REVIEW ARTICLE

The gut microbiota and associated metabolites in multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a severe central nervous system autoimmune inflammatory disease featured by the presence of infiltrated immune cells, demyelination, and degeneration. Recent research has shown that gut microbiota, including some commensal bacteria, is capable of interacting with the host immune system and remarkably influencing the development and outcome of experimental autoimmune encephalomyelitis, a classic animal model of MS. In addition, gut dysbiosis, presented with a significantly altered composition of commensal bacteria, is linked to the immune response and inflammation, such as Th17 activation and B cell function. Moreover, it has been observed that microbiota impacts the immune system by regulating the metabolites in the gut. In this review, we summarize the new research on the relationship and mechanism between the gut microbiota and MS, as well as the implications for developing new strategies in MS by modulating the gut microbiota and metabolites.

Keywords: Gut; Microbiota; Metabolites; Multiple sclerosis; Inflammation

1. Introduction

Multiple sclerosis (MS) is an inflammatory and degenerative disorder of the central nervous system (CNS) with features of demyelination, neuronal loss, permanent axonal damage, and progressive neurological dysfunction. About 2.5 million people are diagnosed with MS, and it is more common in young women than in men[¹³]. Currently, the mechanism of MS is only partially known and still requires further investigation. The pathophysiology of MS is thought to be driven by an autoimmune reaction[⁴]. A key factor in the development of MS is abnormal immune cell activation. The blood-brain barrier (BBB) can be breached by immune cells, cytokines, and inflammatory mediators, allowing for neurological injury. Activation of myelin-specific CD4⁺ T cells, in particular, is crucial in the pathogenesis and is shown in many animal models, including the experimental autoimmune encephalomyelitis (EAE) model. Targeting CNS self-antigens, Th1 and Th17 cells are thought to have a role in the etiology of MS. Interferon (IFN)-γ, which can act on microglia and trigger M1-type polarization, is mostly secreted by Th1 cells, whereas interleukin-17A (IL-17A) and IL-21 are primarily produced by Th17 cells. This may cause the astroglia to release IL-1 and IL-6, which amplify the inflammatory response associated with EAE[⁵⁶].
Intestinal microflora has recently emerged as an important environmental component that may provide important clues to the progression of inflammation and the development of MS. An abnormal intestinal flora was found to be differentially abundant among MS patients compared with healthy controls (HCs), though with little consistency in the bacterial taxa[7-10]. In MS patients, the composition of the intestinal flora is aberrant, including a rise in potentially pathogenic microbes and a reduction in the number of helpful bacteria, according to metagenomic research[11-13]. Another study on how the intestinal microbiota affects people with MS was done by Kadowaki et al. They discovered that the connections between the T cell C-C chemokine receptor type 9 (CCR9) and its ligand C-C chemokine ligand 25 (CCL25) were affected by the gut microbiota. The small intestine epithelium is involved in this interaction, which affects T cell growth and immunity. CCR9 function was shown to be diminished in MS patients. CD4+ T cells increased the expression of CCR9 on T cells. The number of CCR9+ memory T (Tm) cells in peripheral blood was reduced as a result of blocking the CCR9-CCL25 interaction. To ascertain if the gut microbiota has an impact on CCR9+ Tm cells, CD4+ Tm cells from peripheral blood of germ-free (GF) mice conditions have been examined. In GF mice, the number of CCR9+ Tm cells decreased. The researchers also gave wild-type mice short-term antibiotic therapy after inducing EAE in them. Treatment with antibiotics increased the number of CCR9+ Tm cells and significantly reduced the severity of EAE[14]. This finding supports the possibility that gut dysbiosis, through affecting the gut-systemic immunological axis, contributes to the genesis of MS. Furthermore, immunoglobulin A (IgA) protects the mucosal epithelium from invading pathogens, toxins, and food-derived antigens but also regulates gut microbial composition[15]. The effectiveness of anti-B treatments and novel genetic research has highlighted the significance that B cells regulate neuroinflammation in MS[16,17]. Plasma cells of secretory IgA (IgA+ PC)’s gut origins have been shown in the EAE mouse model, and they decreased inflammation of the nervous system under an IL-10-dependent way, underscoring the growing significance of mucosal immune deficiency in MS. IgA+ PC gut-derived intraepithelial lymphocytes suppressed neuroinflammation, prevented EAE, and decreased the number of CD4+ T cells that cause granulocyte-macrophage colony-stimulating factor as they moved from the gut to the periphery and subsequently to the inflamed CNS, which patients with MS have seen a comparable decrease in IgA-bound fecal bacteria following relapse[18]. In all, emerging evidence established a critical link between the gut and MS.

