The role of the gut microbiota in the pathogenesis of multiple sclerosis

Manouk Beuving

Multiple sclerosis (MS) is a chronic, neurodegenerative disease of the central nervous system (CNS), affecting approximately 2.5 million people worldwide. It is characterized by the formation of plaques, caused by inflammation and demyelination, leading to neurodegeneration. Autoreactive T cells cross the blood-brain barrier (BBB) and react against myelin antigens. Th1 and Th17 CD4+ T cells cause inflammation and increase BBB permeability, allowing more immune cells to enter the CNS and cause damage. Anti-inflammatory regulatory T cells are decreased, allowing progression of the disease. Both genetic and environmental risk factors play a role in the onset of MS, with most environmental risk factors being linked to the gut microbiota. The gut microbiota can communicate with the brain via various ways. The gut microbiota evolves over a lifespan, varies between populations and responds to changing lifestyles. The gut microbiota is key contributor to homeostasis of the body, so alterations in the composition and dysbiosis could possibly play a role in MS. The gut microbiota can influence progression of disease by reducing anti-inflammatory immune reactions, mainly a depletion of regulatory T cells and a decrease of short chain fatty acids, and inducing pro-inflammatory immune reactions, mainly Th1 and Th17 differentiation, B cell proliferation and induction of autoreactivity of T cells. Targeting the microbiota might therefore be a good option for treatment of MS.

Introduction

Multiple sclerosis is a chronic, demyelinating and neurodegenerative disease of the central nervous system (CNS). It affects approximately 2.5 million people worldwide and is more prevalent in females than in males (Compston & Coles, 2008). MS is characterized by the formation of plaques: areas of demyelinated axons in both white and grey matter of the brain and spinal cord (Schepici et al., 2019). The plaques are caused by inflammation and demyelination, leading to neurodegeneration in the CNS. This disrupts the ability of neuronal signal conduction, which results in sensory and visual problems, motor impairments, fatigue, pain and cognitive deficits (Compston & Coles, 2008). The current treatment of MS is disease modifying therapy, used to reduce the incidence, prevent and/or reduce the number of relapses and delay the progression of MS (Schepici et al., 2019). Most drugs modulate or suppress the immune and inflammatory processes caused by MS. There is currently no therapy available to reverse the neurological decline and recover from the disease. However, there are some cases reported of MS patients who received fecal microbial transplantation (FMT) and prevented further disease progression (Borody et al., 2011; Makkawi et al., 2018). This implicates a possible role for the gut microbiota in MS pathogenesis.

The role of gut microbiota in several diseases has gained growing interest over the past two decades. Almost all areas of medicine have been shown to be affected by microbiota, including neurology (Sherwin et al., 2018). The microbiota is a complex community of microorganisms in the human intestine. It has a mutualistic relationship with the human body and influences many processes, being a key contributor to host homeostasis (Gill et al., 2006). For this reason, a dysbiosis in the gut microbiota is associated with many gastrointestinal diseases, such as Crohn's disease and irritable bowel syndrome (Lozupone et al., 2012). However, changes in behaviour are also associated with gastrointestinal illness and disorder, and some neurological disorders are impacted by the gut microbiota composition. Indeed, studies in animals have shown the role of the microbiota in key aspects of brain processes like neurodevelopment, neuroinflammation and behaviour. In such manner, a dysbiosis can play a role in neurological disorders and autoimmune diseases (Long-Smith et al., 2019). This led to a growing body of research focusing on the microbiota-gut-brain axis, the bidirectional communication pathway between the gut microbiota and the CNS. Over the past decade, dysregulation of this axis has been

associated with neurological disorders such as Alzheimer's disease, Parkinson's disease, autism spectrum disorder and MS.

The exact cause of MS remains unclear. However, both genetic and environmental factors play a role. Studies have shown that prevalence of MS increases with latitude, and that disease incidence is increased in developed countries (Multiple Sclerosis International Federation, 2013). Developed countries are socioeconomically developed and mostly Westernized, including a change in diet and lifestyle. This has an impact on the microbiota composition, which can have consequences for human health and involvement in the development of diseases (Jörg et al., 2016). Indeed, alterations in the gut microbiota are an environmental risk factor for MS. In this essay, the role of the gut microbiota in MS pathogenesis will be discussed in more detail.

Pathogenesis of MS

In MS, myelin sheaths in the CNS are attacked by T cells of the immune system. Myelin is the protective sheath surrounding axons of neurons, allowing them to increase the rate at which action potentials are passed along the axon (Figure 1). Myelin is formed by oligodendrocytes, which are bound to the axons in the CNS for support and insulation. The damaged myelin sheaths form plaques (also called lesions), clustering around the lateral ventricles and corpus callosum in the cortex and subcortical white matter, the optic nerves and brainstem, and throughout the spinal cord. This leads to a communication breakdown in and between neurons, resulting in sensory, motor and cognitive problems.

