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Review paper

Gut microbiota modification as an option in multiple sclerosis management

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Abstract

Introduction: Multiple sclerosis (MS) is caused by the abnormal activity of the immune system. It is believed that the pathological immune response may be initiated in the intestines, the area of the largest antigen presentation. This is where autoreactive T and B cells are activated, which constitutes the pathomechanism of this disease. In a healthy organism, normal gut microbiota mediates the balance between pro- and anti-inflammatory activity of the immune system.

Aim: This paper aims at describing the healthy gut microbiota, its changes in MS patients, factors that influence its composition and therapeutic corrective possibilities.

Material and methods: The paper is based on available medical literature.

Results and discussion: It has been evidenced that in MS patients the gut microbiota is dominated by pro-inflammatory species. This may be caused by environmental factors, for instance, the diet, antibiotics or stimulants. Methods of the microbiota correction involve dietary change, prebiotics and probiotics as well as fecal microbiota transplantation (FMT). FMT is a particularly safe and promising method that has proven its efficiency on an animal model of MS.

Conclusions: Experimental research has revealed that the correction of the gut microbiota may lead to MS remission or alleviation. FMT utilized in inflammatory bowel disease seems to be presently the most comprehensive intervention. Since only incidental reports of its efficiency in humans are presently available, further clinical studies are necessary.

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1. INTRODUCTION

In multiple sclerosis (MS) the myelin that covers axons is destroyed by the host immune system, consequently leading to neurological deficit. It is known that autoreactive T helper cells (Th cells) are activated in MS.[1] Their population is controlled by the thymus that eliminates them and the activity of the remaining ones (and additionally of autoreactive B cells) is inhibited, among others, by regulatory T cells (T_{regs}).[2] In the case of impaired control, autoreactive lymphocytes differentiate into Th1 and Th17 cells, which, having been activated by an antigen similar to the myelin protein, penetrate into the central nervous system (CNS) thus initiating the inflammatory process.[3] Mucosal-associated invariant T cells (MAIT), that is invariant T cells enriched at mucosal sites and found in the bronchi and gastrointestinal tract, TT CD8+ cells, macrophages, and memory B cells are also involved in the mutual inflammatory response. Additionally, within the CNS the inflammatory process is supported by activated microglia and astrocytes.[2] The reasons why immune response cells destroy its myelin are still unclear. It is believed that genetic factors, environmental factors, lifestyle and pathogens contribute to the risk of MS and affect its course.[4]

The intestine constitutes the region with the largest concentration of immune cells in the organism. This is where the immune system comes into contact with both external antigens and the gut microbiota.[5] The intestine mediates the desired microbiota composition by, among others, the production of IgA – a strong promoter of commensal bacteria within the intestinal lumen; additionally, intestinal cells produce antibodies against specific bacteria species or metabolism products.[6] Recently, it has been discovered that also colonocytes promote the desired anaerobic bacteria via regulating the amount of oxygen in the gastrointestinal tract.[7] An antimicrobial activity is also realized via α-defensins, produced in Paneth cells, cathelicidins, histatins, α-1 antitrypsin or angiogenin-4.[8]

The normal gut microbiota has a positive impact on the intestinal immune system. Bacterial metabolic products, secreted substances and bacterial antigens stimulate pro- and anti-inflammatory processes in the intestinal wall, thus balancing the immune response.[9] Intestinal commensal bacteria produce enzymes that reduce complex carbohydrates to short chain fatty acids (SCFA), mainly, propionate, butyrate and acetate that affect the activity anti-inflammatory particles such as interleukins (IL) 6, 8 and 12B as well as TNF-α.[10] The gut microbiota releases immune antigens – peptidoglycan, lipopolysaccharide and polysaccharide A (PSA) that induce the immune response, for instance, PSA produced by *Bacteroides fragilis* promotes protective T_{reg} cells.[11]

