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Gut-oriented interventions in patients with multiple sclerosis: fact or fiction?

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Abstract. – OBJECTIVE: Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating, disimmune disease of the central nervous system whose etiology and pathogenesis remain poorly understood, due to its complex and multifactorial nature. Evidence of a bidirectional connection linking the gut microbiome with the intestinal barrier and the immune system (the gut-brain axis) may have implications for the pathogenesis of inflammatory demyelinating diseases such as MS. This narrative review summarizes the evidence for the gut-brain axis involvement in the pathogenesis of MS and examines the role of gut-oriented interventions in MS.

PATIENTS AND METHODS: We reviewed all available studies in PubMed concerning gut-directed interventions and MS. This research was conducted using different combinations of pertinent keywords (multiple sclerosis, immune-mediated inflammatory diseases, autoimmune diseases, first demyelinating event, neurocognition, neurological disorders, neurology practice, risk factors, taxonomic biomarkers, nutrition, diet, dietary additives, complementary treatment, gut bacteria, gut microbiome, microbiome, gut-brain axis, epidemiology, alpha-linolenic acid, fermentative metabolites, fat, saturated fat, monoun-

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saturated fat, polyunsaturated fat, omega-3 fatty acids, calorie restricted diet, fasting, fecal microbiome, fecal microbiota transplantation, animal testing).

RESULTS: There is an emerging evidence that alterations in the gut microbiome and increased intestinal permeability may be causative factors in the complex interplay between nutrition, metabolic status and the immune-inflammatory response in patients with MS. This suggests the possibility that modification of lifestyle and the microbiome, for example by specific diets or fecal microbiota transplantation, supplementation with bile acids and intestinal barrier enhancers, may positively influence the pathogenesis of MS.

CONCLUSIONS: Although the role of nutritional factors in the pathogenesis of MS remains to be established, there is evidence that appropriate gut-directed interventions such as diet, nutritional supplementation or fecal transplantation may modulate the inflammatory response and improve the course of MS as a complementary treatment in the disease.

Key Words:

Multiple sclerosis, Dietary interventions, Nutraceuticals, Gut microbiota, Microbiome, Nutrient supplementation, Metabolic pathways, Neuroinflammation.

Introduction

Multiple sclerosis (MS) is a chronic, inflammatory disimmune disease of the central nervous system (CNS), leading to demyelination and axonal damage. The etiology and pathogenesis of MS remain poorly understood due to its complex and multifactorial nature[1].

The bidirectional connection linking the gut microbiome with the intestinal barrier and the immune system (the gut-brain axis) may have implications for the pathogenesis of inflammatory demyelinating diseases such as MS[2-5] (Figure 1). Emerging evidence[2,6] suggests that alterations in the gut microbiome and increased intestinal permeability may be causative factors in the complex interplay between nutrition, metabolic status and the immune-inflammatory response in patients with MS leading to the idea of new therapeutic targets (i.e., modification of lifestyle and the microbiome, by specific diets or fecal microbiota transplantation, supplementation with bile acids and intestinal barrier enhancers). However, the role of nutritional factors in the pathogenesis of MS is yet to be established and the benefits of specific nu-

