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HIGHLIGHTS

Basic

REVIEW

The role of the gut microbiota and microbial metabolites in neuroinflammation

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Recent literature indicates a potential importance of the gut microbiota for immune-mediated diseases. For instance, decreased diversity of commensals or an outgrowth of some bacterial strains, referred to as gut dysbiosis, was recently linked to hypertension, colitis, lupus, rheumatoid arthritis, and multiple sclerosis (MS). Studies in experimental autoimmune encephalomyelitis (EAE) as pivotal animal model of MS revealed a potential importance of microbial metabolites, including short-chain fatty acids or tryptophan metabolites. Both metabolites may influence the disease by modulation of the immune system, mainly by inducing Treg. These studies prompted researchers to investigate the contribution of the gut microbiota and microbial metabolites in the pathogenesis of MS. This review summarizes recent findings on the gut microbiota in MS patients and discusses the potential mechanisms how microbial metabolites may affect neuroinflammation. Many of these studies have been performed in the EAE model and were later reversely translated to humans. We also give a short summary on dietary high-salt effects on microbiota components and discuss the potential relevance of high-salt as a risk factor in MS.

Keywords: Gut microbiota · multiple sclerosis · experimental autoimmune encephalomyelitis · short-chain fatty acids · dietary high-salt

Introduction

Humans are colonized by millions of microorganisms, which behave as symbionts, commensals, or pathogens. This complexity of microorganisms, collectively called the microbiota, is mainly localized in the gastrointestinal tract [1]. The number of bacterial cells at least equals that of human cells within the human body [2], suggesting an enormous impact of the microbiota on host physiology. Indeed, intestinal bacteria may exert major effects on the host, especially via interaction with the immune system. The intestine harbors pivotal cell types and mediators of the innate as well as the adaptive immune system, thus representing an

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important immune organ in the body. The lamina propria and the mesenteric lymph nodes are populated by DCs, macrophages, and lymphocytes [3]. Communication between the microbiota and these lymphocyte populations is enabled by the presentation of cell wall components via APCs, by soluble factors (microbial metabolites), or by the stimulation and modulation of intestinal epithelial cells. The gut microbiota is highly dynamic and can be influenced by the genetic environment of the host and external factors, especially dietary intake. An established microbial imbalance is termed dysbiosis, a state that is related to altered metabolite production. Gut dysbiosis has been linked to local and systemic inflammation, obesity [4], type 2 diabetes [5], inflammatory bowel disease [6], hypertension [7, 8], and also multiple sclerosis (MS) [9, 10]. One integral dietary component that has recently been linked to changes in microbiota composition and function is dietary salt (sodium chloride). According to World Health Organization, most adults from the Western hemisphere consume twice the recommended maximum of salt per day [11]. This increased salt intake was shown to promote local and systemic tissue inflammation and impair intestinal anatomy in both human and animal settings. A large number of studies demonstrate that extracellular high-salt concentrations modulate immune homeostasis by affecting various cell types (reviewed in [12]). In brief, high-salt concentrations favor the activation of pro-inflammatory M1 macrophages [13, 14] and T helper (Th) 17 cells [15, 16], whereas the induction of anti-inflammatory M2 macrophages [17] and the suppressive capacity of regulatory T cells (Treg) [18] are reduced. Thus, high-salt concentrations in the cellular environment shift the immune balance toward a pro-inflammatory state, which provides a potential explanation for the disease-promoting effect of diets rich in salt, which has been observed in various models of immune-mediated diseases [19]. In addition to these direct effects, recent research identified the gut and the gut microbiota as one integral mediator involved in the deleterious effects of high-salt conditions on immune cells.

Relevance of microbial changes in MS

The adult microbiota is largely defined by two dominant phylotypes, namely *Bacteroidetes* and *Firmicutes* [20]. The ratio of these very broad taxa tends to remain stable over months or years, although the composition within these broad phylotypes differs between healthy individuals. This interindividual variety may be attributed to age and gender [21], antibiotic intake [22], and dietary habits [23]. Moreover, the immune system has a profound effect on the microbiota composition, and vice versa, the gut microbiota shapes the immune system [24]. This finding tempted many research to investigate the potential contribution of gut microorganisms in immune-mediated diseases.

