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HIGHLIGHTS

Basic

REVIEW

Diet-microbiome-immune interplay in multiple sclerosis: Understanding the impact of phytoestrogen metabolizing gut bacteria

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Multiple sclerosis (MS) is a chronic and progressive autoimmune disease of the central nervous system (CNS), with both genetic and environmental factors contributing to the pathobiology of the disease. Although HLA genes have emerged as the strongest genetic factor linked to MS, consensus on the environmental risk factors is lacking. Recently, the gut microbiota has garnered increasing attention as a potential environmental factor in MS, as mounting evidence suggests that individuals with MS exhibit microbial dysbiosis (changes in the gut microbiome). Thus, there has been a strong emphasis on understanding the role of the gut microbiome in the pathobiology of MS, specifically, factors regulating the gut microbiota and the mechanism(s) through which gut microbes may contribute to MS. Among all factors, diet has emerged to have the strongest influence on the composition and function of gut microbiota. As MS patients lack gut bacteria capable of metabolizing dietary phytoestrogen, we will specifically discuss the role of a phytoestrogen diet and phytoestrogen metabolizing gut bacteria in the pathobiology of MS. A better understanding of these mechanisms will help to harness the enormous potential of the gut microbiota as potential therapeutics to treat MS and other autoimmune diseases.

Keywords: Diet · Microbial dysbiosis · Microbiota · MS · Phytoestrogen

Introduction

Multiple sclerosis (MS) is a chronic, inflammatory demyelinating disease of the central nervous system (CNS) resulting from an immune-mediated attack on myelinated axons [1]. MS pathogenesis results from multifactorial etiology involving both genetic and environmental factors [2, 3]. However, studies of monozygotic twins have shown that genetic factors contribute only ∼30% of the disease risk [4], with the rest linked to environmental factors. Several environmental factors have been implicated in MS, including vitamin D deficiency, smoking, and exposure to certain infections, such as the EBV [3]. In recent years, the gut microbiota has emerged as an important environmental factor linked with the pathobiology of MS [5–11].

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Microorganisms inhabiting the gastrointestinal tract, collectively known as the "gut microbiota," have evolved with the host over time, forming an intimate and mutually advantageous relationship. The host provides space and nutrients to these microorganisms, whereas the gut microbiota helps with a number of host physiological processes such as the efficient digestion of food to extract energy, maintenance of gut barrier integrity, regulation of host immune responses, protection from pathogens, and so forth [12–15]. During homeostasis, there is a symbiotic relationship between host factors and the microbiota (eubiosis) that helps in maintaining a healthy state. However, alterations in gut microbiota composition and function can perturb this homeostasis, known as gut dysbiosis, which has been reported in multiple diseases, including MS.

Links between the gut microbiome and MS

Multiple studies, including those from our group, have shown that people with MS (PwMS) have microbial dysbiosis [5–11]. Specifically, PwMSs have distinct gut microbiome communities compared to healthy individuals, characterized by an increased abundance of *Pseudomonas, Mycoplasma, Haemophilus, Blautia*, and *Dorea* and a decreased abundance of *Parabacteroides*, *Prevotella, Adlercreutzia, Bacteroides, Faecalibacterium, Anaerostipes, Lachnospiracea*, *Butyricimonas, and Lactobacillus* [5–11]. A recent study from Denmark observed an enrichment of 31 different bacterial species, such as *Blautia, Ruminococcus, Bilophila, Sellimonas*, and so forth in PwMS, and these bacteria were linked to an increase in levels of inflammatory cytokines during disease activity [16]. Moreover, we and others have shown that gut resident fungi (mycobiome) are also modulated in PwMS [17, 18] characterized by a reduced abundance of Saccharomyces [17], a fungus with probiotic-like properties. Thus, results from multiple microbiome studies strengthen the idea that PwMSs have an altered microbiome compared to healthy controls with depletion/reduced abundance of beneficial gut bacteria/fungi.

