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REVIEW ARTICLE

The intestinal barrier in multiple sclerosis: implications for pathophysiology and therapeutics

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Biological barriers are essential for the maintenance of homeostasis in health and disease. Breakdown of the intestinal barrier is an essential aspect of the pathophysiology of gastrointestinal inflammatory diseases, such as inflammatory bowel disease. A wealth of recent studies has shown that the intestinal microbiome, part of the brain-gut axis, could play a role in the pathophysiology of multiple sclerosis. However, an essential component of this axis, the intestinal barrier, has received much less attention. In this review, we describe the intestinal barrier as the physical and functional zone of interaction between the luminal microbiome and the host. Besides its essential role in the regulation of homeostatic processes, the intestinal barrier contains the gut mucosal immune system, a guardian of the integrity of the intestinal tract and the whole organism. Gastrointestinal disorders with intestinal barrier breakdown show evidence of CNS demyelination, and content of the intestinal microbiome entering into the circulation can impact the functions of CNS microglia. We highlight currently available studies suggesting that there is intestinal barrier dysfunction in multiple sclerosis. Finally, we address the mechanisms by which commonly used disease-modifying drugs in multiple sclerosis could alter the intestinal barrier and the microbiome, and we discuss the potential of barrier-stabilizing strategies, including probiotics and stabilization of tight junctions, as novel therapeutic avenues in multiple sclerosis.

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Abbreviations: EAE = experimental autoimmune encephalomyelitis; IBD = inflammatory bowel disease; LPS = lipopolysaccharide; SCFA = short chain fatty acid

Introduction

Biological barriers separate the internal milieu from the external environment and are essential components of maintaining homeostasis. A compromised intestinal barrier function is a prominent feature of many diseases, such as inflammatory bowel disease (Choi *et al.*, 2017; Martini *et al.*, 2017; Mu *et al.*, 2017), graft versus host disease (Nalle and Turner, 2015) and coeliac disease (Schumann *et al.*, 2017), but other biological barriers also fail in a

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myriad of pathological conditions (e.g. renal tubules in glomerulonephritis and lung alveoli in acute respiratory distress syndrome). The CNS is highly sensitive to homeostatic changes, and as such requires its own specialized barrier, the blood–brain barrier, for appropriate functioning. Breakdown of the blood–brain barrier is an essential hallmark of multiple sclerosis pathophysiology. Immune mediated dysregulation of the blood–brain barrier allows for migration of activated inflammatory cells into the brain, which in turn induces demyelination, axonal loss and other tissue damage (Ortiz *et al.*, 2014; Kamphuis *et al.*, 2015). Interestingly, many of the tight junction molecules in endothelial cells of the brain–blood barrier are identical to those in intestinal tissues, such as occludin, claudins and zona occludens-1 (Reinhold and Rittner, 2017). In this review, we examine the multiple lines of evidence, albeit mostly indirect, linking the intestinal barrier function and multiple sclerosis pathophysiology. We also discuss the possible effect of multiple sclerosis disease-modifying therapies and their association with the gut microbiome.

The intestinal barrier

The intestinal barrier maintains homeostasis by preventing the unwanted movement of antigenic molecules and microbes from the lumen of the gastrointestinal tract, while allowing the products of digestion and water to enter the body. The intestinal barrier consists of a physical barrier provided by the inter-epithelial tight junctions, a secretory barrier that includes antimicrobial peptides, mucus and fluid and an immunological barrier, including cells and molecules of the innate and adaptive immune system. The secretory component of the epithelial barrier is regulated by neural mechanisms that integrate this component of barrier function with digestive processes in the gut. Intestinal barrier function refers to ability of the intestinal mucosa and extracellular barrier components (e.g. mucus, antimicrobial peptides) to modulate epithelial permeability and act as a physical and functional limiting step for organism-luminal interactions.

The intestinal lumen and its contents are separated from the rest of the gastrointestinal tissue (and the body) by a single layer of epithelial cells along the length of the gastrointestinal tract. These cells are being constantly renewed and thus require constant proliferation (Delgado *et al.*, 2016). Intestinal stem cells, present in the crypts of the intestinal mucosa, differentiate into both enterocytes, and specialized secretory (Paneth cells and goblet cells) and sensory cells (enteroendocrine cells and tuft cells), a process regulated by complex transcriptional and epigenetic mechanisms (Smith *et al.*, 2017). The intestinal barrier is permeable to water and other small molecules, a property modulated by tight junctions, located around the apical surface of adjacent epithelial cells. Tight junctions consist of a heterogeneous group of transmembrane proteins such as occludins, claudins, junctional adhesion molecules and zona occludens-1, each with specific roles (Gasbarrini and Montalto, 1999; Sturgeon and Fasano, 2016; Volynets *et al.*, 2016; Capaldo *et al.*, 2017; France and Turner, 2017).

The intestinal barrier (Fig. 1) is continuously exposed to a number of immunological and microbiological factors. When the permeability of the intestinal barrier is breached, undesired large molecules, and commensal bacteria, may enter the lamina propria with pathological consequences (Odenwald and Turner, 2017). One of the main causes of increased permeability of the intestinal barrier is inflammation, an event thought to be essential in the pathophysiology of inflammatory bowel disease (IBD) (de Souza *et al.*, 2017; Martini *et al.*, 2017), coeliac disease and sepsis (Yoseph *et al.*, 2016; Schumann *et al.*, 2017). Inflammatory cytokines including interferons, interleukin (IL)-17 and tumour necrosis factor alpha (TNFα), as well as calcium-dependent oxidative stress, have been shown to alter the expression of tight junction proteins and lead to increased intestinal permeability (Reynolds *et al.*, 2012; Yang *et al.*, 2014; Al-Sadi *et al.*, 2016; Gangwar *et al.*, 2017).

Together with intestinal epithelial cells as the first layer of the intestinal barrier are Paneth cells (Fig. 1), which are specialized secretory cells derived from intestinal stem cells. Paneth cells produce antimicrobial peptides, the defensins, which are secreted into the mucus layer (Dupont *et al.*, 2014; Yu *et al.*, 2016; Capaldo *et al.*, 2017). Mucus, secreted from goblet cells, is composed of heavily glycosylated oligomeric mucin proteins, water, ions and secretory IgA. This layer modulates bacterial growth in the intestinal lumen adjacent to the intestinal barrier, prevents bacterial adherence and acts as part of the innate immune response of the organism against microbial pathogens (Dupont *et al.*, 2014).

After the mucus and the epithelial lining of the gastrointestinal tract, the next layer of the intestinal barrier is mostly immunological. Innate lymphoid cells, located in the epithelial layer, can be activated to produce a variety of inflammatory mediators, which play a defensive or a pathogenic role in mammal gut homeostasis (Bostick and Zhou, 2016). Found in close proximity to the single layer of enterocytes, intraepithelial lymphocytes are a heterogeneous population of cells that provide immune protection against pathogens and also regulate immune responses that, if unchecked, could jeopardize the integrity of the barrier (Cheroutre *et al.*, 2011; Olivares-Villagómez and Van Kaer, 2018). The lamina propria (Fig. 1) is populated by B, T and dendritic cells that can initiate and modulate a host of immunological responses (Persson *et al.*, 2013; Gronke *et al.*, 2017). Peyer's patches are secondary lymphoid tissues present in the intestinal mucosa. They are continuously exposed to a variety of antigens, presented to Peyer's patches by microfold epithelial cells and resident dendritic cells (Rochereau *et al.*, 2011; Hashiguchi *et al.*, 2015).

