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Review

Gut microbiome and the risk factors in central nervous system autoimmunity

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ABSTRACT

Humans are colonized after birth by microbial organisms that form a heterogeneous community, collectively termed microbiota. The genomic pool of this macro-community is named microbiome. The gut microbiota is essential for the complete development of the immune system, representing a binary network in which the microbiota interact with the host providing important immune and physiologic function and conversely the bacteria protect themselves from host immune defense. Alterations in the balance of the gut microbiome due to a combination of environmental and genetic factors can now be associated with detrimental or protective effects in experimental autoimmune diseases. These gut microbiome alterations can unbalance the gastrointestinal immune responses and influence distal effector sites leading to CNS disease including both demyelination and affective disorders. The current range of risk factors for MS includes genetic makeup and environmental elements. Of interest to this review is the consistency between this range of MS risk factors and the gut microbiome. We postulate that the gut microbiome serves as the niche where different MS risk factors merge, thereby influencing the disease process.

1. Introduction – multiple sclerosis

Multiple sclerosis (MS) is a demyelinating disease that affects the central nervous system (CNS). In MS, a chronic immune-mediated disease, auto-reactive cells attack the CNS tissue. The specific CNS target molecules are yet to be described. Current genetic information suggests that the critical molecules involved in disease pathogenesis are immunologic, although whether the primary triggering stage is an inflammatory and autoimmune or neurological remains to be elucidated. However, it is evident that MS is characterized by the infiltration of inflammatory cells to the CNS, demyelination, and axonal damage [1]. Lesions appear as demyelinating plaques in both the white and gray matter of the brain and the spinal cord. The most common symptoms of MS are consistent with both gray and white matter disease including fatigue, numbness, coordination worseness, vertigo, bladder, bowel and sexual dysfunction, vision reduction, dizziness, pain, emotional stress and depression [2]. Clinically MS is characterized by either relapsing/remitting or a progressive condition [3].

The disease is most commonly diagnosed at ages 20–50. Interestingly, since the time of its first description by the French neurologist Charcot in the late 1800's the female/male ratio has shifted from 1:1 to its current incidence in which it exceeds 3:1 female to male [4]. This differential gender ratio has arisen the last decades with an apparent differential change, depending on the geographical location [5]. This difference in female/male MS susceptibility is common in other, but not all, autoimmune diseases, such as Sjogren's syndrome, systemic lupus erythematosus (SLE), scleroderma, and rheumatoid arthritis (RA) [6]. The endocrine system might be responsible for the differences in the susceptibility female/male ratio; however, genetic and immunological factors are no doubt part of this complex interaction between the neuronal and immune system. In this article, both consistent and conflicting hypothesis that aim to explain the genetic and or environmental source for this devastating disease will be reviewed. Moreover, we will postulate the importance of the microbial populations that inhabit the gastrointestinal tract as an ecosystem where multiple disease risk factors merge. We hypothesize that the gut microbiome is an adaptable "acquired" immune organ that can modulate the severity MS disease. We speculate with the possible role of specific gut microbes and more importantly specific antigen(s) derived from commensal microbiota as both effectors and regulators of CNS demyelination and perhaps affective disorders as well.

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2. Environmental, genetics and microbial risk factors associated with CNS demyelinating disease

Environmental, genetic, immunological and infectious diseases have been associated with MS [2]. The genetic background is thought to be a critical epidemiologic risk factor. As reviewed by Compton and colleagues, the familial recurrence rate in MS is 20% [7]. Monozygotic twins, sharing the 100% of the genetic information, have an MS lifetime risk of approximately 25–30%, siblings with two affected parents approximately a 20%, whereas dizygotic twins have a 5% lifetime risk, frequencies that suggest that the genetic background could interact with other risk factors.

2.1. Genetic susceptibility and genetic risk factors

Alterations in immuno-regulatory or structural genes might increase the susceptibility to MS. Differences in the human leukocyte antigen (HLA) system—genes in the chromosome 6 that serve as the major histocompatibility complex (MHC) in humans—haplotypes DRBl*1501 and DQ6 have been suggested as genetic risk factors [8]. Recent genome-wide association studies (GWAS) reveal an evident effect of these genetic risk factors, with particular importance of HLA class II region, but also class I [9,10]. Interestingly, the genetic factors associated with MS are involved in immunological processes [11], providing strong theoretical support to the autoimmune nature of MS, and moreover, provides the potential framework that environmental factors may be intimately involved in the modulation and/or perhaps even the trigger for disease susceptibility.