Although cross-sectional studies have helped establish a link between gut microbiota and MS, the question remains whether gut microbiota contributes to disease or disease causes dysbiosis or both. A recent study from Denmark showed a direct correlation between species richness and the number of disease relapses [16]. The clinically nonactive (nonrelapsing) patients showed an enrichment of *Faecalibacterium prausnitzii*, *Gordonibacter urolithinfaciens, Anaerostipes hadrus*, *Gemmiger formicilis*, and *Roseburia inulinivorans* compared to clinically active patients (who had at least one relapse in follow-up period). The clinically active group showed significant enrichment of *Methanobrevibacter smithii* and *Victivallis vadensis*. Interestingly, bacterial species more abundant in clinically active treatment-naïve cases were positively associated with circulating levels of pro-inflammatory cytokines IL-22, IL-17A, IFN-β, IL-33, and TNF-α. Thus, this study provides data that gut microbiota may contribute to MS disease severity by inducing a pro-inflammatory environment.

As microbiome studies suggest an association between gut microbiota and MS, there has been a strong emphasis on understanding the factors affecting the gut microbiota and the mechanism(s) through which gut microbes contribute to MS disease. Therefore, in this review, we will discuss how diet may regulate gut microbiota and affect the pathobiology of MS, using phytoestrogen metabolizing gut bacteria as an example.

Environmental factors, gut microbiota, and MS

Several external factors, such as geographical location, sun exposure, hygiene, and dietary habits, can influence the composition and function of the gut microbiota. Among these factors, diet has emerged as one of the most decisive nonhost factors influencing gut microbiota composition [19, 20]. The human diet and gut microbiota have coevolved over millions of years, with changes in diet and lifestyle driving the evolution of the human gut microbiome. The diets of early humans were primarily composed of plant-based foods, such as fruits, vegetables, nuts, and seeds, and the occasional consumption of animal protein. A study comparing the microbiome of Hadza hunter-gatherers showed seasonal changes in the microbiome, especially wet season (increased plant food), characterized by the presence of bacteria linked with plant carbohydrate utilization [21]. In contrast, mucin/animal carbohydrate-utilizing bacteria dominated the dry-season (increased hunting) microbiome. With the advent of agriculture and the cultivation of grains, legumes, and other crops, humans began consuming more carbohydrates [21–25]. This change in diet favored the growth of bacteria that could break down these new sources of carbohydrates. However, the industrialization of food production has led to the widespread consumption of processed and high-fat/high-sugar foods and the use of antibiotics [24, 25]. This has contributed to decreased bacterial diversity and is associated with increased prevalence of diseases such as obesity, diabetes, inflammatory bowel disease, and MS. Interestingly, the microbiome of individuals from the USA showed a difference in mucin/animal carbohydrate utilization [21] and was similar to the dry season (meat-based diet) but significantly different from the wet-season microbiome of Hadza people, highlighting a loss of plant carbohydrate utilizing gut bacteria in industrialized nations. These evolutionary changes in the microbiome suggest that a diet promoting gut microbiota that utilizes plant carbohydrates might benefit the host, whereas a Western diet promoting mucin degrader/animal carbohydrate-utilizing bacteria might predispose/propagate inflammatory diseases such as MS [22, 24].

This hypothesis aligns with studies in PwMS and its animal model, experimental autoimmune encephalomyelitis (EAE), where a diet rich in fat or carbohydrates was linked with severe disease. Both obese individuals [26, 27] and PwMS [28–30] showed gut dysbiosis with an enrichment of the *Desulfovibrionaceae* family. Interestingly, mice on a high-fat diet showed enrichment of bacteria from the *Desulfovibrionaceae* family, specifically *Desulfovibrio piger* and *Bilophila wadsworthia*. Additionally, high-fat diet-induced obesity increased EAE disease severity in

animal models of MS [31–33]. Besides high fats, Western diets are also rich in sugar, especially high fructose syrup. On a fructose-rich diet, mice lost beneficial bacteria such as *Prevotella, Muribaculum*, and *Bifidobacterium* [34]. These fructose-rich diet mice also showed an enrichment of *Desulfovibrio, Collinsella, Olsenella*, and *Bacteroides* as well as increased frequency of immune cells with pro-inflammatory characteristics [34]. These findings reinforce evidence from extensive population studies suggesting that the Western diet can lead to obesity and gut dysbiosis, which are, in turn, associated with severe disease in PwMS.