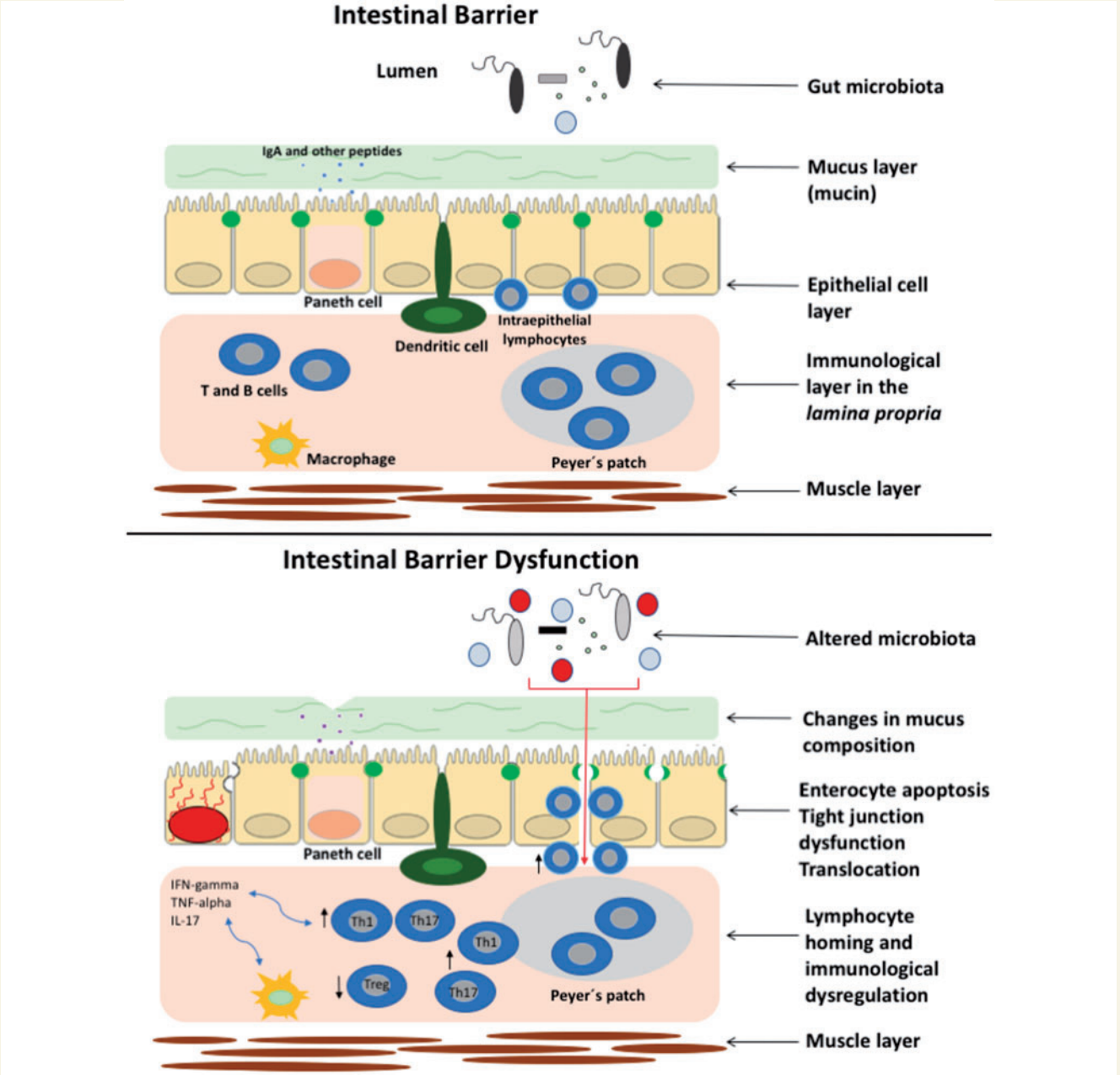

Figure 1 The intestinal barrier and possible mechanisms of barrier dysfunction in multiple sclerosis. The normal intestinal barrier is composed of multiple layers (*top*). From the luminal side outwards, there is a mucus layer in close contact with the commensal microbiota, the single cell epithelial layer (woven together by tight junction proteins depicted here as green closed circles), the lamina propria and submucosa containing the immunological barrier, and finally the muscle and connective tissue layer. Changes in microbiota, mucus composition, epithelial cell death, tight junction function and immunological dysregulation could all lead to breakdown of the intestinal barrier and increased permeability (*bottom*).

CNS demyelination and intestinal barrier breakdown in gastrointestinal disorders: an important link?

An association between multiple sclerosis and IBD has been suggested because of common epidemiological, immunological and genetic patterns (Barcellos *et al.*, 2006). IBD patients have an increased risk for cerebrovascular disease, peripheral neuropathy and demyelinating disease (Casella *et al.*, 2014; Ferro *et al.*, 2014; Morís, 2014), and anti-TNF therapies that are widely used in IBD have also been associated with CNS demyelination (Katsanos and Katsanos, 2014). Indeed, a recent meta-analysis of 10 case-control studies including over 1 million patients found a risk ratio of 1.54 for multiple sclerosis/IBD comorbidity, with no difference between Crohn's disease

and ulcerative colitis (Kosmidou *et al.*, 2017). Certain authors propose that IBD can be conceived as a disorder of the intestinal epithelial barrier, and barrier breakdown is known to be an essential step in the pathophysiology of both Crohn's and ulcerative colitis (for reviews see Jäger *et al.*, 2013; Antoni *et al.*, 2014; Goll and van Beelen Granlund, 2015). Evidence showing white matter involvement in IBD could also provide a link between intestinal barrier breakdown and CNS demyelination.

In an early study, investigators found a 3-fold increase of white matter hyperintensities in the MRIs of patients with IBD (Geissler *et al.*, 1995). A recent estimate suggested that over half of all IBD patients will have white matter hyperintensities in a routine MRI (Ferro *et al.*, 2014). Other findings in IBD include decreased grey matter volume and decreased axial diffusivity in major white matter tracts (Zikou *et al.*, 2014). The aetiology of the white matter lesions found in patients with IBD is uncertain, and some authors suggest that ischaemia and vasculitis might be responsible (Zikou *et al.*, 2014). However, in a report of five cases of patients with Crohn's disease with symptomatic acute white matter lesions suggestive of demyelination, systemic infection, coagulation disorders, or vasculitis were ruled out (de Lau *et al.*, 2009). Additionally, other studies have attempted to describe white matter lesions in patients with Crohn's disease with more detail. White matter lesions suggestive of demyelination were found in 72% of 54 patients compared to 34% in age- and sex-matched controls (Chen *et al.*, 2012). The role of anti-TNF therapy is also debated, and some observational studies have not found an association between therapy and presence of white matter hyperintensities (Chen *et al.*, 2012). In a retrospective analysis of 9095 patients with IBD, anti-TNF therapy was not found to increase the risk of confirmed inflammatory demyelinating CNS lesions (de Felice *et al.*, 2015).

Other gastrointestinal diseases where the intestinal barrier is impaired have also been associated with CNS demyelination. In patients with multiple sclerosis, serological and histological markers of coeliac disease are more frequent than in healthy controls (Rodrigo *et al.*, 2011), although other studies have found inconsistent results (Salvatore *et al.*, 2004). Cases of comorbid coeliac disease and multiple sclerosis are abundant in the literature (Batur-Caglayan *et al.*, 2013; Casella *et al.*, 2016), as are cases of coeliac disease with white matter lesions mimicking multiple sclerosis or other CNS demyelinating diseases (Mirabella *et al.*, 2006; Finsterer and Leutmezer, 2014; Krom *et al.*, 2017). MRI studies in patients with coeliac disease have also shown higher proportion of white matter lesions and grey matter atrophy (Bilgic *et al.*, 2013).

Although a causal link between intestinal barrier breakdown and CNS demyelination cannot be concluded with certainty in these cases, there appears to be an association not solely explained by their shared epidemiological and immunological characteristics. The association between these entities is certainly complex and in need of further study.

Intestinal barrier homeostasis, the microbiome and neuroinflammation: possible mechanisms linking these entities

The interactions between the microbiome and the intestinal barrier, particularly the contribution of the microbiome in maintaining barrier homeostasis, could be central in accounting for its regulation of neuroinflammation (Fig. 2). Several studies have established that there are alterations in the gut microbiome of patients with multiple sclerosis, which has further fuelled the interest in the brain-gut-microbiome connection in multiple sclerosis research.

Early studies showed that, when compared to controls, patients with relapsing-remitting multiple sclerosis have an abundance of *Anaerostipes*, *Faecalibacterium*, *Pseudomonas*, *Mycoplasma*, *Haemophilus*, *Blautia*, and *Dorea* and a relative decrease of *Bacteroides*, *Prevotella*, *Parabacteroides* and *Adlercreutzia* (Cantarel *et al.*, 2015; Miyake *et al.*, 2015; Chen *et al.*, 2016). In paediatric multiple sclerosis, patients have higher levels of members of *Desulfovibrionaceae* and depletion in *Lachnospiraceae* and *Ruminococcaceae* (Tremlett *et al.*, 2016*a*). However, a clear and consistent 'multiple sclerosis microbiome phenotype' has not been described, and a myriad of different species have been implicated. For example, studies have found a significant depletion in *Clostridial* species (Rumah *et al.*, 2013; Miyake *et al.*, 2015), *Butyricimonas* (Jangi *et al.*, 2016), *Roseburia* (Swidsinski *et al.*, 2017) and increases in *Streptococcus* (Cosorich *et al.*, 2017), *Methanobrevibacter*, *Akkermansia* and *Coprococcus* (Cantarel *et al.*, 2015; Jangi *et al.*, 2016). Multicentre studies aiming at defining a 'core microbiome' are underway (Pröbstel and Baranzini, 2018). Furthermore, some of these changes in the microbiome have been associated with immunological derangements, such as differences in the expression of genes involved in interferon and nuclear factor kappa-B (NF-κB) signalling (Jangi *et al.*, 2016), and numbers of pro-inflammatory T helper 17 (Th17) cells in the intestine (Cosorich *et al.*, 2017). At least one study found that differences in the microbiota could predict relapse risk in paediatric multiple sclerosis patients (Tremlett *et al.*, 2016*b*).

