The Role of the Gut Microbiome in Neuromodulation Therapies as a Potential Treatment Adjunct for Multiple Sclerosis

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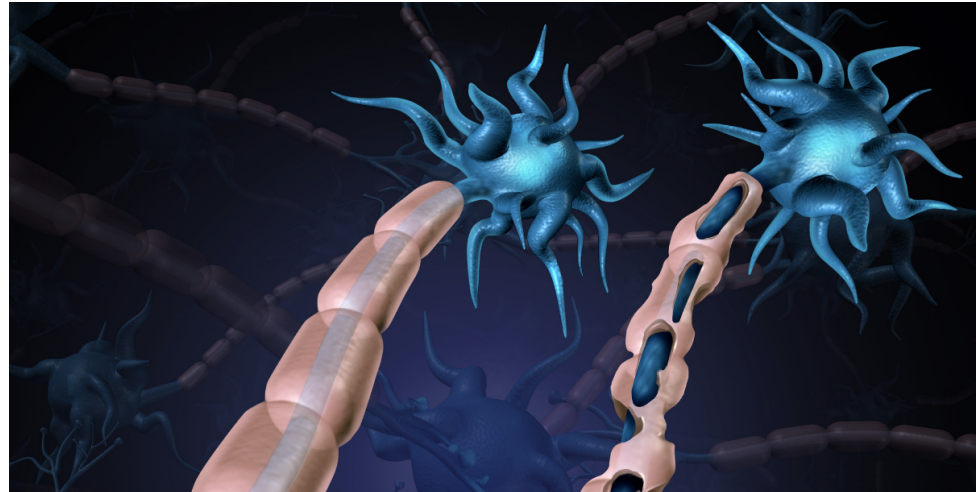
The axon of the neuron, pictured on the right side, begins to degrade following the onset of multiple sclerosis.
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Abstract

Gut microbiome research has surged in popularity over the past decade. These studies have found local and distal effects of micro organisms such as fungi and bacteria on human physiology, including the nervous, immune, and endocrine systems. A number of studies have demonstrated the potential for gut microbiota to combat classical diseases such as clinical depression and autism spectrum disorder. The impact of gut-produced metabolites on the secretion of various cytokines has presented a new-found opportunity for future disease therapy through these micro-organisms. This review examines recent evidence for the use of gut bacteria in neurological rehabilitation, specifically for Multiple Sclerosis (MS) patients. Available data has shown overwhelming support for microbiota-based MS therapy, but the lack of comprehension regarding the specific physiological mechanisms of these microbiota suggests that clinical trials may be far off. Furthermore, there has been minimal research investigating the consequences of using microbiotic therapy in tandem with current therapies such as neurostimulation or drug therapy. Factors including the mechanisms and restorative capability of specific species of microbiota must be studied in depth in order to successfully manipulate the gut microbiome for the treatment of neurological disorders.

Introduction

In recent years, the accessibility and prevalence of research regarding the gut microbiome has increased tremendously. It is common knowledge that the human gut plays an important role in regulating health. The question that remains to be asked is, just how much influence does the gut microbiome hold? Newfound curiosity regarding the effects of manipulation of the bacterial population residing in the gut prompts the scientific community to expand research on ways to harness the power of the gut microbiome. Research on the gut microbiome has advanced considerably since its discovery decades ago with the development of technologies such as 16S rRNA sequencing to assist in mapping the human microbiome (Choileain, 2020; Jangi, 2016; Takewaki, 2020; Zuo, 2018). Furthermore, with advancements in microbiome-related engineering and technology, researchers have come far closer to understanding the specific genes and mechanisms behind microbial functions in the gut and beyond (Azad, 2018; Cani, 2018). However, with a collective human genome including tens of thousands of genes, discovering specific causation from gut-related studies has proven to be exceedingly challenging. A large number of papers studying the human microbiome within the past decade have covered its immense potential to impact human health. However, none have been able to specify the mechanisms, much less the genes behind these microorganisms, which mainly consist of bacteria (Cani, 2018; Han, 2018; Strandwitz, 2018).

In this review, recent evidence showing the impact of general probiotics and specific bacterial implants on human neurophysiology will be examined. The various effects of the gut microbiome on the neuroimmune system will be discussed along with a scrutinization of the current neuromodulatory therapies. This paper explores the possibility of using gut therapy specifically for the clinical treatment of Multiple Sclerosis (MS) patients. Before jumping into this discussion, it is imperative to know the background of this disease and the organisms that may assist in its treatment.

The Gut Microbiome

The term *gut microbiome* refers to the trillions of microbes living within the tract of humans and other animals. Although incredibly small, the microbes in the gut play a huge role in human metabolism, physiology, and immune

system development (Azad, 2018). The gut microbiome encompasses a large scope of symbiotic functions in the body including vitamin synthesis and GI hormone release, the latter of which protects the body from pathogen colonization (Collins, 2012). One aspect of this symbiosis investigated in this review is the effects on the human central nervous system (CNS), where alterations of neuronal signaling by the gut, especially in the brain, have piqued scholarly interest. Particularly, there has been extensive research on microbial impact on the GI tract, as well as its impact on human physiology. Within the past decade, research on the gut microbiome has seen an exponential increase due to the metagenomic revolution - the study of genomes of bacterial species in a specific environment rather than pure laboratory cultures.