2. The gut microbes in the pathogenesis and progression of MS

2.1. Preclinical evidence from the EAE model

Segmented filamentous bacteria (SFB) are important communicators for the differentiation of Th17 cells[19]. It was demonstrated that actively generated EAE is reduced in GF mice yet is recovered through SFB gut colonization[20]. In addition, Berer et al. showed that EAE-resistant GF animals spontaneously acquire EAE without any treatment with adjuvant or pertussis toxin following recolonization with native microbes or a variety of SFB utilizing a T-cell receptor (TCR) transgenic mouse model of EAE[21]. Notably, the majority of SFB-induced Th17 cells respond to SFB antigens, emphasizing the importance of microbial antigens during the activation of effector T cells within the gut[22]. In fact, after SFB colonization, SFB-specific CD4+ T cells of mesenteric lymph nodes (MLNs) are prepared to develop into Th17 cells[23]. Through processes of bystander activation or cross-reactivity between SFB and as of yet uncharacterized CNS antigens, SFB may stimulate T lymphocytes with CNS reactivity. SFB-specific Th17 cells may either recirculate to the CNS after migrating to intestinal mucosa following priming in MLNs or might straight enter the CNS through the systemic circulation. Intriguingly, it has recently been discovered that gut nociceptor neurons, which are often linked to protective reflexes, regulate SFB levels. This suggests that their activity might obliquely govern the development of CNS-reactive Th17 cells[24].

It is worth noting that the impacts of MS on the composition and variety of gut microbes differed in animal models. Omura et al. created an MS model using Thélier’s murine encephalomyelitis virus (TMEV)-infected SJL/J mice and collected feces from TMEV mice on day 4 (pre-onset phase), day 7 (acute phase), and day 35 (chronic phase). According to RNA sequencing, the abundance of individual bacteria genera Marvinbryantia increased on days 7 and 35, while Coprococcus increased on day 35. However, neither the microbial biodiversity nor the overall microbiota pattern was altered[25]. Moreover, after the EAE induction, the gut microbiota composition of EAE-resistant Albino Oxford rats was more stable, whereas the gut bacterial diversity of EAE-susceptible Dark Agouti rats was higher[26]. Constipation-induced intestinal dysbiosis worsens EAE symptoms in C57BL/6 mice while also reducing the abundance and diversity of the intestinal microbiota[27]. These contradictory results hint indirectly at the potential influence of mice species on gut microbes.
2.2. Clinical evidence from MS patients

2.2.1. Akkermansia

A mucus-degrader called *Akkermansia* converts mucin to short-chain fatty acids (SCFAs) which could influence the effects on the immune system[18]. It has been found to have both regulatory and inflammatory activities[19]. Alternately, pro-inflammatory pathways, such as the actuation of complement and coagulation cascades as well as the overexpression of genes related to antigen-presentation, B cell, and TCR signaling, and pro-inflammatory pathways, have been linked to *Akkermansia*[20]. Its capacity to break down mucus, which results in the collapse of the intestinal epithelium barrier as well as a greater baring of local immune cells to microbial antigens, may be the cause of these inflammation-promoting characteristics[20].