Insights into how the microbiome could alter neuroinflammatory responses (reviewed in Colpitts and Kasper, 2017; Wekerle, 2017) have been illuminated by studies in germ-free mice where the microbiome regulates the shift back-and-forth of immune cells from pro- to anti-inflammatory phenotypes (Berer *et al.*, 2011). Mice maintained under germ-free conditions have an attenuated form of experimental autoimmune encephalomyelitis (EAE), an inflammatory model of multiple sclerosis, and show lower levels of IL-17 in both the gut and the CNS, while also

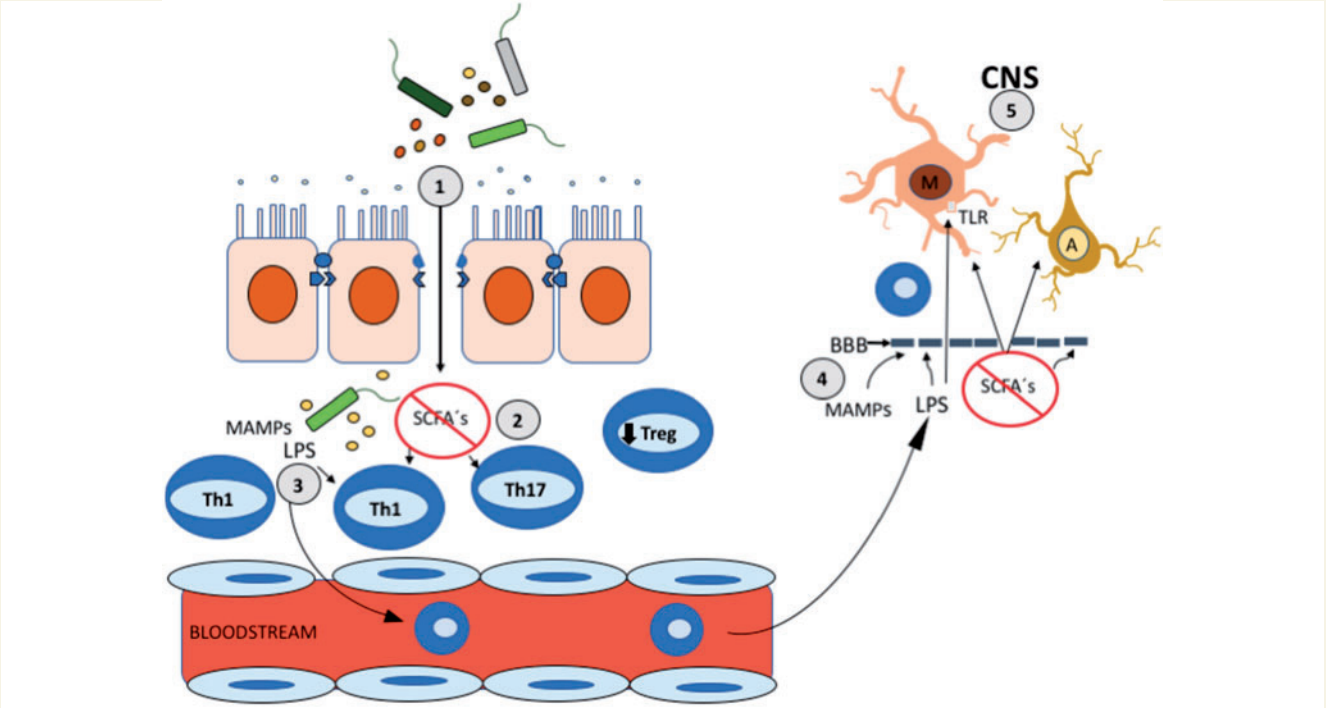

Figure 2 An altered intestinal barrier leads to immune changes in the gut and the CNS. (1) Multiple sclerosis-associated microbiota and immune derangements lead to an altered barrier and increased permeability. (2) Microbiota diversity is reduced, as is production of SCFA's, and some bacteria translocate to the lamina propria. (3) LPS produced by bacteria cause low-grade inflammation and endotoxaemia, and loss of SCFA signalling alters lymphocyte phenotypes. (4) LPS, microbial-associated molecular patterns (MAMPs) and reduced SCFAs alter the blood–brain barrier. (5) LPS and activated lymphocytes reach the CNS, where in absence of normal SCFA concentrations, microglia and astrocyte neuroimmune responses are affected. A = astrocytes; BBB = blood–brain barrier; M = microglia; TLR = Toll-like receptors.

showing an increase in regulatory T cells (Tregs) peripherally (Lee *et al.*, 2011). Colonization with segmented filamentous bacteria in germ-free mice leads to increased production of IL-17 and development of severe EAE. In contrast, other gut commensals such as *P. histicola* are able to suppress EAE severity, by decreasing pro-inflammatory Th1 and Th17 cells, and increasing Tregs and suppressive macrophages (Mangalam *et al.*, 2017). *B. fragilis*, another common commensal strain, can also suppress EAE by expanding Tregs expressing the ectonucleotidase CD39, allowing for increased migration of this regulatory cell type into the CNS (Wang *et al.*, 2014). Microbiota abundant in patients with multiple sclerosis induce the differentiation *in vitro* of human peripheral blood mononuclear cells into Th1 cells while reducing Treg numbers; conversely, microbiota that are decreased in patients with multiple sclerosis stimulate anti-inflammatory IL-10-expressing T cells and FoxP3+ Tregs (Cekanaviciute *et al.*, 2017). Microbiota from patients with multiple sclerosis transplanted to mice prone to develop spontaneous EAE increases their susceptibility to EAE (Berer *et al.*, 2017). Interestingly, multiple sclerosis patient-derived microbiota transplantation did not lead to changes in tight junction protein expression in the mouse recipient gut, but splenic lymphocytes had impaired IL-10 production (Berer *et al.*, 2017).

An altered microbiome also leads to changes in some bacteria-associated products known to influence neuroimmune responses. Short chain fatty acids (SCFAs) such as butyrate, propionate and acetate are produced by bacterial fermentation of dietary carbohydrate and fibre. They play important roles in maintaining intestinal homeostasis, such as mediating sodium transport, serving as the principal energy source of intestinal epithelial cells and modulating gene transcription via inhibition of histone deacetylase activity (Kiela and Ghishan, 2016). Although not focusing on the concentration of SCFAs, CSF metabolomics studies from patients with multiple sclerosis have shown significant differences when compared to controls. SCFAs such as acetate are reduced (Simone *et al.*, 1996; Kim *et al.*, 2017), while others such as formate (Kim *et al.*, 2017) have been found to be elevated in patients CSF. In studies evaluating metabolites in urine, propionate metabolism has also been found to be altered in patients with multiple sclerosis (Gebregiworgis *et al.*, 2016).

In experimental models, eradication of the gut microbiota, or even just limiting the intestinal microbiome diversity, leads to impaired microglia structure and immune function, a process regulated by SCFAs (Erny *et al.*, 2015, 2017). Astrocytes may also be influenced by SCFAs and the microbiome. Dietary tryptophan is metabolized by the gut microbiota into aryl hydrocarbon receptor agonists

such as indoxyl-3-sulfate and indole-3-propionic acid, which can modulate astrocyte inflammatory function trough limiting NF-κB activation in a suppressor of cytokine signalling 2-dependent manner (Rothhammer *et al.*, 2016). SCFAs also reduce T cell proliferation and cytokine production in the gut (D'Souza *et al.*, 2017; Wan Saudi and Sjöblom, 2017). In EAE models, the administration of SCFAs led to amelioration of disease severity in association with a reduction of Th1 cells and an increase in Tregs (Mizuno *et al.*, 2017). Interestingly, an altered microbiota may also alter innate immune responses in the gut favourable for systemic autoimmunity. For example, some types of intraepithelial lymphocytes may act as Tregs that suppress the pathogenic response to the immunizing antigen in EAE (Tang *et al.*, 2007). CD4(+) intraepithelial lymphocytes obtained from transgenic mice prone to develop spontaneous EAE can infiltrate the CNS and ameliorate EAE severity in wild-type mice on transfer, showing regulatory properties (Kadowaki *et al.*, 2016). These same cells proliferate in response to gut-derived antigens, aryl hydrocarbon receptor ligands and microbiota.

SCFAs could also modulate blood–brain barrier permeability. It is well known that SCFAs enhance intestinal epithelial cell barrier function by increasing the expression of tight junction proteins (D'Souza *et al.*, 2017; Wan Saudi and Sjöblom, 2017). Butyrate has also been shown to increase the expression of occludin and zona occludens-1, thus restoring blood–brain barrier permeability in models of traumatic brain injury (Li *et al.*, 2016). In germ-free mice exhibiting an altered blood–brain barrier, butyrate administration led to increased occludin expression and preserved blood–brain barrier permeability (Braniste *et al.*, 2014). Overall, changes in SCFA-producing bacteria in the gut, and the influx of SCFAs into the blood stream, could thus have a distal effect in microglia and astrocyte functions, as well as in modifying blood–brain barrier permeability and the entrance of immune cells into the CNS (Fig. 2).

Besides the above-discussed mechanisms suggesting bystander activation, another possible immunopathogenic link between multiple sclerosis and the gut microbiota is that of molecular mimicry. CNS-specific, self-reactive lymphocytes might be cross-activated by both gut microbiota antigens and myelin (Berer and Krishnamoorthy, 2014). Although there is no conclusive evidence for these mechanisms, commonly found pathogenic and non-pathogenic gut bacteria such as *Bacteroides* spp. and *Enterococcus faecalis* possess potential myelin basic protein encephalitogenic mimics (Westall, 2006).

The intestinal barrier in multiple sclerosis: consequences of a leaky gut

Recent attention in the brain-gut connection in multiple sclerosis research has been focused on the role of the commensal gut microbiome while largely ignoring the interface of the microbiome with the organism, i.e. the intestinal barrier. Therefore, actual evidence for an alteration of the intestinal barrier in multiple sclerosis is limited. In a study of 12 jejunal biopsies from multiple sclerosis patients, Lange and Shiner (1976) found subtle histological changes, such as two cases of villous atrophy, as well as some cases of intestinal inflammatory cell infiltration. A later study found similar infiltrates, and also evidence of intestinal malabsorption in close to 20 of 52 patients with multiple sclerosis (Gupta *et al.*, 1977).

In 1996, Yacyshyn *et al.* (1996) showed that 5 of 20 patients with multiple sclerosis had an altered lactulose/mannitol permeability test, suggesting increased intestinal permeability, a finding also associated with peripheral expression of CD45RO on CD20+ B cells. In the most recent study to date, the lactulose/mannitol permeability test was again used to evaluate intestinal permeability in 22 patients with multiple sclerosis and compared with age- and sex-matched controls (Buscarinu *et al.*, 2017). Investigators found abnormal permeability in 73% of cases versus 28% in controls, but no association between permeability and brain MRI lesion load.