Clinical subtypes of MS

The most common subtype of MS is relapsing-remitting multiple sclerosis (RRMS), affecting 85% of patients. This is characterized by a period of neurological dysfunction in which inflammation and demyelination take place, followed by a period of remyelination and recovery. This process of recurring rounds of relapse and remission cause neurological decline with each relapsing period. Over time, brain disability increases and remyelination processes are hampered, eventually leading to secondary progressive multiple sclerosis (SPMS). In SPMS inflammatory lesions are no longer typical, and progressive neurological decline is accompanied by CNS atrophy: decreased brain volume and increased axonal loss, accompanying largening of the ventricles. Approximately 10% of MS patients is diagnosed with primary progressive MS (PPMS), characterized by progressive neurological decline and complete absence of relapsing periods. This results in steady progression of disability (Dendrou et al., 2015). A small percentage of PPMS patients has progressive relapsing MS (PRMS), the most progressive form of the disease. This is characterized by an ongoing progression, with relapses leading to even faster neurological decline (Weissert, 2013).

Mechanism of disease

In MS, autoreactive T cells cross the blood-brain barrier (BBB) and react against myelin antigens in the CNS. In healthy individuals, potentially autoreactive T cells are suppressed by regulatory T cells, however, in MS the dysfunctional or lower quantity of regulatory T cells allow the autoreactive T cells to cross the BBB. This suggests that these impaired regulatory mechanisms may have an impact on the onset and progression of MS (Compston & Coles, 2008; Dendrou et al., 2015). Experimental autoimmune encephalomyelitis (EAE) studies have shown that regulatory T cells indeed influence the severity and development of MS. EAE studies, which use MS animal models, show the central role of CD4+ T cells (T helper cells). CD4+ T cells are reactivated in the CNS upon infiltrating by antigen-presenting cells, like macrophages or microglia, resulting in inflammatory response (Fletcher et al., 2010). EAE will be discussed in more detail later.

The subtypes Th1 and Th17 CD4+ T cells are the most involved in the initiation of MS. These Th1 and Th17 cells are hypothesized to cause inflammation in the CNS and increase BBB permeability, leading to increased inflammation of the CNS (Dendrou et al., 2015). Th17 cells produce the cytokines IL-17, IL-17A, IL-21 and IL-22, which are involved in inflammation of the brain (Schepici et al., 2019). The inflammation dilates the blood

vessels, leading to an accumulation of cytokines and attraction of more immune cells, including macrophages, natural killer cells and B cells. Moreover, pro-inflammatory cytokines amplify the immune response through activation of microglia, the local immune cells within the brain (Compston & Coles, 2008). Additionally, Th1 cells produce IFN-gamma and TNF-alpha. IFN-gamma activates macrophages and induces production of reactive oxygen and nitrogen species that damage the surrounding tissue. TNF-alpha also causes direct damage to oligodendrocytes, leading to a disruption of myelin production and resulting in lesions (Schepici et al., 2019).
Contrary to CD4+ T cells, the precise role of CD8+ T cells (cytotoxic T cells) remains unclear. However, their quantity is greater in inflamed plaques than that of CD4+ T cells. CD8+ T cells are also able to produce IL-17, are involved in inflammation and cause direct axonal damage by secretion of cytokines, therefore they have a pathogenic role in MS (Dendrou et al., 2015). Besides T cells, B cells potentially also contribute to MS pathogenesis, mostly being involved via antigen-presenting capacity or the ability to produce cytokines (Melbye et al., 2019). All in all, many immune cells are involved in the pathogenesis of MS, leading to demyelination of axons within the CNS.

In early stages, regulatory T cells can suppress the immune response, so oligodendrocytes can recover and start remyelination of the axon. This mainly happens during the acute inflammatory process but can also occur in the progressive phase. The CNS has a pool of oligodendrocyte precursors that can surround the lesions and remyelinate them (Figure 1). However, over time the demyelination process becomes irreversible. The demyelination coexists with axonal and neuronal degeneration, leading to neurological dysfunction (Compston & Coles, 2008).
During chronic inflammation and even in absence of infiltrating immune cells, a chronic neurodegeneration process is triggered. Microglia and astrocytes, local immune cells within the brain, can also produce inflammatory mediators that promote and sustain neuroaxonal damage and thereby contribute further to neurodegeneration. These cells are not only seen in later stages of MS but are even observed in plaques in early disease. They might even contribute to new lesions and general brain atrophy. Both astrocytes and microglia have pro-inflammatory and anti-inflammatory properties, so they can have a neuroprotective function, but can be harmful to the myelin sheaths and axons as well. Dysfunction of these cells may contribute to immune cell infiltration in the CNS and prevent remyelination (Dendrou et al., 2015).

It is still unknown if MS is triggered in the periphery or in the CNS. In the CNS-extrinsic model, activated autoreactive T cells from the periphery cross the BBB and cause inflammation and demyelination within the CNS, leading to neurodegeneration (Dendrou et al., 2015). In the CNS-intrinsic model, neurodegeneration is the start of pathology and the infiltration of immune cells is a secondary event (Dendrou et al., 2015). Another possibility is that both inflammation and neurodegeneration contribute to the process but are completely independent (Compston & Coles, 2008).