MS is an autoimmune disease affecting the central nervous system (CNS). After peripheral activation, immune cells enter the CNS, leading to neuroinflammatory processes, myelin degradation, and axonal loss. Despite growing interest in the role of B cells, T cells are assumed as main pathogenic drivers. Consequently, MS pathology is characterized by increased frequencies of Th17 and Th1 cells, but functionally impaired Treg cells [25]. Pivotal studies in experimental models first suggested that the gut microbiota contributes to MS. EAE is one of the most commonly used animal model of MS. It can be induced by active immunization with myelin peptides or passive transfer of myelin-reactive T cells [26]. Oral antibiotic treatment before EAE induction reduced EAE development in mice, coinciding with an alteration of the gut bacteria composition [27, 28]. The potential relevance of gut microorganisms in EAE was later confirmed in germ-free mice lacking intestinal microbiota. Germ-free mice are protected from EAE development, which could be linked to reduced Th1 and Th17 responses [29, 30]. The transfer of specific bacteria into germ-free mice, such as segmented filamentous bacteria, restored the susceptibility to EAE induction by inducing Th17 cells in the intestine [30]. In contrast, mononcolonization with *Bacteroides fragilis* after oral antibiotic treatment prevented the onset of EAE by increased generation of interleukin (IL)-10 producing Treg cells [31]. These data prompted researchers to perform sequencing analyses of 16S ribosomal DNA isolated from fecal samples obtained from MS patients compared to healthy donors. These studies revealed that the overall diversity of the gut microbiota in MS patients is comparable to that observed in healthy controls. However, the relative abundance of specific bacteria was significantly altered. MS patients show an enrichment of *Clostridium* [32], *Pseudomonas*, *Mycoplana*, *Haemophilus*, *Blautia*, and *Dorea* genera [33] as well as *Methanobrevibacter* and *Akkermansia* [9, 34]. Other studies identified a decreased occurrence of species belonging to Clostridia clusters XIVa and IV and Bacteroidetes [10]. So far, most of these studies were performed in patients with relapsing remitting MS. In contrast, researchers just started to investigate the importance of microbiota changes in patients with different stages of MS [35, 36]. These studies revealed differences in the relative abundance of rare phyla in primary progressive MS patients compared to healthy controls [35]. Secondary progressive MS patients show a relative increase of the *Streptococcuss* genus, which was suggested to correlate with increased oxidative stress in the gut [36]. In contrast, the gut microbiota of relapsing remitting MS patients was characterized by decreased abundance of short-chain fatty acid (SCFA) producing bacteria compared to healthy controls [36], confirming previous studies that demonstrated a relative contribution of microbial SCFAs during neuroinflammation [37]. Potential mechanisms of gut microbiota alterations in MS patients have been studied in the EAE model that might in part be transferred back to the human disease.

Potential mechanism of microbial changes obtained in animal models of MS

Standard approaches for investigation of potential contributions of disease-relevant bacterial strains to MS pathogenesis are bacterial monocolonization or stool transfer experiments in mice subjected to EAE. In one such study, transplantation of human gut