One reason the GI tract is an area of interest is due to its key role in the complex mechanisms of immunoregulation (Joscelyn, 2014; Kirby, 2018; Mangalam, 2017; Ochoa-Repáraz, 2014,2018; Shahi, 2019; Velasquez-Manoff, 2015). At first, interest in the role of the microbiome led researchers to look for any associations regarding innate and adaptive immunity within the human body. For example, gut-associated lymphoid tissue (GALT) represents almost 70% of the entire immune system, and the GI tract hosts around 80% of plasma cells, such as immunoglobulin A (IgA)-bearing cells (Vighi, 2008). This research has since dramatically expanded with the upsurge of discoveries correlating bacteria, such as *Akkermansia muciniphila* or *Prevotella Copri,* with various disease pathologies including: obesity, type II diabetes, and multiple sclerosis (Cani, 2018). For example, researchers now understand that the function of the overall human immune system is deeply influenced by bacteriophages, viruses that parasitize bacteria by infecting them and reproducing inside them. Research indicates that bacteriophages, such as *Caudovirales,* can be manipulated in order to provide relief to patients suffering from *Clostridium Difficile* infection through successful fecal microbiota transplantation. By administering fecal matter from a donor into the intestinal tract of a recipient, the recipient experiences relief of infection symptoms as a result of the change in their gut microbial composition (Zuo, 2018). Furthermore, with the increasing interest in elucidating the potential manipulation of the gut microbiome, there has been an increase in research highlighting its connection with the central nervous system, and its role in maintaining homeostasis (Ochoa-Repáraz, 2009; Wang, 2014; Wang & Kasper, 2014; Winter 2018). However, although research is extensive, there

is an inadequate understanding of the specific mechanisms of the gut on human physiology.

It is widely accepted that the development of the GI microbiome begins at birth. The GI tract is rapidly colonized after childbirth and is impacted throughout life by various external factors such as: illness, antibiotic treatment, and changes in diet (Rodríguez, 2015). It is imperative to acknowledge the consequences of certain lifestyle choices on the gut microbiome, as any shifts in one's microbial genome could affect their entire physiology (Figure 1).
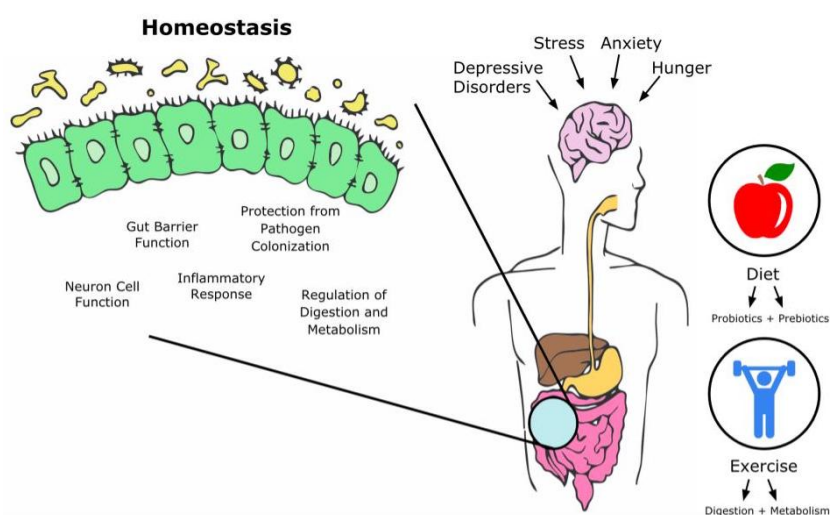

Figure 1. The gut microbiome plays an important role in maintaining host immunity and homeostasis via the gut-brain axis. Since the axis is bidirectional, factors such as hunger, anxiety, stress, and depressive disorders, in addition to lifestyle choices such as diet and exercise, can reshape the gut bacteria's composition and exert an influence on immune function and health.

Diet is a major lifestyle choice that influences gut health. Both short and long-term alterations in diet can impact microbial profiles, and infant nutrition may have lifelong consequences through microbial modulation of the immune system (Harmsen, 2000). In conjunction with the prevalence of malnourishment, with the most common representation being obesity, diet is an ever-growing area of concern within medical research (Manichanh, 2006). Gut microbes produce a large number of bioactive compounds that can be beneficial, such as vitamins. Bacteria, such as *Bifidobacterium,* can generate crucial vitamins such as: vitamin K, B12, biotin, folate, and thiamine (Nicholson, 2012). In addition, digestion is influenced by many enzymes produced by microbes. The microbial diversity in the human gut is

attributable to the spectrum of microbial enzymatic capacity needed to degrade nutrients, particularly the many forms of complex polysaccharides that are consumed by humans (Cantarel, 2012). Nourishing the body properly through a balanced diet is the best way of maintaining a healthy gut microbiota population. The gut microbiome is a complex ecosystem that cycles nutrients between the microbiota and their host cells. This cycling of nutrients dictates the body's immune response to foreign invaders (Azad, 2018). However, the bidirectional nature of this relationship should be noted. Changes in chemical, nutritional, and immunological pathways of the body have also been shown to influence the density and composition of the gut microbiome (Thursby, 2017).

Research has already shown that the gut microbiome is largely dominated by rapidly growing probiotic bacteria because they have the capability to survive in harsh conditions. Some of the most common genera of gut bacteria in adults are *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Clostridium*, *Escherichia*, *Streptococcus,* and *Ruminococcus* (Conlon, 2014). In order to protect the integrity of the gut, some researchers have explored gut modulation by probiotic species, which may be able to improve and restore the gut flora if certain bacterial species of the gut were ever eradicated (Azad, 2018). Two crucial microbial strains discovered for gut microbiome regulation were *Lactobacillus* and *Bifidobacterium.* These quintessential probiotics are target bacterial groups made from short-chain nondigestible carbohydrates (inulin-type fructans, fructo-oligosaccharides [FOS], and galacto-oligosaccharides [GOS]) (Loo, 2009). As it stands, gut regulation is essential in isolating specific bacterial species for clinical therapy, especially for neurological disorders like multiple sclerosis (MS), whose causes are still unknown.