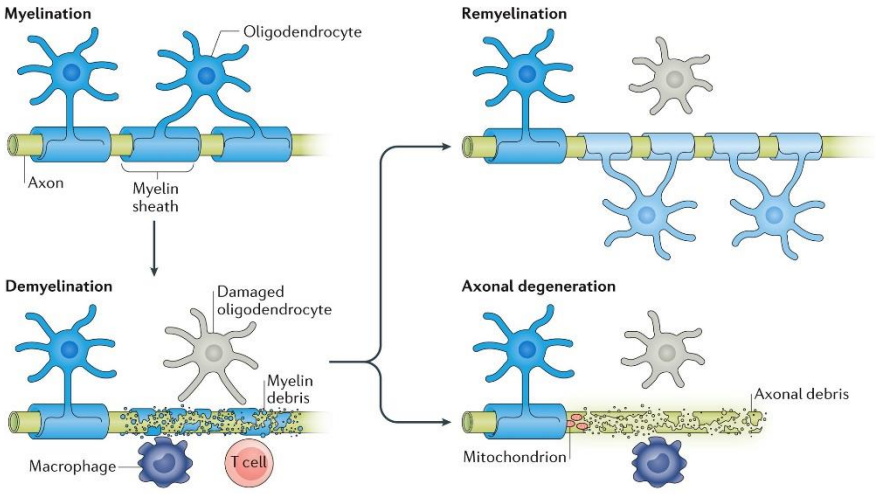

Figure 1. Processes in the CNS during MS. Myelination: healthy axon is protected by myelin sheaths, formed by oligodendrocytes. Demyelination: in MS, T cells and other immune cells like macrophages cause damage to the myelin sheaths and oligodendrocytes. Remyelination: in early disease, new oligodendrocytes can recover the axon, forming new myelin sheaths. Axonal degeneration: oligodendrocytes are not able to recover and protect the axon anymore, leading to degene-ration of the axon. Figure taken from Franklin & Ffrench-Constant, 2017.

Models for MS: experimental autoimmune encephalomyelitis (EAE)

EAE is a demyelinating disease of the CNS and is an animal model for MS. It is induced in susceptible animals by immunization with myelin antigens. EAE is mediated by pro-inflammatory myelin-specific Th1 and Th17 CD4+ T cells, which are activated in the periphery and translocated into the CNS, followed by permeabilization of the BBB. T cells are reactivated within the CNS by antigen-presenting cells, resulting in inflammation and eventually demyelination and axonal damage. EAE can be acute, chronic and relapsing-remitting. It can also be induced by adoptive transfer of activated CD4+ T cells from mice with EAE to healthy mice. This animal model is useful for better understanding the development of MS and underlying processes. However, there are differences between EAE and MS. For example, treatment with IFN-gamma or anti-TNF was protective in EAE, but worsened MS. Therefore, results always have to be carefully interpreted and translated to humans. (Fletcher et al., 2010).

Causes

While the pathogenesis of MS is most likely linked to autoimmunity, as previously described, the exact cause still remains unknown. MS patients have a genetic susceptibility, but environmental factors are also involved, both determining disease risk and probably interacting with each other. The genetically associated risk factors are mainly immunological, while the environmental risk factors are variable.

Genetic predisposition can only explain a fraction of the MS susceptibility risk. MS is inheritable, estimated for around 20%. The firstly discovered MS susceptibility locus on a sex chromosome is located on the X chromosome, which is step toward understanding the genetic contribution of the sex bias, with 3:1 predominance of women being affected with this disease (International Multiple Sclerosis Genetics Consortium, 2019). However, genome-wide associated studies examining the genetic risk for MS showed that MS susceptibility loci are mainly located on genes enriched in immune cell types and tissues. The loci were even located on microglia-specific genes. The genes are related to processes in the development, maturation and differentiation of T, B, NK and myeloid cells. Mainly MHC genes, in particular HLA (human leukocyte antigen) genes, have a strong association with MS. Outside the MHC region, many other susceptible regions were associated with MS, but most of them are also related to the immune system, in particular T cell maturation and T helper cell differentiation. These findings indicate that T cell signaling pathways may be important in the pathogenesis of MS. Implications were not only made for genes coding for immunological genes, but also for involvement of environmental risk factors, like genetic vitamin D pathways. A relative absence was seen of genes relevant to potential pathways for neurodegeneration independent of inflammation. This absence implicates that inflammation leads to neurodegeneration in MS, rather than neurodegeneration being the primary event leading to inflammation, supporting the previously described CNS-extrinsic model (Sawcer et al., 2011; International Multiple Sclerosis Genetics Consortium, 2019).

Recent studies have demonstrated that environmental factors play an important role in MS disease as well, since genetic factors only partly account for the rapid increase in MS incidence and prevalence. For instance, a study has shown that prevalence of MS increases with latitude and that disease incidence is higher in developed countries (Multiple Sclerosis International Federation et al., 2013). A strong biological link is found between MS risk and EBV infection during adolescence. Moreover, an extremely low risk of MS is found in EBV antibody-negative individuals and a decreased risk when infected during childhood. This is attributed to modern-day high hygiene standards that prevent early-life infections, leading to a rise in allergic and immune diseases (Langer-Gould et al., 2017). Next to this, evidence linking cigarette smoking to an increased risk of MS is quite consistent across many studies (Ascherio, 2013).