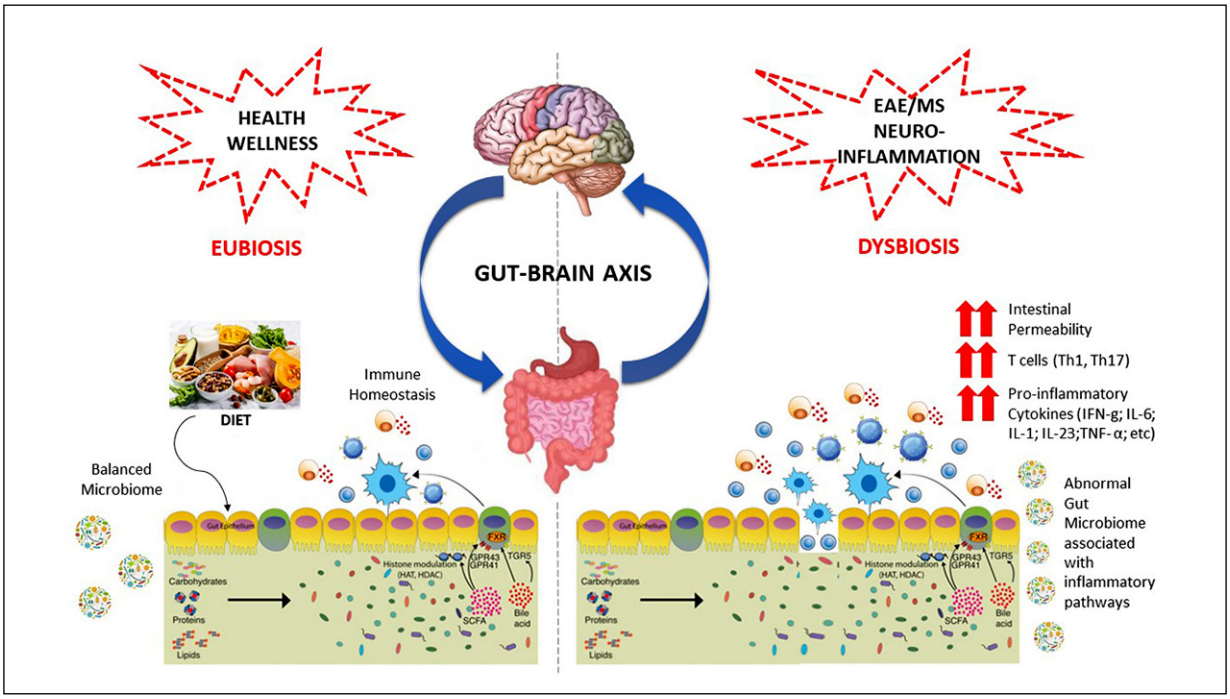

Figure 1. The bidirectional nature of the gut-brain axis, illustrating the potential of the gut microbiome as an amplifier of the immune/inflammatory response and proposing a model of dysbiosis, increased intestinal permeability, microbial translocation and local and systemic inflammation based on animal models of MS. *EAE*, experimental autoimmune encephalomyelitis; *MS*, multiple sclerosis; *IFN*, interferon; IL, interleukin; *Th*, T-helper; *TNF*, tumor necrosis factor.

tritional interventions suggested by pre-clinical trials on the clinical status of MS patients have not been fully determined[4,7].

Despite the current lack of firm evidence, many clinicians already recommend a healthy diet for their MS patients both at the beginning of the disease and in patients with a progressive course[8].

Although at this point dietary strategies must be considered as a complementary approach in conjunction with conventional MS therapies, finding a particular diet with appropriate nutritive interventions may modulate the inflammatory response to improve the course of MS[1]. Many patients with MS already seek complementary and alternative treatments for their condition[9]. Unfortunately, internet remains the main source of information for most patients, frequently leading to choices and behaviors without any scientific support[10]. It is necessary either a scientific analysis of different dietary patterns or other gut-oriented interventions.

To this end, a multidisciplinary group of Italian Neurologists involved in the management of patients with MS assembled to form the MS COMPLETE Project, with the aim of analyzing the existing evidence for different gut-directed interventions (e.g., diet, supplementation, fecal transplant) in MS patients.

Patients and Methods

The project was developed by an Expert (VM), focused on the role of microbiota and nutritional factors in real-world life of MS patients. He gathered a group of 16 Italian Experts in MS, on the basis of their scientific interests. An Expert Gastroenterologist (RS), with documented experience in the treatment of autoimmune conditions of the bowel, also shared experience and proved guidance. A scientific consultancy agency provided technical and scientific support.

The initial step of the project was to review all available studies, both clinical and preclinical, as full papers or abstract, concerning gut-directed interventions and MS. This search was conducted in PubMed, using different combinations of pertinent keywords (multiple sclerosis, immune-mediated inflammatory diseases, autoimmune diseases, first demyelinating event, neurocognition, neurological disorders, neurology practice, risk factors, taxonomic biomarkers, nutrition, diet, dietary additives, complementary treatment, gut bacteria, gut microbiome, microbiome, gut-brain axis, epidemiology, alpha-linolenic acid, fermentative metabolites, fat, saturated fat, monounsaturated fat, polyunsaturated fat, omega-3 fatty acids, calorie restricted diet, fasting, fecal microbiome, fecal microbiota transplantation, animal testing).

