The Journal of Immunology
REVIEW ARTICLE | JANUARY 15 2017

Influence of the Gut Microbiome on Autoimmunity in the Central Nervous System FREE

Sara L. Colpitts; ... et. al

J Immunol (2017) 198 (2): 596–604.
https://doi.org/10.4049/jimmunol.1601438

Brief Reviews

The Journal of Immunology

Influence of the Gut Microbiome on Autoimmunity in the Central Nervous System

Sara L. Colpitts and Lloyd H. Kasper

Autoimmune disorders of the CNS have complex pathogeneses that are not well understood. In multiple sclerosis and neuromyelitis optica spectrum disorders, T cells destroy CNS tissue, resulting in severe disabilities. Mounting evidence suggests that reducing inflammation in the CNS may start with modulation of the gut microbiome. The lymphoid tissues of the gut are specialized for the induction of regulatory cells, which are directly responsible for the suppression of CNS-damaging autoreactive T cells. Whether cause or effect, the onset of dysbiosis in the gut of patients with multiple sclerosis and neuromyelitis optica provides evidence of communication along the gut–brain axis. Thus, current and future therapeutic interventions directed at microbiome modulation are of considerable appeal. ***The Journal of Immunology*****, 2017, 198: 596–604.**

Autoimmune disorders of the CNS have debilitating consequences in afflicted patients. In multiple sclerosis (MS), autoreactive T cells attack the myelin sheath surrounding nerves in the brain and spinal cord by secreting a variety of inflammatory mediators that result in demyelination of both white and gray matter. Although disease symptoms are heterogeneous, such destruction can result in fatigue, numbness or tingling in the extremities, muscle weakness, dizziness and vertigo, bladder and bowel problems, changes in cognitive function, and emotional instability. Neuromyelitis optica (NMO) is an autoimmune disease within the larger family of NMO spectrum disorders that is similar to MS and exhibits some overlapping symptoms, but NMO is more often associated with astrocyte injury and damage to the optic nerve and spinal cord that can cause pain and vision loss in addition to muscle-related deficiencies.

The etiology of MS is complex, involving both genetic and environmental factors. Although the highly polymorphic HLA genes were the first to be identified and remain the most studied, technological advances have allowed for the identification of additional single nucleotide polymorphisms that correlate with disease (most of which are associated with immune function) (1–4). However, the low concordance rate between homozygotic twins highlights the importance of environmental influence on disease onset (5, 6). It has been postulated that bacterial and viral infections acquired from the environment can trigger the development of disease by molecular mimicry and bystander activation mechanisms. The microbiota that colonize the intestine (referred to herein as the gut microbiome), although located within the confines of the body, are foreign organisms that have evolved to live in symbiosis with their human host. Thus, the composition of the gut microbiome has the potential to influence MS pathogenesis. Indeed, germ-free (GF), or gnotobiotic, mice devoid of all commensal gut flora have dramatically attenuated susceptibility to experimental autoimmune (or allergic) encephalomyelitis (EAE), a mouse model of human MS (7, 8). Conversely, the existence of CNS disease has the potential to impact the homeostasis of the gut. Indeed, mice with EAE have increased permeability of the intestinal mucosa allowing for leakage of luminal contents into the body (9). In patients with MS, the existence of a gut–brain connection is further evidenced by increases in constipation, fecal incontinence, and gut permeability (10, 11). Intestinal bowel disease is also more common in MS patients and their families (12, 13). However, gut microbes themselves play an important role in maintaining the integrity of the intestinal epithelium (14). Furthermore, the gut microbiota can influence the permeability of the blood–brain barrier (BBB) by modulating expression of tight junction proteins in endothelial tissues (15).

IL-17–producing Th17 cells drive pathology in CNS autoimmune disease

The generation of Th17 cells is intimately linked to the gut microbiome. GF mice have reduced numbers of Th17 cells, but reconstitution of GF mice with a single commensal called segmented filamentous bacteria can induce Th17 cells and restore susceptibility to EAE (8, 16). The importance of the gut microbiota in the development of Th17 cells and their role in CNS autoimmunity have been reviewed in detail by others (17, 18). Briefly, Th17 cells are a subset of $CD4^+$ effector T cells that express the transcription factor retinoic acid (RA)–related orphan receptor γt and can produce a variety of cytokines such as IL-17A, IL-17F, IL-22, and GM-CSF. The differentiation of Th17 cells is dependent on TGF-β, IL-6, IL-21, and IL-1β

Department of Microbiology and Immunology, Geisel School of Medicine, Dartmouth College, Hanover, NH 03755

Received for publication August 18, 2016. Accepted for publication October 12, 2016.

This work was supported by grants from the National Institutes of Health (AI110170) and the National Multiple Sclerosis Society (RG4662A2/1) (to L.H.K.).

Address correspondence and reprint requests to Dr. Sara L. Colpitts, Geisel School of Medicine, 706 Remsen, 66 College Street, Hanover, NH 03755. Email address: sara.l.colpitts@dartmouth.edu

Abbreviations used in this article: AQP4, aquaporin-4; BBB, blood–brain barrier; B_{reg}, regulatory B cell; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; GF, germ-free; IEC, intestinal epithelial cell; LN, lymph node; MS, multiple sclerosis; NMO, neuromyelitis optica; PSA, polysaccharide A; RA, retinoic acid; SCFA, short-chain fatty acid; T_{reg}, regulatory T cell.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1601438

(present in various combinations) whereas IL-23 is required for their stability and maintenance. In addition to cytokine-derived signals, engagement of the aryl hydrocarbon receptor, known to interact with environmental toxins, drives the generation of Th17 cells and can exacerbate EAE disease (19). Extracellular ATP is yet another factor that can promote Th17 development (20). Ligands of the aryl hydrocarbon receptor and ATP can both be produced by the microbiota, thus affecting the development of Th17 cells (17).

Th17 cells are essential for host defense against bacterial and fungal pathogens, but in the context of autoimmunity, their presence is often destructive. Even before Th17 cells had been identified and characterized, increased IL-17 mRNA was noted in the blood and CSF of MS patients (21). Increased Th17 cells and IL-17 protein were subsequently found in the brain of patients with MS (22). Similar observations regarding increased Th17 cells and IL-17 were noted in patients with NMO (23–25). In both diseases, the highest levels of IL-17 were associated with clinical relapse compared with remission (21, 24). Importantly, Kebir et al. (26) showed that IL-17 receptor expression was upregulated in MS lesions, allowing IL-17 to increase the permeability of the BBB and promote migration of $CD4^+$ lymphocytes into the CNS. In this review, we focus on the regulatory mechanisms that antagonize the pathogenic activity of Th17 cells during EAE and MS.

The immune system coordinates anti-inflammatory responses along the gut–brain axis

The GALT is a unique immune compartment that is associated with the induction of regulatory cells. The GALT comprises 80% of the body's immune system and includes, but is not limited to, the mesenteric lymph nodes (LNs), Peyer's patches, and the lamina propria of the small and large intestine. Due to the constant onslaught of foreign material arriving in the gut as a result of daily food consumption, it is essential for the gut to have extensive mechanisms in place that promote tolerance to food-related Ags. It is not surprising that the microflora that colonizes the gut has taken advantage of these mechanisms to promote its survival by interacting directly with the GALT to influence the emergence of regulatory cells. Even though these regulatory cells are induced in the gut, they have the potential to suppress inflammation at bodily sites far distal from the gastrointestinal tract such as the CNS. Cells of both the innate and adaptive immune systems have essential roles in communication along this gut–brain axis.

Dendritic cells. In general, dendritic cells (DCs) are the sentinels of the immune system. As professional APCs, they patrol the body, including the GALT, taking in both self and foreign matter. DCs are specialized for the processing and presentation of peptide Ags on their surface in the context of MHC class I and MHC class II molecules, which dictate their interaction with $CD8^+$ and $CD4^+$ T cells, respectively. The primary mechanism by which DCs recognize foreign microbes is the expression of pattern recognition receptors specific for evolutionarily conserved microbe-associated molecular patterns. TLRs are one family of pattern recognition receptors that use a common intracellular signaling protein called MyD88. In particular, engagement of TLR2, which recognizes a variety of microbe-associated molecular patterns, has been associated with the induction of tolerogenic DCs (27). DCs can directly sample luminal content by extending processes through the tight junctions between intestinal epithelial cells (IECs) (28). DC probing of the lumen is dependent on MyD88 signaling in neighboring IECs, which suggests an essential role for IEC–DC cross-talk in detection of intestinal microbiota during steady-state and dysbiosis (29). Specialized IECs called M cells can also transport material across the epithelial barrier and deliver luminal content to DCs located in the underlying lymphoid tissue (30). Although IECs have the ability to influence immune cell behavior (reviewed in Ref. 31), their role in CNS autoimmunity is poorly defined. However, Kusu et al. (32) have shown that IECs can directly limit commensal-dependent ATP levels in the small intestine through expression of ectonucleoside triphosphate diphosphohydrolase-7, thus reducing Th17 cells and disease severity in EAE.