2.2. Genetic, hormones and sex differences in multiple sclerosis

The incidence of a number of autoimmune diseases is higher in women than men. Genetic factors define hormonal differences between females and males. Also, the effect of X-chromosome linked mutations has been suggested to affect autoimmune processes [6]. However, genetic factors might not be sufficient to explain the documented rise in the female/male ratio observed in the last decades. In the CNS, immune cells regulate inflammatory processes, but other brain-derived modulatory factors also play a role, by the production of cytokines and hormones, in processes mediated by the autonomic neuronal system. Receptors expressed in immune cells sense cytokines and hormones allowing a critical interaction between the immune, neuronal, and endocrine system. Multiple immune functions such as activation, proliferation, chemotaxis, phagocytic activity and cytotoxic functions are regulated by different endocrine pathways that take place within the CNS. In women with MS, an increased risk factor is conferred when DRB1*1501 is combined with the estrogen receptor alpha-4 (ERα4) polymorphism [12]. Estrogens are sex hormones with well-documented effects in the immune system [6]. Estrogen interaction with its receptors expressed in T cells, B cells, dendritic cells, macrophages, monocytes, natural killer cells and others have been shown to impact the activation and immune function of innate and adaptive immune cells, including regulatory T (Treg) cells [6]. Estrogen administration protects mice against EAE [13] and oral treatment with estriol reduces the number of brain active lesions in female RRMS and SPMS patients, according to a phase I study [14]. A phase II trial studied the effect of a combination of oral estriol with injections of glatiramer acetate for two years, as it was recently reported by Voskuhl and colleagues at the American Academy of Neurology (AAN) 66th Annual Meeting [15]. Treatment with estriol and glatiramer acetate reduced in a 47% the number of relapses after 12 months, and after 24 months the relapse rate reduction was 32% when compared with the placebo plus glatiramer acetate. Although the mechanism of action is not fully understood, in EAE estrogen treatment promotes tolerogenic antigen presenting cells, enhances the expression of the immuno-modulatory programmed death-ligand 1 (PD-L1) on B cells and reduces Th17 levels while regulates the suppressive function of Treg cells [16].

Recently, sex differences have been directly linked to changes in the gut microbiome and to the susceptibility to autoimmune diseases, such as diabetes. In non-obese diabetic (NOD) mice, gut microbes influenced the levels of sex hormones and had a significant effect in pancreatic beta-cell destruction, insulitis and protection against Type 1 Diabetes. Authors hypothesized that the microbiome interacts with genetic risk factors through changes in the endocrine system [17]. Another recently published work suggests that the microbiome determines the different autoimmune susceptibility between females and males [18]. We will further discuss the effect of diet on the gut microbiota and how diet and gut microbes modulate disease severity. It is possible to hypothesize that the increases observed in the female/male ratios are based on the significant changes observed in our lifestyle, including habits such as smoking, consumption of high-fat diets, stress, use of antibiotics and others. More directly related to females is the use of contraceptive methods and their undetermined effect on MS susceptibility [19]. All these factors impact the endocrine and the immune system, but also impact the microbial gut ecosystem.

2.3. Environmental factors

2.3.1. Sun exposure and vitamin D deficiency

A main environmental factor for MS and other autoimmune-related diseases under high scrutiny is the deficiency in Vitamin D uptake and its possible association with insufficient sun exposure. Meta-analysis showed a significant inverse correlation between MS risk and UV radiation [20], and as recently reported, vitamin D insufficiency has a clear association with increased disease in those on immune modulatory therapies for MS such as interferon (IFN)-beta [21]. Experimentally, UV radiation exposure has been shown to reduce the severity of murine EAE [22]. In this report, the continuous treatment with UV radiation protected mice against the disease. Interestingly, vitamin D might not be the mechanism of protection, and direct effect of UV light in immuno-modulatory factors such as IL-10 production and Treg cell induction has been postulated [23].

Several of the risk genetic and environmental factors associated with MS, such as latitude and diet, could promote vitamin D deficiency and affect the immune responses. Over 200 genes are associated to vitamin D function, and suppression of antibody production and enhancement of Th2-type immune responses have been linked to vitamin D [24]. Epidemiological studies showed that high levels of 25-OH Vitamin D in peripheral blood correlated with a reduced risk of MS in Caucasian women, and was indirectly associated with high percentages of Treg cells in MS patients [24]. In northern Europe, areas suffering low UV light exposure and vitamin D deficiency show an inverse correlation with the risk of MS, despite the genetic susceptibility due to the high frequency of HLA-DRB1 associated with these regions [25]. The inverse correlation with MS risk described for these low sun exposure areas could represent an example of the multifactorial nature of the risks associated with MS. As reviewed by Simpson and colleagues [25], diet and specifically vitamin D supplementation in the diet could be a critical element to consider, as suggested by a study showing that the diet used by the population of this Scandinavian area is far, more abundant on vitamin D when compared to other European regions [26].