Multiple Sclerosis

MS has cell-mediated pro-inflammatory effects (also known as type IV hypersensitivities) that result in demyelination of neurons and autoimmune pathogenesis of the disorder, leading to disruptions in brain-body communication. In MS patients, autoreactive T and B lymphocytes enter the CNS, induce inflammation, and undermine the blood-brain barrier (BBB) via cytokine secretion (Ghasemi, 2017). These cytokines begin a signaling cascade that results in oligodendrocytic death and thus, the destruction of neuronal myelin sheaths in the CNS (Ghasemi, 2017). The resultant CNS lesions disturb proper communication between neurons,

and lead to various cortical dysfunctions within MS patients such as modulations to resting motor threshold, short interval cortical inhibition, and central motor conduction time (Ghasemi, 2017). Although microbial therapy holds promise, its implementation as a universal MS therapy remains far off because of the lack of understanding behind the specific mechanisms of gut-brain interactions and early research into this field (Figure 2).
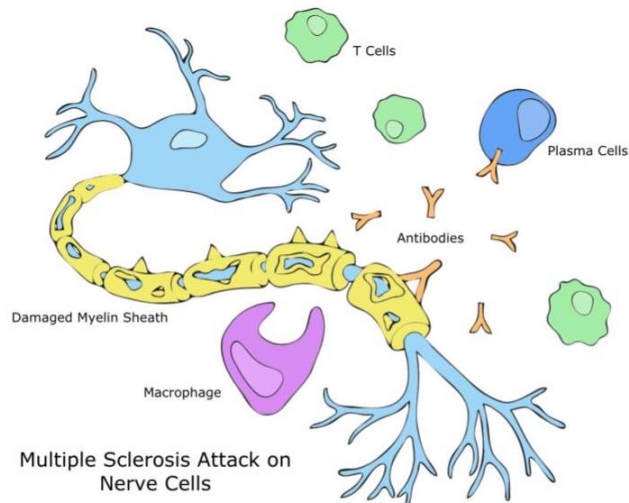

Figure 2. A neuron is made up of a cell body, an axon, and dendrites. The protective coating of the axon, the myelin sheath, are damaged in patients with Multiple Sclerosis. The immune system attacks the myelin sheath and causes injury and inflammation. Neural deficits ensue.

While the specific origin of MS still eludes scientists, most postulate that a combination of genetic and environmental factors plays a significant role in pathogenesis. Environmental elements such as geography, vitamin D deficiency, obesity, diet, smoking, and physical or emotional stress have been shown to be relevant in MS progression (Gianfrancesco, 2016; Ochoa-Repáraz, 2014; Rosso, 2019; Sintzel 2018). When these factors collaborate with adverse genetics, the health of MS patients can quickly deteriorate. One of the most salient genetic factors is sex, with MS diagnosis showing an astounding 2:1 male-to-female ratio (Reynolds, 2018). Women are also diagnosed with an irreversible disability at older ages than men (Confavreux, 2006). Another important genetic factor is the HLA-DRB1*15 gene haplotype. The DR2 haplotype HLA-DRB1*15 gene encodes a protein important in T-lymphocyte reactivity and is also associated with MS disease progression, age at onset, and atrophy of subcortical gray matter (Isobe, 2016).

Types of Multiple Sclerosis

MS patients are categorized into four subtypes including: relapsing-remitting (RRMS), secondary progressive (SPMS), primary progressive (PPMS), and progressive-relapsing (PRMS). RRMS is the most common subtype, affecting approximately 87% of the patient population (Ghasemi, 2017). Autoimmune inflammatory attacks on the nervous system among patients of this subtype occur months or years apart followed by a period of remission, leading to a progressive increase in irreversible disability. This neural damage eventually manifests as vision loss, muscle weakness, and impaired coordination (Weiner, 2008). During periods of remission, patients revert to relatively normal neurological activity (Høglund, 2014). This subtype of MS shows the greatest promise of therapy because intervention is possible during remission periods. Unfortunately, within the population of RRMS patients, around 70% develop SPMS (Kirby, 2018). However, what facilitates the progression from RRMS to SPMS remains unclear. SPMS is characterized by a minimal to complete lack of relapse activity, leading to a constant increase in irreversible neural damage (Høglund, 2014). Although the underlying mechanisms of the progression from RRMS to SPMS are currently unclear, most likely a combination of environmental and genetic factors, SPMS differs from RRMS in that demyelination is restricted to short lengths of disrupted myelin located in aggregates of microglial cells (Prineas, 2001). Patients in this category have a mean age of about 44-63 years at SPMS onset (Confavreux, 2006). PPMS patients see similar effects, but their pathogenesis is slightly different. PPMS patients compose approximately 10-15% of all MS patients and tend to have less brain atrophy but increased spinal cord atrophy (Ghasemi, 2017). In PPMS, regression does not occur. Patients instead experience a steady increase in the debilitating effects of MS (Reynolds, 2018). Lastly, PRMS is the subtype of MS with the fastest increase of disability over time due to periodic immune system attacks along with a steady increase in disability. PRMS is the most devastating subtype, and it comprises approximately 5% of the MS population (Ghasemi, 2017).

MS symptoms are similar among patients, with many resulting from plaque formation after demyelination. These symptoms are notoriously difficult for physicians to predict due to differing plaque locations between patients leading to different symptoms (Kister, 2013). However, common symptoms

in those affected include vision loss, decreased mobility, bowel/bladder dysfunction, sensory loss, impaired coordination, decreased energy, and spasticity (Kister, 2013). Patients often experience chronic pain from conditions such as trigeminal neuralgia (pain triggered by oral activity), dysesthetic pain, back pain, and painful spasms (Solaro, 2004). These conditions are often comorbid with mental health disorders such as depression, further confounding diagnostic efforts (Chwastiak, 2007; Kister, 2013; Vattakatuchery, 2011). Thus, it is critical to pinpoint the specific causes of MS in order to establish a framework for the development of effective microbial therapies.

Multiple Sclerosis Pathogenesis

It is still unclear whether an overactive immune system and acute inflammation are the incipient causes of MS, or if pathogenesis stems from a radically different cause. Similarly, there is still ambiguity regarding autoreactive immune cell leakage into the CNS. The current literature demonstrates that T helper (Th) lymphocytes and various cytokines play a primary role in MS pathogenesis (Williams, 2020; Choileain, 2020; Berer, 2011).