Gene sequencing of the gut microbes in stool samples from patients with MS showed *Acinetobacter calcoaceticus* and *Akkermansia muciniphila* had much higher levels whereas *Parabacteroides distasonis* had significantly lower levels. *A. muciniphila* promoted Th1 cell differentiation, causing pro-inflammatory responses in mononuclear cells of MS patients. When MS patient microbiota was given to GF mice, the animals had more severe EAE symptoms and fewer regulatory T cells (Tregs)[21]. In untreated MS twins, *Akkermansia* species were also found to be increased[22]. *Faecalibacterium* levels were found to be lower in MS patients. They also looked into variations in bacterial makeup between patients who received glatiramer acetate treatment and those who did not (*Bacteroidaceae*, *Faecalibacterium*, *Ruminococcus*, *Lactobacillaceae*, *Clostridium*, and other Clostridiales). Patients with MS, who were not given any treatment, showed a rise among the species *Akkermansia*, *Faecalibacterium*, and *Coproccocus* following vitamin D administration in comparison to the other groups[23]. Although the detailed mechanism of vitamin D is still uncertain, some studies have reported that it binds to the vitamin D receptor and downregulates NLRP3/Caspase-1/GSDMD pyroptosis pathway, which is also activated in gut epithelial cells and associated with gut inflammation[24-26]. A change in intestinal microbes from MS patients was observed by Jangi et al. Rise in *Methanobrevibacter* and *Akkermansia* with lessening in *Butyricimonas* are among the microbiome changes associated with MS, which are also connected to changes in the activation of genes related to dendritic cell (DC) maturation, IFN signaling, as well as Nuclear factor kappa B signaling pathways among circulating T cells and monocytes. When compared to those who are not receiving treatment, patients receiving disease-modified treatment had higher abundances of *Prevotella* and *Sutterella* and lower abundances of *Sarcina*[27]. EAE was alleviated by *Akkermansia* cultured from MS patients, and this improvement was associated with a decrease in γδ+ and IL-17-producing T cells[28].

2.2.2. Clostridia

There were important differences in the species abundance of 21 species noted in the gut of roughly 20 Japanese MS patients. Of the 21 species, a reduction in 19 species was noticeable in MS samples, and 14 of them belonged to Clostridia clusters XIVa and IV. It has been identified that several organisms, including *Parabacteroides* and *Prevotella* (Bacteroidetes), *Adlercreutzia* and *Collinsella* (Actinobacteria), and *Erysipelotrichaceae* (Firmicutes), were decreased in relapsing-remitting MS (RRMS) as compared to HCs[29]. *Prevotella, Parabacteroides*, and *Adlercreutzia* are linked to the metabolism of phytoestrogens as well as the plant-derived xenoestrogen, whereas *Parabacteroides* and *Erysipelotrichaceae* are involved in bile acid metabolism, which also plays a critical role in Th17 inflammation and MS.[30-32]. In addition, there was a study that analyzed the gut microbes of MS patients who had yet to receive therapy in the early stages of the disease and compared them among Caucasians, Hispanics, and African Americans. Early-stage MS patients from all three ethnic groups had an elevated relative abundance of Clostridia, indicating a connection between the etiology of MS and Clostridia. Two studies identified variations in specific *Clostridium* operational taxonomic units between treated and untreated MS people, while no appreciable alterations between all MS patients and controls, raising the possibility that these drugs’ antibacterial capabilities might change the microbiome[32,33]. A strain that proportionally increased in MS, *Collinsella*, has currently been found to be related to the changes in intestinal permeability in MS patients as well as the rise of the pro-inflammatory cytokine IL-17A[34,35].