The gut-associated lymphoid tissue (GALT) is the largest part of the human immune system and, together with the lungs, the main route of contact with the external environment.[12] Here, in Peyer's patches, owing to the mediation of microfold cells (M cells) and dendritic cells, antigens (including bacterial antigens) are taken up from the intestinal lumen and delivered to lymphocytes.[13] When the gut microbiota is normal, T cells subpopulations remain in a dynamic homeostasis. When the gut microbiota becomes dysbiotic, the immune response is transformed into the inflammatory one. Inflammatory mediators, in particular TNF-α, cause tight junctions to leak and give way to bacterial translocatation via the intestinal wall. This initiates a local inflammation and the migration of bacteria to the periphery. The risk of the molecular mimicry increases: the similarity between bacterial antigens translocating through the intestinal lumen and host antigens, e.g., myelin proteins.[4] The activity of the brain-gut axis, a bidirectional communication system between the enteric nervous system (ENS) and the CNS, is affected. It is believed that commensal bacteria via stimulating the autonomic nervous system, and the vagus nerve in particular, affect the activity of the brain. A disruption of the brain-gut axis is assumed to be responsible for such conditions as autism and mood disorders.[14] The theory advocating the relationship between commensal bacteria and autoimmune response against brain antigens has been confirmed in an animal model of MS: experimental autoimmune encephalomyelitis (EAE). Germ-free mice show resistance to EAE induction. However, when exposed to the gut flora from the EAE animals or from MS patients these mice quickly lose this resistance to autoimmune demyelination.[15]

2. AIM

This paper aims at describing the healthy gut microbiota, its changes in MS patients, factors that influence its composition and therapeutic corrective possibilities.

3. MATERIAL AND METHODS

The paper is based on available medical literature.

4. RESULTS AND DISCUSSION

The gut microbiota composition changes in particular sections of the gastrointestinal tract. Since the feces is usually the source of the microbiota sample, there exists the risk of obtaining an imprecise result, especially as regards the quantitative composition. The majority of studies concerning the healthy human gut microbiota report that the stomach is colonized by *Lactobacillus*, *Veillonella* and *Helicobacter*, while the duodenum and small intestine by *Bacilli*, *Streptococcaceae*, *Acinetobacteria*, *Actinomycinaeae* and *Corynebacteriaceae* that become more numerous further from the stomach.[16] The major part of the gut microbiota is host to *Fimicutes* and *Actinobacteria* phylum and *Bacteroides* genus.[17]

Studies of microbiota in MS patients conducted thus far have yielded elusive results.[18] In patients with MS, Chen et al. reported *Pseudomonas*, *Mycoplasma*, *Heamophillus*, *Blautia* and *Dorea* to be more abundant and *Parabacteroi-*

des, *Prevotella*, *Adlercreutzia*, *Lactobacilus*, *Coprobacilus* to be less abundant.[19] In patients with MS, Miyake et al. noticed a depletion of *Faecalibacterium* and *Clostridium XIV*, bacteria known for their anti-inflammatory activity – they are the main SCFA producers.[20] The repeated analysis of this study indicated that in MS patients the microbiota is more diversified in individual patients as compared to healthy controls.[21] Some studies reported an increased percentage of anaerobic bacteria *Methanobrevibacteriaceae* and the colonization with these microbes is associated with a shorter time to relapse in MS.[22] In two studies of MS patients, Berer, Cekanaviciute et al. reported an increase in the number of *Acinetobacter* and *Akkermansia*, bacteria that deplete the population of T_{reg} cells, thus increasing the proliferation of Th1 cells that produce interferon gamma. Also the gut bacteria transplanted from MS patients exacerbated EAE symptoms in mice.[15,23] Another study concerning commensal bacteria revealed the impact of the gut microbiota on the EAE exacerbation. It was demonstrated that *Bacteroides fragilis* produces polysaccharide A, the administration of which reduces EAE symptoms and even delays the disease onset.[24] Apart from *B. fragilis*, also other bacteria have the ability of inhibiting EAE progression and reducing its symptoms. These include, among others: *Lactobacillus*, *Prevotela histicola*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*.[11]

