is associated with a reduction in CNS inflammation in EAE induced Dark Agouti rats (79). Consequently, parasites are recognized as potential risk-reduction factors in the development of MS.

However, a number of studies have provided evidence that alternations in bacteriobiota, the bacterial component of the gut microbiome, are associated with MS development and severity. An early study by Goverman and colleagues found that transgenic mice expressing MBP-specific T cell receptors (MBP-TCR) develop MBP-Complete Freund's adjuvant (CFA) induced EAE when housed in non-sterile conditions, while they remained healthy under specific pathogen-free (SPF) conditions (80). Another study demonstrated that C57BL/6 mice maintained under germ free (GF) conditions after immunization with MOG/CFA exhibited attenuated symptoms of EAE, and that colonization with segmented filamentous bacteria (SFB) promoted EAE development (81). In contrast to this study, Berer and colleagues demonstrated that monocolonization with SFB of GF SJL/J mice expressing MOG-TCR was not effective in the promotion of EAE, and colonization with conventional commensal microbiota prompted EAE development (82). In addition, these authors showed that without induction, EAE spontaneously occurred in SPF-bred animals, while GF-bred animals remained EAE-resistant (82). Importantly, transplantation of an MS patient’s gut microbiota into EAE-induced GF mice resulted in increased EAE activity and severity compared to mice colonized with healthy donors’ gut microbiota (83).

Several studies have revealed connections between antibiotic-induced microbial reduction and EAE development (84, 85). Early studies showed that a short-term oral antibiotics treatment one week prior to immunization leads to gut microbiota alternations associated with a decreased Th17 level in mesenteric lymph nodes and EAE amelioration (84), as well as proinflammatory cytokines depletion, anti-inflammatory cytokines increase, and Treg cell-dependent reduction of disease severity in a $PLP_{139–151}$/$MOG_{35–55}$ induced EAE model (85). Interestingly, our previous results suggested that antibiotics exposure during the prenatal and neonatal period of EAE-susceptible Dark Agouti (DA) rats has long term effects, reflected in increased disease severity after immunization later in their lives, even though gut microbiota was restored (9). Today we know that gut microbiome can affect the host’s immune system, BBB integrity and function, and autoimmune demyelination (86).

Over the years, researchers have uncovered certain bacterial taxa that are associated with both MS and EAE (Figure 2). Several studies reported a significantly increased relative abundance of *Akkermansia* genus in MS patients (83, 87, 88). Considering that commensal species *Akkermansia muciniphila* is involved in mucin turnover in the gut and production of acetate and propionate, one study suggests that its increased abundance in MS is probably a consequence of the disease (89). This is something to keep in mind when studying host-microbiota interactions, because modulation in the gut microbiome

can be either a cause or a consequence of disease. Another species with increased relative abundance in MS is *Acinetobacter calcoaceticus,* known for its role in molecular mimicry of MBP and MOG and reduction of Treg proportion in peripheral blood mononuclear cells (PBMC) *in vitro* (83, 90). Moreover, relative abundances of two genera from family *Lachnospiraceae*, *Blautia*, and *Dorea*, are found to be increased in faecal samples of MS patients (91). A study by Schepici and colleagues showed an increased abundance of the genus *Streptococcus* in MS patients, which is in line with results by other authors (92, 93). Our previous results show increased prevalence of the genus *Romboutsia* and family *Peptococcaceae* in EAE-induced DA rats (94). Besides commonly MS-associated bacteria, several studies have reported an increased abundance of the archeal genus *Methanobrevibacter* in MS patients (88).

On the other hand, the relative abundance of several genera such as *Prevotella*, *Bacteroides*, *Parabacteroides*, *Collinsella*, *Adlercreutzia*, *Lactobacillus*, *Clostridium*, *Anaerostipes*, *Butyricicoccus*, and *Faecalibacterium* decreased in MS patients compared to healthy controls (88, 91, 92). It is interesting that, among these MS negatively associated bacteria, the *Prevotella*, *Parabacteroides*, *Lactobacillus* and *Butyricicoccus* genera are well-known SCFA producers (95–97), while *Faecalibacterium prausnitzii* species are reported to be the main butyrate producers in the gut (98).