Th17 is the most studied T helper cell, and it is considered by many to play a large role in the inflammation observed in MS patients. (Choileáin, 2020; Reynolds, 2010; Mangalam, 2017; Tahmasebinia, 2017). Many studies support the role of Th17 in producing pro-inflammatory interleukins (IL) such as IL-17, IL-17A, IL-17F, IL-21, IL-22, and IL-26 (Ghasemi, 2017; Xu, 2020; Reynolds, 2010). This increased inflammatory response is aggravated further when the normal negative feedback response becomes impaired.

MS patients have displayed a reduced ability to produce T regulatory cells, which normally mediate the inflammatory effects of Th cells that occur as a result of leakiness of the blood-brain barrier (BBB) (Cekanaviciute, 2017). The blood-brain barrier is a semipermeable border composed of endothelial cells (ECs), pericytes, astrocytes, and an extracellular matrix (Abbott, 2010). Recent studies indicate that MS patients have circulating factors and the BBB engages in crosstalk that is mediated by endothelial cells (ECs) and adjustment of astrocytic expression (Williams, 2020; Setiadi, 2019; Zivadinov, 2016). MS patients are further characterized by lymphocytes

undermining BBB permeability by inducing oxidative stress in ECs and enhancing leukocyte transmigration by producing various facilitative proteins: P-glycoprotein, intercellular cell adhesion molecule 1(ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) (Sheikh, 2020). VCAM-1 and ICAM-1 expression is directly correlated to levels of IL-17 and TNF-α, suggesting another mechanism of the enhanced inflammation seen in MS patients (Gao, 2017). By modifying BBB immuno-trafficking, autoreactive cells are readily able to cross into the CNS and cause further damage.

This leakage of immune cells is exacerbated as MS lesions begin increasing B cell transcription ten-fold through promoting the production of B cell-activating factor (BAFF) (Krumbholz, 2005; Kannel, 2015). Furthermore, once pathogen-associated molecules bind to toll-like receptors (TLR) on these lymphocytes, autoreactive T cells are signaled to produce cytokines that induce additional cell differentiation (Reynolds, 2010). MS patients reportedly possess high levels of TLR2 and TLR4 among others, with TLR2 being associated with the defective remyelination seen in MS (Hasheminia, 2014; Wasko, 2020). Microbial manipulation of the immune system gives scientists hope for gut-based therapies, albeit specific causation has not been established.

Current Treatment Options for MS

Due to its complex neurological pathogenesis, MS is currently an incurable disease. Treatment options for patients with MS are divided into three categories: acute relapse management, slowing of disease progression, and treatment of related symptoms (Hart, 2016). Glucocorticoids are the drugs of choice for an acute attack because they downregulate molecules associated with inflammation in the body, such as cytokines and chemokines, and upregulate anti-inflammatory proteins. Therapy with glucocorticoids such as high-dose methylprednisolone should be considered in patients whose relapse is of moderate to severe severity (Doshi, 2016). Ultimately, glucocorticoids are used to shorten the duration of a relapse or acute attack.

It has become increasingly important to focus on slowing disease progression in order to improve the quality of life of MS patients. Disease-modifying treatments (DMTs) have become a key component of comprehensive MS care for this reason (Hart, 2016). RRMS is the most

treatable subtype of MS. Drugs such as beta-interferons, glatiramer acetate, teriflunomide, dimethyl fumarate, and fingolimod have moderate efficacy but are a safer option in patients with RRMS. The human body already produces beta-interferons as a natural response to inflammation. The man-made drug further aids in the downregulation of inflammation and decreases the damage to the nerves in the body. However, since other DMTs can have life-threatening adverse effects, it is necessary to monitor clinical conditions and conduct frequent MRI scans. Alemtuzumab and natalizumab, targeted cancer drugs, have a higher efficacy but present serious side effects such as progressive multifocal leukoencephalopathy (PML). PML, an illness that leads to brain damage, severe disabilities, and death, can develop in patients that are on natalizumab treatment for more than two years with a prior history of chemotherapy or immunosuppression (Doshi, 2016) (Figure 3).
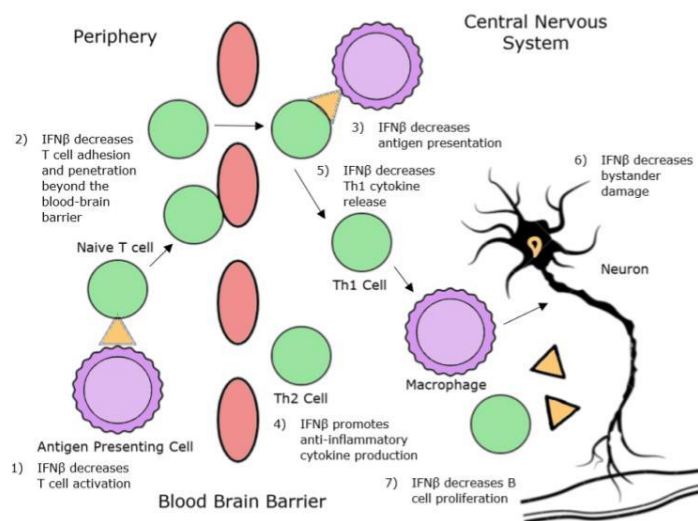

Figure 3. The mechanism of action of the DMT IFNβ. This drug targets immune cells. It decreases T cell activation, B cell proliferation and promotes anti-inflammatory cytokine production.

It is recommended that DMTs are started as soon as possible to increase the likelihood of slowing the progression of the disease before severe neurological deficits can arise. Unfortunately, there is not enough current research indicating that DMTs can aid in slowing progressive MS and there is still a lack of cohesive understanding about DMTs in the research community. More studies are necessary in order to understand the efficacy and safety of drugs while simultaneously slowing the progression of the disease.

Symptomatic treatment of MS is just as important as slowing the progression of the disease or treating an exacerbation. Patients often complain of fatigue, depressive symptoms, urinary frequency, sexual dysfunction, constipation, pain, ataxia, and more (Doshi, 2016). Not all symptoms necessarily require medication. However, it is important that physicians recognize the heterogeneity of this disease when creating a treatment plan for their patients.