An important environmental risk factor of MS is a low level of vitamin D. Humans get vitamin D from three sources: diet, dietary supplements and sunlight. Low levels of the circulating form of vitamin D, 25(OH)D, are already often associated with the risk of MS, but some studies have now even provided evidence for a causal relationship (Jacobs et al., 2020; Rhead et al., 2016).

Related to the incidence and prevalence of MS around the world, is socioeconomic development, urbanization and westernization, including a change in diet and lifestyle (Matveeva et al., 2018). A Western diet is generally associated with high sugar, high salt and high fat levels. Increased childhood BMI and eventually high adult BMI are causal risk factors for MS (Jacobs et al., 2020). Change of diet impacts the composition of the gut microbiota, which is important for the functioning of the immune system: change of nutrients is often discussed as risk factor in autoimmunity. Therefore, changes in the microbiota associated with a western diet could play a role in MS (Jörg et al., 2016).

Most of the causes named above, such as the hygiene hypothesis, lack of vitamin D, obesity and changed diet and lifestyle, are linked to the gut microbiota and can lead to a so-called dysbiosis: an imbalanced microbiota compared with a healthy population. This implicates that the gut microbiota composition can be modified by environmental factors, which could be involved in host immune responses and MS pathogenesis.

The gut microbiota

The human intestine is colonized by a complex community of 100 trillions of microorganisms, together called the microbiota (Lozupone et al., 2012). The microbiota is composed of bacteria, viruses, archaea, fungi and protozoa. The collective genome of all microbiota together is called the microbiome. It contains more than 3 million microbial genes, which is 150 times as many genes as the human genome. Over 99% of the genes are bacterial, the other 0,9% mostly origins from archaea and only 0,1% has a eukaryotic or viral origin (Qin et al., 2010).

The microbiota has a mutualistic relation with the human body: it plays an important role in the homeostasis of the body by influencing many processes. This includes functions such as degradation of complex polysaccharides and synthesis of short-chain fatty acids (SCFAs), production of vitamins and amino acids, deconjugation of bile salts and participation in drug metabolism. SCFAs are an important energy source for the gut microbiota and the intestinal epithelial cells. Furthermore, the gut microbiota is essential for competition with potential pathogens, responding to epithelial cell injury and development and regulation of the immune system. The SCFAs for example have diverse regulatory functions on host physiology and immunity, e.g. playing a role in T and B cell differentiation (Qin et al., 2010; Quigley, 2013). The gut microbiota is a complex and diverse community, which has to respond to environmental changes. Alterations in the microbiota composition can lead to a dysbiosis, implicating consequences for human health (Gill et al., 2006; Quigley, 2013).

Composition of the gut microbiota

A study comparing the gut microbiome of populations worldwide representing different ages and cultural traditions showed that gut microbiota evolves over a lifespan, varies between populations and responds to our changing lifestyles. The composition of the gut microbiota is established at birth, influenced by method of delivery and maternal feeding choice. The composition of the bacterial communities develops towards an adult-like composition within three years after birth. Interpersonal variation in microbial composition and function between infants is greater than in adults. Bacterial diversity and stability increase with age, reaching a maximum diversity during adolescence (Yatsunenko et al., 2012). A diverse and stable microbiota composition is associated with good health.

Metagenomic sequencing studies have shown that the dominating bacterial species in the gut are Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria and Verrucomicrobia (Forster et al., 2019). Bacteroidetes and Firmicutes are the most abundant, representing over 90% of the gut microbiota (Qin et al., 2010). Of the Bacteroidetes, members of the *Bacteroides* and *Parabacteroides* genera are mostly displayed. An important Firmicutes member is *Fecalibacterium prausnitzii* and the most prevalent Proteobacteria member is *Escherichia coli* (Forster et al., 2019). Some other prominent gut species are members of the *Clostridia, Dorea, Eubacterium* and *Ruminococcus* groups and also *Bifidobacteria* and *Lactobacilli* groups (Qin et al., 2010).

The microbiota varies greatly between populations and different cultures. The microbiota composition between Western and non-Western individuals is significantly different. These differences might be explained by environmental exposure, appropriate sanitation, levels of hygiene, antibiotic use and diet. Especially diet greatly influences the composition of the microbiota. To underline this, it is seen that the ratio of *Prevotella* and *Bacteroides* is different for Western and non-Western individuals (Yatsunenko et al., 2012). The microbiota of Western individuals had more *Bacteroides*, associated with long-term diet rich in animal protein, several amino acids and saturated fats. The microbiota of non-Western individuals is more enriched by *Prevotella,* associated with a high-fiber diet and diets dominated by plant-derived polysaccharides, rich in carbohydrates and simple sugars. The *Prevotella/Bacteroides* ratio correlates with the overall diversity among healthy adults, showing a lower diversity in Western individuals (Lozupone et al., 2012). This decreased diversity might promote the onset of a chronic inflammatory state. On the other hand, nutrients such as omega-3 unsaturated fatty acids, fiber and vitamin D can promote microbiota proliferation and its ability to produce anti-inflammatory molecules. Therefore, diet has an impact on the microbiota and health of the host (Schepici et al., 2019).