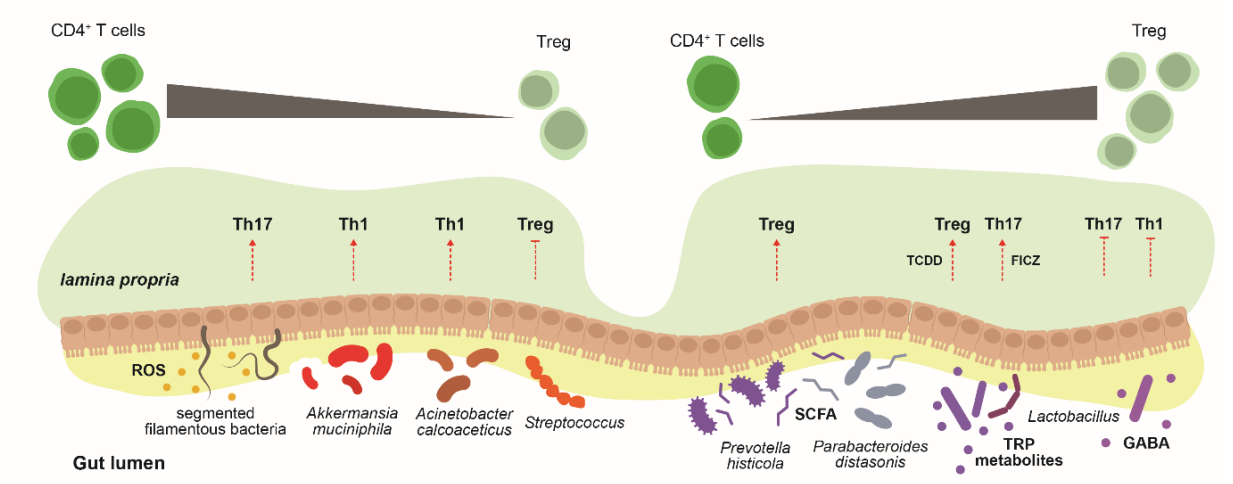

Figure 2. Gut microbiota and its metabolites in multiple sclerosis. ROS, reactive oxygen species; Th, T helper cell; Treg, regulatory T cell; SCFA, short chain fatty acid; TRP, tryptophan; GABA, γ-aminobutyric acid; TCDD - 2,3,7,8-tetrachlorodibenzo-p-dioxin; FITZ - 6-formylindolo(3,2-b)carbazole.

Slika 2. Mikrobiota creva i njeni metaboliti kod multiple skleroze. ROS, reaktivne kiseonične vrste; Th, pomoćničke T ćelije; Treg, regulatorne T ćelije; SCFA, masne kiseline kratkog lanca; TRP, triptofan; GABA, γ-aminobuterna kiselina; TCDD - 2,3,7,8-tetrahlorodibenzo-p-dioksin; FITZ - 6-formilindolo(3,2-b)karbazol.

