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Review Articles

Effects of Gut Microbiome on the Nervous and Immune Systems – a Novel Concept of Gut-Brain Axis in Multiple Sclerosis

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The gut and brain form the gut-brain axis through bidirectional nervous, endocrine, and immune communications. Disorders in the composition and quantity of gut microorganisms can eventually affect both enteric nervous system and central nervous system (CNS), thereby confirming the possible existence of a microbiota-gut-brain axis. Due to the intricate interactions between the gut and the brain, gut symbiotic microorganisms are closely associated with various CNS diseases, such as Parkinson's disease, Alzheimer's disease, chronic fatigue syndrome and irritable bowel syndrome, schizophrenia, autism and multiple sclerosis (MS). Alterations in gut microbiota composition in MS patients under long-term drug treatment have been established. Increased levels of *Bacteroidaceae*, *Faecalibacterium*, *Ruminococcus*, *Lactobacillaceae*, *Clostridium*, and other members of the class *Clostridiales* have been assessed compared to untreated MS individuals. Our understanding is that neuroinflammation might be initiated by gastrointestinal tract (GIT) infections in which the GIT immune system is implicated.

Key words: microbiome-gut-brain axis, neuroinflammation, nervous system diseases, multiple sclerosis

Introduction

The importance of the gut microbiome for the pathogenesis of multiple sclerosis (MS) has been established, although the underlying signaling mechanisms of this relationship have not been studied yet. Many authors suggest that serotonin, a microbial modulated

neurotransmitter (NT) could be a potent candidate for mediator of the bowel and brain in demyelinating disorders [29]. It has been proved that serotonin levels in the gut are controlled by the microbiome, by both secretion and regulation of metabolites. In addition, gut microbiome is supposed to influence the formation of the serotonergic system in the brain. More frequent spread of depression and euphoria is observed in patients with MS. Changes in the serotonergic system have been suggested to be related with the directly altered MS expression of serotonin carriers in the central nervous system (CNS). Serotonin modulating drugs exert indirect beneficial effects in the course of the disease. The role of other microbiome-modulated NTs as γ-aminobutyric acid (GABA) and dopamine in MS has also been discussed. New direction for future research aimed at linking microbiome-regulated NTs to demyelinating disorders is needed [29].

Multiple sclerosis. First described by the French Professor Jean-Martin Charcot in 1868, MS has recently been determined as the primary demyelinating disease with significant loss of myelin and relatively preserved neurons and axons. MS is the most common autoimmune inflammatory neurodegenerative disease of the CNS, characterized by demyelination, neuroaxonal loss and a heterogeneous clinical course [10]. Neuronal and axonal damages have been shown to go along with the demyelination. A person with MS can have almost any neurological symptom, with autonomic, visual, motor, and sensory problems being the most common [8]. In 85% of the patients the disorder is in relapsing-remitting (RRMS) form [15].

There are various methods of treating MS by administering drugs depending on the form and phase of the disease. If the diagnosis and initiation of neuroprotective therapy are timely, the effectiveness of the treatment is more successful. RRMS treatment is expensive and long-lasting, but it is effective in large number of young patients [40]. According to recent studies over 60% of MS patients use complementary and alternative medicine (diet, gymnastics, bee sting in acupuncture points) as helping methods to the conventional treatments. The future of MS treatment should be aimed at combining anti-inflammatory agent and such with a neuroprotective effects. The goal is to prevent demyelination and to reduce axonal loss [25]. It is especially important for young MS patients to lead a normal lifestyle, to work, to have families and children. Any supportive therapy that improves quality of life is of great importance.

In recent years understanding of treatment goals has been changed. The concept of "no evidence of disease activity" (NEDA) has become attractive, not only in the assessment of clinical trial data, but also as a treatment target in clinical practice. This concept focuses on clinical and Magnetic Resonance Imaging (MRI) measures of disease activity. It also reflects patient-reported outcomes - progression of symptoms, adverse effects of treatment, and inability to tolerate injections, which may lead to a switch in treatments. The increasing number of highly active treatments becoming available raises the possibility of treatment election when necessary. That is why truly achieving NEDA will require the development of agents that directly target mechanisms of disease progression. Furthermore, the next revolution in MS therapeutics is remyelination. Such strategies will likely warrant rationally designed combination therapy approaches to both prevent further disease activity and push central nervous system repair [15].