Microbiota-gut-brain axis

A dysbiosis of the gut microbiota composition can play a role in neurological diseases, like Parkinson's disease and MS (Long-Smith et al., 2019). In order to understand this, it is needed to appreciate the connection between the CNS and the gut microbiota, and the way it can influence processes within the brain.

Communication between the gut microbiota and the brain goes via bidirectional signaling, called the microbiota-gut-brain axis. Signaling in the gut-brain-axis can occur via various ways (Figure 2). The vagus nerve is a key mode of communication between the gut and the brain. Further communication systems are the autonomic nervous system, the enteric nervous system and the hypothalamic pituitary adrenal axis. There are many ways the microbiota interacts with the CNS. Microbiota can directly activate the vagus nerve, or interact with enteroendocrine cells to produce bioactive peptides, like neurotransmitters, short-chain fatty acids (SCFAs), amino acids and hormones. SCFAs, including acetate, butyrate, propionate and lactate, can interact with the brain via circulation in the bloodstream. Neurotransmitters and SCFAs can target the enteric nervous system (ENS) via the nerves in the gut wall. Furthermore, microbes contain microbe-associated molecular

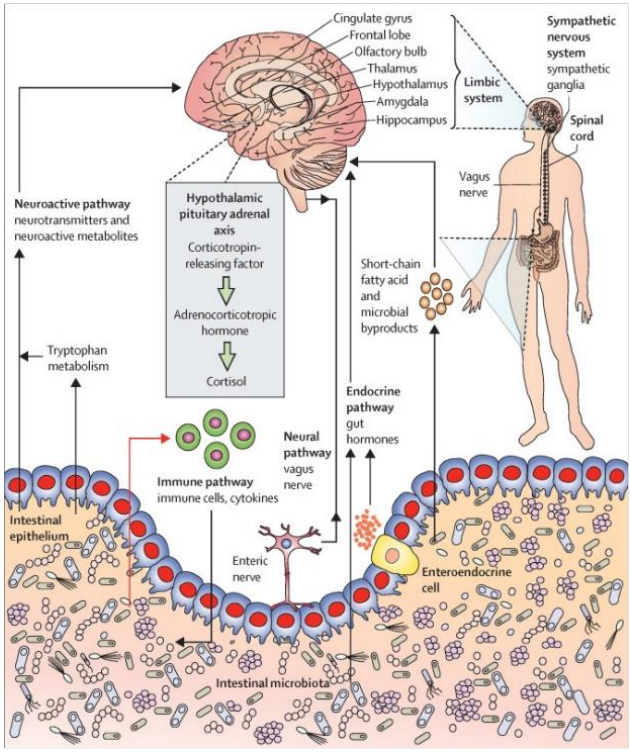

Figure 2. Microbiota-gut-brain axis. Multiple pathways exist for communication between the gut microbiota and the brain. They include the neuroactive pathway (neurotransmitters and neuroactive metabolites); the immune pathway (cytokines and immune cells); neural pathway (vagus nerve, enteric nervous system, spinal nerves); endocrine pathway and the hypothalamic pituitary adrenal axis. The microbiota can produce neurotransmitters and bacterial metabolites, such as short-chain fatty acids. The red arrow indicates immune system stimulation by the luminal content. Taken from Cryan et al., 2020.

patterns (MAMPs), which can be recognized by receptors of the ENS and communicate via immune signaling. The gut contains many immune cells producing pro- and anti-inflammatory cytokines, which can be influenced by the gut microbiota, and can signal to the brain via circulation (Long-Smith et al., 2019). Moreover, these immune signals can activate innate and adaptive immune responses. The other way around, the adaptive immune system influences the microbiota composition and diversity. The immune system plays an important role in communication between the gut and remaining organs, including the brain (Haase et al., 2018).

All in all, the gut microbiota is influenced by many factors, such as geography, culture, age and diet. Mainly diet has an influence on alterations of the microbiota composition, which can impact the health of the host. The gut microbiota can communicate with the brain via many ways and can influence processes within the CNS. An important communication pathway is the immune system. Therefore, it is possible that alterations in the gut microbiota composition can be related to autoimmune diseases, like MS.

The gut microbiota and MS pathogenesis

Alterations in the gut microbiota are an environmental risk factor for MS. The gut microbiota can shape the innate and adaptive immune system, by inducing inflammatory activation of the immune system, via modulation of regulatory and inflammatory responses (Th1 and Th17 cells) (Schepici et al., 2019). Gut bacteria can regulate immune functions involved in MS pathogenesis. In the CNS of animals, the gut microbiota was demonstrated to regulate BBB permeability, activate microglia, limit astrocyte pathogenicity and expression of myelinating genes (Mirza & Mao-Draayer, 2017). Many studies are done to investigate the role of the gut microbiota in MS pathogenesis and to identify the differences between the gut microbiota in MS patients and healthy individuals.