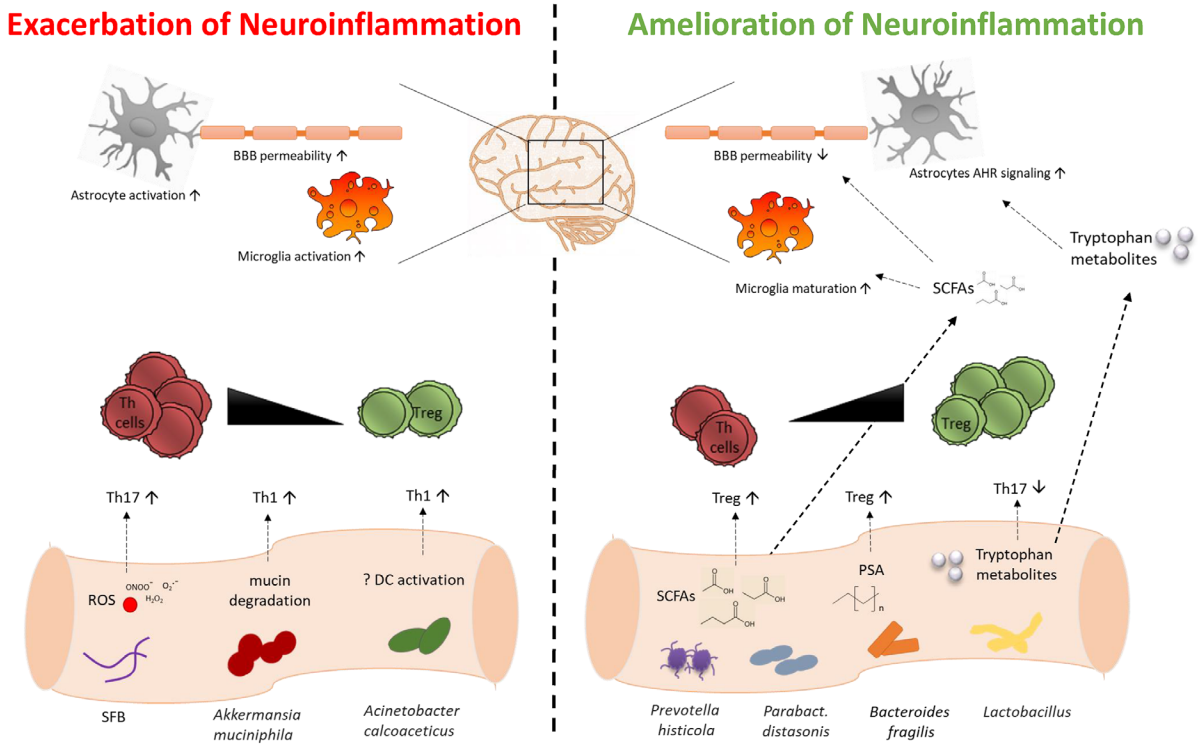

Figure 1. Contribution of the gut microbiota and microbiota metabolites during neuroinflammation in mice.
The transfer of single bacterial strains into germ-free or antibiotic-treated mice can either induce a pro-inflammatory or anti-inflammatory state in the gut during neuroinflammation. Left: Bacterial strains that were shown to be increased in MS patients enhance the differentiation of Th1 and Th17 cells in the gut, whereas regulatory immune cells such as Treg cells are decreased. These bacterial strains include segmented filamentous bacteria (SFB), *Akkermansia muciniphila* and *Acinetobacter calcoaceticus* that might induce alterations in immune cell composition via increased reactive oxygen species (ROS) production, mucin degradation or the activation of DCs. Right: In contrast, bacterial strains that were shown to be reduced in MS patients induce an anti-inflammatory phenotype in the gut when transferred into germ-free or antibiotic-treated mice. Metabolite analysis revealed an increased concentration of short-chain fatty acids (SCFAs), tryptophan metabolites or polysaccharide-A (PSA), thus shifting the immune cell composition towards increased Treg cell frequencies. SCFAs can also directly modulate the blood-brain barrier (BBB) or CNS resident cells such as microglia. Moreover, tryptophan metabolites were shown to affect astrocytes via aryl hydrocarbon receptor (AHR) signaling, thus ameliorating neuroinflammation.

microbial organisms from MS-affected monozygotic twins into the spontaneous relapsing-remitting EAE model resulted in an increased frequency of spontaneous clinical signs compared to the transfer of stool samples from healthy twins [38]. Immunological analysis of these mice revealed a decreased IL-10 production in mice colonized with the stool from MS patients, probably linking decreased anti-inflammatory cells to an increased EAE incidence [38]. Similar results could be obtained by the transfer of single microorganisms to germ-free or antibiotic-treated mice (Fig. 1). The transfer of *Parabacteroides distasonis* (found to be decreased in MS patients) increased the differentiation of $CD4^+$IL-10+ cells, whereas bacteria that were increased in MS patients (*Akkermansia muciniphila* and *Acinetobacter calcoaceticus*) enhanced the differentiation of pro-inflammatory Th1 cells in mice [34]. Further in vitro experiments in human peripheral blood mononuclear cells (PBMCs) confirmed the induction of anti-inflammatory IL-10 secreting $CD4^+CD25^+$ T cells by *P. distasonis*, whereas the stimulation of PBMCs with *A. muciniphila* and *A. calcoaceticus* increased pro-inflammatory immune responses [34]. The modulation of IL-10 producing Treg cells was also observed after the reconstitution of EAE mice with *Prevotella histicola*, a bacterium that is significantly reduced in stool samples of MS patients [39, 40]. These data indicate that differences in specific gut bacteria are functionally associated with a shift toward a pro-inflammatory T-cell profile while decreasing regulatory immune cells in MS. This effect is mainly attributed to the SCFAs butyrate, propionate, and acetate, microbial fermentation products of dietary fiber. SCFAs may directly affect T-cell differentiation. In contrast, propionate treatment decreases the differentiation of Th17 cells [41], whereas treatment with butyrate and propionate increases the differentiation of Treg cells and enhances their suppressive capacity [41–43]. Other studies identified a potential importance of microbial SCFA on blood–brain barrier (BBB) integrity [44]. Germ-free mice showed a reduced expression of tight-junction proteins and an increased BBB permeability compared to mice with a normal gut microbiota. Interestingly, BBB permeability could be decreased by the colonization with SCFA-producing bacteria or direct treatment with butyrate [44]. Other effects of SCFAs have been observed on microglial cells [45]. Germ-free mice displayed altered microglia maturation, differentiation, and function, which could be restored by microbiota recolonization or SCFA treatment. In addition to SCFAs, polysaccharide A