MS can largely impact one's functionality, and patients typically struggle with adapting to the changes in their daily life. Thus, patients are often recommended to start cognitive therapy alongside their medication. Pain and depression are symptoms that have long been overlooked in individuals with MS. One study showed that more than 88% of people with MS experience pain in more than one bodily area (Gromisch, 2020). Another study reported that 22.8% of patients with MS struggle with lifetime major depression (Wang, 2000). Studies have shown that using cognitive behavioral therapy as an adjunct treatment for MS can improve mental health and quality of life in patients (Gromisch, 2020).

Neurostimulation technologies are recent medical advancements that are rapidly being researched as potential symptomatic treatment options for neurologic and psychiatric disorders. An increased understanding of neural circuitry and neurotechnologies has shown promising therapeutic results in patients with neuropsychiatric conditions. These therapies include invasive and noninvasive approaches that target a specific nerve or anatomical region in the body. Some examples include brain temperature control, magnetic stimulation, deep brain stimulation, spinal cord stimulation, and vagus nerve stimulation (Edwards, 2017). Research has shown that gut bacteria communicate with neurons of the enteric nervous system to send signals to the brain via the vagus nerve (Galland, 2014). Currently, an FDA-approved implantable vagus nerve stimulator called LivaNova can treat drug-resistant epilepsy. In addition, vagus nerve stimulation has been used to treat drug-resistant depression (Edwards, 2017). Researchers at the Texas Biomedical Device Center have developed a technology called the RePair System that rewires neural circuitry through stimulation of the vagus nerve, focusing on targeted plasticity therapy. The first human trials involved stroke patients and focused on improving upper and lower limb motor

deficits following a brain injury or disease (Darrow, 2020). Symptoms of MS often include muscle weakness and motor imbalance, leading researchers to believe that this therapy has the potential to change how physicians approach the treatment of MS and other neurological disorders. Another clinical trial tested vagus nerve stimulation in three MS patients in an effort to reduce cerebellar tremor and dysphagia. Symptoms were improved over a period of two to three months and the involvement of the nucleus tractus solitarius, a key visceral component of the vagus nerve, was further studied (Marrasu, 2007). To date, the underlying mechanisms of neuromodulation therapies are not well understood, which is why they are not primary treatment options for most neuropsychiatric diseases. However, a better understanding of neural circuitry in the human body has led to promising technological advancements that may significantly reduce symptoms in patients suffering from various neurological conditions.

Microbial Links to Human Physiology

Lactobacillus is a genus of beneficial gut bacteria that have gained an increasing amount of scholarly attention (Esber, 2020; Zhou, 2015). Specific species of *Lactobacillus* including *L. acidophilus*, *L. casei*, *L. rhamnosus*, and *L. helveticus* have been linked to the prevention of disease in humans and animals (Azad, 2018). *Lactobacillus* can alter the population of microorganisms in the gut microbiome by producing lactic acid, preventing harmful bacteria from colonizing the intestines. A study using a mouse model of hyperlipidemia explored the impact of modulating the gut microbiome by introducing probiotic feeding of *Lactobacillus* into the mice's diet. Significant changes in the microbiota composition were found, including an increased abundance of *Bacteroidetes* and *Verrucomicrobia* and a reduced ratio of *Firmicutes* (Chen, 2014). *L. acidophilus* has even displayed the ability to maintain a homeostatic concentration of inflammatory cytokines, Th17, and regulatory T (Treg) cells (Park 2018). Furthermore, *L. acidophilus* suppressed proinflammatory cytokines such as IL-6, tumor necrosis factor-α (TNF-α), and IL-1β in colon tissues.

Similar to *Lactobacillus*, there has been a research focus on the probiotic genus *Bifidobacterium*. *Bifidobacterium* assists the human body in performing essential functions such as digestion, improving gastrointestinal barrier integrity, preventing harmful bacterial colonization, and suppressing proinflammatory cytokines (Ganz, 2002). It maintains immune homeostasis

by altering the function of dendritic cells in order to protect against foreign bacteria and pathogens (Azad, 2018). Moreover, bifidobacteria increase the proportion of beneficial bacteria in the gut microbiota through cross-feeding, allowing other bacteria to live off of their metabolic products. *Bifidobacterium bifidum* has shown significantly increased metabolic activity when cocultured with *Bifidobacterium breve* (Turroni, 2015). This co-culture of probiotic bacteria affected the metabolic shift in the gut microbiota by increasing the production of short-chain fatty acids, suggesting *Bifidobacterium* could play a role in cognitive function via hormonal signaling (Savignac, 2013). This can lead to improved memory function, including the growth of brain-derived neurotrophic factor (BDNF) and N-methyl-D-aspartate receptor expression (Savignac, 2013).

The prophylactic role of this bacterial species among other probiotic strains was further explored in a mouse model of β-lactoglobulin allergy, finding that the administration of *Bifidobacterium longum subsp. infantis* (*B. infantis*) LA308 for 3 weeks modified the composition of the gut microbiota, signaling a connection between probiotics and the diversity of gut microbiota (Azad, 2018). There was a significant change in forkhead box P3 (FOXP3), transforming growth factor-beta (TGF-β), and IL-10 ileal gene expression, as well as plasma metabolomic alterations in the tryptophan (Trp) pathway. The study concluded that probiotic introduction to the human body led to alterations in immune responses, tolerogenic energy induction, and anti-inflammatory responses (Esber, 2020).