Papers identified during the literature search were presented during two meetings, with the assistance of a professional facilitator. The results were extensively discussed and commented on by the Experts during a free discussion period. The outcomes of these deliberations represent the basis of the present narrative review.

Results

Targeting the Gut Microbiome with Therapeutic Options

Experimental models of MS have found deficits in the function of T regulatory (Treg) cells related to the aberrant gut microbiota composition[3]. Pro-inflammatory T helper 17 (Th17) cells are known to be key players in the pathogenesis of MS, and studies in animals and humans have demonstrated that effector Th17 cells that trigger brain autoimmunity originate in the intestine[11,12]. In conjunction with these findings, detailed fecal microbiome studies[7,13,14] revealed that MS patients have subtle but distinct differences in microbial community profiles, compared with healthy controls especially at a taxonomic level, with consistent patterns emerging across studies. In addition, the bacterial taxa seem to be differentially expressed in different diseases states, with lower species richness in active relapsing-remitting multiple sclerosis (RRMS)[13], indicating the important role of gut microbiota in disease exacerbation. Immunomodulatory therapy for MS was associated with an altered microbiota in treated versus untreated patients, suggesting that treatment with immunological agents may normalize some of the changes in the microbiota related to MS[15]. The alterations in specific taxa are hypothesized to be associated with the up-regulation of inflammatory cytokines and overall inflammation.

Cosorich et al[11] validated in humans the crucial role of the intestinal environment in promoting Th17 cell activation and expansion in patients with MS. They found that increased frequency of Th17 cells were correlated with further disease activity and with specific alterations of the gut mucosa-associated microbiota in MS. They an-

alyzed the microbiota isolated from small intestinal tissues and found that patients with disease activity and increased intestinal Th17 cell had a higher *Firmicutes*/*Bacteroidetes* ratio, increased *Streptococcus*, and decreased *Prevotella* strains compared to healthy controls or patients with no disease activity[11]. Moreover, intestinal Th17 cell frequency was inversely related to the relative abundance of *Prevotella* strains in the human small intestine[11]. All together, these data suggest an association between brain autoimmunity and specific microbiota modifications with excessive Th17 cell expansion in the mucosa of the human intestine.

Although further research is needed, they suggest that the host/microbiota interactions are reciprocal[3], and that therapeutic targeting of the gut microbiome could not only aid in managing the symptoms of MS but might deeply affect disease onset and progression[3].

It is essential to balance positive and negative impacts for each therapeutic option as there are varied potential strategies for modulating the gut microbiome. Gut microbiome-based therapeutic approaches could also have unintended adverse effects and should not be expected to be appropriate for all autoimmune diseases, reinforcing the need to adequately assess possible therapeutics to substantiate their beneficial effects while limiting negative impacts.

Antibiotic Therapy

Antibiotic exposure in early childhood appears to result in lower diversity in the gut microbiome later in life[16]. The usage of broad-spectrum antibiotics, as frequently occurs in patients with severe disability, can also adversely affect the gut microbiome and expose the subject to opportunistic pathogens, such as *Clostridium difficile*[17].

Reports about associations between antibiotic use and MS risk are conflicting, with a comprehensive systematic review of drug exposure in MS patients concluding that antibiotic use was not associated with MS risk in most studies[18]. Furthermore, despite possible adverse events with long-term antibiotic assumption, some evidence suggests that therapeutic targeting of the gut microbiome with antibiotics may be beneficial in patients with MS. For example, the tetracycline-derived antibiotics, including minocycline, have anti-apoptotic, anti-inflammatory and antioxidant properties in addition to antibiotic effects, and have been shown to have neuroprotective activity in experimental models of various diseases of the CNS, including Parkinson's disease and MS[19]. On this basis, a recent controlled clinical study found that minocycline significantly reduced the risk of conversion from a first demyelinating event (clinically isolated syndrome) to MS, compared with placebo, over 6 months[20]. Although the between-group differences were not sustained at 24 months, the results justify further investigation.