Because of the described mechanisms to modulate the immune system, vitamin D may directly affect the gut microbiota composition. Mai and collaborators first described a link between

diet-dependent Vitamin D intake and an altered composition of the gut microbiome [27]. A reduced vitamin D intake could promote the altered immune responses, such as effects in the induction of FoxP3$^+$ Treg cells and reduced T cell gut homing. These immune effects could affect directly the microbial populations of the gut, as suggested by Ly and colleagues [28], and lead to disease. One of the most widely studied gut symbiont, *Bacteroides fragilis*, produces a polysaccharide (PSA) that, as reviewed later in further detail, promotes the induction of gut FoxP3$^+$ Treg cells. As reported by Mazmanian and his group, *B. fragilis* derived PSA and vitamin D interaction appears to exacerbate the induction of FoxP3$^+$ Treg cells and promotes enhanced protection against experimental autoimmunity. Moreover, preliminary studies indicate that PSA can promote the production of colonic 1,25-Dihyroxyvitamin D3 (1,25D) from the 25D precursor, also a precursor for vitamin D [29]. Given the experimental evidence showing that 1,25D activates the induction of Treg cells [30], it is possible that a direct connection between vitamin D and PSA Treg cells induction pathways contribute to the association between diet, vitamin D deficiency, the gut microbiome, and a merged risk factor for autoimmune diseases.

2.3.2. Cigarette smoking

In 1965, a first case-control study reported that the percentage of cigarette smokers among MS diagnosed patients was significantly higher than control individuals [31], although a second study showed no association [32]. More recent studies suggest this correlation, and possibly in the transition from relapsing-remitting MS to secondary-progressive MS forms [33]. The interactions with other risk factors, such as vitamin D deficiency [34], host genetics and viral infections [35,36] have been also suggested. Handel and collaborators published that the combination of genetic susceptibility and environmental factor such as UV light exposure and smoking enhance the risk factor for MS in Europe [37]. Smoking has been described as a factor that influences the gut microbiome in humans, with an increased microbial diversity and overall composition changes observed in the microbiome often volunteers after smoking cessation [38].

2.3.3. Stress

There is a body of unpublished evidence obtained from both patients and clinicians suggesting that stress exacerbates clinical symptoms of MS and augments the number of relapses. One problem that arises when studying stress is its definition. Nevertheless, stress is associated with significant changes in the autonomic nervous system, the hypothalamic–pituitary–adrenal axis, and the vascular system [39]. In EAE, induction of stress has a significant impact on disease severity, as reviewed by Lovera and Reza [40], and case-control studies in humans suggest a link between stress conditions and relapses [41] and disease indicators such as brain lesions evaluated by MRI [42]. Because of the effects that stress has in neuronal, hormonal and vascular systems, the mechanisms that might link stress with altered course of MS might be multifactorial and associated with the immune responses. With this regard, animal studies reported on the association of stress and disturbed composition of the gut microbiome, by mechanisms linked to the gut–brain axis as suggested by Cryan and colleagues [39].

2.3.4. Diet

Although results obtained in several experimental disease models suggest an association between diet and disease, there currently is limited cause-effect information on the role of diet in human autoimmune disease. However, the potential risk of diet in MS in association with the gut microbiome will be further discussed in this manuscript, as well as the interactions between diet and other risk factors, such as vitamin D and its supplementation, also previously proposed [43].

2.4. Microbial infection

The involvement of microbial agents as risk factors in MS and other autoimmune diseases has been widely discussed, and the improvement of the sanitation conditions suggested as the reason behind the significant increase observed in the incidence of these diseases over the last century. The “hygiene hypothesis” states that increased vaccination practices, extended usage of antibiotics, and clean environment may alter the colonization of intestinal pathogenic microorganisms. These scenarios could lead to an imbalance between inflammatory Thl and Th17 and anti-inflammatory Th2 and regulatory cell populations, associated with autoimmune conditions. Commensal bacteria present in the gut, such as *B. fragilis*, have been recently associated to the hygiene hypothesis, traditionally associated to parasitic infections by helminths [44].

The role of viruses as the inducing factor in MS has been widely hypothesized over the past few decades. Different viruses have been suggested as infectious factors that trigger MS. Herpes simplex virus, rabies, Epstein–Barr virus, corona virus, measles, canine distemper virus, and HTLV-1 have been implicated in MS over the past years but to date no conclusive association has yet been established [45]. The presence of Herpes simplex virus-6 (HHV-6) or its DNA has been reported in MS lesions and the cerebrospinal fluid of MS patients [46], without a direct association being established [47]. Epstein–Barr virus (EBV) is a member of the herpes virus family and along with HHV-6, the worldwide spread and one of the most common human viruses, has also been linked to MS as a disease trigger. Interestingly, the EBV nuclear antigen shares the sequence with the myelin basic protein (MBP), which could explain the induction of MBP-specific encephalitogenic T cells in MS, through a molecular mimicry mechanism [2]. Recent epidemiological studies comparing CNS-derived antibodies against EBV versus other neurotropic viruses in childhood and adults MS onset failed to sufficiently establish that EBV is an etiologic agent of MS, although it may regulate the susceptibility to the disease by modifying the immune responses [48].