Similar findings have been recently described in the EAE model, the prototypic inflammatory animal model of multiple sclerosis. Investigators have found altered intestinal permeability, reduced submucosa thickness and altered tight junction expression in intestinal epithelial cells (Nouri *et al.*, 2014). These alterations could also be induced in mice by adoptive transfer of pathogenic T cells. Furthermore, a recent study showed that the degree of intestinal permeability disturbance is closely associated with EAE severity (Secher *et al.*, 2017). Treatment with *Escherichia coli* strain Nissle 1917, a probiotic known to improve intestinal barrier function, preserved tight junction expression and decreased intestinal permeability, leading to reduced EAE severity and decreased secretion of pro-inflammatory cytokines and an increased production of the anti-inflammatory cytokine IL-10 (Secher *et al.*, 2017). This reduction of intestinal permeability led to a reduction of the migration of inflammatory T cells to the CNS, suggesting an impact on blood–brain barrier permeability as well (Secher *et al.*, 2017).

The above studies suggest that there is indeed an alteration in the intestinal barrier in patients with multiple sclerosis and that these changes are at least partly due to an altered intestinal immune response (Buscarinu *et al.*, 2017). The clinical relevance of these findings is unclear, but several possibilities arise. Intestinal barrier dysfunction has been associated with susceptibility to systemic infections (König *et al.*, 2016), and both CNS and systemic infections are a common complication in patients with multiple sclerosis (Venkatesan, 2015). Another possibility is that the intestinal barrier's interplay with commensal microbiota could modulate the immune response pathologically. Finally, alterations in intestinal permeability may modulate or perpetuate neuroimmune dysregulation by increased

transmucosal passage of injurious or immunogenic antigens.

The essential role of the commensal microbiome in the regulation of intestinal immunity is beginning to be recognized, and several recent reviews have been published on this subject (Haak and Wiersinga, 2017; Shi *et al.*, 2017). Commensal bacteria are able to strengthen the gut barrier and regulate intestinal permeability (Lin and Zhang, 2017). A healthy microbiota also preserves intestinal epithelial cell integrity through the production of SCFAs that increase tight junction expression and through toll-like receptor activation (Wells *et al.*, 2017). Intestinal commensal bacteria are recognized by toll-like receptors, a process leading to protection of intestinal epithelium against injury and barrier disruption (Rakoff-Nahoum *et al.*, 2004). Toll-like receptor signalling also promotes epithelial cell proliferation, IgA secretion and expression of antimicrobial peptides in Paneth cells (Abreu, 2010; Wells *et al.*, 2011).

Alterations in the gut homeostatic mechanisms in multiple sclerosis could have as one of its consequences increased bacterial translocation through an impaired intestinal barrier. One recent study found elevated levels of endotoxin [lipopolysaccharide (LPS)] in plasma of patients with multiple sclerosis, and endotoxin concentrations were related to *in vivo* IL-6 production and increased *in vitro* T-helper 17 (Th17)-like responses (Teixeira *et al.*, 2013). Circulating endotoxin was also correlated with the Expanded Disability Status Scale, a measure of clinical disability in multiple sclerosis. In another study, LPS and LPS-binding protein were found to be elevated in the serum of EAE-induced mice; investigators also found increased LPS-binding protein levels in the serum of multiple sclerosis patients compared to healthy controls (Escribano *et al.*, 2017). These studies are evidence of a low-grade endotoxaemia that could be present in patients with multiple sclerosis, possibly due to bacterial translocation in the setting of an altered intestinal barrier.

Besides LPS, enteric bacteria also produce microbial-associated molecular patterns (MAMPs) such as bacterial lipoproteins and double-stranded RNA that can enter the systemic circulation and act through toll-like receptors to modulate the immune system (Patten and Collett, 2013). Toll-like receptors are known to be expressed in microglia and to modulate initiation and severity of EAE in experimental models (Miranda-Hernandez and Baxter, 2013). LPS is a well-known stimulant of microglial responses and is able to disrupt the blood–brain barrier by increasing microglial production of matrix metalloproteinases (Frister *et al.*, 2014). LPS and other MAMPs could constitute another pathway by which an altered intestinal barrier could affect neuroimmune responses in multiple sclerosis.

Finally, the use of oral disease-modifying therapies and/or symptomatic drugs in multiple sclerosis also constitute a concern, as the intestinal barrier is essential in drug absorption (Sánchez-Navarro *et al.*, 2016). On the other hand, there are no currently marketed therapies to improve intestinal barrier function; nutritional, microbial-derived and probiotic agents are being investigated. In the next section, we will discuss the possible effects of currently used disease-modifying therapies on the intestinal barrier as well as other pathophysiological considerations.

Disease-modifying therapies and the intestinal barrier

An interesting aspect of the above mentioned findings is that the microbiome can also be altered by whatever immunomodulatory therapy the multiple sclerosis patient is receiving (Cantarel *et al.*, 2015; Tremlett *et al.*, 2016*b*). The question of whether gut dysbiosis precedes the development of multiple sclerosis or follows the immune alterations (innate, acquired or drug-induced) is also a matter of debate (Ochoa-Repáraz *et al.*, 2017). Disease-modifying therapies are medications that have improved the clinical course of relapsing-remitting multiple sclerosis. While their principal mechanisms are thought to be immune-modulating, their possible effects over the intestinal barrier that may contribute to therapeutic efficacy have not been explicitly evaluated. Below we summarize evidence suggesting that disease-modifying therapies could modulate the intestinal barrier, the gut microbiome and the interaction between the two (Fig. 3). However, the evidence is indirect, and whether this actually plays a meaningful role in clinical response remains to be established.

Interferons

There is evidence suggesting that endogenous interferons could affect the intestinal barrier. Type I interferons, including IFNα and IFNβ, are an integral part of the innate host immune response to gut microbiota, and they modulate bilateral interactions between epithelial cells and commensal flora (Giles and Stagg, 2017). For example, IFNβ has shown stabilizing properties in biological barriers (such as the intestinal, blood–brain and blood–lung barriers), partly through the upregulation of tight junction proteins in endothelial cell layers (Kraus *et al.*, 2004; LeMessurier *et al.*, 2013; Long *et al.*, 2014). The commensal microbiota also stimulates dendritic cell IFNβ production, which increases the proliferation of Tregs in the intestine, a process itself inhibited by intestinal epithelial cell apoptosis (Nakahashi-Oda *et al.*, 2016). Type I interferons also inhibit the continuous proliferation of the intestinal epithelium by activating the p53 pathway and inducing epithelial cell apoptosis (Katlinskaya *et al.*, 2016), and mice lacking type I interferon receptor on Paneth cells show an altered microbiota (Tschurtschenthaler *et al.*, 2014).

Glatiramer acetate

Various studies have shown that glatiramer acetate reduces colonic injury in animal models of colitis, through reduction of TNFα signalling, elevation of regulatory T cells and