The ability of DCs to promote tolerance is highly dependent on their ability to produce the anti-inflammatory cytokine IL-10. The presence of IL-10 in the cytokine milieu at the time of a DC–T cell interaction will direct the T cell toward a regulatory fate. In addition to IL-10, production of TGF-β is essential for the tolerance-inducing potential of DCs (33). A specialized subset of nonlymphoid CD103-expressing DCs is present in the GALT that are developmentally related to conventional $CD8\alpha^+$ DCs requiring expression of ID2, IFN regulatory factor 8, and Batf3 (34, 35). In particular, $CD103^+$ DCs in the small intestine can express high levels of the enzyme aldehyde dehydrogenase, allowing them to metabolize dietary vitamin A to RA and preferentially drive regulatory T cell (T_{reg}) generation (36–38). Although it has previously been shown that treatment with exogenous RA or a synthetic RA receptor agonist is sufficient to significantly reduce the severity of EAE (39, 40), a recent study suggests that RA may also reduce disease by directly inhibiting IL-17 production not only from $CD4^+$ T cells but also γδ T cells (41). Increased expression of IDO is yet another mechanism used by $CD103^+$ DCs to promote regulatory cell development and tolerance (42), and IDO-deficient mice develop exacerbated EAE (43).

Note that macrophages can also adopt a phenotype that promotes the induction of immunosuppressive cells. These macrophages, termed alternatively activated macrophages, have a unique phenotype but also produce IL-10. Whereas a role for alternatively activated macrophages has been demonstrated in EAE and MS (reviewed in Ref. 44), their role in communication along the gut–brain axis is less defined.

Regulatory T cells. The primary role of T_{regs} is to maintain peripheral tolerance. Most autoreactive T cells are removed from the T cell repertoire by deletion in the thymus in a process termed central tolerance. However, low frequencies of T cells specific for self proteins escape deletion and enter the peripheral circulation. T_{regs} have the capacity to counteract the proinflammatory activity of autoreactive T cells, including the production of IL-17. During EAE, T_{regs} migrate to the CNS where they suppress inflammation (45). T_{regs} are most notably identified by the expression of CD25 (the high-affinity receptor for IL-2) and Foxp3, but populations of $Foxp3^-$ cells with regulatory function have been described (46). T_{regs} mediate the suppression of autoreactive T cells through the expression of inhibitory molecules such as CTLA-4 and GITR and cytokine production (IL-10 and TGF-β). A subset of T_{regs} expresses the ectonucleotidase CD39 (also known as ectonucleoside triphosphate diphosphohydrolase-1) (47, 48). CD39 acts in concert with CD73 to break down ATP to

adenosine. Because ATP has proinflammatory properties whereas adenosine promotes anti-inflammatory IL-10 production, particularly during EAE (49, 50), CD39 expression promotes regulatory function by T cells. Interestingly, $CD4^+$ T cells have proven to be somewhat plastic regarding their differentiation potential such that Th17 cells, even those specific for CNS Ags, can acquire regulatory properties specifically within the gut (51). Similarly, sequestration of pathogenic Th17 cells in the intestine can significantly reduce CNS inflammation (52), but the role of the microbiota has not been examined in these processes.

Unfortunately, although T_{regs} are present in MS patients, they exhibit inferior functional capacity compared with healthy controls. Viglietta et al. (53) were the first to describe the reduced ability of T_{regs} isolated from MS patients to suppress activated T cells, which was later confirmed by others (54, 55). Indeed, FOXP3 expression is reduced in $CD25^+$ T_{regs} isolated from MS patients (55–57). The reduction in the functional capacity of T_{regs} from patients with MS could also be the result of reduced IL-10 production (58, 59). Furthermore, Fletcher et al. (60) identified a reduction in the frequency of $CD39^+$ T_{regs}, which were also impaired in their ability to suppress IL-17 production from activated T cells. Importantly, both FOXP3 expression and IL-10 production were restored in patients treated with IFN-β (57). However, studies also suggest that differences may exist in the suppressive potential of T_{regs} in patients with relapsing-remitting MS versus secondary progressive MS (55, 57, 58, 60).

Regulatory B cells. It is now appreciated that regulatory B cells (B_{regs}) also play a significant role in immune suppression during EAE (61, 62). Although phenotypically diverse, B_{regs} can be loosely defined as any B cell capable of producing IL-10 (63). IL-35 has also been implicated in the regulatory activity of B cells during EAE (64). Similar to T_{regs}, B cells can also use the CD39/CD73 axis to regulate inflammation through the reduction of ATP levels, but this has not been tested in the EAE model (65). Unlike T_{regs}, studies suggest that B_{regs} assert their immunosuppressive activity locally (in the draining LN) but not within the CNS itself (66). However, a recent study showed that the adoptive transfer of in vitro–activated pro–B cells (bone marrow B cells stimulated with CpG-B) could significantly reduce EAE symptoms when transferred therapeutically (67). The authors found that these cells matured into IL-10–producing B_{regs} that were able to traffic to the spinal cord. B_{regs} are capable of directly responding to PAMPs via TLRs, and their ability to reduce EAE is dependent on TLR2/4 expression (68). Furthermore, despite normal numbers of B_{regs} in MyD88 knockout mice (based on extracellular phenotype), the absence of MyD88 signaling significantly reduced the overall regulatory function of B cells based on cytokine production (69). Taken together, these findings suggest that direct TLR engagement on B cells, potentially deriving from the microbiome, is essential for B_{reg} induction and function during EAE.

The contribution of B_{regs} to the suppression of autoimmune disease in patients with MS and their role in current and novel therapeutics are actively being explored. A corresponding population of B_{regs} has been identified in humans capable of suppressing activated T cell proliferation (70, 71). In MS patients, B_{regs} are deficient in their ability to produce IL-10 when stimulated in vitro (72, 73). Although inflammatory B cells have also been implicated in the pathogenesis of EAE and MS (62, 74, 75), immunomodulatory approaches that shift that balance between these two populations by reducing inflammatory B cells while promoting regulatory populations could prove therapeutic (76, 77). Furthermore, B cells may also prove beneficial based on their ability to enhance the T_{reg} population (78).

Microbial dysbiosis in patients with CNS autoimmunity

Several recent studies have addressed the following critical question: are there significant differences in the microbial contents of the gut between patients with CNS autoimmunity and healthy controls? Although authors have found subtle differences in the exact composition of gut microflora within the patient versus control populations (as would be expected considering differences in geographical location), the overwhelming conclusion is that, indeed, microbial dysbiosis is present in the intestine of MS patients.

In a cohort of 31 patients with relapsing-remitting MS compared with 36 healthy controls, Chen et al. (79) found significant differences in microbiota structure between patients with MS and healthy controls. There was no difference in overall species richness (α diversity) between healthy controls and MS patients, but within the MS patient cohort, there was a trend toward reduced species richness in patients with active disease whereas patients in remission were similar to the healthy controls. Such changes in α diversity could suggest a role for the gut microbiome in disease exacerbation, but future longitudinal studies are needed to establish correlation. At the community level, relapsing-remitting MS patients exhibited an enrichment of *Pseudomonas* and *Mycoplana* (Proteobacteria), *Blautia* and *Dorea* (Firmicutes), and *Pedobacter* (Bacteroidetes) with a decreased abundance of *Adlercreutzia* and *Collinsella* (Actinobacteria), *Lactobacillus* (Firmicutes), and *Parabacteroides* (Bacteroidetes). In a second study with a cohort of 60 MS patients and 43 healthy controls, *Methanobrevibacter* (Euryarchaeota) and *Akkermansia* (Verrucomicrobia) were identified as increased and *Butyricimonas* (Bacteroidetes) was decreased in MS patients (80). Interestingly, Jangi et al. (80) performed secondary analysis separating treated versus untreated MS patients as independent cohorts ($n = 32$ and 28, respectively). They found certain genera such as *Prevotella* (Bacteroidetes) and *Sutterella* (Proteobacteria) that were reduced in untreated patients but restored to normal levels with treatment. Furthermore, they identified *Sarcina* (Firmicutes) as being reduced only in treated patients, which highlights the potential of MS therapies to influence the gut microbiome as well. *Prevotella* and *Sutterella* species were also significantly reduced in a Japanese cohort of MS patients (81). However, in this study, 14 of the 19 species with reduced prevalence were located in *Clostridia* cluster XIVa or IV with three additional species identified from the genus *Bacteroides*. Thus, although dysbiosis is clearly evident in MS patients, particularly those naive to treatment, the cause-and-effect relationship between gut dysbiosis and CNS autoimmunity is still unclear. Changes in the microbiome (i.e., the environment) could play a role in predisposistion to the development of MS and/or act as a trigger for initiating disease in genetically predisposed individuals, but additional studies are required to determine whether the altered microbiome drives changes in immunity or the onset of immunological disease induces modifications in the microflora.