Gut microbiota-related metabolites in multiple sclerosis

Short-chain fatty acids play a major role in multiple sclerosis

SCFAs are small organic molecules with important physiological properties in the context of gut microbiome-host interactions. They are the products of fermentation of complex indigestible polysaccharides facilitated by certain bacterial species in the host colon. The most abundant SCFAs in the MGBA are acetic acid, propionic acid, and butyric acid (99). A myriad of properties have been ascribed to these structurally simple molecules, from being important energy sources for the colon epithelium to exhibiting potent immunomodulatory effects, in addition to strengthening the intestinal barrier (100). Besides gut barrier protection, SCFAs can cross the BBB as signal molecules, regulate its permeability and modulate GBA (101). Several studies reported a reduction of SCFAs level in fecal samples of RR-MS patients (102, 103). Acetate, propionate and butyrate levels in particular were observed to be significantly lower or even depleted in MS patients (102). Relative fecal SCFA levels in patients have also been shown to correlate with the severity of disease, expressed in terms of the level of MS-induced disability, resulting in lower relative abundance of butyric and caproic acids and higher relative abundance of acetic acid in patients with higher Expanded Disability Status Scale scores (EDSS). Reduced SCFA levels have also been observed in sera of these patients (104). Among gut microbiome members, it is known that different bacterial taxa are involved in SCFAs production from dietary fibers, such as genera *Prevotella* (105), *Butyricimonas* (106), *Bifidobacterium, Veillonella* and species *Faecalibacterium prausnitzii*, *Eubacterium hallii* and *Phascolarctobacterium succinatutens* (107). For example, *Prevotella* deficiencies are claimed to be unique features of the gut microbiota of MS patients, and a direct correlation has been observed between the abundance of the *Prevotella* genus in the gut and the levels of fecal acetate and propionate (88, 91, 102). Many studies associated fecal and serum levels of SCFAs with MS progression and immune cell differentiation in MS patients (102, 104, 108). Thus, significant negative correlations between the *Streptococcus* and *Prevotella* genera abundance and peripheral Treg (pTreg) and Th17 cells have been revealed respectively (Figure 2). Finally, total SCFA levels have been shown to positively correlate with the proportion and function of pTreg cells (102, 104).

The effects of SCFA supplementation as a therapeutic approach for treating MS have been studied. Propionic acid supplementation in particular was shown to have immunomodulatory and neuroprotective effects (104). These disease-ameliorating properties of propionic acid, when taken as an oral supplement, stem from the enhanced IL-10 mediated suppressive function of Treg cells. It additionally led to a decreased proportion of Th1 and Th17 cells, and an increased presence of pTreg cells. A decrease in relapse rates and a stabilization of disability were also observed in long-term supplementation. Keeping in mind the fact that propionic acid is mainly produced by gut bacteria, these results suggest a significant impact of the gut microbiome on the pathophysiology of MS.

Similar results have been achieved with oral supplementation of *Prevotella histicola*, a known producer of propionic acid among other SCFAs. A recent study has shown that the administration of this bacterium can lead to the suppression of EAE in mice, as evidenced by a decrease in Th1/Th17 cells, an increase in Treg cells, tolerogenic dendritic cells and suppressive macrophages, and reduced demyelination in the CNS (109).

Protective role of intestinal γ-aminobutyric acid in multiple sclerosis

GABA is an inhibitory mediator of the CNS and its levels are decreased in MS patients (110, 111). Certain bacteria in the gut are capable of producing GABA, granting the gut microbiome a particularly potent role in regulating both the CNS and PNS (112). *Lactobacillus* species have emerged as especially relevant gut microbiome constituents when it comes to the GABA-mediated regulation of the host's nervous system, with the most important GABA producer in the gut being *Lactobacillus brevis* (113). Our previous results showed that oral administration of *L. brevis* BGZLS10-17 alleviates EAE symptoms in DA rats (114). Finally, the neuroprotective properties of GABA are assumed to stem from its immunosuppressive potential, as suppression of APCs by GABA through decreased MAPK signaling leads to a dampening of the inflammatory immune response to myelin antigens (115).

Role of tryptophan derivatives and bile acids in multiple sclerosis

L-tryptophan is an essential amino acid found in foods like meat and legumes, and it is subject to biotransformation in the gut, both by the host and the gut microbiota (116). TRP and end products of its transformation are transported out of the gut into circulation. TRP metabolism is a complex network of metabolic pathways, but it can be roughly grouped into three branches: the kynurenine pathway, the serotonin pathway and the indoles pathway (117). Most of the production of the metabolites of the first two pathways is attributed to the host, while gut bacteria produce most of the indole and indole derivatives. Many of these metabolites can act as aryl hydrocarbon receptor (AhR) ligands, granting them immuno- and neuroprotective properties.