Human Gut-Immune Interactions

The immune system plays a pivotal role as the intermediary between the gut microbiome and CNS, especially in the regulation of autoimmune responses. The presence or absence of several components of the gut microbiota regulates T and B cell activation within the brain, leading to the enhanced inflammatory response associated with autoimmune diseases like MS. For instance, the presence of gut-residing bacteria *Faecalibacterium prausnitzii* caused various symptoms in patients with Crohn's disease, an autoimmune disease characterized by heightened inflammation in the digestive tract (Velasquez-Manoff, 2015). Patients who lacked this bacteria experienced inflammatory bowel syndrome and asthma, while those possessing them displayed a negative correlation with inflammatory

autoimmune diseases (Velasquez-Manoff, 2015). The potential therapeutic applications of this bacteria's anti-inflammatory nature are currently under research for autoimmune disorders (Figure 4) (Velasquez-Manoff, 2015).
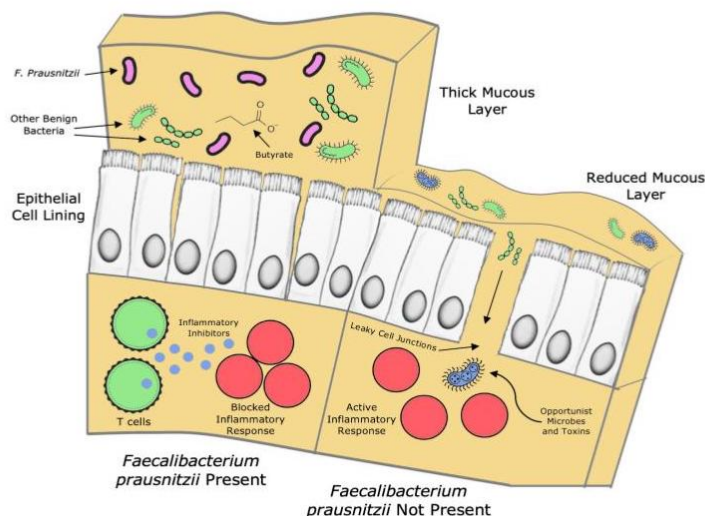

Figure 4. The presence of bacteria *F. Prausnitzii* in the gut maintains the integrity of the epithelial gut lining and inhibits the T cell inflammatory response. The absence of this materia creates leaky cell junctions in the gut lining, which allow for opportunistic microbes and toxins to enter and activate the inflammatory response.

Human-Gut Immune Interactions - Metabolites

Further studies on gut-immune interactions have emphasized modifications to inflammatory cytokine pathways. These interactions vary from cytokine-specific to stimulant-specific responses or sometimes, both concurrently. Studies revealed that 10% of the variability of cytokine responses associated with inflammation can be accounted for by variations in the gut microbiome (Schirmer, 2016). Moreover, the interaction between the bacterial component of the gut microbiome and immune cells does not occur directly, but through metabolites, small molecules referring to the intermediates or end products of a metabolic pathway (Wirthgen, 2018).

One such pathway leads to the formation of tryptophol, a metabolite and common inhibitor of TNF-α. The degradation of the essential amino acid Trp to tryptophol is negatively associated with the interferon gamma (IFNγ) inflammatory pathway (Schirmer, 2016). Several other Trp-derived metabolites, particularly the TRYP-6 neuroactive compounds kynurenine, quinolinate, serotonin, indole, and indole derivatives, may have a critical role in bacterial signaling (Kaur, 2019). Low levels of these compounds are associated with disorders like major depression, autism spectrum disorder,

and Parkinson's disease. Researchers have seen extensive modulation of these metabolic pathways from several genera of gut bacteria which break down Trp like *Clostridium*, *Burkholderia*, *Streptomyces*, *Pseudomonas*, and *Bacillus* (Kaur, 2019).

In addition, it is believed that gut microbiota contribute to the availability of Trp and the neurotransmitter 5-hydroxytryptamine, which regulates neuroendocrine signaling (Martin, 2018). 5-hydroxytryptamine, also known as serotonin, is important in combating the comorbidities that often accompany MS. Namely, major depression and anxiety disorder (Kelly, 2016; Zheng, 2016; Winter, 2018). However, there are a multitude of additional disorders that may be associated with gut microbiota including obesity, diabetes mellitus, schizophrenia, and autistic disorders (Evrensel, 2015). Changes in gut microbiota are relevant to mood states due to their contribution to the production of neurotransmitters (Mittal, 2017). Regulation of this pathway appears to be extremely important for the development of gut-based MS therapy.

However, while the formation of Trp metabolites is a key pathway, there are various other important metabolites that should be considered. Secondary bile acids (2BAs) are strongly influenced by microbial activity and activate the intestinal L cell's surface G protein-coupled bile acid receptors (Martin, 2018). 2BAs derived from spore-forming bacteria of the gut regulate a significant percentage of 5-HT synthesis and release from enterochromaffin cells, introducing another intriguing connection between gut metabolites (Yano, 2015).

Short-chain fatty acid (SCFA) synthesis is another metabolic pathway that is affected by gut bacteria, often through bacterial fermentation or host protein glycosylation. Microbial fermentation (primarily by *Bacteroidetes*) leading to SCFA production in the colon and blood is believed to play a critical role in immunoregulation (Choileáin, 2020). Furthermore, the biochemical conversion of nutrients into SCFAs and other amino acid-derived metabolites like 5-HT are often conducted by intestinal microbes (Hemarajata, 2013). These peptides function as immunomodulators through mechanisms including (Park, 2019):

1. metabolic integration, or the integration of two or more metabolites
2. microbiota regulation

3. histone deacetylase (HDAC) inhibition, which is involved in epigenetic

or non-epigenetic regulation of cancer cells

4. G-protein coupled receptor (GPCRs) activation that plays a role in the cellular signal transduction pathway

The proinflammatory cytokines tumor necrosis factor-alpha (TNF-α) and IL-1β even see an increased level after SCFA induction of the gut-immune system (Galland, 2014). SCFAs have been shown to upregulate or downregulate primary immune cells such as CD4+ effector cells and IL10+ Tregs in mice with experimental autoimmune encephalitis (EAE), a disease with pathogenesis similar to MS (Höftberger, 2015). Some SCFAs can contribute to the anti-inflammatory response by stimulating IL-10 production while others, for example, the G-protein coupled receptors GPR41 and GPR43, may initiate a pro-inflammatory response. However, further research must occur to determine the exact relationship between SCFAs and related immune cells, as well as the pathways through which this inflammation occurs (Park, 2019).