Both MS and NMO are driven by pathogenic Th17 cells reactive against self-proteins. Unlike MS, in patients with NMO, the target of autoreactive T cells has been identified as aquaporin-4 (AQP4), a water channel protein that transports water across cell membranes. AQP4 is expressed by astrocytes in the brain. The dominant peptide epitope of AQP4 recognized by T cells shares significant sequence homology with an ATP-binding cassette transporter permease from *Clostridium perfringens*, a human gut commensal, and AQP4-specific T cells cross-react with *C. perfringens* (82). When comparing the microbiome of patients with NMO to healthy controls, principal component analysis revealed significant compositional differences. *C. perfringens* was the second most enriched taxon and was overabundant compared with either healthy controls or patients with MS (83). Indeed, others have found that *C. perfringens* type A is significantly reduced in MS patients compared with healthy controls (84). Importantly, the increase in *C. perfringens* could not be attributed to the use of immune-modulating therapy because a subset of patients in both the NMO and MS groups were treated with rituximab. Similar to MS and EAE, it is unknown whether the overrepresentation of *C. perfringens* in the gut of NMO patients is the cause or effect of autoimmune disease. The presence of *C. perfringens* in the gut has the potential to act as a molecular mimic. Furthermore, other bacteria, such as Fibrobacteres, were also enriched in NMO patients and could contribute to the overall disease state independently or in collaboration with *C. perfringens*.

Emergence of microbiome-directed therapies for EAE and MS

Current therapies available for patients with MS can be categorized as immune-modulating drugs and immunosuppressants (i.e., corticosteroids). In general, the goal of the former is to slow nervous system degeneration whereas the latter can often improve symptoms to help maintain quality of life. There are currently eight Food and Drug Administration–approved immune-modulating drugs (some available in multiple forms) that can be used alone or in combination to treat MS. Regardless, there is a great need for novel approaches to combat CNS destruction. Considering the now well-defined link between the gut microbiota and brain physiology, it is not surprising that new therapies are being developed that target the gut microbiome.

Oral antibiotics. In our laboratory, we have shown that modulation of the gut microbiota using orally administered broad-spectrum antibiotics is sufficient to provide significant protection against EAE (85). This treatment regimen was effective when the drugs were given orally (by gavage or in the drinking water) but not i.p. The frequency and total number of Foxp3$^+$ T_{regs} was significantly increased in both mesenteric and CNS-draining LNs when mice with EAE were treated with antibiotics, and there was a corresponding increase in IL-10 production as well. Furthermore, CD103$^+$ DCs isolated from the GALT of antibiotic-treated mice were superior in their ability to induce T_{regs} from naive T cells in vitro. These findings provide strong evidence in support of anti-inflammatory communication along the gut–brain axis following modulation of the microbiome.

We have also previously shown a connection between modulation of the gut microbiome and the induction of B_{regs}. When mice were treated orally with broad-spectrum antibiotics, there was a significant increase in the frequency and total number of CD5$^+$CD1d$^+$ B cells (86). Importantly, antibiotic-induced B_{regs} had potent immunosuppressive activity in vivo that was significantly greater than B_{regs} isolated from control treated animals, despite similar levels of IL-10 production in vitro. Importantly, note that a second study found conflicting results showing that antibiotic treatment significantly reduced the number and frequency of B_{regs} as measured by IL-10 production, but these differences could be the result of the different antibiotic mixtures used in the two studies, different vendors, housing, and/or diet (87). Regardless, additional preclinical studies are required to determine whether microbiome-altering therapeutics are effective in the absence of B_{regs}.

Several other studies have described the beneficial effects of oral antibiotic treatment. Yokote et al. (88) used a unique mixture of antibiotics to significantly reduce EAE disease. Similar to our studies, they found an increase in total IL-10 production from the mesenteric LN, but no increase in the frequency of Foxp3$^+$ T_{regs}. Alternatively, they found that the success of oral antibiotic treatment was dependent on a subset of invariant NK T cells. Minocycline, an oral tetracycline antibiotic commonly used for the treatment of acne, has also been used in a rat model of EAE to reduce disease severity both prophylactically and therapeutically (89). Subsequently, minocycline has been used in three clinical trials alone or in combination with current therapies to treat patients with MS. When administered to patients in combination with glatiramer acetate, minocycline showed a trend toward reduced CNS deterioration (90), but when combined with IFN-β, there was no significant difference with placebo-treated controls (91). However, the therapeutic window for minocycline may be earlier in disease rather than later because treatment with minocycline significantly reduced conversion to MS when treatment was initiated at the time of the first clinical demyelinating event (92). Importantly, note that whereas minocycline may promote autoimmune suppression by modulation of the intestinal microflora to repair the dysbiosis observed in MS patient, it can also have multiple direct immune-modulating effects that could contribute to the observed protection. Although minocycline may or may not ultimately prove beneficial in the context of MS, other oral antibiotics or combinations of antibiotics could be considered or developed in the future to promote anti-inflammatory communication along the gut–brian axis. Indeed, vancomycin was shown to improve the symptoms of autism in 8 out of 10 children studied (93).

Probiotic usage. By definition, a probiotic is any live microorganism that confers a significant health benefit on the host. This term can refer to both commensal microbes that normally reside in the gut and exogenous, possibly food-borne, microbes that travel through the intestine following consumption. Numerous bacterial strains given alone or in combination have been shown to improve CNS inflammation, including *Lactobacillus* species, *Pediococcus acidilactici*, *Bifidobacterium bifidum*, *Bifidobacterium animalis*, and *Streptococcus thermophiles* (94–97). Genetically engineered bacterial strains such as *Lactococcus lactis* expressing heat shock protein 65 from *Mycobacterium leprae* have also been used to reduce clinical symptoms of EAE (98, 99). Even one type of yeast, *Candida kefyr*, commonly found in fermented foods, significantly reduced EAE disease (100). In almost all of the studies in which probiotics significantly improved EAE disease, the reduction in

CNS inflammation was attributed to the induction of T_{regs} and/or IL-10 production (95–97, 100). Furthermore, when healthy volunteers were fed *Bifidobacterium infantis* 35624 for 8 wk, the frequency of FOXP3$^+$ CD4 T cells in the blood was significantly increased compared with pretreatment measurements (101).

Probiotics also have an effect on B_{regs}. Mercadante et al. (102) have shown that *L. lactis* can promote the generation of IL-10–producing B cells that mediate tolerance in a model of graft-versus-host disease. *Clostridium butyricum*, when given in combination with specific immunotherapy, significantly increases the frequency of IL-10$^+$ B cells in both mice and humans (103, 104).

In some cases, efficacy has been observed using heat-killed bacteria (97, 100). Although heat-killed bacteria do not classify as probiotics (because they are not alive), it suggests that bacterial-derived products can nonetheless have therapeutic potential. Indeed, *Bacteroides fragilis* functions as a probiotic because it significantly reduces the severity of EAE in mice (105), but outer membrane vesicles from *B. fragilis* can also induce immunomodulatory effects and prevent inflammation (106). Furthermore, we found that the ability of *B. fragilis* to function as a probiotic was dependent on expression of a single capsular polysaccharide (polysaccharide A [PSA]) (105). We found that PSA purified from *B. fragilis* can both prophylactically and therapeutically reduce EAE disease when administered to mice via oral gavage (107). Both *B. fragilis* and PSA can condition DCs to generate T_{regs} in a TLR2-dependent manner (105–109). In our studies with human PBMCs, we have shown that naive CD4 T cells can acquire a CD39$^+$FOXP3$^+$ regulatory phenotype when cocultured with DCs and purified PSA in vitro (110). This augmentation of T_{reg} function suggests supplementation with PSA could be an attractive novel therapeutic for patients with MS. Importantly, the ability of gut microbes and microbial products to influence CNS activity applies not only in the context of autoimmunity but also in murine models of depression and autism spectrum disorder, further supporting the importance of the gut–brain axis (111–113).

Helminth therapy. Whereas the gut microbiome usually refers to those microbes that exist in a symbiotic relationship with their human host, certain microbes maintain a parasitic relationship with their host, many of which exist in the intestine. The presence or absence of parasites can thus contribute to the onset of autoimmune disease as suggested in the hygiene hypothesis. The hygiene hypothesis proposes a negative correlation between the decrease in parasitic infections and the increase in autoimmune diseases in the developed world as a result of increased hygiene.

Parasitic infections are often long-lasting chronic infections that require a certain degree of immunosuppression to promote and maintain their longevity. Parasites, in particular helminths or worms, accomplish this feat by driving the induction of Th2 cells that produce anti-inflammatory cytokines, including IL-4, IL-10, IL-13, and TGF-β. By reducing the production of Th2 cytokines in vivo, the balance is shifted toward the activity of Th1/Th17 responses that drive autoimmunity. This has significant bearing on MS, as evidenced by a negative correlation between high rates of helminth infection and high MS prevalence worldwide (114). In one study, the authors followed 12 MS patients actively infected with intestinal helminths during a 4-y period and found a significant reduction in disease progression compared with uninfected patients as measured by multiple parameters (115). These studies implicated increased IL-10 from both T_{regs} and B_{regs} in disease attenuation (115, 116).