Multiple human epidemiological studies have revealed the effects of environmental factors on the prevalence of MS and demonstrated that viral infections, lack of sun exposure, vitamin D and vitamin A deficiency, active or passive smoking, season of birth, obesity, dietary habits (especially high levels of salt and fat), stress and the intestinal microflora play a significant role in the initiation of the disease. Epstein–Barr virus (EBV) is a widely recognized risk factor. A seroepidemiological study of MS found that nearly 100% of the patients were infected with EBV. If EBV infection occurs in late childhood, it is considered the largest risk factor for the development of MS.

There is a strong EBV-specific CD8+ response in the blood during the onset of MS; the intensity of the response decreases during the course of the illness [1].

Nowdays, it is accepted that MS is a CD4+ T helper (Th) 1-mediated autoimmune disease; however, CD8+ T cells may be involved in the pathogenesis as they are the predominant lymphocyte found in MS lesions. The cells that form myelin in the CNS (oligodendrocytes) are thought to be the main target for attack in MS, and the inflammatory demyelinating lesions characteristic of MS can occur in the optic nerve, brainstem, spinal cord and periventricular white matter [12, 15]. MS has also been suggested to arise from a negative interaction between genetic and environmental factors. A large number of genes promote autoimmune response against brain cells [8, 40]. New experimental and clinical studies indicate that autoimmune attacks are triggered by interaction between brain immune cells and gut microbiota. The pathophysiology of MS involves several aspects of abnormalities: redox, inflammatory/autoimmune, vascular, neuroendocrine and neurodegenerative [15].

Life with MS. Patients with MS are often best cared for in multidisciplinary approach [23, 24]. The diet has been associated with improvements in the majority of the population in terms of mood, fatigue and cognitive impairment - all issues that factor significantly in MS. Such results, particularly concerning mood modification, are reported in a study on a Mediterranean-style diet [12].

Microbiome. Commensal and symbiotic microbes densely populate the human body, the majority of which being bacteria. These microbes occupy different habitats such as gut, skin, vagina and oral cavity. The types and abundance of microbes differ not only in various organs, but also in the different individuals. The genome of these microorganisms and their ecosystems constitute the microbiome. Factors such as diet, environment, host genetics and way of birth may be factors influencing the wide microbial diversity [11]. Diet and gut microbiota composition are probably related to the leaky gut phenomenon [1, 20].

Probiotics and Prebiotics. About 100 000 billion bacteria are present in the human intestines [21, 32]. The beneficial and harmful bacteria in the gut are in balance.

Probiotics are living bacteria in the group of good ones – they maintain the electrolyte balance in the body and the good microflora in the gut [21, 32, 33].

Prebiotics are indigestible dietary fibers that stimulate the growth of good bacteria inhabiting the colon. They pass through the gastrointestinal tract unchanged and stimulate the growth and development of beneficial bacteria in the gut. Prebiotics are resistant to heat amplitudes and the increased stomach acidity, i.e. they are the perfect food for the existence and growth of beneficial gut microorganisms [32]. Probiotics and prebiotics play key role in prevention and neutralization of the harmfull effects of many pathogenic bacteria as *Helicobacter pylory* and *Escherichia coli*.

Gut-brain axis. Hippocrates has wisely remarked: “all diseases begin in the gut” and “death sits in the bowel”, thereby creating the hypothesis that gut is responsible for many disorders including neurodegenerative diseases [27]. Nowadays, the main concept of the gut-brain axis represents a major shift of our understanding about the brain health [30]. Gastrointestinal tract (GIT) infections could be initiated by neuroinflammation in which the GIT immune system is implicated.