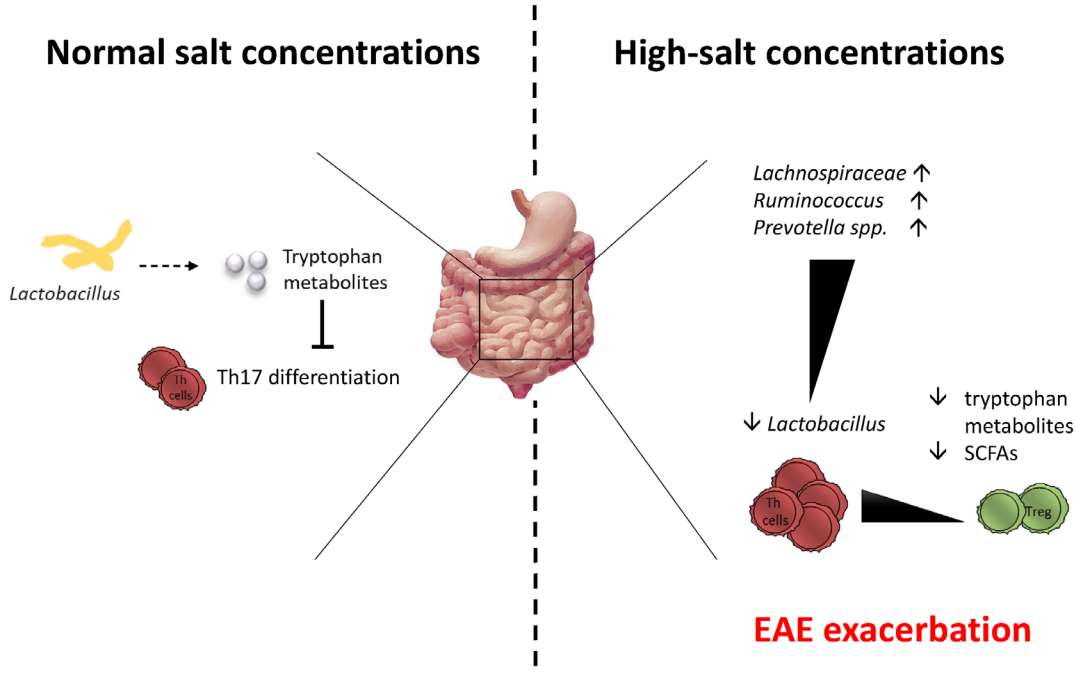

Figure 2. Potential mechanism of high-salt induced EAE exacerbation via modulations in the gut.
Under normal salt conditions, the gut commensal *Lactobacillus* produces tryptophan metabolites that can block Th17 cell differentiation in the gut (left). After high-salt intake, some bacterial species are increased in mice, including *Lachnospiraceae*, *Ruminococcus* and *Prevtolla spp*., whereas *Lactobacillus* are depleted in the gut microbiota. Decreased *Lactobacillus* was shown to coincide with increased Th17 cell frequencies in the gut, probably mediated by decreased production of tryptophan metabolites. Moreover, high-salt concentrations reduce short-chain fatty acid (SCFA) concentrations in the gut, thereby decreasing Treg cell differentiation. The shift toward pro-inflammatory Th17 cells in the gut due to high-salt-induced microbiota changes was shown to exacerbate EAE, the animal model of MS.