Considering the regulatory potential of helminth infections, multiple laboratories have similarly shown that in vivo infection with helminths can reduce the severity of EAE by inducing a combination of IL-10, T_{regs}, and B_{regs} (117–121). Furthermore, exposure to parasite products and/or Ags can reduce EAE disease, suggesting that live infection is not a requirement for immunosuppression similar to probiotics (114, 122, 123). However, in a follow-up to the aforementioned study, when anti-helminth treatment was initiated in 4 of the 12 patients due to the onset of parasitosis symptoms, the parasites were eliminated, but the severity of MS disease quickly progressed to mirror the uninfected patient cohort (124). Regardless, the preclinical and correlation studies have encouraged the development of helminth-based therapeutics to treat MS. One strategy has been to infect MS patients with eggs from *Trichuris suis*, a helminth unable to establish long-lived infection in humans. Unfortunately, two small studies have presented conflicting results, although both found no adverse symptoms associated with treatment (125, 126). Larger studies are required to determine whether *T. suis* egg therapy will prove successful in significantly reducing the progression of MS disease. Alternatively, the identification and development of new helminth-derived Ags has the potential to shift the balance from a proinflammatory to an anti-inflammatory milieu, thus reducing CNS autoimmunity.

Dietary modification. In the absence of any exogenous manipulation or ingested therapeutics, modulation of the gut microbiome can occur simply by changing one's diet. Studies have shown that the gut microbiome is significantly different between obese and lean individuals (127), but microbial composition can change in as little as 1–2 d following dietary intervention (128). In EAE, consumption of a calorie-restricted diet can improve disease symptoms whereas a high-salt diet exacerbates disease by promoting Th17 differentiation (129–131). A recent study also found a positive correlation between salt intake and both exacerbation rates and radiological activity in patients with relapsing-remitting MS (132). Interestingly, dietary Ags can impact immunity independent of the gut microbiome based on studies in GF mice fed an elemental diet (133).

Overall, increased microbial diversity is associated with an increase in fiber-rich foods (134). In particular, a high-fiber diet promotes specific species of microbes within the Firmicutes and Bacteroidetes phyla. These microbes are responsible for the breakdown of nondigestible fiber in the colon and produce short-chain fatty acids (SCFAs) as part of the fermentation process. SCFAs, including propionate, acetate, and butyrate, play a critical role in suppressing inflammation by inducing T_{regs} (135). Conversely, a diet rich in long-chain fatty acids can promote Th17 differentiation and exacerbate disease (136). SCFAs also play an important role in the maintenance of CNS integrity. As mentioned above, GF mice exhibit increased permeability in the BBB (15). However, when GF mice were monocolonized with either *Clostridium tyrobutyricum* or *Bacteroides thetaiotaomicron*, both of which produce SCFAs, the defects in BBB permeability were restored. Interestingly, both MS and NMO patients have a

distinct urinary metabolic signature compared with healthy controls (137). Intermediates involved in propionate metabolism were significantly decreased in patients with MS, which could be influenced by changes in the gut microbiota. Thus, the presence or absence of SCFA-producing microbes is important to consider when assessing the dysbiosis of autoimmune patients and also when developing probiotic strategies. Furthermore, recent advances in personalized nutrition (i.e., the development of individual diet plans based on gut microbiota and other parameters) have been used to control blood glucose levels, which has significant applications in the prevention and/or treatment of type II diabetes (138). It would be of distinct appeal to use a similar approach to not only correct microbial dysbiosis through diet but to also establish a long-term dietary approach to reduce inflammatory activity and relapses in patients with MS (139, 140).

Conclusions

The gut–brain axis provides for the critical exchange of biologic information that affects both the physiology and immunology of the host. The bidirectional activity between the gut microbiome and the GALT allows for the establishment of a systemic homeostatic balance. Immune regulation in CNS demyelinating diseases reflects a balance between those cells driving disease, such as Th17 cells, and regulatory cells from both T and B cell lineages that are influenced by the microbiome. Improved balance of the dysregulated immune function in experimental CNS demyelinating disease can be achieved by a variety of approaches that alter colonization of the gut microflora. Thus, novel approaches to treating human MS, as well as other autoimmune conditions, aimed at modulation of the microbiota may represent a major paradigm shift in how we approach treating human disease.

Disclosures

The authors have no financial conflicts of interest.

References

1. Sawcer, S. 2008. The complex genetics of multiple sclerosis: pitfalls and prospects. *Brain* 131: 3118–3131.
2. Sawcer, S., G. Hellenthal, M. Pirinen, C. C. Spencer, N. A. Patsopoulos, L. Moutsianas, A. Dilthey, Z. Su, C. Freeman, S. E. Hunt, et al.; International Multiple Sclerosis Genetics Consortium.; Wellcome Trust Case Control Consortium. 2011. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 476: 214–219.
3. Beecham, A. H., N. A. Patsopoulos, D. K. Xifara, M. F. Davis, A. Kemppinen, C. Cotsapas, T. S. Shah, C. Spencer, D. Booth, A. Goris, et al.; International Multiple Sclerosis Genetics Consortium. 2013. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nat. Genet.* 45: 1353–1360.
4. Lill, C. M., F. Luessi, A. Alcina, E. A. Sokolova, N. Ugidos, B. de la Hera, L. Guillot-Noël, S. Malhotra, E. Reinthaler, B. M. Schjeide, et al. 2015. Genome-wide significant association with seven novel multiple sclerosis risk loci. *J. Med. Genet.* 52: 848–855.
5. Mumford, C. J., N. W. Wood, H. Kellar-Wood, J. W. Thorpe, D. H. Miller, and D. A. Compston. 1994. The British Isles survey of multiple sclerosis in twins. *Neurology* 44: 11–15.
6. Willer, C. J., D. A. Dyment, N. J. Risch, A. D. Sadovnick, and G. C. Ebers, Canadian Collaborative Study Group. 2003. Twin concordance and sibling recurrence rates in multiple sclerosis. *Proc. Natl. Acad. Sci. USA* 100: 12877–12882.
7. Berer, K., M. Mues, M. Koutrolos, Z. A. Rasbi, M. Boziki, C. Johner, H. Wekerle, and G. Krishnamoorthy. 2011. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature* 479: 538–541.
8. Lee, Y. K., J. S. Menezes, Y. Umesaki, and S. K. Mazmanian. 2011. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. USA* 108(Suppl. 1): 4615–4622.
9. Nouri, M., A. Bredberg, B. Weström, and S. Lavasani. 2014. Intestinal barrier dysfunction develops at the onset of experimental autoimmune encephalomyelitis, and can be induced by adoptive transfer of auto-reactive T cells. *PLoS One* 9: e106335.
10. Yacyshyn, B., J. Meddings, D. Sadowski, and M. B. Bowen-Yacyshyn. 1996. Multiple sclerosis patients have peripheral blood CD45RO$^+$ B cells and increased intestinal permeability. *Dig. Dis. Sci.* 41: 2493–2498.
11. Nusrat, S., E. Gulick, D. Levinthal, and K. Bielefeldt. 2012. Anorectal dysfunction in multiple sclerosis: a systematic review. *ISRN Neurol.* 2012: 376023.
12. Kimura, K., S. F. Hunter, M. S. Thollander, E. V. Loftus, Jr., L. J. Melton, III, P. C. O'Brien, M. Rodriguez, and S. F. Phillips. 2000. Concurrence of inflammatory bowel disease and multiple sclerosis. *Mayo Clin. Proc.* 75: 802–806.
13. Gupta, G., J. M. Gelfand, and J. D. Lewis. 2005. Increased risk for demyelinating diseases in patients with inflammatory bowel disease. *Gastroenterology* 129: 819–826.
14. Kozakova, H., M. Schwarzer, L. Tuckova, D. Srutkova, E. Czarnowska, I. Rosiak, T. Hudcovic, I. Schabussova, P. Hermanova, Z. Zakostelska, et al. 2016. Colonization of germ-free mice with a mixture of three lactobacillus strains enhances the integrity of gut mucosa and ameliorates allergic sensitization. *Cell. Mol. Immunol.* 13: 251–262.
15. Braniste, V., M. Al-Asmakh, C. Kowal, F. Anuar, A. Abbaspour, M. Tóth, A. Korecka, N. Bakocevic, L. G. Ng, P. Kundu, et al. 2014. The gut microbiota influences blood-brain barrier permeability in mice. *Sci. Transl. Med.* 6: 263ra158.
16. Ivanov, I. I., Rde. L. Frutos, N. Manel, K. Yoshinaga, D. B. Rifkin, R. B. Sartor, B. B. Finlay, and D. R. Littman. 2008. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 4: 337–349.
17. Chewning, J. H., and C. T. Weaver. 2014. Development and survival of Th17 cells within the intestines: the influence of microbiome- and diet-derived signals. *J. Immunol.* 193: 4769–4777.
18. Dos Passos, G. R., D. K. Sato, J. Becker, and K. Fujihara. 2016. Th17 cells pathways in multiple sclerosis and neuromyelitis optica spectrum disorders: pathophysiological and therapeutic implications. *Mediators Inflamm.* 2016: 5314541.
19. Veldhoen, M., K. Hirota, A. M. Westendorf, J. Buer, L. Dumoutier, J. C. Renauld, and B. Stockinger. 2008. The aryl hydrocarbon receptor links T_H17-cell-mediated autoimmunity to environmental toxins. *Nature* 453: 106–109.
20. Atarashi, K., J. Nishimura, T. Shima, Y. Umesaki, M. Yamamoto, M. Onoue, H. Yagita, N. Ishii, R. Evans, K. Honda, and K. Takeda. 2008. ATP drives lamina propria T_H17 cell differentiation. *Nature* 455: 808–812.
21. Matusevicius, D., P. Kivisäkk, B. He, N. Kostulas, V. Ozenci, S. Fredrikson, and H. Link. 1999. Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. *Mult. Scler.* 5: 101–104.
22. Tzartos, J. S., M. A. Friese, M. J. Craner, J. Palace, J. Newcombe, M. M. Esiri, and L. Fugger. 2008. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. *Am. J. Pathol.* 172: 146–155.
23. Ishizu, T., M. Osoegawa, F. J. Mei, H. Kikuchi, M. Tanaka, Y. Takakura, M. Minohara, H. Murai, F. Mihara, T. Taniwaki, and J. Kira. 2005. Intrathecal activation of the IL-17/IL-8 axis in opticospinal multiple sclerosis. *Brain* 128: 988–1002.
24. Wang, H. H., Y. Q. Dai, W. Qiu, Z. Q. Lu, F. H. Peng, Y. G. Wang, J. Bao, Y. Li, and X. Q. Hu. 2011. Interleukin-17-secreting T cells in neuromyelitis optica and multiple sclerosis during relapse. *J. Clin. Neurosci.* 18: 1313−1317.
25. Li, Y., H. Wang, Y. Long, Z. Lu, and X. Hu. 2011. Increased memory Th17 cells in patients with neuromyelitis optica and multiple sclerosis. *J. Neuroimmunol.* 234: 155–160.
26. Kebir, H., K. Kreymborg, I. Ifergan, A. Dodelet-Devillers, R. Cayrol, M. Bernard, F. Giuliani, N. Arbour, B. Becher, and A. Prat. 2007. Human T_H17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat. Med.* 13: 1173–1175.
27. Manicassamy, S., R. Ravindran, J. Deng, H. Oluoch, T. L. Denning, S. P. Kasturi, K. M. Rosenthal, B. D. Evavold, and B. Pulendran. 2009. Toll-like receptor 2-dependent induction of vitamin A-metabolizing enzymes in dendritic cells promotes T regulatory responses and inhibits autoimmunity. *Nat. Med.* 15: 401–409.
28. Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J. P. Kraehenbuhl, and P. Ricciardi-Castagnoli. 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat. Immunol.* 2: 361–367.
29. Chieppa, M., M. Rescigno, A. Y. Huang, and R. N. Germain. 2006. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. *J. Exp. Med.* 203: 2841–2852.
30. Ohno, H. 2016. Intestinal M cells. *J. Biochem.* 159: 151–160.
31. Peterson, L. W., and D. Artis. 2014. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat. Rev. Immunol.* 14: 141–153.
32. Kusu, T., H. Kayama, M. Kinoshita, S. G. Jeon, Y. Ueda, Y. Goto, R. Okumura, H. Saiga, T. Kurakawa, K. Ikeda, et al. 2013. Ecto-nucleoside triphosphate diphosphohydrolase 7 controls Th17 cell responses through regulation of luminal ATP in the small intestine. *J. Immunol.* 190: 774–783.
33. Chen, W., W. Jin, N. Hardegen, K. J. Lei, L. Li, N. Marinos, G. McGrady, and S. M. Wahl. 2003. Conversion of peripheral CD4$^+$CD25$^-$ naive T cells to CD4$^+$ CD25$^+$ regulatory T cells by TGF-β induction of transcription factor Foxp3. *J. Exp. Med.* 198: 1875–1886.
34. Ginhoux, F., K. Liu, J. Helft, M. Bogunovic, M. Greter, D. Hashimoto, J. Price, N. Yin, J. Bromberg, S. A. Lira, et al. 2009. The origin and development of nonlymphoid tissue CD103$^+$ DCs. *J. Exp. Med.* 206: 3115–3130.
35. Edelson, B. T., W. Kc, R. Juang, M. Kohyama, L. A. Benoit, P. A. Klekotka, C. Moon, J. C. Albring, W. Ise, D. G. Michael, et al. 2010. Peripheral CD103$^+$ dendritic cells form a unified subset developmentally related to CD8α$^+$ conventional dendritic cells. *J. Exp. Med.* 207: 823–836.