Gut-Immune Interactions - Proteins

An important part of gut-immune interactions is the effect that they have on toll-like receptors (TLRs). A few studies have suggested there is a connection between TLR2, and its signaling pathway, that ties MS to the microbiome (Wang, 2014; Wasko, 2020). TLR2 can be stimulated by bacterial lipopeptides. In murine models of MS, microbial injections inducing TLR2 tolerance have shown inhibition of CNS inflammation while improving remyelination (Wasko, 2020). Another protein, TLR4, has also displayed responses to lipopolysaccharides (LPS) from gram-negative bacteria (Park, 2009). In practice, lack of exposure to LPS from gut bacteria resulted in a lack of TLR4 tolerance, resulting in deficient regulation of innate immune TLR responses and enhanced autoimmunity (Wasko, 2020).

Gut-Immune Interactions - Cytokines

When researching the gut-brain axis relationship, it is important to understand how each micro or macromolecule affects both sides of this affiliation. With the importance of cytokines in the formation of MS lesions, these proteins are particularly important in understanding how the gut can impact the brain. Numerous studies have found that dysbiosis of the gut may induce proinflammatory responses, suggesting a potential avenue for gut-based MS treatment (Choileáin, 2020; Galland, 2014;

Monteleone, 2011; Martin, 2018; Adamcyzk-Sowa, 2017; Shahi, 2017). Proinflammatory interleukins such as IL-6 and IL-17 have shown evidence of being affected by microbial modifications in the gut. Interestingly, mesenteric lymph nodes (MLN) of antibiotic-treated animals produced less IL-6 while significantly increasing levels of the anti-inflammatory IL-13 and IL-10 compared to controls (Ochoa-Repáraz, 2009). Furthermore, research regarding the gut-brain axis has shown that artificial activation of aryl hydrocarbon receptor (AhR) ligands, a part of the tryptophan metabolite pathway, decreased the concentration of IFN-γ while up-regulating proinflammatory IL-22 in the gut of inflammatory bowel disease patients (Monteleone, 2011). These gut-based cytokine alterations are only compounded with the modifications to peripheral blood mononuclear cells (PBMCs) within the body.

Gut-Immune Interactions-Peripheral Blood Mononuclear Cells

PBMCs such as T cells among others all see concentration adjustments due to the gut. Exposure of healthy PBMCs to *Parabacteroides distasonis,* a common gut bacteria, significantly increased the percentage of IL-10 expressing CD4+CD25+ T cells and IL10+FoxP3+ Tregs within the CD4+CD3+ population (Cekanaviciute, 2017). Furthermore, treatment of mice with *Prevotella histicola* also showed an increase in CD4+FOXP3+ Tregs in addition to a decrease in proinflammatory Th1 and TH17 immune cells (Mangalam, 2017). These correlations display bacterial importance in the regulation of CD4+ and CD8+ T cells; which have shown an increased expression of CXCR3+ in MS patients, contributing to greater leakage in the blood-brain barrier (Choileáin, 2020). Protection of this barrier will be crucial in the development of gut-based MS therapies in order to combat the reduced expression of regulatory proteins seen in RRMS patients such as occludin and vascular endothelial cadherin (Sheikh, 2020).

While T lymphocyte regulation is extremely important in achieving this goal, so are other PMBCs like NK cells and B cells, the latter of which is believed to be involved with MS because of their association with immunoglobulin presence (Høglund, 2014). MS usually involves the depletion of B cell numbers and an increase in T lymphocytes (Krumbholz, 2012). NK cells are important in their role of target cell lysis and can also be a therapeutic target due to their role in cytokine and chemokine secretion (Høglund, 2014). Additionally, they have the ability to modify or lyse T

cells, an interaction that can be investigated in the future to improve the understanding of the dysregulation of immune systems in MS patients (Høglund, 2014).

The gut-immune relationship is a two-way street. In the future, research must take into account that the gut does not affect human physiology unidirectionally. While the composition of the gut microbiome heavily influences the autoimmune response by regulating the interactions between immunoregulatory cells and metabolites, the immune system is also critical in cultivating healthy bacteria and destroying harmful bacteria in our gut (Velasquez-Manoff, 2015). The key players in shaping the gut microbiome are nucleotide-binding and oligomerization domain-like receptors (NLRs or NOD-like receptors). For instance, the NOD2 bacterial sensor regulates inflammation caused by the growth of the commensal *Bacteroides vulgatus* (Ramanan, 2014). This regulation promotes epithelial stem cell survival and regeneration in the gut. Furthermore, NLRs that assemble into multiprotein complexes, known as inflammasomes, activate inflammatory caspases and regulate microbial diversity in the gut. The NOD-, LRR (leucine-rich repeat)- and pyrin domain-containing 6 complex (NLRP6) is a type of inflammasome co-modulated by microbiota-derived metabolites that is linked with intestinal homeostasis, intestinal antiviral innate immunity, and the regulation of epithelial IL-18 secretion and AMP expression profiles (Zheng, 2020). To fully grasp the intricacies around this bidirectional relationship and the pathways that associate the two, researchers continue to explore this vital frontier.

Discussion

It is clear that the gut-brain axis has an impact on human physiology, so taking advantage of this non-invasive approach will be the crucial next step to treating and/or preventing MS. There has been prior evidence showcasing the relevance of gut microbiota in the treatment of neurological and motor disorders similar to MS including major depression and Parkinson's disease (Bremner, 2020; Haney, 2018; Han, 2018; O'reardon, 2006; Tian, 2020; Winter, 2018; Zhou, 2015; Martin, 2018). Yet, even though extensive research has been conducted over the past decade, the specific mechanisms of the gut-brain axis in relation to MS have yet to be found. However, a few notable considerations for these mechanisms include (1) systemic cytokine activation, (2) neurotransmitter synthesis, and/or (3) neuronal circuitry alterations (Galland, 2014). One could interpret this as

academia leaning toward investigations on the nervous, immune, and endocrine systems.