(PSA) produced by *B. fragilis* may induce the differentiation of IL-10-producing Treg cells [46] and PSA treatment during EAE can protect mice from disease development [31]. Moreover, tryptophan metabolites may modulate T-cell subsets by either promoting Th1 and Th17 differentiation or by the induction of Treg cells (reviewed in [47]). Interestingly, tryptophan metabolites can be produced by different strains from the bacteria genera *Lactobacillus* [48], and Lactobacilli were recently shown to ameliorate EAE by reducing Th1 and Th17 cells [49–51]. In addition, neurotransmitters such as gamma amino butyric acid, serotonin, or norepinephrine can be produced by gut microorganisms [52–54] and may have a potential role in MS pathogenesis [55]. Yet, the here discussed gut bacterial metabolites represent a small fraction of metabolites that were shown to influence animal models of MS. Besides SCFAs, tryptophan metabolites, PSA or neurotransmitters, lactic acid, poly-γ-glutamic acid, or cell wall components such as peptidoglycan may also be considered as potential contributors in MS pathology [56–59].

Modulations of the gut microbiota and its metabolites by high-salt concentrations

Dietary factors can disturb and alter the microbiota composition and function, including the production of microbial metabolites. This is not surprising, assuming that dietary components first pass the gastrointestinal tract before being absorbed and distributed to tissues [60]. Several studies identified alterations of the microbial composition by high-salt ingestion. In humans and mice, high-salt intake was associated with changes in the gut microbiome reflecting an increased ratio of *Firmicutes* to *Bacteroidetes* [61] (Fig. 2). More in detail, bacteria enriched by high-salt primarily belong to the family *Lachnospiraceae* [61] or *Prevotella spp*. in mice and humans [62]. *Prevotella* have been associated with chronic inflammation in rheumatoid arthritis [63] but had a positive effect in the animal model of MS [40]. Yet, *Prevotella* comprise a large number of species, a fact that probably explains these contradictory data. In contrast, elevated salt intake was associated with a reduction of *Lactobacillus* [50, 61], a genus that was beneficially linked to effects in colitis [64], salt-induced hypertension [50], and neuroinflammation [49–51]. During neuroinflammation, the high-salt-induced depletion of *Lactobacillus* was paralleled by the induction of Th17 cells in the gut and spleen, coinciding with deteriorating EAE symptoms [50]. These data confirmed previous studies, indicating that high-salt concentrations may affect neuroinflammation via Th17 cell induction [16, 65]. Interestingly, oral administration of *Lactobacilli* during EAE prevented the salt-induced aggravation of EAE and decreased Th17 cell differentiation in the gut [50]. Independent of high-salt concentrations, recently published studies confirmed the beneficial effect of *Lactobacillus* treatment during EAE [49, 51]. One possible mechanism of high-salt-induced Th17 differentiation via

modulation of the gut microbiota may be the high-salt-induced alteration of microbial metabolites. High-salt intake in mice was paralleled by reduced concentrations of the fecal tryptophan metabolite indole-3-lactic acid [50], a tryptophan metabolite produced by *Lactobacilli* [48, 50]. Supplementation of indoles during EAE reduced CNS inflammation, probably by activating aryl hydrocarbon receptor signaling in astrocytes [66]. Moreover, indole-3-lactic acid inhibited Th17 cell polarization in vitro and may thus link the high-salt diet-induced suppression of *Lactobacillus* to the induction of Th17 cells in EAE [50]. In addition to decreased tryptophan metabolite concentrations, high-salt intake in mice also decreased the concentration of SCFAs [64, 67]. Recent studies have shown that SCFAs exert beneficial effects in EAE mice [41, 68]. The administration of the SCFA propionate during EAE ameliorated disease symptoms by increasing Treg cell frequencies in the small intestine, thus leading to a more anti-inflammatory environment in the gut [41]. This beneficial effect on EAE severity has also been demonstrated for high-fiber intake, resulting in increased SCFA concentrations in the gut [68].