36. Coombes, J. L., K. R. Siddiqui, C. V. Arancibia-Cárcamo, J. Hall, C. M. Sun, Y. Belkaid, and F. Powrie. 2007. A functionally specialized population of mucosal CD103$^+$ DCs induces Foxp3$^+$ regulatory T cells via a TGF-β and retinoic acid-dependent mechanism. *J. Exp. Med.* 204: 1757–1764.
37. Mucida, D., Y. Park, G. Kim, O. Turovskaya, I. Scott, M. Kronenberg, and H. Cheroutre. 2007. Reciprocal T_H17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 317: 256–260.
38. Sun, C. M., J. A. Hall, R. B. Blank, N. Bouladoux, M. Oukka, J. R. Mora, and Y. Belkaid. 2007. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J. Exp. Med.* 204: 1775–1785.
39. Klemann, C., B. J. Raveney, A. K. Klemann, T. Ozawa, S. von Hörsten, K. Shudo, S. Oki, and T. Yamamura. 2009. Synthetic retinoid AM80 inhibits Th17 cells and ameliorates experimental autoimmune encephalomyelitis. *Am. J. Pathol.* 174: 2234–2245.
40. Zhan, X. X., Y. Liu, J. F. Yang, G. Y. Wang, L. Mu, T. S. Zhang, X. L. Xie, J. H. Wang, Y. M. Liu, Q. F. Kong, et al. 2013. All-*trans*-retinoic acid ameliorates experimental allergic encephalomyelitis by affecting dendritic cell and monocyte development. *Immunology* 138: 333–345.
41. Raverdeau, M., C. J. Breen, A. Misiak, and K. H. Mills. 2016. Retinoic acid suppresses IL-17 production and pathogenic activity of γδ T cells in CNS autoimmunity. *Immunol. Cell Biol.* 94: 763–773.
42. Matteoli, G., E. Mazzini, I. D. Iliev, E. Mileti, F. Fallarino, P. Puccetti, M. Chieppa, and M. Rescigno. 2010. Gut CD103$^+$ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut* 59: 595–604.
43. Yan, Y., G. X. Zhang, B. Gran, F. Fallarino, S. Yu, H. Li, M. L. Cullimore, A. Rostami, and H. Xu. 2010. IDO upregulates regulatory T cells via tryptophan catabolite and suppresses encephalitogenic T cell responses in experimental autoimmune encephalomyelitis. *J. Immunol.* 185: 5953–5961.
44. Jiang, Z., J. X. Jiang, and G. X. Zhang. 2014. Macrophages: a double-edged sword in experimental autoimmune encephalomyelitis. *Immunol. Lett.* 160: 17–22.
45. McGeachy, M. J., L. A. Stephens, and S. M. Anderton. 2005. Natural recovery and protection from autoimmune encephalomyelitis: contribution of CD4$^+$CD25$^+$ regulatory cells within the central nervous system. *J. Immunol.* 175: 3025–3032.
46. Roncarolo, M. G., S. Gregori, M. Battaglia, R. Bacchetta, K. Fleischhauer, and M. K. Levings. 2006. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol. Rev.* 212: 28–50.
47. Deaglio, S., K. M. Dwyer, W. Gao, D. Friedman, A. Usheva, A. Erat, J. F. Chen, K. Enjyoji, J. Linden, M. Oukka, et al. 2007. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J. Exp. Med.* 204: 1257–1265.
48. Borsellino, G., M. Kleinewietfeld, D. Di Mitri, A. Sternjak, A. Diamantini, R. Giometto, S. Höpner, D. Centonze, G. Bernardi, M. L. Dell'Acqua, et al. 2007. Expression of ectonucleotidase CD39 by Foxp3$^+$ Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood* 110: 1225–1232.
49. Tsutsui, S., J. Schnermann, F. Noorbakhsh, S. Henry, V. W. Yong, B. W. Winston, K. Warren, and C. Power. 2004. A1 adenosine receptor upregulation and activation attenuates neuroinflammation and demyelination in a model of multiple sclerosis. *J. Neurosci.* 24: 1521–1529.
50. Yao, S. Q., Z. Z. Li, Q. Y. Huang, F. Li, Z. W. Wang, E. Augusto, J. C. He, X. T. Wang, J. F. Chen, and R. Y. Zheng. 2012. Genetic inactivation of the adenosine A_{2A} receptor exacerbates brain damage in mice with experimental autoimmune encephalomyelitis. *J. Neurochem.* 123: 100–112.
51. Esplugues, E., S. Huber, N. Gagliani, A. E. Hauser, T. Town, Y. Y. Wan, W. O'Connor, Jr., A. Rongvaux, N. Van Rooijen, A. M. Haberman, et al. 2011. Control of T_H17 cells occurs in the small intestine. *Nature* 475: 514–518.
52. Berer, K., M. Boziki, and G. Krishnamoorthy. 2014. Selective accumulation of pro-inflammatory T cells in the intestine contributes to the resistance to autoimmune demyelinating disease. *PLoS One* 9: e87876.
53. Viglietta, V., C. Baecher-Allan, H. L. Weiner, and D. A. Hafler. 2004. Loss of functional suppression by CD4$^+$CD25$^+$ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* 199: 971–979.
54. Haas, J., A. Hug, A. Viehöver, B. Fritzsching, C. S. Falk, A. Filser, T. Vetter, L. Milkova, M. Korporal, B. Fritz, et al. 2005. Reduced suppressive effect of CD4$^+$ CD25^{high} regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. *Eur. J. Immunol.* 35: 3343–3352.
55. Venken, K., N. Hellings, K. Hensen, J. L. Rummens, R. Medaer, M. B. D'hooghe, B. Dubois, J. Raus, and P. Stinissen. 2006. Secondary progressive in contrast to relapsing-remitting multiple sclerosis patients show a normal CD4$^+$CD25$^+$ regulatory T-cell function and FOXP3 expression. *J. Neurosci. Res.* 83: 1432–1446.
56. Huan, J., N. Culbertson, L. Spencer, R. Bartholomew, G. G. Burrows, Y. K. Chou, D. Bourdette, S. F. Ziegler, H. Offner, and A. A. Vandenbark. 2005. Decreased FOXP3 levels in multiple sclerosis patients. *J. Neurosci. Res.* 81: 45–52.
57. Venken, K., N. Hellings, M. Thewissen, V. Somers, K. Hensen, J. L. Rummens, R. Medaer, R. Hupperts, and P. Stinissen. 2008. Compromised CD4$^+$ CD25^{high} regulatory T-cell function in patients with relapsing-remitting multiple sclerosis is correlated with a reduced frequency of FOXP3-positive cells and reduced FOXP3 expression at the single-cell level. *Immunology* 123: 79–89.
58. Soldan, S. S., A. I. Alvarez Retuerto, N. L. Sicotte, and R. R. Voskuhl. 2004. Dysregulation of IL-10 and IL-12p40 in secondary progressive multiple sclerosis. *J. Neuroimmunol.* 146: 209–215.
59. Astier, A. L., G. Meiffren, S. Freeman, and D. A. Hafler. 2006. Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. *J. Clin. Invest.* 116: 3252–3257.
60. Fletcher, J. M., R. Lonergan, L. Costelloe, K. Kinsella, B. Moran, C. O'Farrelly, N. Tubridy, and K. H. Mills. 2009. CD39$^+$Foxp3$^+$ regulatory T cells suppress pathogenic Th17 cells and are impaired in multiple sclerosis. *J. Immunol.* 183: 7602–7610.
61. Fillatreau, S., C. H. Sweenie, M. J. McGeachy, D. Gray, and S. M. Anderton. 2002. B cells regulate autoimmunity by provision of IL-10. *Nat. Immunol.* 3: 944–950.
62. Matsushita, T., K. Yanaba, J. D. Bouaziz, M. Fujimoto, and T. F. Tedder. 2008. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J. Clin. Invest.* 118: 3420–3430.
63. Tedder, T. F. 2015. B10 cells: a functionally defined regulatory B cell subset. *J. Immunol.* 194: 1395–1401.
64. Shen, P., T. Roch, V. Lampropoulou, R. A. O'Connor, U. Stervbo, E. Hilgenberg, S. Ries, V. D. Dang, Y. Jaimes, C. Daridon, et al. 2014. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* 507: 366–370.
65. Kaku, H., K. F. Cheng, Y. Al-Abed, and T. L. Rothstein. 2014. A novel mechanism of B cell-mediated immune suppression through CD73 expression and adenosine production. *J. Immunol.* 193: 5904–5913.
66. Matsumoto, M., A. Baba, T. Yokota, H. Nishikawa, Y. Ohkawa, H. Kayama, A. Kallies, S. L. Nutt, S. Sakaguchi, K. Takeda, et al. 2014. Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation. *Immunity* 41: 1040–1051.
67. Korniotis, S., C. Gras, H. Letscher, R. Montandon, J. Mégret, S. Siegert, S. Ezine, P. G. Fallon, S. A. Luther, S. Fillatreau, and F. Zavala. 2016. Treatment of ongoing autoimmune encephalomyelitis with activated B-cell progenitors maturing into regulatory B cells. *Nat. Commun.* 7: 12134.
68. Lampropoulou, V., K. Hoehlig, T. Roch, P. Neves, E. Calderón Gómez, C. H. Sweenie, Y. Hao, A. A. Freitas, U. Steinhoff, S. M. Anderton, and S. Fillatreau. 2008. TLR-activated B cells suppress T cell-mediated autoimmunity. *J. Immunol.* 180: 4763–4773.
69. Yanaba, K., J. D. Bouaziz, T. Matsushita, T. Tsubata, and T. F. Tedder. 2009. The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals. *J. Immunol.* 182: 7459–7472.
70. Iwata, Y., T. Matsushita, M. Horikawa, D. J. Dilillo, K. Yanaba, G. M. Venturi, P. M. Szabolcs, S. H. Bernstein, C. M. Magro, A. D. Williams, et al. 2011. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 117: 530–541.
71. Kessel, A., T. Haj, R. Peri, A. Snir, D. Melamed, E. Sabo, and E. Toubi. 2012. Human CD19$^+$CD25^{high} B regulatory cells suppress proliferation of CD4$^+$ T cells and enhance Foxp3 and CTLA-4 expression in T-regulatory cells. *Autoimmun. Rev.* 11: 670–677.
72. Duddy, M., M. Niino, F. Adatia, S. Hebert, M. Freedman, H. Atkins, H. J. Kim, and A. Bar-Or. 2007. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J. Immunol.* 178: 6092–6099.
73. Hirotani, M., M. Niino, T. Fukazawa, S. Kikuchi, I. Yabe, S. Hamada, Y. Tajima, and H. Sasaki. 2010. Decreased IL-10 production mediated by Toll-like receptor 9 in B cells in multiple sclerosis. *J. Neuroimmunol.* 221: 95–100.
74. Miyazaki, Y., R. Li, A. Rezk, H. Misirliyan, C. Moore, N. Farooqi, M. Solis, L. G. Goiry, O. de Faria Junior, V. D. Dang, et al; CIHR/MSSC New Emerging Team Grant in Clinical Autoimmunity; MSSRF Canadian B cells in MS Team. 2014. A novel microRNA-132-sirtuin-1 axis underlies aberrant B-cell cytokine regulation in patients with relapsing-remitting multiple sclerosis [corrected]. [Published erratum appears in 2014 *PLoS One* 9: e109041.] *PLoS One* 9: e105421.
75. Li, R., A. Rezk, Y. Miyazaki, E. Hilgenberg, H. Touil, P. Shen, C. S. Moore, L. Michel, F. Althekair, S. Rajasekharan, et al; Canadian B cells in MS Team. 2015. Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. *Sci. Transl. Med.* 7: 310ra166.
76. Chen, D., M. Blazek, S. Ireland, S. Ortega, X. Kong, A. Meeuwissen, A. Stowe, L. Carter, Y. Wang, R. Herbst, and N. L. Monson. 2014. Single dose of glyco-engineered anti-CD19 antibody (MEDI551) disrupts experimental autoimmune encephalomyelitis by inhibiting pathogenic adaptive immune responses in the bone marrow and spinal cord while preserving peripheral regulatory mechanisms. *J. Immunol.* 193: 4823–4832.
77. Li, R., A. Rezk, L. M. Healy, G. Muirhead, A. Prat, J. L. Gommerman, and A. Bar-Or, MSSRF Canadian B Cells in MS Team. 2016. Cytokine-defined B cell responses as therapeutic targets in multiple sclerosis. *Front. Immunol.* 6: 626.
78. Ray, A., S. Basu, C. B. Williams, N. H. Salzman, and B. N. Dittel. 2012. A novel IL-10-independent regulatory role for B cells in suppressing autoimmunity by maintenance of regulatory T cells via GITR ligand. *J. Immunol.* 188: 3188–3198.
79. Chen, J., N. Chia, K. R. Kalari, J. Z. Yao, M. Novotna, M. M. Soldan, D. H. Luckey, E. V. Marietta, P. R. Jeraldo, X. Chen, et al. 2016. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci. Rep.* 6: 28484.
80. Jangi, S., R. Gandhi, L. M. Cox, N. Li, F. von Glehn, R. Yan, B. Patel, M. A. Mazzola, S. Liu, B. L. Glanz, et al. 2016. Alterations of the human gut microbiome in multiple sclerosis. *Nat. Commun.* 7: 12015.
81. Miyake, S., S. Kim, W. Suda, K. Oshima, M. Nakamura, T. Matsuoka, N. Chihara, A. Tomita, W. Sato, S. W. Kim, et al. 2015. Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to clostridia XIVa and IV clusters. *PLoS One* 10: e0137429.
82. Varrin-Doyer, M., C. M. Spencer, U. Schulze-Topphoff, P. A. Nelson, R. M. Stroud, B. A. Cree, and S. S. Zamvil. 2012. Aquaporin 4-specific T cells in neuromyelitis optica exhibit a Th17 bias and recognize *Clostridium* ABC transporter. *Ann. Neurol.* 72: 53–64.
83. Cree, B. A., C. M. Spencer, M. Varrin-Doyer, S. E. Baranzini, and S. S. Zamvil. 2016. Gut microbiome analysis in neuromyelitis optica reveals over-abundance of *Clostridium perfringens*. *Ann. Neurol.* 80: 443–447.