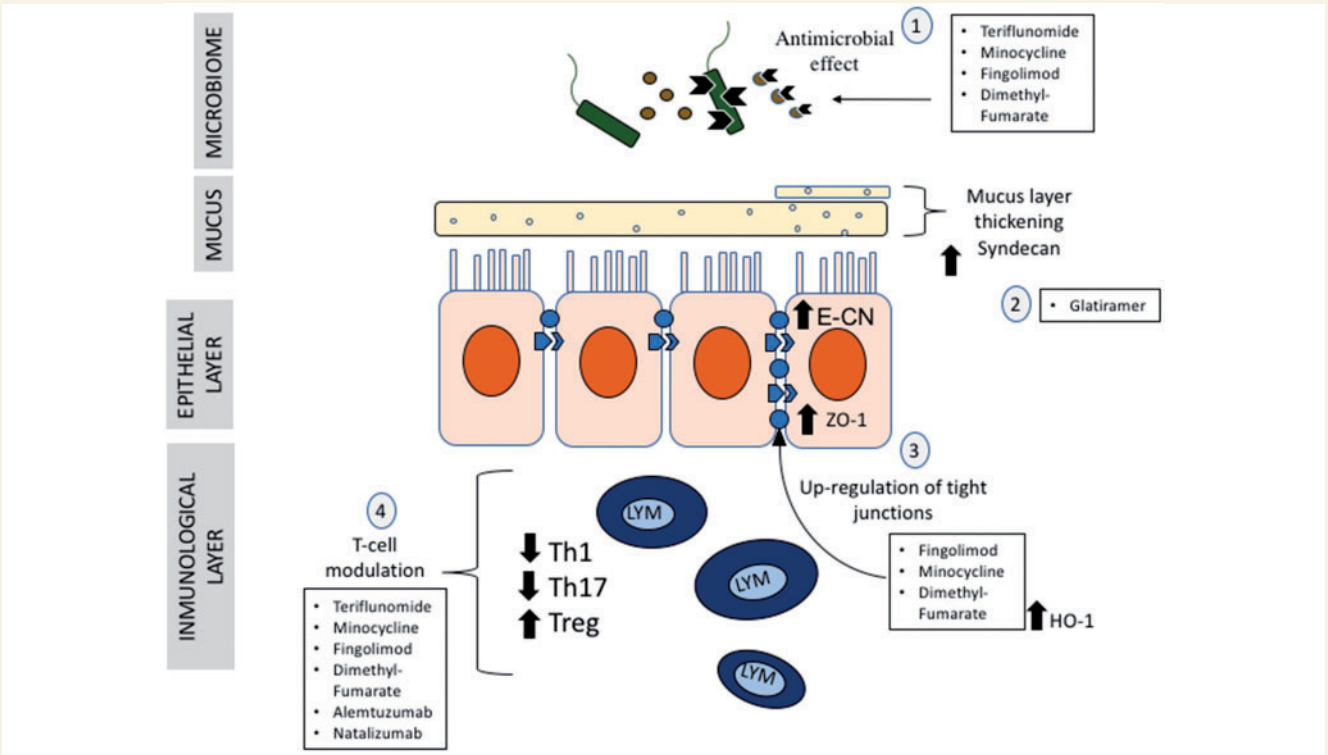

Figure 3 Disease-modifying therapies can modulate the intestinal barrier. Different disease-modifying therapies in clinical use may beneficially modulate intestinal barrier function through a variety of mechanisms. (1) Oral disease-modifying therapies have antimicrobial properties, while minocycline is a tetracycline antibiotic. Dimethyl fumarate acts as a Michael acceptor and can deplete bacterial nucleophilic thiols. (2) Glatiramer acetate has been shown to increase syndecan, the most abundant heparan sulphate proteoglycan in the gastrointestinal tract. (3) Fingolimod, dimethyl fumarate and minocycline increase tight junction expression. Dimethyl fumarate increases zona occludens-1 (ZO-1) in a heme-oxygenase-1 (HO-1) dependent pathway, while S1P signalling increases E-cadherin (E-CN). (4) Most disease-modifying therapies modulate lymphocyte (LYM) populations and functions in non-neurological tissues, such as in the lamina propria. Whether any of these effects have a mechanistic relevance for their therapeutic action is unknown.

increase in anti-inflammatory mediators such as IL-10 and TGFβ (Aharoni *et al.*, 2005, 2007). In one such study, glatiramer acetate attenuated colitis severity and prevented the destabilization of the intestinal epithelial barrier (Yablecovitch *et al.*, 2011). There is also evidence suggesting that patients with multiple sclerosis treated with glatiramer acetate have different microbiota composition. In a small study, glatiramer-treated patients had stool taxonomic units (evaluated by hybridization of 16S rRNA to a DNA microarray) of *Bacteroidaceae*, *Faecalibacterium*, *Ruminococcus*, *Lactobacillaceae*, *Clostridium*, and other *Clostridiales* that were significantly different than those of untreated patients (Cantarel *et al.*, 2015).

Natalizumab

Dysregulated recruitment of leucocytes into the intestine is one of the components of the immune response responsible for barrier breakdown in IBD (Danese *et al.*, 2005; Fiorino *et al.*, 2010). Integrins are expressed on intestinal lymphocytes and are essential in their homing to intestinal lymphoid tissues and trafficking through the intestinal mucosa (Hamann *et al.*, 1994; Tanaka *et al.*, 1995; Miura *et al.*, 1996; Farstad *et al.*, 1997; Bradley *et al.*, 1998; Fujimori *et al.*, 2002). Natalizumab, which blocks the activity of integrins (both $\alpha 4\beta 1$ and $\alpha 4\beta 7$), has shown effectiveness in reducing the severity of IBD (Fiorino *et al.*, 2010; Bamias *et al.*, 2013). However, its association with JC virus-related CNS complications has led to the development of specific $\alpha 4\beta 7$-antibodies such as vedolizumab, now routinely used in the treatment of IBD (Zundler *et al.*, 2017).

Nonetheless, the effects of natalizumab on integrins and lymphocyte trafficking in the gut suggests it could modulate the inflammatory response in this site in multiple sclerosis. A potential role for intestinal lymphocytes and integrins in multiple sclerosis pathophysiology has been suggested by results from mouse EAE models. Th17 cells, prominent drivers of EAE, are controlled and redirected in the small intestine. Th17 cells, which are normally pro-inflammatory, acquire a regulatory phenotype in the intestine and are ultimately eliminated through the intestinal lumen (Esplugues *et al.*, 2011). In EAE, there is infiltration of proinflammatory Th1/Th17 cells and reduction of Tregs in the gut, in association with functional and morphological changes (Nouri *et al.*, 2014). Furthermore, mice lacking integrin α show a loss of Th17 cells in the intestine and resistance

against EAE (Acharya *et al.*, 2010; Melton *et al.*, 2010). In spontaneously EAE resistant B10.S mice, blocking α4β7 integrin leads to peripheral availability of Th17 cells and increased severity of EAE (Berer *et al.*, 2014). In patients with multiple sclerosis, natalizumab treatment reduces the populations of integrin α-4-positive Th1, Th17 and Tregs differentially, while affecting the immune function of residual integrin α-4-positive T cells (Kimura *et al.*, 2016). The gut might act as a checking point, a reservoir and an activation site for Th17 and other T cells, a process regulated in part by intestinal integrins. Natalizumab and its non-selective integrin blockade could lead to changes in the way lymphocytes interact with the intestinal tissue. Considering the abovementioned findings, it is possible that natalizumab's therapeutic properties in multiple sclerosis could depend, at least in part, on these intestinal effects, besides those seen in blood–brain barrier, integrins and lymphocyte trafficking.

Fingolimod

Another drug that acts through the regulation of leucocyte trafficking is fingolimod, a functional antagonist of the sphingosine 1-phosphate receptor (S1P). S1P1 receptors are highly expressed on lymphocyte membranes and are critical for T and B cell egress from secondary lymphoid organs. S1P can affect the intestinal barrier by modulating tight junction proteins (Greenspon *et al.*, 2011; Pászti-Gere *et al.*, 2016), particularly under inflammatory conditions (Dong *et al.*, 2015). For instance, fingolimod reduces endothelial barrier dysfunction in blood vessels and lung epithelium in experimental models of sepsis and haemorrhagic shock (Lundblad *et al.*, 2013; Bonitz *et al.*, 2014). Fingolimod also sequesters and alters the activation of lymphocytes in intestinal tissues (Chiba *et al.*, 1998; Yanagawa *et al.*, 1998; Henning *et al.*, 2001; Halin *et al.*, 2005; Sugito *et al.*, 2005; Daniel *et al.*, 2007), an effect thought to be mechanistically relevant in multiple sclerosis therapeutics. In the mouse EAE model, development of EAE was associated with increased accumulation of T cells in Peyer's patches, a process increased by fingolimod (Spirin *et al.*, 2014). Fingolimod can also directly affect the microbiota. Both sphingosine and fingolimod inhibit *C. perfringens* growth and endotoxin production *in vitro*, suggesting an intrinsic antibacterial property (Rumah *et al.*, 2017).

Dimethyl fumarate

Dimethyl fumarate (DMF) is derived from the simple organic acid fumaric acid, and it acts as an immunomodulator by promoting T cell apoptosis, shifting to a Th2 response and acting as an antioxidant. There is limited but interesting evidence suggesting DMF could beneficially affect both the intestinal barrier and the gut microbiota. DMF alleviates experimentally induced colitis and reduces the Th1 response in mouse models and protects human intestinal epithelial cells against oxidative barrier dysfunction by preserving zona occludens-1 and occludin expression *in vitro* (Casili *et al.*, 2016). DMF also preserves intestinal mucosa morphology after mycotoxin exposure and decreases intestinal permeability by strengthening tight junctions (Ma *et al.*, 2017). In this model, DMF also led to increased microbiome diversity, with more abundance of bacteria producing SCFAs, such as *Gemella*, *Roseburia*, *Bacillus* and *Bacteroides*. DMF can also directly reduce *C. perfringens* growth and exhibits anti-mildew and antibacterial properties (Ma *et al.*, 2017; Rumah *et al.*, 2017).

Alemtuzumab

Alemtuzumab is an anti-CD52 antibody that causes depletion of mainly lymphocytes and is highly effective in the clinical management of multiple sclerosis (Hartung *et al.*, 2015). Despite its specific mechanism of action, there is evidence suggesting it has detrimental effects over the integrity of the intestinal barrier and might alter the gut microbiome.

In mice, anti-CD52 antibodies induce increased intestinal barrier permeability (Qu *et al.*, 2009) and lead to reductions in epithelial cell populations and to altered tight junction ultrastructure (Shen *et al.*, 2013, 2015). In macaques, alemtuzumab-induced intestinal barrier disruption is associated with epithelial cell apoptosis as well as with increased circulating levels of D-lactate and endotoxin, indirect markers of intestinal barrier breakdown and bacterial translocation (Li *et al.*, 2011; Qu *et al.*, 2015). Lymphocyte depletion with alemtuzumab treatment in macaque models also resulted in dramatic changes in the gut microbiota (Li *et al.*, 2010). *Lactobacillales*, *Enterobacteriales*, *Clostridiales*, and the genus *Prevotella* and *Faecalibacterium* were primarily responsible for the variations of the gut microbiota after lymphocyte depletion (Li *et al.*, 2013). The diversity of fungal microbiota was similarly affected (Li *et al.*, 2014). Despite this preclinical evidence, alemtuzumab-induced intestinal barrier disruption is infrequent in clinical practice. However, a case of spontaneous pancolitis was described in a patient with multiple sclerosis treated with alemtuzumab recently (Vijiaratnam *et al.*, 2016), and historically, the use of alemtuzumab in haematological malignancies has been associated with the development of diarrhoea and opportunistic intestinal infections (Goteri *et al.*, 2006; Ronchetti *et al.*, 2014).