In contrast to humans, whose digestive systems generate roughly 17 gastrointestinal enzymes primarily for breaking down starch, the gut microbiota has thousands of supplementary enzymes [35, 36]. This enables them to break down and ferment dietary polysaccharides into short-chain fatty acids and other metabolites the host can absorb. Thus, a plant diet rich in microbiota-accessible carbohydrates promote the growth of diverse beneficial gut microbiota linked with health. However, without plant-based carbohydrates, microbiota may utilize the gp-rich mucus layer as an alternative energy source. Only a select few gut bacteria, including *Bifidobacterium* and *Akkermansia*, have evolved the capacity to utilize mucin as a nutrient source [37–39]. As the mucosal layer and underlying epithelial cells play an important role in maintaining the barrier integrity [40], plant carbohydrate-deprived microbiota would gradually begin to consume this protective barrier, resulting in inflammation and/or heightened vulnerability to pathogens [41]. In contrast, a plant-based diet rich in fiber/fermentable carbohydrate, phytoestrogen, tryptophan, and their metabolites, such as short-chain fatty acid and equol, are linked with health. Thus, a well-balanced diet based on plant and unprocessed food can provide many health benefits by correcting gut dysbiosis. Multiple dietary regimens such as Mediterranean diet, Paleolithic diet, modified paleo diet (Wahls diet), ketogenic diet, Swank diet, McDougall diet, and fasting-mimicking diet are being explored as possible therapeutic strategies in PwMS [42–45]. However, the analysis of the gut microbiome in ongoing dietary intervention studies is needed to help establish whether these diets correct gut dysbiosis.

The gut bacteria-induced phytoestrogen metabolism and its impact on the regulation of host immunity

Phytoestrogens are plant-derived polyphenols with structural similarities to human estrogens and comprised several classes of chemical compounds, including isoflavones (soy) and lignans (flaxseed) [46, 47]. Humans do not have the capacity to metabolize phytoestrogen, but certain gut bacteria can metabolize them to produce metabolites such as S-equol (equol) from isoflavones, which shows significantly higher estrogenic activity than the original isoflavones [48]. Estrogens have also been shown to possess immunomodulatory properties [49–54] and ameliorate MS during pregnancy [55].

Several gut bacteria lacking in PwMS such as *Prevotella, Parabacteroides, Adlercreutzia*, and *Bifidobacterium* can metabolize dietary phytoestrogen. The significance of phytoestrogen and phytoestrogen metabolizing gut microbiota in disease protection was confirmed in EAE, as mice on a diet with isoflavones (a type of phytoestrogen) are protected from EAE [56]. Most importantly, disease protection depended on the presence of gut microbiota as antibiotic depletion of microbiota abrogated the disease protective effect, and supplementation with phytoestrogen metabolizing bacteria restored disease protective function [56]. Additionally, mice on an isoflavone diet showed a lower inflammatory response as measured by antigen-specific CD4 T-cell proliferation, pro-inflammatory cytokine production [56], and increased levels of sera metabolites linked with an anti-inflammatory nature such as polyunsaturated fatty acids [57]. Additionally, the ability of exogenous equol to protect mice on a phytoestrogen-free diet confirmed the importance of gut bacteria-induced phytoestrogen metabolism in the EAE disease protection [56].