As discussed later through this review, the multiple and various risk factors associated with MS may suggest that more that the combination, rather than a single etiological factor, might be responsible for the induction of the disease. For instance, a recent study showed that blood obtained from individuals prior their first clinical manifestations had reduced 25(OH)-D levels and increased EBV reactivity [49]. EBV and high latitudes, linked to low 25(OH)-D levels have been also associated with a functional deficiency of CD8$^+$ T cells in their response to the virus and exacerbated expansion of auto-reactive B cells that are infected with EBV [50].

3. The association of the various environmental, genetic, and microbial risk factors in CNS demyelination and the gut microbiome

The human body maintains a physical and chemical separation with the exterior through the skin, and mucosal surfaces of the conjunctiva, the respiratory, gastrointestinal (GI), and the urogenital tracts. However, and despite their role as barriers, these areas constitute essential locations for the interaction between us and the environment where we live. The skin and all mucosal tissues constitute the ecosystem for a highly heterogeneous and abundant community of microorganisms that represent the microbiota. The genomic pool that constitutes this community in association with host genome is collectively referred to as the microbiome. First described by Joshua Lederberg [51], the microbiome comprises a widely diverse community of bacteria, archaea, fungi, protozoa and viruses. Evolutionarily, vertebrates and their microbiome have evolved since developing a

digestive system that allows for the freedom of movement whether in water, air or land. Microbial symbiont communities are essential for the vertebrate's immune system development, for the appropriate digestion of food and processing of nutrients such as fibers, and also to serve as combat forces against infections [52]. The microbiome is heterogeneous in its composition [52], 100 times larger than the human genome, and in terms of individual organisms, ten times more numerous than our individual cell counts [53]. *Actinobacteria*, *Firmicutes*, and *Proteobacteria* members dominate the skin microbiota. In the oral cavity *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* are most abundant. The urogenital tract is primarily dominated by *Firmicutes*, whereas the respiratory tract shows an abundance of *Bacteroidetes* and *Firmicutes*. The GI tract shows a predominance of *Bacteroidetes* and *Firmicutes*. However, there are qualitative and quantitative differences depending on the location within the GI tract [52]. The GI tract is the most extensive site for exposure and interaction with the microbiome and also pathogens that utilize the mucosa as invasion and infection sites. The host's immune system, determined by its genome, is responsible for the control of symbiont, pathobiont, and pathogenic microorganisms. In turn, the microbiota modulates the environment by influencing the normal function and development of the GI tract. Rodent germ-free (GF) (born and raised sterile) studies showed that the monocolonization with *B. fragilis* was sufficient to stimulate early development of the gut-associated lymphoid tissue (GALT), to induce normal organogenesis in the spleen and thymus and balanced immune development [54].

The microbiome is also heterogeneous temporally: microbes colonize vertebrates soon after birth, and a more stabilized microbiome is established early in life. The gut microbiota is affected by numerous stimuli, in particular diet, but also our own immune and endocrine systems that can alter its composition. Recent evidences show that the adult microbiome is not as stable as previously believed, and that diet can promote significant and rapid changes in the overall microbial abundances [55]. Moreover, diet and geographical factors merge affecting selectively the gut microbiome composition [56], and combined have been proposed as risk factors in IDB [57]. The multifactorial nature of the triggering process of MS has been also experimentally linked with symbiont microbes of the GI tract. The interactions of microbes with the host have evolved into a complex balance of host genes, environment and microorganisms. TLR5 deficiency, which recognizes the flagella of bacteria, led to metabolic disorders such as hyperlipidemia, with enhanced levels of triglycerides and cholesterol, hypertension, enhanced adiposity, as well as a 20% increase in the average body weight of mice when compared to TLR5 competent mice [58]. Another murine TLR bacterial ligand, lipopolysaccharide (LPS), recognized by TLR4 was linked with a permanent low-level inflammatory status and increases levels of serum LPS when conventional mice were fed with a high-fat diet (HFD). These mice became obese and suffered from metabolic unbalances and diseases, such Type 2 Diabetes (T2D) [59].