2.2.3. Prevotella

Cosorich et al. investigated the potential relationship with changes in the intestinal microbiota of MS patients. They examined the microbes that were separated from small intestinal tissues and noticed that in comparison to HCs and MS patients without clinical symptoms, those with increased disease activity and a rise in the number of intestinal Th17 cells had an elevated Firmicutes/Bacteroidetes ratio, a larger relative abundance of *Streptococcus*, and fewer *Prevotella* strains. It showed that the relative frequency of *Prevotella* strains in the human small intestine is negatively correlated with the frequency of gut Th17 cells. It demonstrates that abnormal Th17 cell growth in the human gut and certain microbiome changes are linked to cerebral autoimmunity[10]. Mangalam et al. also report that *Prevotella histicola*
can restrain EAE symptoms in HLA class-II transgenic model. *P. histicola* restraints disease by the alteration of systemic immune responses, leading to a decrease in pro-inflammatory Th1 and Th17 cells as well as a rise in the frequencies of CD4+FoxP3+ regulatory T cells, tolerogenic DC, and suppressive macrophage. These findings point to functional relationships between variations in certain gut bacteria and a change toward a pro-inflammatory T-cell profile that may amplify or sustain autoimmune responses, perhaps discovering a previously unidentified environmental factor to MS pathogenesis.

### 2.2.4. Other evidence

Although immunological markers (such as Th2, Th17, and Treg) did not change between cases and controls in research of 24 kids (15 in relapsing remission and 9 in controls), gut microbiota associations did. Species richness and Th17 showed a positive correlation for relapsing remission patients. Bacteroidetes had a negative correlation with Th17 in relapsing remission patients, whereas *Fusobacteria* had a positive correlation with Tregs in control patients. Another study that included 18 pediatric RRMS cases and 17 controls discovered that, in comparison to controls, MS cases had a significant depletion in *Lachnospiraceae* and *Ruminococcaceae* as well as enrichment in members of the *Desulfovibrionaceae* (Bilophila, *Desulfovibrio*, and *Christensenellaceae*). Microbial genes with expression higher in MS than in control are involved in glutathione metabolism, and this result remains the same regardless of the administration of immunomodulatory drugs. Additional studies have shown that a lower abundance of butyrate-producing microbes, such as *Butyricicoccus desmolans* and *Odoribacter*, is linked to a higher risk of MS disease recurrence among children.

### 3. Microbial metabolites in the pathogenesis of MS

#### 3.1. SCFAs

With chain lengths ranging from one to six carbon atoms, SCFAs are the primary component of dietary fiber fermentation in the colon. Butyrate, acetate, and propionate are the most common. SCFA helps to keep the intestinal barrier intact, and butyrate, in particular, improves intestinal barrier function by regulating the expression of tight junction proteins. Intracellular butyrate, propionate, and acetate inhibit histone deacetylases (HDACs) activity and promote histone hyperacetylation; SCFAs appear to inhibit monocyte, macrophage, and DC maturation by suppressing HDACs, reduce pro-inflammatory cytokine production, and promote T cell differentiation into Th1 cells, Th17 cells, IL-10+ T cells, as well as Treg cells. Among them, butyrate increases cellular metabolism and improves the memory capacity of activated CD8+ T cells. It is worth noting that SCFAs can move across the BBB and there are functional SCFA receptors in the CNS. It is also notable that some SCFA-producing bacteria, including *A. muciniphila*, *Roseburia inulinivorans*, *Butyricimonas*, and *Faecalibacterium praunitzii*, have been characterized.

Acetate, propionate, and butyrate concentrations in feces and blood samples are lower in MS patients than in HCs, possibly pointing to a protective role for SCFAs in MS. Notably, compared to healthy people, secondary progressive MS patients had lower blood levels of acetate, propionate, and butyrate. The feces sample of RRMS patients likewise revealed identical findings, indicating that these changes happen independently of the disease types. Decreased relative abundance of recognized SCFA-producers among the MS microbes, such as *Roseburia*, *Caproococcus*, *Blautia*, *Faecalibacterium*, *Dorea*, *Butyricicoccus*, and *Clostridium XIVb*, is correlated with decreases in fecal SCFAs.