Role of microbiota in onset of MS

In the first research describing the role of the gut microbiota in initiation of MS, Berer et al. (2011) showed that the gut microbiota is essential in triggering immune processes, leading to a relapsing-remitting autoimmune disease driven by myelin-specific CD4+ T cells in absence of the microbiota in EAE. Their results support the hypothesis that pathogenesis of MS originates in the peripheral immune system, instead of MS resulting from neurodegenerative changes in the CNS. This is shown in an experiment in which specific pathogen-free (SPF) relapse-remitting mice (RR) mice developed EAE within a few months, while germ-free mice did not develop EAE. RR mice which had been germ-free for a while (6-12 weeks) immediately developed EAE when re-colonized with healthy microbiota. Re-colonization of germ-free mice restored T cell cytokine production but also led to overproduction reactions. Germ-free RR mice lack activated autoimmune cells due to lack of microbial stimuli. Full clinical EAE required the participation of both myelin-specific T cells and myelin-reactive B cells. The requirement of B cells being available shows that B cells play an important role in MS pathogenesis, although activation B cells depend on the expression of the target myelin autoantigen. RR mice missing this myelin autoantigen did not form antibodies despite having healthy microbiota (Berer et al., 2011).

The role of microbiota in the onset of MS was shown again in a later study by Berer et al. (2017), in which they compared the gut microbial composition of monozygotic twins. One of the twins had MS and the other was healthy. Interestingly, a transfer of human MS-derived microbiota to germ-free mice triggered spontaneous development of EAE. This suggests a causal role of the microbiota in the onset of MS. Increased incidence of spontaneous EAE in the germ-free mice could be attributed to activation of autoimmune T cells or depletion of regulatory mechanisms. The study showed EAE mice suffered from a decrease in production of IL-10, a protective cytokine against CNS autoimmune disease. Treatment with anti-IL-10 led to an increased incidence of spontaneous EAE in the germ-free mice, supporting a protective role for IL-10 and implicating that IL-10 may have a regulatory role in human spontaneous CNS autoimmunity and MS (Berer et al., 2017). Another study transplanted microbiota from MS patients into myelin immunized germ-free mice, resulting in more severe symptoms of EAE and reduced frequency of regulatory T cells compared to mice transplanted with

healthy microbiota. This shows that the gut microbiota is able to transfer the MS phenotype between humans and a MS model, suggesting a potentially causal role for the microbiota in MS (Cekanaviciute et al., 2017).

Alterations in MS gut microbiota

Most studies comparing the gut microbiota of MS patients with healthy individuals observed little variation in species diversity between the two groups. However, the relative abundance of bacterial species significantly differs between the two groups, resulting in an altered gut microbiota (Chen et al., 2016; Miyake et al., 2015). Still, the observed altered bacterial species were different among many studies. This can mostly be explained by differences between the participants, like age and geography, and distinct methodological problems, like the sample size of cohorts and sequencing methods. Reynders et al. (2020) found another explanation for distinct results among studies, showing that gut microbiota variations differ between different MS subtypes, as a consequence of heterogeneity of the disease. Therefore, interindividual variation of microbiota could be explained by assigning them to a MS subtype (Reynders et al., 2020).

Miyake et al. (2015) observed a reduction in proportion of several *Bacteroidetes* species in RRMS patients. Furthermore, a depletion of *Clostridium* species was found, which have the ability to produce SCFAs, in particular butyrate. Jangi et al. (2016) also showed a decreased abundance of butyrate-producing bacteria, namely *Butyricimonas.* Butyrate stimulates anti-inflammatory responses through regulatory T cell induction. Furthermore, a decreased butyrate level can disrupt barrier function and promote inflammation. Next to this, an increase of *Methanobrevibacter,* involved in inflammatory processes, and *Akkermansia* in RRMS patients was found. *Akkermansia* is involved in transformation of mucus into SCFA, which causes damage to the gut barrier, so immune cells are more exposed to microbial antigens in the gut. Pro-inflammatory activity of *Akkermansia* is also involved in upregulation of genes involved in antigen presentation, Th1 cell differentiation, B and T cell signaling and activation of complement and coagulation cascades (Jangi et al., 2016). This same result was found by Cekanaviciute et al. (2017), next to an increase of *Acinetobacter*, which can produce myelin-like components, promoting autoimmunity against myelin. *Parabacteroides* abundance was reduced, normally stimulating differentiation of anti-inflammatory CD4+ T cells and regulatory T cells (Cekanaviciute et al., 2017). These results show the role of the gut microbiota in the generation of pro-inflammatory immune cells.

Further stimulation of immune cells by the gut microbiota was observed by Cosorich et al. (2017), who found that in MS patients, increased abundance of Th17 cells correlates with high disease activity and specific alterations of the gut microbiota. The rise of Th17 cells was associated with higher relative abundance of *Streptococcus* strains, which already have been shown to be capable of inducing Th17 cell differentiation in humans. In mice, a pro-inflammatory intestinal environment promotes Th17 cell expansion and self-reactive T cell pathogenicity. These T cells migrate to the CNS where they contribute to MS pathogenesis by attacking myelin sheaths. Next to this, a reduction in *Prevotella* was found, which is capable of producing the anti-inflammatory metabolite propionate, that limits intestinal Th17 cell expansion in mice (Cosorich et al., 2017). Taken together, the intestinal environment of MS patients is moving towards a pro-inflammatory state.