Fecal Microbiota Transplantation

The possible impact of fecal microbiota transplantation (FMT) on the disease was investigated by Berer et al[21] on 34 monozygotic twin pairs who were discordant for MS. MS twin-derived microbiota transplanted into a transgenic mouse model of spontaneous brain autoimmunity induced a significantly higher incidence of autoimmunity than in the case of a healthy twin-derived microbiota[21]. While there was high intra-individual and temporal stability in the microbial profiles of colonized mice, there was a marked reduction in *Sutterella*, a bacterial genus associated with protective immunoregulatory activity *in vitro*. Levels of expression of interleukin (IL)-10, but not other cytokines, were reduced in mice colonized with microbiota from MS-twin samples, compared with healthy-twin samples, suggesting a weakened regulatory mechanism in MS. The findings of Berer et al[21] suggest that particular elements of MS-derived microbiota can trigger MS-like autoimmune disease in a humanized transgenic mouse model, and support investigations into protective and pathogenic microbial factors in human MS.

Nowadays, few preliminary studies have investigated the impact of FMT in patients with MS. A case study[22] showed that treatment with FMT for constipation resolved the neurological symptom of three patients with MS for 2-15 years. Another patient, with RRMS, received FMT following neurological deterioration related to episodes of refractory *C. difficile* enterocolitis. Her Expanded Disability Status Scale (EDSS) immediately stabilized, without other treatment or lifestyle modifications. Her functional status improved over the subsequent 10 years of follow up[23]. Although the composition of the patient's microbiome prior to *C. difficile* infection or after FMT was not recorded, a potential role of FMT on the systemic immune system could be hypothesized.

Another patient[24] voluntarily underwent FMT, with her spouse as donor. FMT ameliorated deambulation, increased anti-inflammatory gut

bacteria, such as the butirrate producing Faecalibacterium prausnitzii, increased fecal SCFA, and serum Serum Brain-Derived-Neurotrophic-Factor (BDNF) levels. It is well known that BDNF is an important factor for neuronal survival and that it decreases in MS[25].

FMT therapy is not without safety concerns. In a systematic review[26] of 50 studies of FMT, predominantly for refractory *C. difficile* infection or inflammatory bowel disease (IBD), the overall incidence of adverse events was 28.5%, predominantly consisting of abdominal discomfort, transient fever, nausea and/or vomiting, or constipation. Furthermore, in patients with IBD, FMT could lead to disease flare, possibly related to unrecognized pathogens from the donor stool.

Thus, concerns regarding the safety of FMT remain to be addressed, including issues relating to the screening protocols for donor microbiota, preparation of the stool sample, optimal method of administration, the frequency of applications, antibiotic pretreatment, etc. The utility of FMT in MS has yet to be established. Several clinical trials are currently assessing its safety and efficacy in patients with RRMS (clinicalTrials.gov Identifiers: NCT03594487; NCT03183869).

Immunomodulatory Therapy

Despite the important role that the gut microbiome plays in immune function in disimmune disorders, such as MS, there are limited data on the effect of disease-modifying therapies (DMT) on MS treatment-associated taxa. Jangi et al[15] used gene sequencing to investigate the structure of the fecal microbiome in 60 subjects with MS and 43 healthy controls. They identified microbiome alterations in MS that included changes in a number of organisms known to drive inflammatory processes or that have been associated with autoimmunity. Specifically, increases of *Methanobrevibacter* and *Akkermansia* and decreases in *Butyricimonas* correlated with changes in the expression of genes involved in dendritic cell maturation, interferon (IFN) signaling and nuclear factor (NF)-κB signaling pathways in circulating T cells and monocytes[15]. Patients on disease-modifying treatment showed increased abundances of *Prevotella* and *Sutterella*, species found to be also increased in healthy controls, and decreased *Sarcina*, compared with untreated MS patients.

Two DMT, glatiramer acetate (GA) and dimethyl fumarate (DMF) were associated with differences in gut microbial composition in patients with MS compared to treatment-naïve patients. Both decreased relative abundance of the Lachnospiraceae and Veillonellaceae families. In addition, DMF was associated with decreased relative abundance of the phyla Firmicutes, Fusobacteria, the order Clostridiales. Moreover, it was observed an increase in the phylum Bacteroidetes[27].

Taken together, these studies suggest interesting relationships between gut microbiota and disease status in patients undergoing disease-modifying therapies. However, it remains unclear whether the observed alterations in microbiota composition were causal in the development of IBD and MS or a consequence of the disease pathogenesis.