84. Rumah, K. R., J. Linden, V. A. Fischetti, and T. Vartanian. 2013. Isolation of *Clostridium perfringens* type B in an individual at first clinical presentation of multiple sclerosis provides clues for environmental triggers of the disease. *PLoS One* 8: e76359.
85. Ochoa-Repáraz, J., D. W. Mielcarz, L. E. Ditrio, A. R. Burroughs, D. M. Foureau, S. Haque-Begum, and L. H. Kasper. 2009. Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. *J. Immunol.* 183: 6041–6050.
86. Ochoa-Repáraz, J., D. W. Mielcarz, S. Haque-Begum, and L. H. Kasper. 2010. Induction of a regulatory B cell population in experimental allergic encephalomyelitis by alteration of the gut commensal microflora. *Gut Microbes* 1: 103–108.
87. Rosser, E. C., K. Oleinika, S. Tonon, R. Doyle, A. Bosma, N. A. Carter, K. A. Harris, S. A. Jones, N. Klein, and C. Mauri. 2014. Regulatory B cells are induced by gut microbiota-driven interleukin-1β and interleukin-6 production. *Nat. Med.* 20: 1334–1339.
88. Yokote, H., S. Miyake, J. L. Croxford, S. Oki, H. Mizusawa, and T. Yamamura. 2008. NKT cell-dependent amelioration of a mouse model of multiple sclerosis by altering gut flora. *Am. J. Pathol.* 173: 1714–1723.
89. Popovic, N., A. Schubart, B. D. Goetz, S. C. Zhang, C. Linington, and I. D. Duncan. 2002. Inhibition of autoimmune encephalomyelitis by a tetracycline. *Ann. Neurol.* 51: 215–223.
90. Metz, L. M., D. Li, A. Traboulsee, M. L. Myles, P. Duquette, J. Godin, M. Constantin, and V. W. Yong, GA/Minocycline Study Investigators. 2009. Glatiramer acetate in combination with minocycline in patients with relapsing–remitting multiple sclerosis: results of a Canadian, multicenter, double-blind, placebo-controlled trial. *Mult. Scler.* 15: 1183–1194.
91. Sørensen, P. S., F. Sellebjerg, J. Lycke, M. Färkkilä, A. Créange, C. G. Lund, M. Schluep, J. L. Frederiksen, E. Stenager, C. Pfleger, et al; RECYCLINE Study Investigators. 2016. Minocycline added to subcutaneous interferon β-1a in multiple sclerosis: randomized RECYCLINE study. *Eur. J. Neurol.* 23: 861–870.
92. Metz, L. M., D. Li, A. Traboulsee, P. Duquette, V. W. Yong, M. Eliasziw, G. Cerchiaro, J. Greenfield, A. Riddehough, M. Yeung, et al. 2015. Minocycline reduces the relative risk of multiple sclerosis in people experiencing their first clinical demyelinating event by 44.6%: results of a phase III double-blind placebo controlled Canadian multicentre clinical trial. *Mult. Scler.* 21 (Supp. 11): 780–781 (Abstr. 227).
93. Sandler, R. H., S. M. Finegold, E. R. Bolte, C. P. Buchanan, A. P. Maxwell, M. L. Väisänen, M. N. Nelson, and H. M. Wexler. 2000. Short-term benefit from oral vancomycin treatment of regressive-onset autism. *J. Child Neurol.* 15: 429–435.
94. Ezendam, J., A. de Klerk, E. R. Gremmer, and H. van Loveren. 2008. Effects of *Bifidobacterium animalis* administered during lactation on allergic and autoimmune responses in rodents. *Clin. Exp. Immunol.* 154: 424–431.
95. Kwon, H. K., G. C. Kim, Y. Kim, W. Hwang, A. Jash, A. Sahoo, J. E. Kim, J. H. Nam, and S. H. Im. 2013. Amelioration of experimental autoimmune encephalomyelitis by probiotic mixture is mediated by a shift in T helper cell immune response. *Clin. Immunol.* 146: 217–227.
96. Lavasani, S., B. Dzhambazov, M. Nouri, F. Fåk, S. Buske, G. Molin, H. Thorlacius, J. Alenfall, B. Jeppsson, and B. Weström. 2010. A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. *PLoS One* 5: e9009.
97. Takata, K., M. Kinoshita, T. Okuno, M. Moriya, T. Kohda, J. A. Honorat, T. Sugimoto, A. Kumanogoh, H. Kayama, K. Takeda, et al. 2011. The lactic acid bacterium *Pediococcus acidilactici* suppresses autoimmune encephalomyelitis by inducing IL-10-producing regulatory T cells. *PLoS One* 6: e27644.
98. Ochoa-Repáraz, J., C. Riccardi, A. Rynda, S. Jun, G. Callis, and D. W. Pascual. 2007. Regulatory T cell vaccination without autoantigen protects against experimental autoimmune encephalomyelitis. *J. Immunol.* 178: 1791–1799.
99. Rezende, R. M., R. P. Oliveira, S. R. Medeiros, A. C. Gomes-Santos, A. C. Alves, F. G. Loli, M. A. Guimarães, S. S. Amaral, A. P. da Cunha, H. L. Weiner, et al. 2013. Hsp65-producing *Lactococcus lactis* prevents experimental autoimmune encephalomyelitis in mice by inducing $CD4^+LAP^+$ regulatory T cells. *J. Autoimmun.* 40: 45–57.
100. Takata, K., T. Tomita, T. Okuno, M. Kinoshita, T. Koda, J. A. Honorat, M. Takei, K. Hagihara, T. Sugimoto, H. Mochizuki, et al. 2015. Dietary yeasts reduce inflammation in central nerve system via microflora. *Ann. Clin. Transl. Neurol.* 2: 56–66.
101. Konieczna, P., D. Groeger, M. Ziegler, R. Frei, R. Ferstl, F. Shanahan, E. M. Quigley, B. Kiely, C. A. Akdis, and L. O'Mahony. 2012. *Bifidobacterium infantis* 35624 administration induces Foxp3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. *Gut* 61: 354–366.
102. Mercadante, A. C., S. M. Perobelli, A. P. Alves, T. Gonçalves-Silva, W. Mello, A. C. Gomes-Santos, A. Miyoshi, V. Azevedo, A. M. Faria, and A. Bonomo. 2014. Oral combined therapy with probiotics and alloantigen induces B cell–dependent long-lasting specific tolerance. *J. Immunol.* 192: 1928–1937.
103. Liao, H. Y., L. Tao, J. Zhao, J. Qin, G. C. Zeng, S. W. Cai, Y. Li, J. Zhang, and H. G. Chen. 2016. *Clostridium butyricum* in combination with specific immunotherapy converts antigen-specific B cells to regulatory B cells in asthmatic patients. *Sci. Rep.* 6: 20481.
104. Shi, Y., L. Z. Xu, K. Peng, W. Wu, R. Wu, Z. Q. Liu, G. Yang, X. R. Geng, J. Liu, Z. G. Liu, et al. 2015. Specific immunotherapy in combination with *Clostridium butyricum* inhibits allergic inflammation in the mouse intestine. *Sci. Rep.* 5: 17651.
105. Ochoa-Repáraz, J., D. W. Mielcarz, L. E. Ditrio, A. R. Burroughs, S. Begum-Haque, S. Dasgupta, D. L. Kasper, and L. H. Kasper. 2010. Central nervous system demyelinating disease protection by the human commensal *Bacteroides fragilis* depends on polysaccharide A expression. *J. Immunol.* 185: 4101–4108.