3.1. Obesity, CNS demyelination and the gut microbiome

Evidence suggests an association between obesity, gut microbiota, and the induction of Th17 responses that could relate obesity with various autoimmune disorders. In accordance with the experimental data, the worldwide increase in the incidence of obesity and metabolic diseases such as T2D has been associated with changes in dietary habits [60]. The direct effect of hormones in diet and obesity may also play a role in disease severity. Adipose-derived hormones and adipokines, cytokines produced by adipose cells, might constitute which diet/obesity regulate the immune system. Leptin is a hormone produced by adipose tissue, but also by the stomach, skeletal muscles and placenta. The proportion of fat mass in the tissue regulates leptin production, thatcontrols food intake, influences the autonomic nervous system and regulates the production of other hormones. Mice that are deficient in leptin or in the receptors for leptin are obese and suffer from severe immune system alterations. Interestingly, leptin-deficient mice do not develop EAE [61] while neutralizing leptin in EAE mice reduces disease severity [62]. In MS patients, high levels of leptin are observed in active CNS lesions [63], and in sera and cerebrospinal fluid (CSF) [64]. Thus, the neuroendocrine system, tightly regulated by nutritional factors and habits, affects significantly the immune system [65]. Fasting for instance, that reduces the levels of circulating leptin, impacts proinflammatory responses and regulates EAE severity [66]. Other hormones of the gut–brain axis have shown modulatory functions in autoimmunity: Neuropeptides, such as vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY) affect the induction of Treg cells and migration patterns of immune cells. VIP has been shown to induce FoxP3$^+$ Treg cells and be protective against experimental rheumatoid arthritis [67] and diminish the severity of murine EAE [68]. In mice, diet-induced gut microbiome alterations promote significant changes in the inflammatory responses elicited by the adipose tissue [69]. Moreover, the connection between adipose-derived hormones and the gut microbiome has been seen to influence the fat-gene regulation in the CNS [70]. These effects of the gut microbiome on the neuroendocrine system would then be essential in the regulation of inflammation and disease severity.

It is known now that the composition of the gut microbiota of obese donors is significantly different to that from lean individuals [71], and that diet impacts the composition of the gut microbiome [55]. Westernized diet increases the relative abundance of *Firmicutes* and reduces *Bacteroidetes* in mice [72]. In humans, obesity was associated with reduced bacterial diversity and reduced abundance of *Bacteroidetes* [71]. When fed with a western diet for eight weeks, GF mice gain significantly less weight and fat mass than conventional mice and are protected against diet-induced glucose intolerance [73]. When conventional mice receive a high-fat diet the levels of plasma free fatty acids increase. Plasma free fatty acids enhancement during HFD consumption and obesity has been associated with inflammatory syndrome and insulin resistance, exacerbating inflammation, and promoting oxidative stress by releasing of reactive oxygen species [74]. These enhanced free fatty acid levels while subjected to high-fat diets are reduced by the treatment with antibiotics against the gut microbiome [75]. In EAE, HFD administration exacerbates EAE severity [76] through the activation of Th17 cells whereas a caloric-restricted diet ameliorates disease [77]. Protection by the administration of caloric-restricted diet to SJL mice was associated with enhanced plasma levels of corticosterone and adiponectin and reduced IL-6 and leptin.

The disruption in the balanced gut microbiota is termed "dysbiosis". Dysbiosis has been suggested to be responsible for intestinal inflammation that is observed in patients suffering from irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). In IBS patients, the gut microbiome composition differs significantly from healthy individuals [78]. In IBD patients, the analysis of the gut microbiota revealed statistically significant differences between the populations of Crohn's disease patients and ulcerative colitis patients and healthy individuals [79]. To date, no studies on the gut microbiome of multiple sclerosis patients have been documented, however, experimental data support the importance of the gut microbiome in the control of the severity of the disease [80].

3.2. Commensal bacteria in the effector/regulatory network in CNS demyelination

Given the importance of diet in the modeling of the gut microbiome and the effect of gut microbes controlling inflammation, it is

reasonable to hypothesize that it is in the gut where a pool of risk factors merge, including those directly associated with the genetics of the host. Studies performed using germ-free (GF) animals demonstrated that presence of gut symbionts is essential in the development of the immune system. GF mice show a default Th2 biased immune system, with a significant reduction of lamina propria Th17 cells [81], the main body reservoir for these cells. Mono-colonization of GF mice with *Segmented Filamentous Bacterium* (*SFB*) is sufficient to restore the Th17 responses in these mice [82].

By contrast, the colonization with *B. fragilis* rebalances the biased immune responses in GF mice and promotes regulatory cell subpopulations [54]. *B. fragilis* is a gram-negative anaerobic bacterium present in the gut of most mammals, including humans. *B. fragilis* produces eight different capsular polysaccharides, essential for the survival and proliferation in this ecosystem [83]. One of these polysaccharides, polysaccharide-A (PSA), is a zwitterionic tetrasaccharide with varying number of repeating units per macromolecule that activates and induce the proliferation of $CD4^+$ T cells through a TLR2-mediated antigen presenting cell recognition and MHC class II-based presentation. PSA produced by *B. fragilis* can balance the Th2 biased immune responses in GF mice, and promotes strong IL-10-producing T cell responses that involve Trl cells, $FoxP3^+$ Treg cells, and $CD39^+$ Treg cells. The essential role of *B. fragilis* and PSA in immunoregulation and control of autoimmunity will de discussed in further detail.