It is commonly recognized that dietary habits, environmental factors and medication are significant stimuli that change the microbiota.[25] Dietary changes during the human development enforce the adaptation of the gut microbiota. For instance, a transition to solid food after the neonatal period leads to the decrease in *Bifidobacteria* and *Enterobacteria* populations.[26] The same mechanism is responsible for changes in the gut microbiota in adulthood owing to new dietary habits. New gut microbiota leads to new interactions with the immune system.[27] With a long-term specific diet, bacteria that are best adapted to digest the consumed food appear, e.g., *Bacteroides* in a meat diet and *Prevotella* with a great intake of carbohydrates.[28] The gut microbiota composition gradually changes with dietary habits and adapts to them after many months.[29] Periodical dietary plans do not result in evident changes, although a rapid short-term dietary change results in quick but short lasting changes in the microbiota.[30] The depletion of *B. fragilis* and *Lactobacillus* was observed in vegetarians and lacto-vegetarians. A gluten free diet decreases the quantity of the commensal *Lactobacillus* and *Bifidobacterium*, and increases the population of opportunist *Enterobacteriacea*.[31] A vegetarian diet also decreases the number of *Clostridium* geni.[32] A high-fat diet stimulates a pro-inflammatory response of the immune system as a result of an increased activity of the white adipose tissue that produces inflammatory mediators, including TNF-α, IL-6, leptin, and C-reactive protein. This observation led to the association of the so-called 'Western Diet' with autoimmune diseases such as MS, rheumatoid arthritis, IBD and type 1 diabetes.[33] It appears that the intake of antibiotics leads to changes in the microbiota composition, but the total number of bacteria remains unchanged.[34] Qualitative and species changes depend on the type of antibiotic and the manner of administration; targeting anaerobic bacteria, vancomycin, clindamycin and metronidazole have the most dramatic effect.[35] The intake of coffee does not lead to significant modifications in the major groups of bacteria, but increases the numbers of *Bifidobacterium* species.[36] Products of smoking may facilitate the activation of autoreactive B and T cells; on the other hand, rather than on commensals, tobacco may impact toxically on intestinal pathogens, thus reducing the local inflammatory process and stimulating the brain-gut axis via the vagus nerve.[37]

Therapeutic attempts to change the gut microbiota in MS may involve a few methods, including, the administration of prebiotics and probiotics. Moreover, the efficiency of the replacement of the gut microbiota has been discussed for a few years.[38] This is the method for the modification of the gut microbiota that has been used for several years for various autoimmune diseases that are associated with dysbiotic conditions. Unfortunately, only incidental reports have been available thus far for MS patients. Prebiotics stimulate the proliferation or activity of beneficial bacteria found in the colon and may be fermented by the commensal gut microbiota.[39] They include, for instance, galacto-oligosaccharides (GOS), the so-called resistant starch, and fructooligosaccharides (FOS) and are reduced to SCFA.[40] Thus prebiotics selectively impact on the gut microbiota. Moreover, via the SCFA diffusion to the blood, prebiotics may also impact on the brain-gut axis. As a result of reduced lipogenesis they protect the cardiovascular system, and due to inducing the increase of IL-4, IL-8 and IL-10, they impact on the immune system.[41] The effectiveness of prebiotics in immune diseases is elusive. Although they diminish the symptoms of irritable bowel syndrome, no clinical benefit has been revealed in Crohn's disease, despite an observed decrease in pro-inflammatory cytokines.[42] Typically, probiotics are produced from *Lactobacillus* and *Bifidobacterium* that, as already mentioned, activate T_{reg} cells in the peripheral nervous system, and then reduce pro-inflammatory IFN-γ, TNF-α and IL-17. Similar effects, however, have been revealed with the administration of *Clostridium* genera.[43] It is also known that probiotics consisting of many genera and species of bacteria lead to more significant changes in the gut microbiota than monoculture products.[44]

Studies on the animal model of MS have provided grounds for the administration of probiotics. Mice with EAE that were on a probiotic diet were more resistant to autoimmunity, and animals that developed the disease had milder symptoms.[45] In a double blind study with the administration of the probiotic containing *Lactobacillus acidophilus*, *L. casei*, *Bifidobacterium bifidum*, and *L. fermentum* a significant functional improvement was obtained in the EDSS score and in Beck's depression inventoryin patients with MS.[46] The impact of immunomodulation in MS on the gut microbiota composition is of interest. Patients treated with interferon or glatiramer acetate the population of *Prevotella* and *Suterella* bacteria was larger, but the percentage of *Sarcina* decreased, which may prove that immunomodu-

lation normalizes the microbiota.[47] It was also reported that MS patients treated with glatiramer acetate present more *Bacteroidaceae*, *Lactobacillaceae*, *Faecalibacterium*, *Clostridium* and *Ruminococcus* as compared to the control group.[18] Comparing the gut microbiota of untreated MS patients and healthy volunteers, Castillo-Alvarez et al. found significant differences in the proportion of *Furmicutes*, *Actinobacteria* and *Lentisphareae*, in particular, which however normalized in patients who underwent immunomodulation therapy.[48] In a recent study, propionic acid (PA) was supplemented in MS patients. After 2 weeks of PA intake a significant and sustained increase of functionally competent T_{reg} cells was observed. In parallel, Th1 and Th17 cells decreased significantly. Post-hoc analyses revealed a reduced annual relapse rate, disability stabilization, and reduced brain atrophy after 3 years of PA intake by MS patients.[49]