A decrease in abundance of microbes involved in fatty acid metabolism and increase in microbial pathways involved in defense mechanisms was observed by Chen et al. (2016). To be more specific, a decrease in bacteria associated with phytoestrogen metabolism was observed in RRMS patients, with lower abundance of *Prevotella, Adlercreutzia* and *Parabacteroides*. Phytoestrogens are molecules of plant origin, with main sources as legumes (particularly soybeans), many fruits, whole grains and other vegetables. Decreased abundance of phytoestrogen-converting bacteria has been implicated in an increase in oxidative stress and inflammatory cytokines (Schepici et al., 2019). Estrogens can suppress and/or protect animals from disease in EAE, so reduced levels of metabolizing bacteria might implicate regulation of estrogen receptor signaling by gut microbiota and their metabolites in RRMS (Chen et al., 2016). Furthermore, when comparing active-RRMS patients with remission-RRMS patients and healthy individuals, a loss of species diversity was found. This implicates that the gut microbiota plays an important role in the progression of MS.

To explore the alterations in gut microbiota of MS patients with less other potential environmental exposures, Tremlett et al. (2016) conducted a study with children with RRMS. Just as in adults, there was little species diversity between MS patients and healthy patients, but alterations were characterized by taxonomic enrichments and depletions. This suggests that recent MS onset may be associated with subtle changes in a small number of microbial taxa, rather than large shifts in composition. The found alterations of the microbiota were an increase in relative abundance of *Streptococcus, Bifidobacterium* and *Methanobrevibacter*, and a decreased relative abundance of the *Clostridia* order, among which butyrate-producing species. Altogether, this indicates that early dysregulation of the microbiota stimulates pro-inflammatory and inhibits anti-inflammatory responses of the gut (Tremlett et al., 2016).

The role of short-chain fatty acids (SCFAs)

The pathogenesis of MS is not only influenced by the gut microbiota, but also by the microbiota-derived metabolites like SCFAs, as described in most of the aforementioned studies. As discussed earlier, the microbiota produces SCFAs as a side product of fermentation of dietary fiber. These SCFAs can interact with the CNS via the microbiota-gut-brain axis. The type and amount of fibers consumed affect the composition of the gut microbiota and consequently changes the type and amount of SCFAs produced. The SCFAs are mostly produced by Firmicutes, Bacteroidetes and Actinobacteria. Bacteroidetes members produce high levels of acetate and propionate, whereas Firmicutes produce mainly butyrate (Haase et al., 2018). SCFAs regulate the integrity of the epithelial barrier by regulating mucus production and tight junction expression (Figure 3). Increased permeability of this barrier and related changes in mucosal immunity may be a risk factor for immune-mediated diseases. SCFAs were shown to affect epigenetic enzymes in the gut by promoting the differentiation of naive T cells into regulatory T cells by inhibiting histone deacetylase activity (Melbye et al., 2019). They also interact with the BBB and are able to cross it and modulate brain function, although it is not completely understood how. Germ-free mice develop BBB leakage due to loss of tight junctions between CNS endothelial cells. When colonized with fecal microbiota from normal mice, the process was reversed, indicating gut microbiota influences BBB integrity. Butyrate administration restored the BBB in germ-free mice (Mirza & Mao-Draayer, 2017).

SCFAs can induce expansion of regulatory T cells and have anti-inflammatory effects by inducing anti-inflammatory phenotypes of immune cells (Figure 3). Butyrate was shown to induce IL-10 production, a cytokine implicated in regulatory immune functions. The suppressive capacity of regulatory T cells is disturbed in MS patients, so decreased abundance of SCFA-producing bacteria may represent a possible mechanism driving inflammation (Haase et al., 2018). This is supported by animal studies that showed that the number of