Interestingly, the gut microbiome of mice on an isoflavone diet showed similarities with the microbiome of healthy controls, whereas the microbiome of mice on a phytoestrogen-free diet resembled the microbiome of PwMS characterized by a higher abundance of *Akkermansia muciniphila* [56]. Therefore, in a follow-up study [58], we tested whether switching the diet from a phytoestrogen-free diet to an isoflavone diet would restore gut microbial homeostasis. Four- to six-week-old mice on standard chow were switched to an isoflavone-diet or phytoestrogen-free diet, and after 6 weeks on special diets, isoflavone-diet mice were switched to a phytoestrogen-free diet, and vice versa. We observed that the change from an isoflavone diet to a phytoestrogen-free diet reduced the overall richness and evenness of the microbial communities. However, a significant change in richness and evenness of the gut microbiota was not detected when the diet was changed from phytoestrogen-free to isoflavone-diet, suggesting that the absence of phytoestrogen strongly impacts the diversity of the gut microbiota.

Although the *A. muciniphila* levels did not change when switching diets, the change in diet from an isoflavone diet to a phytoestrogen-free diet significantly reduced the abundance of *Bifidobacterium* species, specifically *Bifidobacterium adolescentis* and *Bifidobacterium longum* [58]. As *Bifidobacterium* is designated as beneficial "probiotic" species [59–62], losing these species after the diet change to phytoestrogen-free indicates overall deterioration of gut microbiome homeostasis. Interestingly, the phytoestrogen metabolizing genes and equol levels were lost within a week after switching the diet from an isoflavone diet to a phytoestrogen-free diet [58]. In contrast, when switching the diet from a phytoestrogen-free to an isoflavone diet, the phytoestrogen metabolizing genes and functions were restored after only 28 days of diet switch [58]. Thus, switching to a phytoestrogen-free diet is associated with an inflammatory phenotype characterized by the loss of beneficial bacteria, especially *Bifidobacterium* species, without changes in the abundance of *A. muciniphila*. These data highlight the importance of diet in influencing the composition of gut microbiota. The next logical question is how these isoflavone-diet-induced changes in the composition of gut microbiota affect inflammation and disease.