Gut metabolites have been also proposed as essential regulators in autoimmunity. Fermentation is a hallmark that defines a gut microbe as symbiont. Microbial processing of fiber generates short-chain fatty acids (SCFA), known to promote strong anti-inflammatory effects in IBD models, and to exacerbate the regulatory function of Treg cells. One of the mechanisms described for the anti-inflammatory effect of SCFA in immune cells is by their interaction with the G-Protein coupled Receptor 43 (GPR43) [84]. Stimulation of GPR43 with SCFA promotes the induction of colonic IL-10-producing $FoxP3^+$ Treg cells. Treatment of GF mice with SCFA restores the unbalanced frequency of colonic Treg cell populations and their function, and promoted the protection against colitis [85]. A fermentation product produced by *Clostridia* clusters, butyrate, has been also shown to induce colonic Treg cells and reduce the severity of experimental colitis in mice [86]. Remarkably, preliminary studies from Weiner *et al.* presented at the 2014 Joint ACTRIMS and ECTRIMS meeting suggest and differential in both archaea and butyrate producing organism abundances in the gut microbiota of MS patients versus healthy donors [87]. Gut symbionts are essential for the host organism's nutrient uptake. Moreover, their processing products are determinant in local and systemic immune-regulation. Thus, diet, considered an environmental risk factor in MS, appears as a central regulator of gut microbes, with known experimental importance in CNS demyelinating inflammation.

4. Mechanisms of regulation of central nervous system by gut microbes

Oral tolerance has been associated with the control of experimental autoimmune diseases including EAE [88]. Genetically attenuated strains of *Salmonella* have been successfully used to induce tolerance and protection in EAE and rheumatoid arthritis models [89]. In EAE, *Salmonella enterica* serovar Typhimurium expressing the CFA/I fimbriae of Enterotoxigenic *Escherichia coli* confers prophylactic [90] and therapeutic [91,92] protection against EAE after a single oral dose, in a TGF-β-producing $FoxP3^+$ Treg cells-dependent mechanism [91]. In vivo neutralization of TGF-β ablates the protection conferred by *Salmonella-CFA* induced Treg cells [92]. These findings suggest a connection between the immune responses elicited in the gut and the immune consequences that may take place within the CNS.

Different pathways of gut-brain axis in the context of CNS inflammation have been proposed. The CNS releases signaling peptides that modulate dietary patterns and food intake. These signals have direct effects in biochemical and physical processes that control nutrient absorption, and regulate endocrine pathways. As recently reviewed by Cryan and colleagues [39], the endocrine and nutritional regulation is mediated through the autonomic nervous system and the hypothalamus–pituitary adrenal axis, constituting an essential regulatory component of the brain–gut axis that is influenced by different factors, such as stress and diet. These mechanisms of control may influence gut microbial communities. In turn, gut microbes modulate brain function by the release of metabolites such as SCFA, antigens (peptidoglycan, LPS, PSA) with immunological effects, and endocrine factors such as 5-hydroxytryptamine produced by enteroendocrine cells [52]. Recent experimental evidences found in mice suggest that the GI ecosystem modulates neuronal functions through the enteric nervous system (ENS). The ENS is essential in controlling the integrity and functions of the GI. Gut symbionts such as PSA-producing *B. fragilis* and Lactobacillus rhamnosus (JB-1) reach murine ENS afferent neurons and influence their function [93].

In MS, auto-reactive cells respond to inflammatory stimuli and attack the CNS causing demyelination and axonal damage. In MS patients, there is a primary role for interleukin-17 (IL-17) and pro-inflammatory Th17 cells in its pathogenesis. IL-17 expressed by lymphocytes found in cerebrospinal fluid and plaques of MS patients was enhanced when compared to healthy individuals [94]. The expression of IL-17 receptor (IL-17R) and IL-22 receptor (IL-22R) in the vessels of MS lesions are enhanced when compared to the vessels of healthy individuals [95]. Peripheral blood mononuclear cells of patients with MS exacerbation have enhanced levels of Th17 cells ($CCR6^+CD161^+$) when compared to patients treated with interferon (IFN)-β and glatiramer acetate [96]. As earlier discussed, CNS inflammation may be controlled by Treg cells. Interestingly, no significant differences in Treg cell frequencies between MS patients and healthy individuals were found, however, the suppressive function of Treg cells from MS patients appears to be reduced when compared to those obtained from healthy individuals [97]. Approved MS therapeutics such as IFN-β and glatiramer acetate, and currently evaluated alemtuzumab potentiate the anti-inflammatory function of Treg cells in EAE and MS [98,99].

Th17 cell-suppressive Treg subset expressing CD39 is disrupted in MS patients [100]. Similar mechanism has been observed in autoimmune hepatitis [101]. In patients suffering from autoimmune hepatitis, $CD39^+$ Treg cells have enhanced expression of CD127 and produce IFN-γ and IL-17. We have recently shown that PSA purified from *B. fragilis* protects EAE mice through a CD39-based mechanism [102]. Upon oral administration of PSA $CD39^+$ Treg cells express higher levels of migratory signaling molecules and accumulate within the CNS of EAE mice [103]. We now have evidence that PSA also enhances the frequency of CD39 in human Treg cells [104].