Microbiome is shaped by host factors such as genetics and nutrients but in turn is able to influence host biology in health and disease. Gut microbiome is probably plaing a crucial role in the bidirectional gut-brain axis that integrates both gut and CNS activities, and thus the concept of microbiome-gut-brain axis has emerged. Many studies reveal how diverse forms of neuro-immune and neuro-psychiatric disorders are correlated with or modulated by variations of microbiome, microbiota-derived products

as well as antibiotics and probiotics. The microbiome probably poises the peripheral immune homeostasis and predisposes the host susceptibility to CNS autoimmune diseases such as MS [39].

Larger studies are necessary to investigate the changes within the gut microbiome and MS, in order to find potential disease biomarkers and therapies. These findings might contribute to development of new therapeutic strategies that modulate gut microbiota.

Therefore, the aim of the current review article is to summarize the available literature data about the impact of microbiome on MS development.

How does the microbiome influence the MS? GIT is a point of combined influence of the body's largest concentration of immune cells, a vast network of 500 million neurons and the gut microbiota. It also serves as a primary barrier between the outside world of potential pathogens and the internal environment of the body [19]. This interaction is constituted as early as the postnatal period of life. The postnatal period is a critical developmental phase of the GIT. Various GI functions, such as intestinal epithelial barrier (IEB) role and motility undergo profound changes during this period.

The human gut microbiome may influence human brain health by several different mechanisms. Structural components of bacterial cell wall such as lipopolysaccharides provide low-grade tonic stimulation of the innate immune system. Excessive stimulation due to bacterial dysbiosis, small intestinal bacterial overgrowth, or increased intestinal permeability may lead to inflammation in the CNS. Dysfunctional responses of the adaptive immunity by cross-reaction with human antigens could be caused by bacterial proteins. The functions of different bacterial enzymes may produce neurotoxic metabolites as D-lactic acid and ammonia. [14]. Some beneficial metabolites as short-chain fatty acids may also cause neurotoxic effects. Production of neurotransmitters and other hormones from some gut microbes are identical or similar to the produced by humans. In this way bacterial receptors for these substances may influence microbial growth and virulence. Direct stimulation of afferent neurons in the enteric nervous system by gut bacteria makes them able to send signals to the brain via the *vagus nerve.* Through these different pathways gut microbes shape the architecture of sleep and stress reactivity of the hypothalamic-pituitary-adrenal axis. In such a way these microorganisms influence the memory, mood and cognition. Thus, they are clinically and therapeutically relevant to a variety of disorders, including alcoholism, chronic fatigue syndrome, fibromyalgia and restless legs syndrome [14].

The bidirectional communication via the gut-brain axis is suggested to play a substantial role in neurologic diseases, including anxiety, depression, autism, MS, Parkinson's disease (PD), Alzheimer's disease (AD), etc. [9]. Although subtle differences in the exact composition of gut microflora have been found within the patient versus control populations (as would be expected considering the differences in geographical location), the overwhelming conclusion is that, indeed, microbial dysbiosis is present in the intestine of MS patients [7]. The intestinal microflora in MS patients has greater inter-individual variability than that of healthy controls. A decrease in the percentage of several *Bacteroides* species, including *B. stercoris*, *B. coprocola,* and *B. coprophilus* in the intestinal microflora in patients with MS has been shown [1]. Significant differences in microbiota structure between patients with MS and healthy controls have been found in a cohort of 31 patients with RRMS compared with 36 healthy controls. According to several studies, MS patients have distinct microbial profile compared to healthy controls. Increased abundance of *Pseudomonas*, *Mycoplana*, *Haemophilus*, *Blautia*, and *Dorea* genera in MS patients have been established, whereas control group has shown increased abundance of *Parabacteroides*, *Adlercreutzia* and *Prevotella* genera [6, 19, 30].

This is consistent with the hypothesis that MS patients have gut microbial dysbiosis and further study is needed to better understand their role in the etiopathogenesis of

MS [6]. However, there was no difference in overall species richness (α-diversity) between healthy controls and MS patients but within the MS patient cohort there was a trend toward reduced species richness in individuals with active disease whereas those in remission were similar to the healthy controls. Such changes in α-diversity could suggest a role of the gut microbiome in disease exacerbation but future longitudinal studies are necessary to establish correlation [7].