Researchers of the nervous system believe the vagus nerve, the cranial nerve that controls the signaling between the brain and GI tract, plays a key role in the interactions of the gut-brain axis (Figure 5).
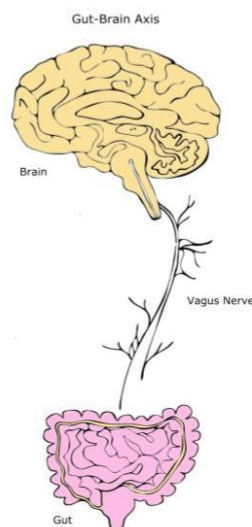

Figure 5. The vagus nerve is the tenth cranial nerve and extends from the brainstem through the neck and the thorax down to the abdomen. It carries signals from the digestive system and organs to the brain and vice versa. In addition, it modulates inflammation, maintains homeostasis, and regulates many body sensations.

One study found that the vagus enhances neural plasticity (post-stroke) with improvements in both cognitive and motor function, both of which are largely diminished in MS patients (Liu, 2016). The vagus nerve is also important for neurogenesis through the modulation of brain-derived neurotrophic factor (O'Leary, 2018). Further research also correlates neuroelectrical stimulation of the vagus nerve with a decrease in symptoms of various neurological disorders in mice (Zhou, 2015; Haney, 2018). As it stands, gut-brain signaling appears to be relatively dependent on vagus nerve activity, but this may only be true in certain experimental systems (Bercik, 2011).

Impacts on chemical communication in the brain have also illustrated the value of the gut-brain axis in MS treatment. It has been noted that more than 90% of the body's 5-hydroxytryptamine (5-HT), or serotonin, is produced in the gut (Yano, 2015). 5-HT receptors are critical in the

mediation of gut-brain axis activity in MS patients (Malinova, 2018). Gut microbiota have also shown success in the alteration of host serotonin levels through the mediation of small molecules like SCFAs or secondary bile acids (2BAs) (Yano, 2015). SCFAs in particular are important in mediating host-microbe communication via enteroendocrine and enterochromaffin cells, the latter of which also play a role in tryptophan metabolism (Figure 6) (Martin, 2018).
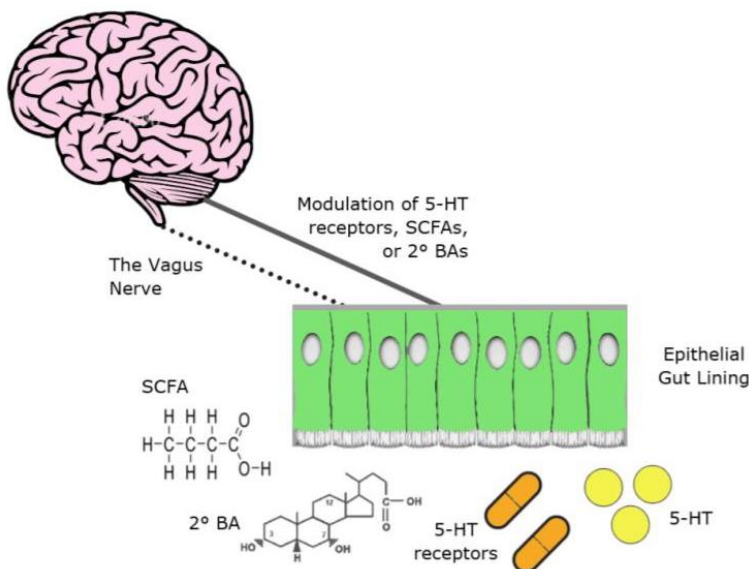

Figure 6. Stimulation of the vagus nerve has shown efficacy in modulating 5-HT receptors, SCFAs, and 2° BAs in the gut microbiome.

Although the gut-brain axis shows great potential for MS therapy, research on the functions of specific bacterial species on the gut-brain axis is limited. There is significant evidence to suggest that few genera such as *Bacteroides* and *Firmicutes* are affected by mental and physiological stress, but their specific effect on MS pathology is unclear (Tian, 2020; Choileáin, 2020). Studying the effects of general probiotics versus specific bacterial species on MS symptoms is necessary to determine the direction of future research on MS therapy via the gut-brain axis. Furthermore, future research directed toward the integration of current MS therapies and their effect on the gut-brain axis is needed. For example, in regards to neuromodulation technology, the development of closed-loop adaptive systems which use predictive models of neural circuitopathies to alter neurostimulation parameters without clinical supervision is a promising avenue of research (Drew, 2019; Edwards, 2017; Lozano, 2019). Another promising

development is the creation of minimally invasive, wireless neuromodulation technology to alter biological parameters in real-time (Tanabe, 2017; Iodice, 2017).

In recent years, there has been an increase in clinical trials exploring the connection between the vagus nerve and neurological deficits. For example, a current clinical trial is exploring how stimulating the transcutaneous vagus nerve could improve cognitive function (University of Ostrava, 2019). The clinical trial is using non-invasive stimulation provided by a transcutaneous electrical nerve stimulation device for four hours a day at 25Hz, 250 μs pulse width placed on the tragus. This novel area of preventive medicine could provide an interesting perspective on MS therapies. If the technological device results in clinically significant applications, it could improve executive neurological function and potentially help prevent demyelination.

Conclusion

There has been minimal research regarding many areas of concern such as the effects on the blood-brain barrier by B cells, the specific mechanisms of lymphocytes and cytokines on the gut-brain axis, and the association between MS and the microbiome. It is unclear whether or not the gut microbiome will become a viable therapy specifically for neurodegenerative disorders, but the association between human physiology and microbiota cannot be ignored. With a greater understanding of symbiotic human-microbial interactions, there is no doubt that future research will be useful in building a greater comprehension that may result in clinically significant therapies.

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