Colon Hydrotherapy

Colon hydrotherapy (hydrocolontherapy; rectal lavage; colonic irrigation) consists of retrograde large bowel irrigation with warm water through a tube gently inserted into the ano-rectum. It has been shown to have a positive role in the treatment of neurogenic intestinal dysfunction, without the potential risk of side effects. Few authors support rectal irrigation[28-31], but it is not yet understood how the gut microbiota composition and homeostasis are affected by this procedure. It seems that in normal individuals, a high-volume polyethylene glycol bowel cleansing preparation has a long-lasting effect that may alter and distort bacterial homeostasis. For example, a decrease in the abundance of *Lactobacillaceae*, a population of protective bacteria has been noted in some studies[28,29], which may have negative implications for immune function. However, no relevant publications were identified for the use of colon hydrotherapy as a disease-modifying intervention in MS. Further studies are required to assess whether the reported changes for the treatment have any metabolic, immunological or clinical consequence in autoimmune disorders like MS.

Dietary Interventions

The species profile of the gut microbiome is shaped to a large extent by dietary intake; manipulation of the composition of nutrients and other components, the gut is exposed to, may modify the composition and function of the gut microbiome. The typical "Western diet" rich in processed foods, refined carbohydrates, saturated fats, red meat, and salt can contribute to inducing chronic inflammatory states[4]. A better understanding of the impact of dietary factors on the chronic inflammatory states of diseases, such as

MS could lead to the development of nutritional interventions. They can contribute to ameliorate some of the symptoms, to slow disease progression and ultimately to influence the course of MS by modulating the inflammatory status of the disease[4]. A survey[32] of MS patients showed that the large majority of them were interested in dietary modifications that might benefit the course of their disease, supporting the feasibility of trials of dietary interventions. Many of them are based on an oxidative stress, which is an important component of the inflammatory process in the disease states, leading to myelin degradation and axonal damage in MS[33]. It is unknown whether dietary antioxidants display other biological properties beyond simple antioxidant activity in MS. The role of antioxidants and vitamins, together with trace elements and minerals, has been the focus of considerable research over recent years. Findings in this field remain inconclusive, except for the presence of higher vitamin D concentrations, that appears to slow disease progression in MS[34]. Over the last decade, the role of a low-fat diet and supplementation with polyunsaturated fatty acids (PUFAs)[35,36], the role of minerals, antioxidants[33,35], and vitamins[34,35] on MS pathogenesis and clinical status has received increased attention, but it remains inconclusive. A Cochrane Collaboration systematic review[35] concluded that consistent evidence for benefits and risks of vitamin supplementation and antioxidants in MS is lacking.

A pilot study[37] investigated the effects of a calorie-restricted, semi-vegetarian diet and supplementation with vitamin D and other nutritional compounds (fish oil, lipoic acid, omega-3 PUFAs, resveratrol and multivitamin complex) in 33 patients with RRMS and 10 patients with primary-progressive MS. After 6 months, the markers of inflammatory status, such as serum gelatinase matrix metalloproteinase-9 levels and n-3/n-6 PUFA concentrations, improved in over half of the patients[37]. However, no significant changes in neurological signs were observed at this time, suggesting that a "short" duration of dietary intervention was insufficient to reveal any clinical outcomes on disease course. It is remarkable that levels of vitamin D were insufficient in all patients at baseline and continued to decline over time, despite supplementation. This study highlights that a "healthy" nutritional intervention can be well accepted in MS patients, and may ameliorate their inflammatory status.

Several critical points should be considered: how the gut microbiota can be characterized in the clinical setting, how a diet can be personalized or reproduced on the basis of microbiota composition, and how the effects on pathology and clinical outcomes of MS patients can be measured.

There is a high level of interest in the potential role of Mediterranean diet in MS community, but so far, evidence coming from randomized-controlled trial is scarce. A 6-month intervention trial[38] with 30 MS patients showed statistically significant reduction in the Neurological Fatigue Index-MS scores (p = 0.01) and in the EDSS (p = 0.01) in the intervention group. These preliminary data suggest the need of larger dietary intervention trials.

Nutraceuticals

A wide range of natural compounds, including various antioxidants, probiotics and biologicals, dietary fats, salmon proteoglycans, phytoestrogens, soybean components, trace elements, and vitamins, including vitamins D, A, and E have been proposed as components of disease-modifying nutraceutical formulations with potential anti-inflammatory activity. However, there is limited scientific evidence for the use of specific nutraceuticals in the management of MS. Many interventional trials have had contradictory results, perhaps related to a lack of well-designed and appropriately-controlled studies.