106. Shen, Y., M. L. Giardino Torchia, G. W. Lawson, C. L. Karp, J. D. Ashwell, and S. K. Mazmanian. 2012. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. *Cell Host Microbe* 12: 509–520.
107. Ochoa-Repáraz, J., D. W. Mielcarz, Y. Wang, S. Begum-Haque, S. Dasgupta, D. L. Kasper, and L. H. Kasper. 2010. A polysaccharide from the human commensal *Bacteroides fragilis* protects against CNS demyelinating disease. *Mucosal Immunol.* 3: 487–495.
108. Wang, Y., K. M. Telesford, J. Ochoa-Repáraz, S. Haque-Begum, M. Christy, E. J. Kasper, L. Wang, Y. Wu, S. C. Robson, D. L. Kasper, and L. H. Kasper. 2014. An intestinal commensal symbiosis factor controls neuroinflammation via TLR2-mediated CD39 signalling. *Nat. Commun.* 5: 4432.
109. Round, J. L., and S. K. Mazmanian. 2010. Inducible $Foxp3^+$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl. Acad. Sci. USA* 107: 12204–12209.
110. Telesford, K. M., W. Yan, J. Ochoa-Reparaz, A. Pant, C. Kircher, M. A. Christy, S. Begum-Haque, D. L. Kasper, and L. H. Kasper. 2015. A commensal symbiotic factor derived from *Bacteroides fragilis* promotes human $CD39^+Foxp3^+$ T cells and Treg function. *Gut Microbes* 6: 234–242.
111. Desbonnet, L., L. Garrett, G. Clarke, J. Bienenstock, and T. G. Dinan. 2008. The probiotic *Bifidobacteria infantis*: an assessment of potential antidepressant properties in the rat. *J. Psychiatr. Res.* 43: 164–174.
112. Desbonnet, L., L. Garrett, G. Clarke, B. Kiely, J. F. Cryan, and T. G. Dinan. 2010. Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience* 170: 1179–1188.
113. Hsiao, E. Y., S. W. McBride, S. Hsien, G. Sharon, E. R. Hyde, T. McCue, J. A. Codelli, J. Chow, S. E. Reisman, J. F. Petrosino, et al. 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* 155: 1451–1463.
114. Peón, A. N., and L. I. Terrazas. 2016. Immune-regulatory mechanisms of classical and experimental multiple sclerosis drugs: a special focus on helminth-derived treatments. *Curr. Med. Chem.* 23: 1152–1170.
115. Correale, J., and M. Farez. 2007. Association between parasite infection and immune responses in multiple sclerosis. *Ann. Neurol.* 61: 97–108.
116. Correale, J., M. Farez, and G. Razzitte. 2008. Helminth infections associated with multiple sclerosis induce regulatory B cells. *Ann. Neurol.* 64: 187–199.
117. La Flamme, A. C., K. Ruddenklau, and B. T. Bäckström. 2003. Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. *Infect. Immun.* 71: 4996–5004.
118. Walsh, K. P., M. T. Brady, C. M. Finlay, L. Boon, and K. H. Mills. 2009. Infection with a helminth parasite attenuates autoimmunity through TGF-β-mediated suppression of Th17 and Th1 responses. *J. Immunol.* 183: 1577–1586.
119. Wu, Z., I. Nagano, K. Asano, and Y. Takahashi. 2010. Infection of non-encapsulated species of *Trichinella* ameliorates experimental autoimmune encephalomyelitis involving suppression of Th17 and Th1 response. *Parasitol. Res.* 107: 1173–1188.
120. Wilson, M. S., M. D. Taylor, M. T. O'Gorman, A. Balic, T. A. Barr, K. Filbey, S. M. Anderton, and R. M. Maizels. 2010. Helminth-induced $CD19^+CD23^{hi}$ B cells modulate experimental allergic and autoimmune inflammation. *Eur. J. Immunol.* 40: 1682–1696.
121. Reyes, J. L., A. F. Espinoza-Jiménez, M. I. González, L. Verdin, and L. I. Terrazas. 2011. *Taenia crassiceps* infection abrogates experimental autoimmune encephalomyelitis. *Cell. Immunol.* 267: 77–87.
122. Sewell, D., Z. Qing, E. Reinke, D. Elliot, J. Weinstock, M. Sandor, and Z. Fabry. 2003. Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization. *Int. Immunol.* 15: 59–69.
123. Zheng, X., X. Hu, G. Zhou, Z. Lu, W. Qiu, J. Bao, and Y. Dai. 2008. Soluble egg antigen from *Schistosoma japonicum* modulates the progression of chronic progressive experimental autoimmune encephalomyelitis via Th2-shift response. *J. Neuroimmunol.* 194: 107–114.
124. Correale, J., and M. F. Farez. 2011. The impact of parasite infections on the course of multiple sclerosis. *J. Neuroimmunol.* 233: 6–11.
125. Benzel, F., H. Erdur, S. Kohler, M. Frentsch, A. Thiel, L. Harms, K. P. Wandinger, and B. Rosche. 2012. Immune monitoring of *Trichuris suis* egg therapy in multiple sclerosis patients. *J. Helminthol.* 86: 339–347.
126. Voldsgaard, A., P. Bager, E. Garde, P. Åkeson, A. M. Leffers, C. G. Madsen, C. Kapel, A. Roepstorff, S. M. Thamsborg, M. Melbye, et al. 2015. *Trichuris suis* ova therapy in relapsing multiple sclerosis is safe but without signals of beneficial effect. *Mult. Scler.* 21: 1723–1729.
127. Turnbaugh, P. J., M. Hamady, T. Yatsunenko, B. L. Cantarel, A. Duncan, R. E. Ley, M. L. Sogin, W. J. Jones, B. A. Roe, J. P. Affourtit, et al. 2009. A core gut microbiome in obese and lean twins. *Nature* 457: 480–484.
128. David, L. A., C. F. Maurice, R. N. Carmody, D. B. Gootenberg, J. E. Button, B. E. Wolfe, A. V. Ling, A. S. Devlin, Y. Varma, M. A. Fischbach, et al. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505: 559–563.
129. Piccio, L., J. L. Stark, and A. H. Cross. 2008. Chronic calorie restriction attenuates experimental autoimmune encephalomyelitis. *J. Leukoc. Biol.* 84: 940–948.
130. Kleinewietfeld, M., A. Manzel, J. Titze, H. Kvakan, N. Yosef, R. A. Linker, D. N. Muller, and D. A. Hafler. 2013. Sodium chloride drives autoimmune disease by the induction of pathogenic T_H17 cells. *Nature* 496: 518–522.
131. Wu, C., N. Yosef, T. Thalhamer, C. Zhu, S. Xiao, Y. Kishi, A. Regev, and V. K. Kuchroo. 2013. Induction of pathogenic T_H17 cells by inducible salt-sensing kinase SGK1. *Nature* 496: 513–517.
132. Farez, M. F., M. P. Fiol, M. I. Gaitán, F. J. Quintana, and J. Correale. 2015. Sodium intake is associated with increased disease activity in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* 86: 26–31.