Besides the microorganisms, other environmental factors as toxins might start a pathological process within enteric nerve cell plexus, causing mucosal inflammation and oxidative stress. In this way gut leakiness could be initiated which could disrupt the integrity of the blood brain barrier (BBB) and thus increase its permeability [1]. Numerous data are emerging on the effect of gut microflora on the immune system in human and mouse models of MS. According to recent studies there are data of probiotic influence on stress markers and immunoregulatory responses on healthy volunteers both *in vivo* and *in vitro*. In many animal models and in human studies the probiotics have been found to influence the intestinal microbiota and immunity, and in this way to stabilize the mucosal barrier. It has recently been shown that probiotics influence the 'gut-brain' axis modeling the behavior in the way that significantly reduces anxiety, depression, and stress [35]. There is evidence that epsilon toxin causes BBB permeability and in this way kills the brain myelin producing cells oligodendrocytes - the same cells that die in MS lesions [28, 38]. Epsilon toxin may be responsible for triggering MS. It is produced by certain strains of *Clostridium perfringens*, a spore-forming bacterium that is one of the most common causes of foodborne illness in the United States [1]. *C. perfringens* is a spore-forming gram-positive bacterium found in many environmental sources as well as in the intestines of humans and animals. It prefers to grow in conditions with very little or no oxygen, and under ideal conditions can multiply very rapidly. There is a relation between the GIT and physiological stress expressed by the levels of cortisol and immunoglobulin A (IgA) in the saliva in patients with MS [1, 38].

Typical microbial agents associated with some neurodegenerative autoimmune disorders in humans. Prevalence of Haptoglobin I (88%) in patients with Alzheimer Disease and 46.7% in controls both infected with *H. pylori* have been established [27]. Probably this is the main reason for the assessed iron deficiency in patients with autism. Very strong correlation of *Desulfovibrio* with the severity of autism manifestations has been noted, represented by the Autism Spectrum Disorders restricted/repetitive behavior subscale score [35]. Toxins B and D from *C. perfringens* have also been suggested to influence the neurological symptoms in MS patients [1]. A lot of alterations in gut microflora composition have been observed in patients with MS. Increased levels of *Methanobrevibacter* and *Akkermansia* have been assessed but decreased amounts of *Butyricimonas* and *Lachnospiraceae* have been found [17, 18]. Alterations in gut microflora composition in MS patients under treatment with glatiramer acetate (long-term drug) have been noted. There are increased levels of *Bacteroidaceae*, *Faecalibacterium*, *Ruminococcus*, *Lactobacillaceae*, *Clostridium*, and other members of the class *Clostridiales* compared to untreated MS patients [1]. Epsilon Toxin from *C. perfringens* has been determined as a candidate-trigger for new lesion formation in MS [38]. *Campylobacter jejuni* has been found in 154 patients with Guillain-Barre Syndrome (GBS), 63 patients with other neurological diseases, and 50 normal controls [16]. Using quantitative PCR, alterations in gut microflora composition have been found in patients with PD compared to normal controls. Decreased levels of *Bacteroidetes* and *Prevotella* and increased amounts of *Enterobacteriaceae* in PD have been assessed [36]. Additionally, fecal short chain fatty acids concentrations were significantly reduced in PD patients compared to controls.