Teriflunomide

Teriflunomide selectively and reversibly inhibits dihydroorotate dehydrogenase, leading to a reduction in the number of activated lymphocytes that enter the CNS (Miller, 2015). Teriflunomide could alter the microbiome and the host response to enteral pathogens. Treatment of porcine intestinal epithelial cells with teriflunomide led to reduced capacity to fight bacterial infection through suppression of STAT-6

signalling (Yi *et al.*, 2016). Teriflunomide could also directly inhibit *C. perfringens* growth *in vitro* (Rumah *et al.*, 2017). Animals treated with teriflunomide in a mouse model of EAE had fewer antigen-presenting cells in Peyer's patches as well as an increase in gut-specific CD39(+) Treg cells that could protect against EAE when used in an adoptive transfer regimen (Ochoa-Repáraz *et al.*, 2016).

Minocycline

Minocycline is a second-generation tetracycline that was first introduced over half a century ago. Besides its antibiotic effects, it also has anti-inflammatory, immune-modulating and anti-apoptotic properties, all of which have been proposed as possible pathways towards neuroprotection (Yong *et al.*, 2004; Giuliani *et al.*, 2005). A recent randomized, double-blind, placebo controlled trial showed that oral minocycline could delay the appearance of a new demyelinating events in patients with clinically isolated syndrome, as well as reduce the appearance of T_2 lesions in the brain (Metz *et al.*, 2017).

Minocycline's immune-modulating and anti-inflammatory properties have also been observed in intestinal tissues. In a chemically-induced colitis model in mice, minocycline reduced intestinal inflammation, mucosal injury, restored microbiota and preserved tight junction protein expression (Huang *et al.*, 2009; Garrido-Mesa *et al.*, 2011*a*, *b*). As an antibiotic, minocycline also alters the gut microbiome. A recent study evaluated the effects of various commonly used antibiotics, including minocycline, on the salivary and gut microbiome in 66 healthy adults. Antibiotic exposure led to reductions in health-associated butyrate-producing species as well as proliferation of potentially resistant strains in the gut microbiome, although the changes were more robust after amoxicillin and ciprofloxacin administration (Zaura *et al.*, 2015). Other studies have shown that some gut commensals such as *Bifidobacteria* and *E. coli* are susceptible to minocycline (Moubareck *et al.*, 2005; Kirchner *et al.*, 2014). Minocycline thus presents an intriguing option in dual modulation of the intestinal barrier function. It could have protective anti-inflammatory properties while also altering the composition of the gut microbiome.

Treating the diseased intestinal barrier

Current treatments for a diseased intestinal barrier are limited, but there are various interesting avenues of research. One of the main therapeutic targets are tight junctions. Larazotide acetate, also known as AT-1001, is a synthetic octapeptide related to the zonula occludens toxin produced by *Vibrio cholera*, developed as a treatment for coeliac disease. It acts locally to decrease tight junction permeability by blocking zonulin receptors and thus preventing actin rearrangement in response to stimuli, and *in vitro* it can stabilize tight junctions and decrease intestinal permeability (Paterson *et al.*, 2007; Gopalakrishnan *et al.*, 2012; Khaleghi *et al.*, 2016). However, clinical trials in coeliac disease have yielded conflicting results, despite showing a beneficial effect over intestinal permeability (Kelly *et al.*, 2013; Leffler *et al.*, 2012, 2015).

Another approach in improving intestinal barrier function is enrichment of the mucus layer, a strategy being explored in IBD (Stange, 2017). Lecithin, or phosphatidylcholine, accounts for the majority of the phospholipids in the intestinal mucus layer, and is available as a delayed release oral formulation. In randomized phase II controlled studies, delayed-release lecithin was proven to be clinically and endoscopically effective in ulcerative colitis, and phase III studies are underway (Stremmel and Gauss, 2013; Stange, 2017). Recent interest has also been placed on stem cell-based therapies to regenerate the intestinal epithelium, through luminal transplantation (Holmberg *et al.*, 2018), but these approaches are still in an experimental phase.

There has also been recent interest in the effects of vitamin D over intestinal barrier function and immune homeostasis (Dimitrov and White, 2017). In a model of experimental colitis, mice overexpressing vitamin D receptor in the intestinal epithelium show preserved intestinal permeability, reduced caspase expression and less induction of apoptosis (Liu *et al.*, 2013). Vitamin D also attenuates TNFα-induced apoptosis in human colonic cells through reduction of NF-κB activation and mucosal IKK kinase activity, thereby preserving barrier function (see Li *et al.*, 2015 for a review). Vitamin D signalling also preserves the mucosal barrier integrity by abrogating myosin light chain kinase dependent tight junction dysregulation during colonic inflammation through suppression of NF-κB *in vitro* (Du *et al.*, 2015). Cultured colonic samples from patients with ulcerative colitis have altered expression of the tight junction claudin as well as increased pro-inflammatory cytokine expression; these changes were reversed by incubation with vitamin D (Stio *et al.*, 2016). A recent small, randomized and placebo controlled study reported improvements in intestinal permeability [assessed by excretion of oral sugars (lactulose and mannitol were used as markers of small intestine permeability, sucrose as a marker of gastro-duodenal permeability, and sucralose as marker of combined small- and large-bowel permeability)] as well as serum immune markers in patients with IBD after vitamin D treatment (Raftery *et al.*, 2015). Vitamin D appears to be important in the regulation of the intestinal barrier function, a mechanism not yet thoroughly evaluated in multiple sclerosis research.

Probiotics have emerged as an interesting option in regulating intestinal barrier function, fuelled by research in both *in vitro* and *in vivo* models that show that some microbiota can stabilize the intestinal barrier (Bron *et al.*, 2017). However, small clinical studies in necrotizing

Table 1 Possible therapeutic interventions to improve barrier function

Intervention	Target
AT-1001 (larazotide)	Tight junction proteins
Lecithin	Mucus layer composition
Probiotics/faecal transplantation	Pleiotropic
Vitamin D	Epithelial and immunological homeostasis
Dietary/nutritional	Pleiotropic. 'High' short chain fatty-acid diet?

enterocolitis, irritable bowel syndrome and IBD have shown only modest effects. There are no large randomized, placebo controlled studies and there is no obvious standardization of the quantities and composition of a given therapeutic probiotic 'agent', making trials difficult (Bron *et al.*, 2017). There has been growing interest in the use of faecal microbiota transplantation (the ultimate microbiome modification) for the treatment of patients with chronic gastrointestinal infections and IBD (Smits *et al.*, 2013), with excellent results observed in *C. difficile* colitis. It is also a safe procedure. Its effectiveness in autoimmune diseases and multiple sclerosis is unknown at this time.

There are other sources of interest in probiotics in multiple sclerosis. Probiotic administration is known to modulate the immune response in the mouse EAE model. Different formulations have been shown to reduce EAE duration (Ezendam *et al.*, 2008), inhibit the pro-inflammatory Th1/Th17 polarization (Kwon *et al.*, 2013), induce IL-10 producing Treg cells (Ochoa-Repáraz *et al.*, 2010*a*, *b*; Takata *et al.*, 2011) and enhance CD103 expression in dendritic cells (Ochoa-Repáraz *et al.*, 2010*b*), all while preventing, delaying or attenuating EAE. *E. coli* strain Nissle 1917 has been shown to reduce EAE-induced intestinal barrier dysfunction, while also reducing disease severity and beneficially modifying T cell functions (Secher *et al.*, 2017).

Despite these encouraging studies, few clinical trials have been performed using probiotics in multiple sclerosis. In one early trial, investigators used the non-pathogenic helminth *Trichuris suis* (Fleming *et al.*, 2011). Five newly diagnosed patients with relapsing-remitting multiple sclerosis were given *T. suis* orally for 3 months, and favourable trends were seen in MRI outcomes (reduction in enhancing lesions from baseline) and immunological assessments (increased IL-10). A recent double-blind, placebo-controlled trial randomized 60 multiple sclerosis patients to receive a probiotic capsule or placebo for 12 weeks (Kouchaki *et al.*, 2017). Probiotic treatment mildly improved Expanded Disability Status Scale (an absolute 0.4-point difference) and depression and anxiety symptoms, reduced high-sensitivity C-reactive protein and improved other metabolic measures such as insulin sensitivity and high-density lipoprotein-cholesterol levels. Probiotics also downregulated the gene expression of some pro-inflammatory cytokines in patients' peripheral blood-derived mononuclear cells (Tamtaji *et al.*, 2017). In these studies, the treatment was safe and tolerable, but follow-up was too short to show any meaningful benefit in radiological or clinical outcome measures. Nonetheless, the encouraging results seen in the EAE model will surely promote further clinical trial development.

SCFAs are bacterial fermentation products from indigestible diet components. The most common SCFAs are acetate, propionate and butyrate. SCFAs could have a beneficial effect over the intestinal barrier. Butyrate was shown to be able to accelerate tight junction protein assembly and preserve permeability in a single enterocyte layer *in vitro* model, a process mediated by AMP-activated protein kinase activity (Peng *et al.*, 2009). SCFAs could also increase prostaglandin-dependent mucin expression in intestinal epithelial cells, enhancing their mucoprotective properties (Willemsen *et al.*, 2003). In an EAE model, dietary SCFA ameliorated the course of EAE through expanded Treg cell populations in the lamina propria, through suppression of the JNK1 and p38 pathway (Haghikia *et al.*, 2015). CD44 knockout mice that show attenuated EAE also have increased microbiota diversity and SCFA production in the gut (Chitrala *et al.*, 2017).