SCFAs have implications for MS disease. Serum caproic acid (CA) concentrations increased in MS patients as butyrate and acetate concentrations decreased. CA was also found to be positively associated with CD4+IFN-γ+ T cells. Similarly, another study discovered that acetate concentration decreased in MS patients, which was associated with a negative relationship with the pro-inflammatory biomarker IFN. In addition, it decreased the count of effector T cells in the intestine and increased the release of IL-10 by regulatory B cells, which improved EAE.

Current studies on propionic acid confirm that it can improve the severity of MS. With propionate supplementation, the number of peripheral Tregs and their ability to inhibit MS symptoms *ex vivo* and *in vitro* were found to increase with propionic acid in a way that was IL-10-dependent. Furthermore, Tregs treated with propionate were transferred to EAE mice to lessen the severity of the illness. Last but not least, propionate also raises the quantity of Treg in the spleen and spinal cord. According to these findings, propionate reduces CNS autoimmunity by raising the number of Treg cells throughout the body, including the CNS, where they are likely to suppress continuing inflammation.

Notably, Th17 cells treated with acetate exacerbated the EAE progression. As a result, this might tip the immune response in favor of a Th17 response. Surprisingly, elevated levels of acetate were found in the plasma of MS patients and were connected to higher numbers of CD8+ IL-17+ T cells and greater neurological impairment. It was
also discovered that MS patients have a higher proportion of butyrate and propionate levels that are compared to HCs and that both butyrate and valerate are positively correlated with pro-inflammatory cytokines\(^59\). These findings may suggest a labyrinthine function of SCFAs in the regulation of CNS autoimmune inflammation.

### 3.2. Tryptophan

Tryptophan is a naturally occurring monoamine alkaloid with a function as an agonist of the aromatic hydrocarbon receptor and is also produced by gut microbiota metabolism. The metabolic products of tryptophan include indole-3-lactic acid (ILA), indole-3-acetic acid (IAA), and indole-3-carboxaldehyde (IAld). Furthermore, other metabolic products (kynurenine, kynurenic acid, and xanthurenic acid) act as ligands for the aryl hydrocarbon receptor (AhR) and can have immune- and neuro-modulatory impacts.

When compared to HCs, serum tryptophan concentrations were lower and kynurenine levels were higher in MS patients, indicating that tryptophan metabolism may be disturbed in this condition\(^60\). Dietary tryptophan shortage exacerbated the clinical course of EAE in mice. When tryptophan was added back into the diet, it alleviated the condition in wild-type mice but not in AhR\(^{-}\) mice\(^61\). The analysis of tryptophan derivatives now includes AhR ligands as a result of recent investigations. Elevated blood indole-3-propionic acid (IPA) and IAA concentrations in children with MS are linked to higher rates of cognitive processing and less severe illness\(^62\). In addition, a lower risk of recurrence was linked to the fecal microbiota's enrichment of tryptophan catabolism-related genes. This is supported by the finding that AhR ligand levels in the blood are lower in RRMS patients compared to HCs, exception for patients with benign disease (long-standing diagnosis, but mild clinical symptoms) and those who are actively relapsing, where they might be upregulated in an anti-inflammatory feedback loop\(^63\).

Laquinimod, a synthetic indole-containing substance, reduced EAE by activating AhR-dependent signaling in astrocytes. Laquinimod clinical trials, however, have had conflicting outcomes\(^64,65\). Laquinimod did not significantly slow the course of RRMS despite a considerable reduction in brain shrinkage (Clinical Trial NCT01707992)\(^66\). When *Lactobacillus murinus* and *Lactobacillus reuteri* are administered as probiotics, fecal levels of tryptophan metabolites such as ILA, IAA, and IAld are raised and ameliorate EAE. Although this impact was not investigated with the other metabolites, it was hypothesized that ILA would have a protective role by lowering Th17 polarization and IL-17A production from myelin oligodendrocyte glycoprotein peptide (MOG)-reactive T cells\(^67\).