Numerous publications have focused on the role of B cells in MS pathogenesis [105]. $CD20^+$ B cells have been targeted with MS therapeutics showing promising effect in reducing relapse rates and lesions in RR-MS patients [106]. However, the need for the depletion of B cells remains controversial due to the existence of B cell subpopulations with regulatory phenotypes that are involved in the protection to EAE. B cell-deficient C57BL/6 mice suffer an exacerbated EAE form [107] and IL-10-producing B cells transferred into IL-10 deficient mice protect them against the disease [108]. Splenic $CDld^{high}CD5^+$ B cells that produce IL-10 are protective in EAE through TLR2/4 and MyD88 signaling pathway, implicating a potential role for bacterial antigens in the EAE regulation

by B cells [109]. The effect of diet in obesity and diabetes has been linked to B cells. CD20 treatment attenuates diet-induced diabetes in mice [110], and B cells isolated from obese mice and T2D humans have a proinflammatory cytokine pattern, and are defective in the production of interleukin-10 (IL-10). By contrast, B cell-null mice are protected from pathogenic outcomes of obesity and inflammation [111]. In this work, DeFuria and colleges showed that the effect of B cells in diet-induced diabetes is mediated through their proinflammatory profile of cytokines released and also by potentiating the autoreactive proinflammatory effect of T cells. The connection between the administration of HFD, the induction of autoimmunity and gut microbes may relay on the effect of the gut microbiome in B cell functions. In the murine gut, B cells undergo development influenced by the microbiome [112], and we have shown that alterations of the gut microbiome modulate B cell function in EAE [113]. The adoptive transfer of CDld$^+$ B cells from EAE mice treated with antibiotics transferred protection to EAE recipient mice. Flow cytometry analysis of recipient mice showed reduced Thl/Th17 profile after the transfer of B cells, suggesting a secondary effect of B cells in modulating T cell responses as observed in diabetes experimental models [111]. The importance of B cells in MS pathogenesis, the evidences suggesting that different phenotypes might be beneficial or detrimental in the disease course, and the potential of these cells to be modulated by diet and changes in the gut microbiome make these cells highly targetable and a relevant focus of future studies.

4.1. Gut helminths and CNS demyelinating diseases

Early exposure to helminths could be protective against the disease [114]. Antigens from helminths induce TLR2-dependent immunomodulatory phenotypes in cells obtained from MS patients [115]. Large studies are currently evaluating the beneficial effect of helminths' infection in MS patients. A study that followed 12 relapsing-remitting MS patients naturally infected with helminths compared to uninfected matched controls demonstrated remarkable disease stability [116]. Immunological read-outs showed that peripheral blood mononuclear cells obtained from infected MS patients produced enhanced levels of IL-10 and TGF-β when compared to uninfected donors, whereas inflammatory IL-12 and IFN-γ where reduced. A pilot study showed how five relapsing-remitting MS patients treated orally with *Trichuris suis ova* (TSO) every two weeks presented reduced gadolinium-enhancing lesions after three months of treatment [117]. These numbers increased two months after the termination of the treatment. A Th2 switch and a regulatory cytokine pattern were observed in serum. An association between infection with helminthes and their effect on the established commensal microbiota has not been investigated.

4.2. Gut bacteria and CNS demyelinating diseases

Studies performed in different EAE models in GF mice demonstrate that the induction and severity of CNS demyelinating inflammation depend on the presence of the gut microbiota [118,119]. Gut microbes, such as SFB, have been implicated in the induction of potent proinflammatory Th17 cell responses in the gut that ultimately restore the severity of EAE in GF-mice [118]. Altering gut microbiota abundances impacts EAE severity in mice [120,121]. Oral treatment of mice with antibiotics reduced the EAE severity by diminished pro-inflammatory responses and enhanced FoxP3$^+$ Treg cells that accumulated in mesenteric and cervical lymph nodes (LN). FoxP3$^+$ Treg cells obtained from the cervical LN of mice treated with antibiotics produced elevated IL-10 levels and conferred protection against EAE after adoptive transfer. When mice treated with antibiotics were injected with anti-CD25 antibody to neutralize the effect of CD25$^+$ T cells, a significant increase in the disease severity was observed. However, the disease severity was significantly lower than in EAE mice not subjected to the oral administration of antibiotics but treated with anti-CD25 antibody. These results suggested that other regulatory cell populations that might be important in the protection observed, such as a role for iNKT cells [121] and IL-10 producing CD19$^+$ B cells [113].