Dietary interventions that increase the availability of SCFAs and reduce other types of fatty acids could be an interesting therapy in improving the intestinal barrier function in multiple sclerosis, with the additional possibility of beneficial immunological effects. However, evidence showing a benefit for any kind of dietary interventions in multiple sclerosis is scarce, despite widespread acceptance that a 'healthy' diet is probably best (Altowaijri *et al.*, 2017; Esposito *et al.*, 2017). Some probiotic species are also rich sources of SCFAs, suggesting the possibility of a combination approach.

Concluding remarks

The recent interest in the role of the gut microbiota in multiple sclerosis has not been accompanied by a similar interest in the intestinal barrier. The intestinal barrier is the physical and functional zone of interaction between the luminal microbiome and the organism, and it is also responsible for modulating multiple biochemical processes and immune modulation of the mucosa. It appears that besides dysbiotic changes in the gut microbiome, the intestinal barrier function is also altered both in EAE models and in patients with multiple sclerosis, but the precise consequences of this alteration are unclear. Evidence of CNS demyelination in gastrointestinal disorders where there is barrier breakdown and basic studies showing how the intestinal barrier homeostasis can directly influence microglia and neuroinflammation provide some insights. Furthermore, most disease-modifying therapies appear to also impact on the intestinal barrier and the gut

microbiome. To advance the understanding of this complex interaction, future studies will have to take into consideration the microbiome, the intestinal barrier and the downstream neuroimmunological changes to accommodate for them in a single integrative model. Both the precise mechanisms involved in the breakdown of the intestinal barrier, and the value, if any, of therapeutic modulation of the intestinal barrier in multiple sclerosis, also require further study.

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References

Abreu MT. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. Nat Rev Immunol 2010; 10: 131–44.

Acharya M, Mukhopadhyay S, Païdassi H, Jamil T, Chow C, Kissler S, et al. αv Integrin expression by DCs is required for Th17 cell differentiation and development of experimental autoimmune encephalomyelitis in mice. J Clin Invest 2010; 120: 4445–52.

Aharoni R, Kayhan B, Arnon R. Therapeutic effect of the immunomodulator glatiramer acetate on trinitrobenzene sulfonic acid-induced experimental colitis. Inflamm Bowel Dis 2005; 11: 106–15.

Aharoni R, Sonego H, Brenner O, Eilam R, Arnon R. Therapeutic effect of glatiramer acetate in a murine model of inflammatory bowel disease is mediated by anti-inflammatory T-cells. Immunol Lett 2007; 112: 110–19.

Al-Sadi R, Guo S, Ye D, Rawat M, Ma TY. TNF-α modulation of intestinal tight junction permeability is mediated by NIK/IKK-α axis activation of the canonical NF-κB pathway. Am J Pathol 2016; 186: 1151–65.

Altowaijri G, Fryman A, Yadav V. Dietary interventions and multiple sclerosis. Curr Neurol Neurosci Rep 2017; 17: 28.

Antoni L, Nuding S, Wehkamp J, Stange EF. Intestinal barrier in inflammatory bowel disease. World J Gastroenterol 2014; 20: 1165–79.

Bamias G, Clark DJ, Rivera-Nieves J. Leukocyte traffic blockade as a therapeutic strategy in inflammatory bowel disease. Curr Drug Targets 2013; 14: 1490–500.

Barcellos LF, Kamdar BB, Ramsay PP, DeLoa C, Lincoln RR, Caillier S, et al. Clustering of autoimmune diseases in families with a high-risk for multiple sclerosis: a descriptive study. Lancet Neurol 2006; 5: 924–31.

Batur-Caglayan HZ, Irkec C, Yildirim-Capraz I, Atalay-Akyurek N, Dumlu S. A case of multiple sclerosis and celiac disease. Case Rep Neurol Med 2013; 2013: 576921.

Berer K, Boziki M, Krishnamoorthy G. Selective accumulation of pro-inflammatory T cells in the intestinecontributes to the resistance to autoimmune demyelinating disease. PLoS One 2014; 9: e87876.

Berer K, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, Xia Z, et al. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. Proc Natl Acad Sci USA 2017; 114: 10719–24.

Berer K, Krishnamoorthy G. Microbial view of central nervous system autoimmunity. FEBS Lett 2014; 588: 4207–13.

Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johner C, et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature 2011; 479: 538–41.

Bilgic B, Aygun D, Arslan AB, Bayram A, Akyuz F, Sencer S, et al. Silent neurological involvement in biopsy-defined coeliac patients. Neurol Sci 2013; 34: 2199–204

Bonitz JA, Son JY, Chandler B, Tomaio JN, Qin Y, Prescott LM, et al. A sphingosine-1 phosphate agonist (FTY720) limits trauma/hemorrhagic shock-induced multiple organ dysfunction syndrome. Shock 2014; 42: 448–55.

Bostick JW, Zhou L. Innate lymphoid cells in intestinal immunity and inflammation. Cell Mol Life Sci 2016; 73: 237–52.

Bradley LM, Malo ME, Fong S, Tonkonogy SL, Watson SR. Blockade of both L-selectin and alpha4 integrins abrogates naive CD4 cell trafficking and responses in gut-associated lymphoid organs. Int Immunol 1998; 10: 961–8.

Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M, et al. The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med 2014; 6: 263ra158.

Bron PA, Kleerebezem M, Brummer RJ, Cani PD, Mercenier A, MacDonald TT, et al. Can probiotics modulate human disease by impacting intestinal barrier function? Br J Nutr 2017; 117: 93–107.

Buscarinu MC, Cerasoli B, Annibali V, Policano C, Lionetto L, Capi M, et al. Altered intestinal permeability in patients with relapsing-remitting multiple sclerosis: a pilot study. Mult Scler 2017; 23: 442–6.

Buscarinu MC, Romano S, Mechelli R, Pizzolato Umeton R, Ferraldeschi M, Fornasiero A, et al. Intestinal permeabiliy in relapsing-remitting multiple sclerosis. Neurotherapeutics 2018; 15: 68–74.

Cantarel BL, Waubant E, Chehoud C, Kuczynski J, DeSantis TZ, Warrington J, et al. Gut microbiota in multiple sclerosis: possible influence of immunomodulators. J Investig Med 2015; 63: 729–34.

Capaldo CT, Powell DN, Kalman D. Layered defense: how mucus and tight junctions seal the intestinal barrier. J Mol Med 2017; 95: 927–34.

Casella G, Bordo BM, Schalling R, Villanacci V, Salemme M, Di Bella C, et al. Neurological disorders and celiac disease. Minerva Gastroenterol Dietol 2016; 62: 197–206.

Casella G, Tontini GE, Bassotti G, Pastorelli L, Villanacci V, Spina L, et al. Neurological disorders and inflammatory bowel diseases. World J Gastroenterol 2014; 20: 8764–82.

Casili G, Cordaro M, Impellizzeri D, Bruschetta G, Paterniti I, Cuzzocrea S, et al. Dimethyl fumarate reduces inflammatory responses in experimental colitis. J Crohns Colitis 2016; 10: 472–83.

Cekanaviciute E, Yoo BB, Runia TF, Debelius JW, Singh S, Nelson CA, et al. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. Proc Natl Acad Sci USA 2017; 114: 10713–18.

Chen J, Chia N, Kalari KR, Yao JZ, Novotna M, Soldan MM, et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. Sci Rep 2016; 6: 28484.

Chen M, Lee G, Kwong LN, Lamont S, Chaves C. Cerebral white matter lesions in patients with Crohn's disease. J Neuroimaging 2012; 22: 38–41.

Cheroutre H, Lambolez F, Mucida D. The light and dark sides of intestinal intraepithelial lymphocytes. Nat Rev Immunol 2011; 11: 445–56.

Chiba K, Yanagawa Y, Masubuchi Y, Kataoka H, Kawaguchi T, Ohtsuki M, et al. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. J Immunol 1998; 160: 5037–44.

Chitrala KN, Guan H, Singh NP, Busbee B, Gandy A, Mehrpouya-Bahrami P, et al. CD44 deletion leading to attenuation of experimental autoimmune encephalomyelitis results from alterations in gut microbiome in mice. Eur J Immunol 2017; 47: 1188–99.

Choi W, Yeruva S, Turner JR. Contributions of intestinal epithelial barriers to health and disease. Exp Cell Res 2017; 358: 71–7.

Colpitts SL, Kasper LH. Influence of the gut microbiome on autoimmunity in the central nervous system. J Immunol 2017; 198: 596–604.

Cosorich I, Dalla-Costa G, Sorini C, Ferrarese R, Messina MJ, Dolpady J, et al. High frequency of intestinal TH17 cells correlates with microbiota alterations and disease activity in multiple sclerosis. Sci Adv 2017; 3: e1700492.

Danese S, Semeraro S, Marini M, Roberto I, Armuzzi A, Papa A, et al. Adhesion molecules in inflammatory bowel disease: therapeutic implications for gut inflammation. Dig Liver Dis 2005; 37: 811–18.

Daniel C, Sartory N, Zahn N, Geisslinger G, Radeke HH, Stein JM. FTY720 ameliorates Th1-mediated colitis in mice by directly affecting the functional activity of CD4 + CD25 + regulatory T cells. J Immunol 2007; 178: 2458–68.

De Felice KM, Novotna M, Enders FT, Faubion WA, Tremaine WJ, Kantarci OH, et al. Idiopathic inflammatory demyelinating disease of the central nervous system in patients with inflammatory bowel disease: retrospective analysis of 9095 patients. Aliment Pharmacol Ther 2015; 41: 99–107.

de Lau LM, de Vries JM, van der Woude CJ, Kuipers EJ, Siepman DA, Sillevis Smitt PA, et al. Acute CNS white matter lesions in patients with inflammatory bowel disease. Inflamm Bowel Dis 2009; 15: 576–80.

de Souza HSP, Fiocchi C, Iliopoulos D. The IBD interactome: an integrated view of aetiology, pathogenesis and therapy. Nat Rev Gastroenterol Hepatol 2017; 14: 739–49.