The start of EAE in mice is paradoxically prevented by a complete lack of dietary tryptophan before vaccination due to the microbiota-dependent harm to brain T cells\(^68,69\). However, mice fed with a control diet started to show a reduction in EAE symptoms, whereas clinical disease worsens if dietary tryptophan is withdrawn after the onset of EAE\(^65\). Another study showed that IFN-\(\beta\) causes the AhR expression of astrocytes, which might lead to a therapeutic IFN-\(\beta\)s role in MS through increasing glial cell responsiveness to anti-inflammatory AhR ligands. In addition, the AhR ligands indoxyl 3-sulfate, IPA, and IAld all alleviated EAE via AhR signaling and restricted astrocyte production of IL-6, tumor necrosis factor (TNF)-\(\alpha\), CCL2, and inducible nitric oxide synthase\(^68\).

### 3.3. Phytoestrogens

Phytoestrogens are dietary substances generated from plants that share structural similarities with 17-estradiol. Given this, phytoestrogens may also affect immune function in MS\(^70,71\), *Prevotella*, *Parabacteroides*, *Adlercreutzia*, *Slackia*, and *Lactobacillus*, which metabolize phytoestrogens as well as improve bioavailability, diminished among MS patients, hence demonstrating that phytoestrogen is linked to the etiology of MS\(^71,72\). Recently, an eating plan that contains phytoestrogen decreased EAE disease from a manner that depended on phytoestrogen-metabolizing bacteria. In addition, it is demonstrated that mice given this diet and devoid of phytoestrogen had intestinal flora compositions that were strikingly comparable to those of MS patients. These findings imply that commensal bacterially generated food phytoestrogen metabolites may have an impact on CNS autoimmunity.

Isoflavones are phytoestrogens that are only metabolized by the human body via gut microbes. It was discovered that the number of bacteria capable of metabolizing isoflavones was low in MS patients, and further research revealed that isoflavone diet mice had an altered gut microbial composition as well as an anti-inflammatory phenotype that inhibited EAE\(^73,74\).

### 4. Therapeutic implications for microbiota in MS

#### 4.1. Probiotics

Much research in recent years has focused on probiotics to restore the balance of the gut microbiota. It is thought that these live microorganisms work by altering the gut microbes to encourage intestinal barrier integrity as well as the differentiation and activation of immunoregulatory cell subsets over inflammatory cell subsets\(^75,76\). Oral probiotics improved gut microbiota diversity by increasing the abundance of many species,
including Bacteroides, Odoribacter, Lactobacillus, Sutterella, and Bifidobacterium. Probiotic use also reduced the abundance of strains previously linked to MS intestinal dysbiosis such as Akkermansia and Blautia. Two commercial multi-species probiotics, Lactibianeiki and Vivomixx, induce peripheral immune tolerance in EAE mice by regulating DC number and phenotype. The Lactibianeiki group had a higher frequency of Tregs with a lower frequency of plasma cells. Furthermore, oral Vivomixx inhibited the proliferation of microglia, astrocytes, and leukocyte infiltration, while activating the proliferation of regulatory B cells (Bregs) in the CNS of TMEV mice. It is worth noting that Lactibianeiki and Vivomixx are commercial products that can be quickly translated into clinical use. In mice, a kind of probiotic, Lactobacillus acidipiscis, induced the generation of γδ Treg cells and CD4+ Foxp3+ Treg cells while inhibiting the differentiation of cerebrospinal Th1 as well as Th17 cells. Oral probiotics also can modulate immune cells, inhibit pro-inflammatory cytokine expression such as IL-1/6/17, and IFN-γ, and promote the anti-inflammatory cytokine IL-10 expression in EAE mice.