Effects of probiotics/prebiotics on the nervous and immune systems in healthy volunteers. Significantly decreased levels of cortisol in saliva in administration of *Lactobacillus plantarum* 299v [4] and in urine – in administration of strains *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 [31] have been observed, compared to placebo treated individuals. According to other studies, attenuated increases in cortisol levels and subjective anxiety in response to acute stressor have been observed in application of *B. longum* 1714 [3]. No overall effect has been noted in treatment with *Lactobacillus rhamnosus* (JB-1) [22]. In another group of healthy controls in administration of *Bifidobacterium infantis* increased secretion of IL-10 and enhanced *Foxp3* expression in peripheral blood has been assessed [26]. This gene codes protein FOXP3 (forkhead box P3) also known as scurfin, which is involved in immune system responses. It appears to function as a master regulator of the regulatory pathway in the development and function of regulatory T cells (T_{regs}), which generally turn the immune response down [26]. The same authors have established activated immunoregulatory responses on dendritic cells from healthy donors in *in vitro* studies. Decreased serum concentrations of the inflammatory markers CRP, IL- 1β, and TNF-α have been noted in application of *Lactobacillus salivarius* UBL S22 fructooligosaccharides (FOS). In *in vitro* study of whole blood from healthy human individuals appearance of Zwitterionic polysaccharides, up-regulated immunologic synapse components, as well as, activated induction on $CD39^{+}Foxp3^{+}$, IL-10, IFNγ and increased frequency of CD39-expressing T_{regs} have been observed compared to patients with gastro-intestinal problems [34]. One of two prebiotics (FOS or Bimuno®-galactooligosaccharides – B-GOS) has led to decreased salivary cortisol awakening response after B-GOS intake. However, no effects after administration of FOS have been shown [32]. According to many authors in treatment of children with autism by *Lactobacillus acidophilus*, *L. rhamnosus* and *L. acidophilus* strain *Rosell*-11 have been noted decreased body weight and BMI as well as decreased severity in administration together with *B. longum* [33, 35]. Probiotic capsule significantly improves ability of concentration and carrying out orders in autistic children [21].

Probiotics/prebiotics therapeutic strategies in MS. Treatment paradigms based on the hygiene hypothesis include probiotic strategies, that is directed to administration of non-pathogenic live microorganisms which, when given in adequate amounts, confer a health benefit on the host. There are statistically significant differences in the microbiome composition of MS patients versus healthy volunteers. Many experiments of probiotic/prebiotic treatment were carried out on animal models and the reported effects are:

– Increased effectiveness in combined treated stress-induced visceral pain than with single probiotic;
– Less stress-induced anxiety-like behaviour and prevented deficits in social interaction with conspecifics are available;
– Low intestinal permeability *in vivo* under basal conditions and in response to MS or WAS (water avoidance stress);
– Attenuated rise of corticosterone levels in response to MS or WAS by the probiotic;
– Increased IFN-γ levels;
– Higher locomotion and exploratory behavior;
– No alterations in the development of aggressor avoidance following social defeat [2, 5, 37].

The role of microbe-based interventions in stress-related disorders has been shown in mouse model [5]. Usability of *Lactobacillus fermentum* as a novel tool for the prevention

and/or treatment of gastrointestinal disorders associated with altered intestinal epithelial barrier (IEB) functions in the newborn rats has been suggested [37]. In patients with MS the combination of *L. helveticus* and *B. longum* has been more effective in regulating glucocorticoid negative feedback on the HPA axis than probiotic alone [2].

Fecal microbiota transplantation has a 1700-year history [13]. This forgotten treatment method has been put into use again during the last 50 years. There are case reports that it is effective in the treatment of MS, and also of autism, PD, chronic fatigue syndrome and irritable bowel syndrome [13].

As the etiology and pathogenesis of MS are not fully understood, an individual approach is needed. Since it is an autoimmune disease, it is of great importance to reduce the immune system stimuli. For all patients, an appropriate diet, clean water consumption, rest and a longer stay in clean air, providing a suitable lifestyle, is important [23, 24, 25].

Conclusions

The type of diet has been characterized as critically important for patients with MS. From this point of view, the microbial-related therapies can be suggested as supportive treatment in parallel with the accepted medical protocol. As far as the etiology and the pathogenesis of MS are still unknown, it would be reasonable to forward the research in the direction of the gut-brain axis. If such clue is proved, a new, probiotic approach could be developed in addition to the unified MS protocol. Having in mind the positive influence of probiotics on MS aspects of human health, such approach might improve the quality of life of patients with this disorder.

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