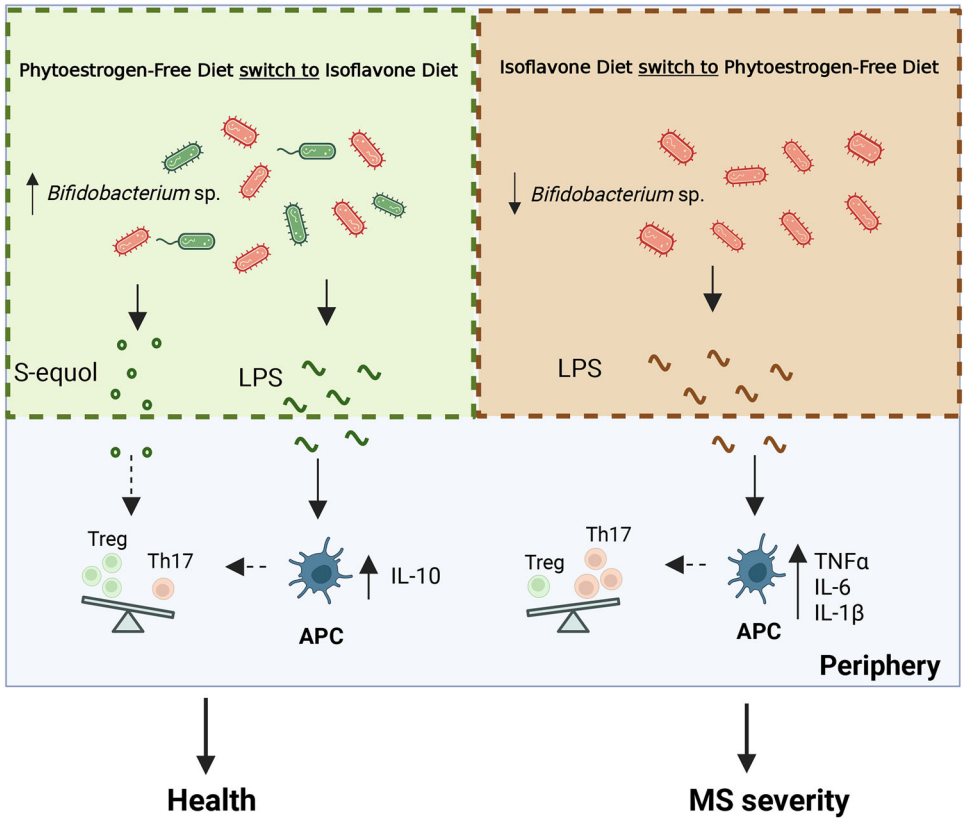

Figure 1. Diet regulates host immune response by regulating gut microbiota and their metabolites. A healthy diet such as isoflavone promotes a diverse and balanced gut microbiota that can maintain a healthy state by inducing immunoregulatory cytokines and cells. In contrast, the lack of isoflavones in diet induces a dysbiotic gut microbiota characterized by the loss of beneficial gut bacteria and the acquisition of immunostimulatory bacterial molecules such as modified LPS by symbionts to become pathobiont. This dysbiotic gut microbiota can predispose or propagate the disease by inducing a pro-inflammatory response. Thus, the ratio between symbionts and pathobionts can determine the disease severity outcome through fine-tuning bacterial LPS. *Source*: Created with BioRender.com.

Dietary change alters gut microbial lipopolysaccharide biosynthesis and modulates pro- and anti-inflammatory cytokine production

Analysis of bacterial functional pathways between the two diet groups showed a reduction in glycosyltransferase activity and an increase in polysaccharide lyase when the diet was changed from isoflavone to phytoestrogen-free diet [58]. Both the glycosyltransferase and polysaccharide lyase family of enzymes are involved in lipopolysaccharide (LPS) synthesis pathways, implying that isoflavone dietary conditions may contribute to the formation of structurally different LPSs compared to a phytoestrogen-free diet. Interestingly, the LPS derived from the feces of mice kept on the phytoestrogen-free diet induced significantly higher levels of TNF-α, IL-1β, IL-6, IL-12/23, and CXCL1 compared to the LPS derived from feces of mice kept on the isoflavone-diet. On the other hand, IL-10 production was significantly higher in the mice kept on an isoflavone diet suggesting an immunoregulatory effect. Higher levels of pro-inflammatory cytokines TNF-α, IL-6, IL-1β, and IL-12/23 and lower levels of anti-inflammatory/immunoregulatory IL-10 indicate that the phytoestrogen-free diet may contribute to a pro-inflammatory effect in the gut. Finally, mice switched from an isoflavone diet to a phytoestrogen-free diet showed increased EAE severity compared to mice switched from a phytoestrogen-free to an isoflavone diet [58]. Thus, an isoflavone-rich diet may protect from EAE by the induction of an anti-inflammatory response through the enrichment of beneficial bacteria, equol production, and modulation of LPS biosynthesis pathways.

Interestingly, these data point toward a potential mechanism where the pro-inflammatory nature of a potential pathobiont such as *A. muciniphila* is determined by the presence or absence of other beneficial bacteria such as *Bifidobacterium spp*., modulation of LPS biosynthesis, and immunostimulatory capacity (Fig. 1). This is an important finding regarding the symbiont versus pathobiont role of *A. muciniphila* and might explain the conflicting role *of A. muciniphila* reported in MS versus obesity. Although multiple studies, including the recent iMSMS study [63], showed an enrichment of *A. muciniphila* in PwMS, *A. muciniphila* has also been shown to be a beneficial bacterium in obesity [64, 65]. Interestingly, most *Bifidobacterium spp*. metabolize plant carbohydrates as a main food source, but some, including *B. adolescentis*, can also utilize mucin as a food source [39, 66, 67]. In contrast, *A. muciniphila* is known for its ability to degrade and consume

the mucin layer of the gut, but sometimes it can also metabolize other nutrients, including plant carbohydrates [68]. Thus, a complex interaction among microbial community members foraging on similar food sources may influence the symbiont versus pathobiont nature of specific bacterial strains. This might also explain the findings where the disease-protective effect of miR-30D was linked with the expansion of *A. muciniphila* [69]. Based on our data, we hypothesize that the pathogenicity of *A. muciniphila* is determined by multiple factors, including diet and the absence of beneficial bacteria such as *Bifidobacterium* species, including *B. adolescentis and B. longum*.