Probiotic treatment has only been tested in three research addressing MS, and there are few human studies on the subject. In two small double-blinded randomized controlled trials (RCTs), the MS group receiving a combination of Lactobacillus and Bifidobacterium every day for 12 weeks displayed meaningful ameliorations among disability score, depression, anxiety, and inflammatory markers, with decreased IL-8 and TNF-α expression of peripheral blood mononuclear cells. Similarly, Tankou et al. discovered a reduction of CD80 protein production in peripheral monocytes after giving MS patients as well as HCs a probiotic combination including Lactobacillus, Bifidobacterium, and Streptococcus twice daily for 2 months. After taking probiotics, the documented modifications to the immune system and gut microbiota composition were not sustained.

An analysis of GF mice mono-colonized with a new strain of the Erysipelotrichaceae family, however, revealed that Lactobacillus reuteri treatment increased MOG-specific responses. It was suggested that the molecular similarity between MOG and the uvrA gene product of Lactobacillus reuteri could be the mechanism causing EAE exacerbation. Following the study, it is important to take into account the synergistic impact that these microorganisms have when determining the pathogenicity of MS. Similarly, another study discovered that Lactobacillus reuteri aggravated EAE in mice with certain genetic predispositions.

4.2. Antibiotics
In mice models, antibiotic intervention in the gut microbiota is currently yielding positive results. Researchers report that oral prophylactic antibiotics during the presymptomatic transition phase, in particular, prevented motor dysfunction in TMEV mice and reduced susceptibility to EAE; however, antibiotic treatment after an EAE episode did not reduce the severity of the illness. Clostridium butyricum and norfloxacin, as gut microbiota interventions, may alleviate EAE by inhibiting the Th17/Treg-related pathway. Treatment with Clostridium butyricum reduced the number of Th1 cells in the spleen. According to a recent study, oral ampicillin therapy reduced the severity of EAE. Researchers also discovered two molecules produced by Lactobacillus reuteri and a newly discovered strain of the Erysipelotrichaceae family that work together to cause the accumulation of MOG-specific Th17 cells in the small bowel. The data also support a link of the gut-microbiota-CNS axis.

There is not much research on the impact of antibiotic therapy on MS. In comparison to IFN-β-only therapy, two small studies looking at the effects of doxycycline and IFN-β in MS found lower rates of relapse, better indices of disability, and fewer gadolinium-enhancing lesions. Minocycline delayed the conversion of patients with clinically isolated syndrome (CIS) to MS during a 6-month but not a 24-month timeframe, according to another double-blind, randomized trial. A bigger investigation into those with CIS is continuing (Clinical Trial NCT04291456). Indeed, one study found that using ampicillin during clinical EAE worsened the disease, whereas using vancomycin had no clinical effect. There are dangers associated with continuous antibiotic treatment for MS, including the growth of fungus, opportunistic pathogens such as Clostridium difficile, and antibiotic-resistant infections.

4.3. Diet
Diet has a significant impact on the gastrointestinal tract, and dietary treatments can help to correct intestinal microflora imbalances. A study in marmosets showed that a targeted dietary intervention lowers pro-inflammatory T cells that respond to recombinant human MOG and improves brain remyelination. Another study, by taking mice on intermittent fasting (IF), found that IF improved the clinical course and pathology of EAE in mice by improving intestinal dysregulation, which increased microbial abundance and enrichment, increasing ketone formation and glutathione metabolism, and enhancing antioxidant pathways, leading to less inflammation, demyelination, and axonal damage.
Different diet preferences may have an impact on MS. MS relapse rates and the expanded disability status scale scores showed a decrease in the high-vegetable/low-protein diet group, and both Th17 cells and programmed cell death protein 1-expressing CD4+ T cells were reduced\cite{100}. In addition, the Mediterranean diet was found to have a favorable impact on MS in a multi-center, cross-sectional investigation\cite{101}. An RCT found that 15 days of a periodic calorie limitation diet was highly tolerable and showed a lower level of the proinflammatory adipokine leptin without changes in adiponectin among 16 MS patients recovering from relapsing MS as compared to an ad libitum diet. This study found that dietary restriction enriched Faecalibacterium, Lachnospiraceae incertae suis, and Blautia populations in the gut and that adiponectin levels were positively linked with Faecalibacterium\cite{99}. The metabolic and gut microbiota alterations between mice and MS patients receiving IF showed a consistent pattern and highlighted the possibility for translation of IF. Nonetheless, there are drawbacks to the human study, including limited sample size, brief research duration, and the impossibility of a blind clinical trial regarding the participating patients’ diet allocation.