Clinical relevance of microbial restoration in patients with MS

It is controversially discussed whether high-salt intake might represent a risk factor in MS. Many studies revealed a positive correlation of high-salt intake and EAE severity [16, 50, 65, 69–71]. However, others identified that high-salt-mediated aggravation of EAE is sex and gene dependent in mice [72]. One observational study in humans found higher relapse rates and increased numbers of MRI lesions in two cohorts of relapsing remitting MS patients with high-salt intake [73]. In contrast, another study revealed no effects of high-salt consumption on the conversion from clinically isolated syndrome to MS [74]. Moreover, two studies in pediatric MS found that high-salt consumption does neither increase the risk of MS development nor affect the time to relapse [75, 76]. Yet, this limited number of studies in different forms of MS and partly different methods for the quantification of salt intake are insufficient to confirm or exclude any high-salt effects in MS. Considering the number of studies demonstrating salt effects on immune cell subsets [12] and immune-related diseases [19], it seems likely that high-salt intake may represent a risk factor for autoimmunity. Yet, further studies in MS patients will be needed to proof this assumption. However, there are newly published studies showing that reconstitution of high-salt altered microbiota metabolites during MS can impact disease progression. Indicating a potential relevance of high-salt-induced depletion of Lactobacilli in humans, we showed that a short-term high-salt challenge in healthy humans affects the survival of intestinal *Lactobacillus*, alongside increased frequencies of Th17 cells in the blood [50]. Yet, it is unclear whether *Lactobacillus*-mediated high-salt effects can be transferred from the EAE model to MS. However, a potential importance has been suggested in an earlier study, showing that *Lactobacillus* spp. abundance is decreased in patients with relapsing–remitting MS [33]. Moreover, a pilot study in humans demonstrated the relevance of probiotics as immunomodulatory agents in MS patients and healthy controls [77]. Administration of a probiotic cocktail of eight bacteria containing *Lactobacillus* spp. induced changes in the gut microbiota composition that were associated with anti-inflammatory immune responses and decreased frequencies of intermediate monocytes in the periphery [77, 78]. The probiotic treatment restored gut bacteria that were depleted in MS patients, including *Lactobacillus*, and decreased the frequency of inflammatory monocytes, indicating a potential benefit of probiotic treatment as add-on to established MS therapies. Further clinical trials are necessary to prove this concept.

A further benefit in MS therapy has been observed for SCFAs. Analysis of human gut microbial metabolites revealed an altered SCFA concentration in MS patients compared to healthy controls [43, 79]. Based on our insights obtained in EAE mice, we recently investigated the impact of propionate administration in MS patients and healthy controls [43]. In a proof-of-concept study, propionate was supplemented to treatment naïve MS patients or as add-on to their immunotherapy for 2 weeks. In both settings, propionate intake resulted in a significant increase of Treg cells, while Th1 and Th17 cells significantly decreased. Moreover, retrospective analyses of a long-time propionate intake revealed a reduced annual relapse rate and stabilization of disability [43]. Interestingly, the Treg inducing effect of propionate was connected to the gut microbiota. In a sophisticated gut explant model, intestinal colonization with microbiome derived from MS patients who received propionate compared with pre-therapy microbiome led to an upregulation of gene expression patterns associated with Treg development [43]. These data place propionate as a potential add-on therapy to currently existing MS drugs.

Summary and conclusion

A diet containing high amounts of salt results in alterations of gut microbiota composition and function that may induce a pro-inflammatory immune phenotype. In particular, a suppression of *Lactobacillus spp*. may be a contributing factor. On a functional level, such salt-induced alterations to the community may result in a reduced intestinal production of SCFAs and indole metabolites. The direct supplementation of metabolites of bacterial origin, such as propionate, may circumvent gut dysbiosis and exert beneficial effects by increasing regulatory immune mechanisms, as shown in MS. Thus, exploiting microbiota-mediated mechanisms, either by re-shaping the gut microbiome or by direct administration of bacterial metabolites, is a promising approach to reverse salt-induced pathomechanisms. The use of prebiotics (e.g. indigestible carbohydrates), probiotics (targeted administration of live bacteria), or postbiotics (specific metabolites of bacterial origin) may therefore be a novel therapeutic avenue to restore immune cell homeostasis in immune-mediated diseases such as MS.

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Abbreviations: **BBB**: blood–brain barrier · **PSA**: polysaccharide A · **SCFA**: short-chain fatty acid

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