MS has been associated with altered levels of TRP metabolites (118). Quinolinic acid (QA) and kynurenic acid (KA) have both been shown to be elevated in MS, with QA exhibiting neurodegenerative and neuroinflammatory effects, and KA potentially playing a neuroprotective role (119). Both metabolites have been detected in increased levels in MS patients, but with QA being increased significantly more than KA, leading to accumulation of damage to the nervous system.

TRP metabolites shed light on the importance of gut microbiota. Higher levels of TRP byproducts containing indole, primarily generated by gut bacteria, have been linked to milder disease symptoms. Additionally, having more genes related to TRP breakdown in the gut microbiota is connected to a reduced risk of experiencing disease relapses (118). Certain indole derivatives, like indole-3-propionic acid (IPA), have been shown to improve intestinal epithelial barrier integrity by promoting tight junction formation (120).

Secondary bile acids are another type of important bacterially derived metabolites that have been linked to MS. Decreased serum levels of certain secondary bile acids have been reported both in mouse models of EAE and in MS patients (121, 122). A decrease in the abundance of *Clostridium* cluster XIVa species in MS patients accompanies this change in secondary bile acids levels (123). MS-specific neuroprotection mediated by secondary bile acids and/or their producers in the gut is an ongoing topic of research, and the link mostly extends to statistical correlations.

Conclusion

Considering further directions in the gut microbiome-MS association research, it is important to note that the differences in levels of bacterially derived metabolites and microbiota composition between MS patients and healthy controls are not universal across different geographic locations and further depend on factors such as diet, body-mass index, sex, age and ethnicity (88, 91, 103). Efforts are underway to accumulate data with these important considerations in mind, in order to come to more relevant conclusions, which will help determine the true role of bacterially derived metabolites in MS pathophysiology. Importantly, experimental models should be developed to investigate the potential causative relationship between metabolites levels and gut microbiota composition and MS pathogenesis. Finally, an emerging group of probiotics commonly referred to as neurobiotics, which could be used in treatment of neurodegenerative disorders, might revolutionize the treatment of specific psychiatric and neurodegenerative disorders.

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Mikrobiom-crevo-mozak osovina kod multiple skleroze

Dušan Radojević, Svetlana Soković Bajić, Miroslav Dinić, Aleksandar Bisenić, Jelena Đokić, Nataša Golić*

Grupa za interakcije probiotika i mikrobiote sa domaćinom, Laboratorija za molekularnu mikrobiologiju, Institut za molekularnu genetiku i genetičko inženjerstvo, Vojvode Stepe 444a, 11042 Beograd 152, Srbija

*Autor za korespondenciju: Nataša Golić, e-mail: natasag@imgge.bg.ac.rs

Kratak sadržaj

Mikrobiom-crevo-mozak osovina (MGBA) predstavlja blisku dvosmernu vezu između creva i centralnog nervnog sistema (CNS) posredovanu imunskim sistemom, enteričnim nervnim sistemom (ENS), nervom vagusom i mikrobiomom creva. Posredstvom metabolita koje produkuju, mikroorganizmi creva, uključujući bakterije, gljive i viruse, komuniciraju sa CNS-om i tako utiču na funkcije mozga, zbog čega je mikrobiota creva prepoznata kao veoma važan faktor održavanja homeostaze MGBA. Takođe, veliki broj podataka ukazao je na povezanost disbioze mikrobioma creva i nastanka i težine simptoma različitih neurodegenerativnih i psihijatrijskih bolesti, uključujući multiplu sklerozu (MS), autoimunsku bolest nervnog sistema. MS je hronična bolest CNS-a povezana sa više genetskih faktora, kao i sa različitim sredinskim faktorima i životnim navikama. Najvažnija obeležja MS su neuroinflamacija i demijelinizacija u mozgu i kičmenoj moždini, a veliki broj istraživanja je ukazao i na specifične mikrobijalne markere ove bolesti. Cilj ovog rada je da pruži pregled najvažnijih podataka o povezanosti promena u sastavu i funkciji mikrobiote creva i patoloških promena karakterističnih za MS.

Ključne reči: mikrobiom creva, multipla skleroza, crevo-mozak osovina, metaboliti bakterija, disbioza