As an example, promising studies in experimental autoimmune encephalomyelitis (EAE), an animal model of Th1 and Th17-mediated MS, suggested that PUFAs may reduce markers of inflammation and modify the gut microflora in beneficial ways. Well-designed studies of omega-3 fatty acids (omega-3 FAs) supplementation in MS patients have not produced improvements in robust clinical outcomes[33,39,40]. It can be expected that the research for novel nutritional compounds with anti-inflammatory, immune-modifying and antioxidative properties which could modify the disease course in MS will continue. Nowadays there is a growing interest in compounds derived from scorpion toxins, able to block specific potassium channels of T cells, epigallocatechin-3-gallate, a green tea flavonoid with antioxidant properties, and other plants, such as curcumin, glycosides derived from mustard oil, and *Gingko biloba* extracts[33,39,40]. It is noteworthy the immunomodulatory value of probiotics, which may im-

prove the gut microbial balance, changing the composition of the microbiota and correcting dysbiosis[4,41].

The probiotic *Enterococcus faecium* L3 was comparable in immunomodulatory activity in EAE to glatiramer acetate. There was no protective action when L3 was administered simultaneously with glatiramer acetate, suggesting that different components of the immune system were being stimulated[41]. However, immune responses induced by probiotics in animal models of MS have not necessarily been beneficial[33] and, again, evidence from clinical trials in MS is limited. In a recent pilot study[42], administration of the probiotic VSL3, a mixture of 8 different bacteria, to MS patients increased the abundance of some taxa, predominantly *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* species[42]. An anti-inflammatory peripheral innate immune response was also observed. Among MS patients a probiotic containing Lactobacillus, Bifidobacterium, and Streptococcus decreased the abundance of dysbiosis associated taxa, and induced an anti-inflammatory peripheral immune response[43]. The therapeutic challenges of MS are unlikely to be solved by nutraceuticals. Moreover, the use of nutraceuticals as add-on interventions may lead at least to a better understanding of the associations between specific dietary components and disease activity. This may ultimately suggest therapeutic targets of interest.

Ketogenic Diet

The ketogenic diet (i.e., one poor in carbohydrates and rich in fat, especially saturated) has been proposed as a potential complementary treatment of progressive MS. In fact, *in vitro* and *in vivo* studies[44-46] suggest that neurodegeneration underlies the pathogenesis of progressive MS and that mitochondrial dysfunction may lead to reduced availability of adenosine triphosphate (ATP), thus lowering the effect of ATP in suppressing inflammation. In this regard, it has been shown that ketogenic diet increases ATP production and promotes mitochondrial biogenesis *in vitro* and in animal models, while bypassing dysfunctional steps within the mitochondrial bioenergetic process, increasing antioxidant levels and reducing oxidative damage[44]. Nothwithstandig, there are currently limited data supporting the benefits of ketogenic diet in progressive MS patients[45,46].

In one small study[46] in 25 MS patients, 10 of whom received a ketogenic diet for 6 months, and 14 healthy controls, a ketogenic diet normalized the stool microbiome after 6 months. At baseline, no microbiome pattern typical of MS was apparent in the MS patients, but concentrations and diversity of numerically substantial bio-fermentative bacteria (particularly *Roseburia*, *Bacteroides* and *F. prausnitzii*) were lower, compared with healthy controls ($p < 0.001$). In the patients receiving the ketogenic diet, bacterial concentrations initially decreased substantially, before starting to increase by week 12, reaching values typical of the healthy controls by 6 months[46].

Owing to the lack of consistent human data, the ketogenic diet deserves further investigations as a potential therapeutic intervention in MS.

Intermittent Fasting

Intermittent fasting (IF) is another approach to altering the clinical course of MS, that has been reported to increase gut bacteria diversity, enrich levels of the *Lactobacilli*, *Bacteroides*, and *Prevotella* genera and enhance antioxidative protective changes in gut microbiome metabolic pathways in animal models of MS[6]. In this study, the clinical course of EAE was less severe in mice on the IF regimen, accompanied by a reduction of inflammatory infiltrates and reduced demyelination in the spinal cord. The proportion of pro-inflammatory IL-17-producing T cells in the gut was reduced, whereas regulatory T cells were enhanced. FMT from mice in the IF group ameliorated EAE in naïve recipient mice on a normal diet, suggesting that modulation of the gut microbiome may play a mechanistic function in the beneficial effects related to IF.