Fecal microbiota transplantation (FMT) involves the transfer of the gut microbiota from a healthy donor to a recipient with a disease in order to restore the patient's microbiota to its healthy status.[50] For years, FMT has been successfully used to treat recurrent *Clostridium difficile* infection. Fecal transplant quickly restores balance in the gut microbiota.[51] Unfortunately, in autoimmune diseases such as ulcerative colitis and Crohn's disease, the success of FMT is not as spectacular and the effectiveness is measured as the period of the obtained remission.[52] Some role may be played by the compatibility of the donor and the recipient, dependent on genetic factors, innate immune responses or even their diets.[53] Presently, FMT seems to be a promising therapy also in non-intestinal autoimmune diseases: rheumatoid arthritis, Sjogren's syndrome, Hashimoto's thyroiditis and MS. Unfortunately, these assumptions, based on experimental trials, have not yet been extensively confirmed by clinical observations. Only a few cases of the impact of FMT on MS have been described thus far.[38] Borody et al. reported a series of three patients with relapsing-remitting MS, treated with FTM due to accompanying constipation, who demonstrated long-term remissions of the primary disease.[54] A case of 10-years of stability was reported in a patient with secondary progressive MS, who underwent FMT to treat resistant *C. difficile* infection.[55] The review of the ClinicalTrial.gov database indicates that only seven studies assessing the safety and efficiency of FMT in MS have been registered. To our best knowledge, no clinical trial with humans, devoted to this issue, has been completed. One study was finished before the completion of the trial for non-medical reasons, and the study group was too small for statistical analysis.[56] Significantly, the same research center has designed and registered another study with the use of FMT in MS, which will be the first double blind study.[57] The registered studies are presented in Table. Unfortunately, the absence of presented partial results does not allow for the evaluation of the progress in those studies.

5. CONCLUSIONS

Experimental research has revealed that the correction of the gut microbiota may lead to MS remission or alleviation. FMT utilized in inflammatory bowel disease seems to be presently the most comprehensive intervention. Since only incidental reports of its efficiency in humans are presently available, further clinical studies are necessary.

Table 1. The list of published studies devoted to FMT in MS patients.

Author	Study group	Planned duration	FMT (*n*, type)	Outcome measures	Comments
Gelfand J[58]	*n* = 30 RRMS	2018–2020	*n* = 1, colonoscopy	Patients who completed the study protocol, change in fecal microbiota, AE, T and B cells subtypes levels, new abnormalities in MRI, immunoglobulin	Partial results unavailable
Afanasyev B[59]	*n* = 20 RRMS, PPMS, SPMS with AutoHSCT	2019–2021	*n* = 2, capsules orally	Effectiveness (overall survival), AE, QoL, assessmet of T cells population, brain MRI	Partial results unavailable
Keshavarzian A[60]	*n* = 1 case-only, RRMS	2018–2020	unknown	SCFA, BDNF, MRI, changes in cytokines	Partial results unavailable
NG S[61]	*n* = 450 different diseases including MS	2019–2024	unknown	Efficiency, AE	Partial results unavailable
Guarnaccia J, Browne F[62]	*n* = 15 CDMS	2019–2020	*n* = 1, capsule orally	Microbiota changes, funcional changes, changes in B and T cells population, changes in immunoglobulin level, intracellular cytokines, AE	Partial results unavailable
Kremenchutzky M[56]	*n* = 14 RRMS	2017–2019	*n* = 6, enema	Peripheral blood cytokines, gut microbiome, gut permeability, MRI, EDSS	Finished before completion due to non-medical reasons
Silverman M[57]	*n* = 34 RRMS	2020–2023	*n* = 2, capsules orally	MRI, BBB assessment, gut permeability, gut microbiome, metabolomics	Planned

Comments: AE – adverse events; MRI – magnetic resonance imaging; AutoHSCT – autologous hematopoietic stem cell transplantation; BDNF – brain-derived neurotrophic factor; EDSS – expanded disability status scale; BBB – blood–brain barrier; RRMS – relapsing-remitting MS; CDMS – clinically definite MS; PPMS – primary progressive MS; SPMS – secondary-progressive MS.

Conflict of interest
None declared.

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