133. Kim, K. S., S. W. Hong, D. Han, J. Yi, J. Jung, B. G. Yang, J. Y. Lee, M. Lee, and C. D. Surh. 2016. Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science* 351: 858–863.
134. Cotillard, A., S. P. Kennedy, L. C. Kong, E. Prifti, N. Pons, E. Le Chatelier, M. Almeida, B. Quinquis, F. Levenez, N. Galleron, et al; ANR MicroObes Consortium. 2013. Dietary intervention impact on gut microbial gene richness. *Nature* 500: 585–588.
135. Smith, P. M., M. R. Howitt, N. Panikov, M. Michaud, C. A. Gallini, M. Bohlooly-Y, J. N. Glickman, and W. S. Garrett. 2013. The microbial metabolites, short-chain fatty acids, regulate colonic T_{reg} cell homeostasis. *Science* 341: 569–573.
136. Haghikia, A., S. Jörg, A. Duscha, J. Berg, A. Manzel, A. Waschbisch, A. Hammer, D. H. Lee, C. May, N. Wilck, et al. 2015. Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. [Published erratum appears in 2016 *Immunity.* 44:951–953] *Immunity* 43: 817–829.
137. Gebregiworgis, T., H. H. Nielsen, C. Massilamany, A. Gangaplara, J. Reddy, Z. Illes, and R. Powers. 2016. A urinary metabolic signature for multiple sclerosis and neuromyelitis optica. *J. Proteome Res.* 15: 659–666.
138. Zeevi, D., T. Korem, N. Zmora, D. Israeli, D. Rothschild, A. Weinberger, O. Ben-Yacov, D. Lador, T. Avnit-Sagi, M. Lotan-Pompan, et al. 2015. Personalized nutrition by prediction of glycemic responses. *Cell* 163: 1079–1094.
139. Riccio, P., R. Rossano, M. Larocca, V. Trotta, I. Mennella, P. Vitaglione, M. Ettorre, A. Graverini, A. De Santis, E. Di Monte, and M. G. Coniglio. 2016. Anti-inflammatory nutritional intervention in patients with relapsing-remitting and primary-progressive multiple sclerosis: a pilot study. *Exp. Biol. Med. (Maywood)* 241: 620–635.
140. Bisht, B., W. G. Darling, R. E. Grossmann, E. T. Shivapour, S. K. Lutgendorf, L. G. Snetselaar, M. J. Hall, M. B. Zimmerman, and T. L. Wahls. 2014. A multimodal intervention for patients with secondary progressive multiple sclerosis: feasibility and effect on fatigue. *J. Altern. Complement. Med.* 20: 347–355.