Translating such findings to MS in humans requires caution, as the EAE model does not fully represent human MS. However, the results are of interest, and a small pilot study in MS patients confirmed that intermittent energy restriction over 2 study weeks altered blood adipokines and the gut flora resembling the protective changes, observed in the mice study[6].

The effects of IF on the gut microbiome are also corroborated by a controlled feeding study in which three different calorie-restricted diets (intermittent or continuous calorie restriction; weight-stable diet) were undertaken over 8 weeks by 36 people with MS. Changes in lipid metabolites related to neurological and immunological function were observed over time, accompanying weight loss[47]. These changes were similar to those after short-term aggressive calorie reduction (i.e., 25% *vs.* 100% calorie needs/day).

Fasting-mimicking Diet

A fasting-mimicking diet (FMD) consists of macronutrients and micronutrients with a very low calorie and low protein content but rich in unsaturated fats. This diet is useful to reduce the glucose and insulin-like growth factor 1 (IGF-1) levels and to increase ketone bodies. Compared to IF, a FMD may be more practical and easier to be adopted by patients. Periodic 3-day FMD cycles reduced the clinical severity and symptoms of EAE in a murine model of MS[48]. They were effective in ameliorating demyelination and in promoting oligodendrocyte precursor cell regeneration and axon remyelination. In addition, they lowered pro-inflammatory cytokines and antigen-presenting cells involved in activating T lymphocytes associated with immune dysfunction[48].

In a human clinical trial, patients with RRMS were randomized to a single 7 days cycle of FMD followed by a Mediterranean diet, a ketogenic diet, or a control diet for 6 months[48]. Preliminary data indicated that the FMD and ketogenic diets were safe, feasible, and potentially effective. Both dietary interventions improved health-related quality of life and reduced disability symptoms[48]. However, whether or not the FMD will reduce MS pathology in humans remains unclear.

Omega-3 and -6 Enriched Diet

As discussed before, a diet rich in processed foods, refined carbohydrates, saturated fats, red meat and salt (the "Western diet") can contribute to chronic inflammatory states[37]. Trying to avoid a high fat intake, it appears that the type of fatty acid may produce both beneficial as well as harmful effects, depending on aliphatic chain length[49]. The potential anti-inflammatory effects of PUFAs in MS and other autoimmune inflammatory disorders have attracted some interest, and there is evidence of a modest association between the consumption of low levels of unsaturated fat in the diet and an increased risk of MS[50]. Higher consumption of fish and omega-3 supplementation was linked to better quality of life and less disability in MS people surveyed in a large international study[51,52]. In these patients, flaxseed oil supplementation, a source of alfa-linoleic acid, was associated with a 52.6% relapse rate reduction at 12 months, in univariate analysis. Furthermore, omega-3 and omega-6 PUFA supplementation reduces immune-cell activation in *in vitro* and *in vivo* studies, with a number of complex metabolic pathways implicated[50]. Various clinical trials of PUFA supplementation in MS patients have, however, provided mixed results, possibly related to important design limitations and diverse selection of outcome measures. A previous Cochrane Collaboration review of dietary interventions for MS concluded that data from randomized controlled trials of PUFAs (omega-3 FA, omega-6 FA, and linoleic acid) did not support a significant effect for PUFAs on MS disease progression, either for chronic progressive MS or RRMS[35]. There were potential benefits in relapse outcomes associated with omega-6 FAs in some studies, but in general, no judgments about safety or patient-reported outcomes could be made. Available data were insufficient to assess any real benefit/harm from PUFA supplementation.

There is some evidence[53] for the immunomodulatory effects of omega-3 FAs on the pro-inflammatory cytokines, IL-1β, IL-2, TNF-α, and IFN-γ and on matrix metalloproteinase-9 (MMP-9) production by immune cells in MS. Serum pro-inflammatory cytokine levels and oxidative stress markers (TNF-α, IL-1β, IL-6, nitric oxide metabolites) were found to be reduced at 12 months in 50 patients with RRMS treated with fish oil supplementation, compared with placebo ($p \leq 0.001$)[54]. Although no differences in EDSS and relapse rates were observed in this study, another randomized placebo-controlled trial found that omega-3 FA and vitamin D co-supplementation improved EDSS and metabolic status of patients with MS[55]. Fifty-three patients, aged 18 to 55 years and matched for EDSS scores, gender, medications, BMI, and age were randomized to co-supplementation or placebo for 12 weeks. Patients taking omega-3 FA plus vitamin D had significant improvements in EDSS compared with placebo. Other oxidative stress markers were also significantly improved in the supplemented group, including serum high-sensitivity C-reactive protein (hs-CRP), plasma total antioxidant capacity, total glutathione, and malondialdehyde concentrations[55].