4.4. Fecal microbiota transplantation (FMT)

Many intestinal illnesses, such as Clostridium difficile infection\cite{102}, ulcerative colitis\cite{103}, and irritable bowel syndrome\cite{104}, respond well to FMT. FMT has mostly been used in animal models in MS investigations. Transplanting the intestinal bacteria of EAE-resistant mice into EAE-susceptible or EAE-provoked mice can alleviate EAE\cite{105,106}. However, there is evidence of the opposite effect, where the transfer of the gut microbes from PWD mice, which is resistant to EAE, to EAE-susceptible B6 mice exasperates EAE, whereas the transfer of the gut microbes from B6 mice to PWD mice increases susceptibility to EAE; this could be due to the presence of genetic susceptibility, making the role of gut microbes less important in EAE, implying that animal models with different genetic background may interfere to some extent with the study of gut microbial facets of MS\cite{87}.

### Table 1. The change and functions of gut microbiota in multiple sclerosis

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Family</th>
<th>Genus/species</th>
<th>Functions in MS</th>
<th>References</th>
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<td>Firmicutes</td>
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<td>Blautia, Dorea, Roseburia inulinivornans</td>
<td>Produce SCFAs</td>
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</tbody>
</table>

Abbreviations: MS: Multiple sclerosis, SCFAs: Short-chain fatty acids.
5. Conclusion

In summary, gut microbes play a crucial role in the progression of MS together with the exacerbation of neuroinflammation by acting as environmental factors through the gut-brain axis. Multiple microbiome species, particularly *Akkermansia* as well as *Collinsella*, are more prevalent in MS patients. The number of SCFA-producing genera, many of which are *Firmicutes* or Bacteroidetes, is decreasing (Table 1). To further understand the molecular connections between gut and brain functions as well as how they affect CNS autoimmunity, more study is required. An experimental idea of FMT is to transplant gut microbiota from the group in the experiment that received the active intervention. EAE improved in FMT mice when compared to those given 60 min of strength training\(^ {107} \); similar results were seen in FMT mice compared to mice given delta-9tetrahydrocannabinol (THC) and Cannabidiol (CBD) treatment (THC and CBD are drugs used to treat muscle spasms in MS patients), and the improvement in EAE may be attributed to microbiota changes in the mice through bacterial transplantation\(^ {108} \). This could reveal novel MS treatment pathways. Pomegranate peel extract is a natural compound that acts as a prebiotic and has anti-EAE properties. Intestinal flora from PPE-treated mice was transplanted into EAE mice, which prevented illness development\(^ {109} \). These studies demonstrated that fine-tuning the gut bacteria may be an interesting approach to MS treatment.

Studies showed that MS is influenced by the changes in microbiota-derived metabolites, including SCFAs, phytoestrogens, and tryptophan derivatives. In preclinical models and clinical trials, interventions that affect the microbe, such as probiotics, antibiotics, diet, and FMT, are currently being studied. In a case study, an RRMS patient that was given FMTs from five donors daily showed raised levels of propionate, butyrate, and brain-derived neurotrophic factor as well as decreased levels of pro-inflammatory cytokines and relative abundance of *F. prausnitzii* in the weeks after the transplant. Clinical tests revealed that the patient’s ability to walk and maintain balance had improved\(^ {110} \). The above studies suggest that FMT might be an emerging treatment for MS. It may complement currently available therapeutic choices for MS patients (Figure 1).

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Conflict of interest
The authors declare that they have no competing interests.

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