Open questions and future research directions

Several challenges remain in understanding how diet-microbiota interactions, especially the role of phytoestrogen-metabolizing gut bacteria, regulate the pathobiology of MS. Here, we identify three key questions for the field to consider on this topic that apply to all studies focused on determining the mechanism through which diet-microbiota interactions influence health and disease. First, what dietary factors influence gut microbiome composition, particularly the balance between symbionts (gut bacteria linked with a healthy state) and pathobionts (bacteria linked to a disease state)? Second, how does the gut microbiota impact the host's balance of immunoregulatory and pro-inflammatory responses? Third, can a beneficial diet alone or in combination with symbionts correct gut dysbiosis in PwMS?

Potential approaches to answer these open questions include combining in vitro experiments utilizing human biospecimens and in vivo studies using mouse models. To understand the dietary and microbial factors regulating symbiont or pathobiont characteristics, *A. muciniphila* can be used as a model organism. *A. Muciniphila* may behave differently in the context of an isoflavone-rich diet (anti-inflammatory) compared to an isoflavone-depleted diet (pro-inflammatory). To identify the microbial factors regulating the symbiont versus pathobiont balance, germ-free mice can be colonized with *A. muciniphila* and a second bacterial species, such as one from the genus *Bifidobacterium*, to examine how these two bacteria influence isoflavone-dependent immunomodulatory activity and disease outcomes. Germ-free mice can also be colonized with complex microbiomes from either mice or humans that are naturally deficient in *A. muciniphila* and *Bifidobacterium* species [70, 71]. Such mice can then be colonized with either *A. muciniphila* alone, *Bifidobacterium* alone, or the combination of both and then fed isoflavone-containing diets to systematically identify the diet-microbe interactions influencing immune responses and EAE severity. To determine the specific gut bacterial components that may regulate immune responses during isoflavone consumption, studies should examine the importance of various gut bacteria-derived PAMPs, including LPS, in the induction of pro-inflammatory cytokines. Such approaches could also allow further investigation into how anti-inflammatory properties are abrogated when feeding an isoflavone-rich diet and whether LPSs from specific gut microbiota members have high versus low inflammatory capacities that modulate EAE in the presence/absence of isoflavones. Taken together, these approaches and others can improve our understanding of how gut bacterial metabolism of dietary isoflavones promotes a disease-resistant state with a healthy symbiont/pathobiont balance and whether the pathogenic features of a phytoestrogen-free diet are caused by a pro-inflammatory ecology. Similar approaches can be applied to other diet-microbiome studies to dissect the mechanisms through which interactions among symbionts, pathobionts, and diet affect disease pathology in MS as well as other inflammatory diseases such as rheumatoid arthritis, colitis, and atherosclerosis.

Conclusion

The gut microbiome is known to influence the pathogenesis of many human diseases, and there is much increasing evidence supporting gut microbiota alterations in PwMS. Thus, targeted interventions to modulate gut microbiota toward a "healthier" composition remain an attractive potential therapy. However, defining what constitutes a "healthy" microbiome with the potential to suppress inflammation and MS remains a challenge. Future studies on understanding the dynamics among different gut bacteria modulated in PwMS will help define a disease-modulating gut microbiome. This will also help in defining whether diet plus microbiota (synbiotics) might be a better approach than bacteria (probiotics) or diet (prebiotics) alone as a treatment option for PwMS.

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Conflict of interest: AKM is one of the inventors of a technology claiming the use of *Prevotella histicola* to treat autoimmune diseases. AKM received royalties from Mayo Clinic (paid by Evelo Biosciences). However, no fund or product from the patent was used in the present study. All other authors declare no commercial or financial relationships that could be a potential conflict of interest.

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Abbreviation: **PwMS**: people with MS

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