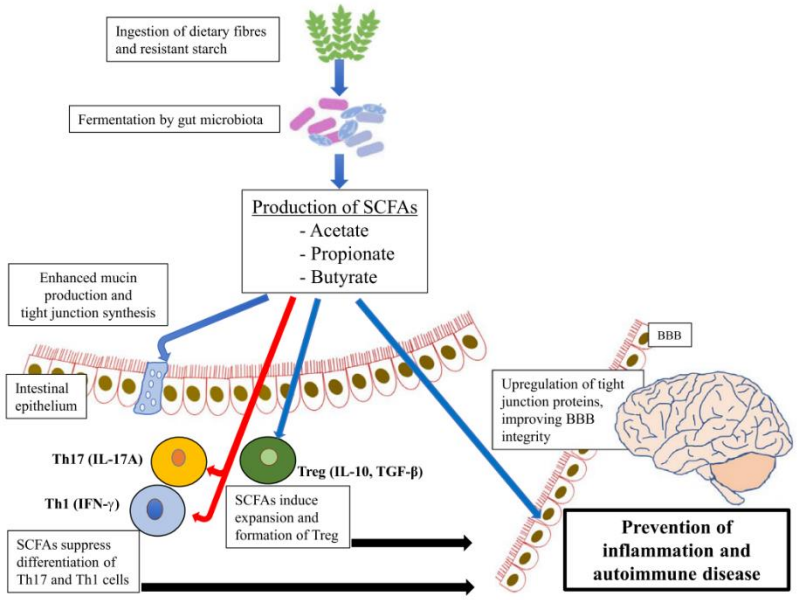

Figure 3. SCFAs have regulatory effect on immune functioning and the involvement of SCFAs in the microbiota-gut-brain axis. SCFAs are produced by bacterial fermentation of dietary fibers and resistant starch. They trigger mucin production and tight junction synthesis; upregulate tight junction proteins, improving the BBB integrity; induce expansion and formation of regulatory T cells and suppress differentiation of Th1 and Th17 cells. The balance between these different T cell types drives pathogenic or protective responses in the CNS. Figure taken from Melbye et al., 2019.

colonic regulatory T cells increased in mice fed with a butyrylated starch diet, along with improved gut integrity, as indicated by increased expression of tight junction proteins and modified autoreactive T cell and regulatory T cell populations (Melbye et al., 2019). SCFAs may also stimulate the production of retinoic acid by epithelial cells, a metabolite that enhances regulatory T cell differentiation and prevents Th17 cell differentiation (Haase et al., 2018). A decrease of SCFAs eventually leads to a pro-inflammatory state of the intestines and eventually the CNS, contributing to the pathogenesis of MS.

Discussion

In this essay, the relationship between the gut microbiota and the pathogenesis of MS is described. MS is an inflammatory and neurodegenerative disease of the central nervous system (CNS). Autoreactive T cells cross the BBB and attack the myelin sheaths surrounding axons in the CNS. Mainly the subtypes Th1 and Th17 CD4+ cells are involved by causing inflammation and increasing BBB permeability, allowing more immune cells to enter the CNS and cause damage. The abundance of regulatory T cells is decreased, leading to an imbalance of pro-inflammatory and anti-inflammatory immune cells. This eventually leads to demyelination and neurodegeneration. MS incidence and prevalence increases with latitude and is higher in developed countries, which are associated with lower levels of vitamin, higher prevalence of obesity and a changed diet and lifestyle. All these risk factors are somehow related to the gut microbiota, showing a role for the microbiota in the pathogenesis of MS. The microbiota composition evolves over a lifespan and is affected by many factors, like age, geographic location, mode of delivery and diet. A dysbiotic gut microbiota composition can influence the CNS via many ways, leading to neurological disease.

The altered microbiota in MS patients could be a cause or consequence of MS. The studies conducted in EAE models, where a transfer of MS microbiota triggered spontaneous EAE, suggest that the microbiota may be the cause for the onset of MS. This supports the CNS-extrinsic model, hypothesizing that MS is triggered in the periphery, where autoreactive T cells are activated and cause inflammation and demyelination within the CNS, leading to neurodegeneration. However, EAE in mice is not completely the same as in MS in humans, so results should be interpreted and translated carefully. Most studies with MS patients are association studies and do not show a causal relation between the microbiota and the onset of the disease. Nevertheless, they do confirm a characteristic dysbiosis of the intestine during MS and show the significant influence on the progression of the disease. They show that the overall microbiota in MS patients is altered, with differences in relative abundance of bacterial species. This results in a decrease of anti-inflammatory events, mainly downregulation of regulatory T cells and decrease of SCFAs production, and increase of pro-inflammatory events, mainly Th1 and Th17 cell differentiation, B cell proliferation and induction of autoreactivity of T cells. A decrease of SCFAs production leads to a higher risk of immune-mediated disease, like MS. All in all, it is highly likely that the gut microbiota plays an important role in the pathogenesis of MS. Therefore, targeting the microbiota for treatment of MS might be a good option.

There are two possible treatment options targeting the gut microbiota. The first is FMT, a transfer of intestinal microbiota with the goal being to introduce or restore a stable microbial composition in the gut. This therapy has been succesful in a few MS patients, however evidence for this treatment is little and more trials in patients are needed to demonstrate the effect of FMT in MS (Borody et al., 2011; Vendrik et al., 2020). A second strategy could be modification of diet. Intake of vitamin D supplements seems to be a good dietary suggestion, since this is an important risk factor for MS. It is also important to prevent overweight, since this is a causal factor for MS. Western people have a lower microbial diversity than non-Western people, explained by a diet high in saturated fat, salt and animal protein (Yatsunenko et al., 2012). Therefore, a shift to a healthy and balanced diet, with vegetables, fruits, unsaturated fats, simple sugars and rich in plant-derived polysaccharides (a substrate for SCFA synthesis), could play a protective role in the course of the disease. Better knowledge of the underlying factors that trigger and drive MS may lead to the development of better and possibly personalized dietary and lifestyle interventions that may prevent and control the development and progression of MS.

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