The periodontal *Porphyromonas gingivalis* potentiates glial activation, enhances proinflammatory responses and exacerbates EAE [122,123] whereas *Lactobacillus* and *Bifidobacterium* strains regulate EAE severity. *Bifidobacterium animalis* reduces EAE in Lewis rats [124] and the administration of a mixture of *Lactobacillus* protects mice against the disease [125]. C57BL/6 mice treated prophylactically with *Lactobacillus paracasei* and *Lactobacillus plantarum* strains showed reduced EAE clinical symptoms and proinflammatory responses, and the combination of three different strains of *L. plantarum* reduced the severity in established EAE, in a mechanism associated with the induction of IL-10 producing FoxP3$^+$Treg cells. Probiotic *Pediococcus acidilactici* strain R037 [126] and *Lactococcus lactis* engineered to produce the Heat shock protein 65 (Hsp65) [127] also reduce the severity of EAE. R037 induced IL-10-producing Trl cells whereas *L. lactis*-Hsp65 promoted inducible Treg cells and Latency-associated peptide (LAP)$^+$ CD4$^+$ Treg cells expressing the membrane-bound TGF-β. A clinical trial in RRMS using probiotics associated with disease protection in EAE is currently underway (Weiner et al., personal communication).

PSA produced by *B. fragilis* is, to date, the only isolated symbiont factor that can induce strong regulatory cell populations, either FoxP3$^+$ Treg cells [128,129], CD39$^+$FoxP3$^+$ Treg cells [102], or Tr-1 cells [54,130,131] and that is protective against EAE [102,128,132]. The presence or absence of the polysaccharide determines the protective or pathogenic outcome of *B. fragilis* in EAE [133]. A strain of *B. fragilis* deficient in the production of PSA restores the susceptibility of EAE in mice previously treated with antibiotics, whereas mice recolonized with the intact strain of *B. fragilis* remain protected. PSA determined the cytokine profile of colonized mice and the conversion of effector CD4$^+$ T cells into IL-10 producing Treg cells *in vitro*. In mice colonized with PSA-deficient *B. fragilis* enhanced levels of systemic IL-17 and IL-6, and reduced IL-10 production were observed when compared to mice colonized with intact *B. fragilis.* Moreover, FoxP3$^+$ Treg cells converted *in vitro* from CD4$^+$FoxP3$^-$ cells obtained from mice recolonized with PSA-producing *B. fragilis* produced enhanced levels of IL-10 and induced EAE protection after adoptive transfer. The production of suppressive IL-10 producing FoxP3$^+$ Treg cells by outer membrane vesicles (OMV) released by PSA-producing *B. fragilis* was also documented, in processes dependent on TLR2 signaling and not found when OMV's were isolated from *B. fragilis* deficient in the production of PSA [134].

In EAE, prophylactic and therapeutic protection in SJL/J and C57BL/6 was achieved by the oral treatment with a highly purified preparation of PSA [102,128,132]. PSA promoted a significant accumulation of CD103$^+$ dendritic cells and FoxP3$^+$ Treg cells in the cervical LN of EAE mice, but not in non-EAE animals subjected to treatment, suggesting that an inflammatory stimulus might be required in order for PSA to promote an immunological shift [133]. PSA induced an enhanced regulatory function of CD103$^+$ dendritic cells. EAE protection by PSA was completely abrogated in IL-10 deficient mice. Recently, it has been shown that gut-derived plasmacytoid dendritic cells (pDC) play a significant role in the immunomodulatory effects of PSA-producing *B. fragilis* [132]. PSA recognition by dendritic cells and plasmacytoid dendritic cells in mice appear to be TLR2 dependent [102,135]. Recently, our laboratory has demonstrated that PSA promotes the expansion of a CD39$^+$ Treg population through TLR2 activation [102]. Oral treatment with PSA promotes the acquisition of a

$CD39^+$ phenotype in $CD4^+$ T cells that, independent of FoxP3 expression have an exacerbated regulatory phenotype with high levels of IL-10 produced. The role of CD39 in the protection conferred by PSA was confirmed when CD39 deficient mice remained unprotected to sub-optimal EAE after treatment. Moreover, PSA promotes an enhanced migratory phenotype in $CD39^+$ Treg cells with elevated levels of CCR5, CCR6, and CXCR3 [103], and adhesion molecules such as CD49b and CD29 [102]. *In vitro*, $CD39^+$ Treg cells from PSA-treated EAE mice migrated more efficiently towards inflamed EAE CNS tissue, and a significant accumulation of $CD39^+$ Treg cells were observed in EAE mice treated with PSA when compared to untreated EAE mice [103].

5. Conclusions

Epidemiological and experimental data suggest the interaction of multiple risk factors associated with multiple sclerosis. The gut microbiome is the largest "acquired immune organ" and could act as the reservoir where various and widely distinct factors, including genetic and environmental, merge. We hypothesize that the gut microbiome is the principal risk factor for multiple sclerosis, and perhaps for other autoimmune diseases as well. The clinical implications of recently published works support an important and novel role for gut symbiont organism and antigen(s) in regulating peripheral immune homeostasis. Accordingly, adjustment to the gut microbiota may be a reasonable pathway by which to control disease pathogenesis and offer an important pathway for the treatment of multiple sclerosis and perhaps other autoimmune conditions.

Conflict of interest

The authors have no conflicting financial interests.

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