A recent review has suggested that omega-3 supplementations may have beneficial effects on reducing the relapsing rate, inflammatory markers, and ameliorating the quality of life for MS patients[56]. Further investigation is required to confirm the useful supplementation with PUFAs in patients with MS.

Short Chain-Fatty Acids

Short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate, are metabolites

produced by gut microbiota through fermentation of indigestible fibers, with important immunomodulatory properties. SCFAs supplementation in an animal model suppressed EAE in association with an increase of Tregs and a decrease in Th1. SCFAs reduction seems to have a causal role in MS pathogenesis[57]. In MS patients, SCFAs are reduced and branched SCFAs were inversely correlated with clinical disability[58].

Paleolithic Diet

The Paleolithic (Paleo) diet is intended to mimic the diet of the early hunter-scavengers and is a low processed, low-grain foods diet consisting primarily of game meats, green vegetables, and a high intake of PUFAs. A longitudinal study on 18 patients with progressive MS investigated the effect of a modified Paleolithic diet-based, multimodal, intervention on the associations of lipid profiles and fatigue over 12 months[59]. Fatigue, measured at baseline and every 3 months using a Fatigue Severity Scale (FSS), decreased from a mean FFS of 5.51 ± 1.31 at baseline to 3.03 ± 1.6 at 12-months. Improvements in FSS were associated with those in lipid profile (increased high-density lipoprotein cholesterol (HDL-C) and lower total cholesterol at 12 months. FSS improvements with the Paleo diet have also been seen in other small studies in patients with MS[60,61]. However, in the absence of additional studies, conclusions on the value of the Paleo diet in MS cannot be made.

Conclusions

There is a common belief that the human microbiota is relevant for the modulation of the immune system and, most likely, for the expression (onset) and reactivation of disease. It is possible that an increased diversity and different protective compositions of microbiota might be beneficial to different autoimmune diseases.

The high diversity of the microbiota may be of significance either in relation to anti-inflammatory patterns (mainly in patients with RRMS) or for patterns that strengthen the energy production with anti-degenerative effects (relevant in patients with progressive/degenerative course). The results appear generally more promising in the animal models, probably in relation to the greater "simplicity" and to the possibility of isolating a single factor or effect, with respect to the setting of human MS, which is enormously more complex and multifactorial[5].

Focusing research on probiotic organisms that produce beneficial metabolites or induce changes in diet or other environmental factors with subsequent dysbiosis can offer novel preventive and adjunctive treatments against many inflammatory autoimmune diseases. The types of diet that appear to have the main support in the scientific literature are those with the potential property of reducing oxidative stress, inflammation, and mitochondrial function. The advantage of these dietary interventions is that they are noninvasive, generally well-tolerated, and sometimes consistent with dietary recommendations (i.e., the Mediterranean diet) aimed at improving other aspects of physical health. Unfortunately, most of the cited studies in our revision are poorly designed (multiple arms, multiple interventions, without control groups), have used different methodologies to analyze microbiota and are usually performed on small groups of patients to reduce the ability to draw firm conclusions[7]. It is essential to identify a simple methodology for the analysis of microbiota, but with high sensitivity, so that the reproducibility of reported observations among different studies can be compared. In this way, microbiota changes can be easily detected in prospective studies. There is also a strong need to regularly screen the scientific literature on this topic since the volume of papers published each month makes continuous in-depth update essential to identify relevant advances and to maintain an updated knowledge on this challenging issue. Finally, it should be reiterated that, to date, despite the abundance of scientific evidence in favour of the involvement of the gut-brain axis in the pathogenesis of MS, the various diets and regimes to change microbiota composition and to ameliorate the disease status can not absolutely replace the conventional therapies and can be only considered complementary to established pharmacological treatments.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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