Delgado ME, Grabinger T, Brunner T. Cell death at the intestinal epithelial front line. FEBS J 2016; 283: 2701–19.

Dimitrov V, White JH. Vitamin D signaling in intestinal innate immunity and homeostasis. Mol Cell Endocrinol 2017; 453: 68–78.

Dong J, Wang H, Zhao J, Sun J, Zhang T, Zuo L, et al. SEW2871 protects from experimental colitis through reduced epithelial cell apoptosis and improved barrier function in interleukin-10 gene-deficient mice. Immunol Res 2015; 61: 303–11.

D'Souza WN, Douangpanya J, Mu S, Jaeckel P, Zhang M, Maxwell JR, et al. Differing roles for short chain fatty acids and GPR43 agonism in the regulation of intestinal barrier function and immune responses. PLoS One 2017; 12: e0180190.

Du J, Chen Y, Shi Y, Liu T, Cao Y, Tang Y, et al. 1,25-Dihydroxyvitamin D protects intestinal epithelial barrier by regulating the myosin light chain kinase signaling pathway. Inflamm Bowel Dis 2015; 21: 2495–506.

Dupont A, Heinbockel L, Brandenburg K, Hornef MW. Antimicrobial peptides and the enteric mucus layer act in concert to protect the intestinal mucosa. Gut Microbes 2014; 5: 761–5.

Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci 2015; 18: 965–77.

Erny D, Hrabě de Angelis AL, Prinz M. Communicating systems in the body: how microbiota and microglia cooperate. Immunology 2017; 150: 7–15.

Escribano BM, Medina-Fernández FJ, Aguilar-Luque M, Agüera E, Feijoo M, Garcia-Maceira FI, et al. Lipopolysaccharide binding protein and oxidative stress in a multiple sclerosis model. Neurotherapeutics 2017; 14: 199–211.

Esplugues E, Huber S, Gagliani N, Hauser AE, Town T, Wan YY, et al. Control of TH17 cells occurs in the small intestine. Nature 2011; 475: 514–18.

Esposito S, Bonavita S, Sparaco M, Gallo A, Tedeschi G. The role of diet in multiple sclerosis: a review. Nutr Neurosci 2017, in press. doi: 10.1080/1028415X.2017.1303016.

Ezendam J, de Klerk A, Gremmer ER, van Loveren H. Effects of Bifidobacterium animalis administered during lactation on allergic and autoimmune responses in rodents. Clin Exp Immunol 2008; 154: 424–31.

Farstad IN, Halstensen TS, Kvale D, Fausa O, Brandtzaeg P. Topographic distribution of homing receptors on B and T cells in human gut-associated lymphoid tissue: relation of L-selectin and integrin alpha 4 beta 7 to naive and memory phenotypes. Am J Pathol 1997; 150: 187–99.

Ferro JM, Oliveira SN, Correia L. Neurologic manifestations of inflammatory bowel diseases. Handb Clin Neurol 2014; 120: 595–605.

Finsterer J, Leutmezer F. Celiac disease with cerebral and peripheral nerve involvement mimicking multiple sclerosis. J Med Life 2014; 7: 440–4.

Fiorino G, Correale C, Fries W, Repici A, Malesci A, Danese S. Leukocyte traffic control: a novel therapeutic strategy for inflammatory bowel disease. Expert Rev Clin Immunol 2010; 6: 567–72.

Fleming JO, Isaak A, Lee JE, Luzzio CC, Carrithers MD, Cook TD, et al. Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. Mult Scler 2011; 17: 743–54.

France MM, Turner JR. The mucosal barrier at a glance. J Cell Sci 2017; 130: 307–14.

Frister A, Schmidt C, Schneble N, Brodhun M, Gonnert FA, Bauer M, et al. Phosphoinositide 3-kinase γ affects LPS-induced disturbance of blood-brain barrier via lipid kinase-independent control of cAMP in microglial cells. Neuromolecular Med 2014; 16: 704–13.

Fujimori H, Miura S, Koseki S, Hokari R, Komoto S, Hara Y, et al. Intravital observation of adhesion of lamina propria lymphocytes to microvessels of small intestine in mice. Gastroenterology 2002; 122: 734–44.

Gangwar R, Meena AS, Shukla PK, Nagaraja AS, Dorniak PL, Pallikuth S, et al. Calcium-mediated oxidative stress: a common mechanism in tight junctiondisruption by different types of cellular stress. Biochem J 2017; 474: 731–49.

Garrido-Mesa N, Camuesco D, Arribas B, Comalada M, Bailón E, Cueto-Sola M, et al. The intestinal anti-inflammatory effect of minocycline in experimental colitis involves both its immunomodulatory and antimicrobial properties. Pharmacol Res 2011a; 63: 308–19.

Garrido-Mesa N, Utrilla P, Comalada M, Zorrilla P, Garrido-Mesa J, Zarzuelo A, et al. The association of minocycline and the probiotic Escherichia coli Nissle 1917 results in an additive beneficial effect in a DSS model of reactivated colitis in mice. Biochem Pharmacol 2011b; 82: 1891–900.

Gasbarrini G, Montalto M. Structure and function of tight junctions. Role in intestinal barrier. Ital J Gastroenterol Hepatol 1999; 31: 481–8.

Gebregiworgis T, Nielsen HH, Massilamany C, Gangaplara A, Reddy J, Illes Z, et al. A urinary metabolic signature for multiple sclerosis and neuromyelitis optica. J Proteome Res 2016; 15: 659–66.

Geissler A, Andus T, Roth M, Kullmann F, Caesar I, Held P, et al. Focal white-matter lesions in brain of patients with inflammatory bowel disease. Lancet 1995; 345: 897–8.

Giles EM, Stagg AJ. Type 1 interferon in the human intestine-A coordinator of the immune response to the microbiota. Inflamm Bowel Dis 2017; 23: 524–33.

Giuliani F, Hader W, Yong VW. Minocycline attenuates T cell and microglia activity to impair cytokine production in T cell-microglia interaction. J Leukoc Biol 2005; 78: 135–43.

Goll R, van Beelen Granlund A. Intestinal barrier homeostasis in inflammatory bowel disease. Scand J Gastroenterol 2015; 50: 3–12.

Gopalakrishnan S, Tripathi A, Tamiz AP. Larazotide acetate promotes tight junction assembly in epithelial cells. Peptides 2012; 35: 95–101.

Goteri G, Rupoli S, Tassetti A, Pulini S, Morichetti D, Filosa A, et al. Severe diarrhoea during Campath-1H treatment for refractory cutaneous T-cell lymphoma. Ann Hematol 2006; 85: 617–19.

Greenspon J, Li R, Xiao L, Rao JN, Sun R, Strauch ED, et al. Sphingosine-1-phosphate regulates the expression of adherens junction protein E-cadherin and enhances intestinal epithelial cell barrier function. Dig Dis Sci 2011; 56: 1342–53.
Gronke K, Kofoed-Nielsen M, Diefenbach A. Isolation and flow cytometry analysis of innate lymphoid cells from the intestinal lamina propria. Methods Mol Biol 2017; 1559: 255–65.
Gupta JK, Ingegno AP, Cook AW, Pertschuk LP. Multiple sclerosis and malabsorption. Am J Gastroenterol 1977; 68: 560–5.
Haak BW, Wiersinga WJ. The role of the gut microbiota in sepsis. Lancet Gastroenterol Hepatol 2017; 2: 135–43.
Haghikia A, Jörg S, Duscha A, Berg J, Manzel A, Waschbisch A, et al. Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. Immunity 2015; 43: 817–29.
Halin C, Scimone ML, Bonasio R, Gauguet JM, Mempel TR, Quackenbush E, et al. The S1P-analog FTY720 differentially modulates T-cell homing via HEV: T-cell-expressed S1P1 amplifies integrin activation in peripheral lymph nodes but not in Peyer patches. Blood 2005; 106: 1314–22.
Hamann A, Andrew DP, Jablonski-Westrich D, Holzmann B, Butcher EC. Role of alpha 4-integrins in lymphocyte homing to mucosal tissues *in vivo*. J Immunol 1994; 152: 3282–93.
Hartung HP, Aktas O, Boyko AN. Alemtuzumab: a new therapy for active relapsing-remitting multiple sclerosis. Mult Scler 2015; 21: 22–34.
Hashiguchi M, Kashiwakura Y, Kojima H, Kobayashi A, Kanno Y, Kobata T. Peyer's patch innate lymphoid cells regulate commensal bacteria expansion. Immunol Lett 2015; 165: 1–9.
Henning G, Ohl L, Junt T, Reiterer P, Brinkmann V, Nakano H, et al. CC chemokine receptor 7-dependent and -independent pathways for lymphocyte homing: modulation by FTY720. J Exp Med 2001; 194: 1875–81.
Holmberg FEO, Pedersen J, Jørgensen P, Soendergaard C, Jensen KB, Nielsen OH. Intestinal barrier integrity and inflammatory bowel disease: stem cell-based approaches to regenerate the barrier. J Tissue Eng Regen Med 2018; 12: 923–35. 10.1002/term.2506.
Huang TY, Chu HC, Lin YL, Lin CK, Hsieh TY, Chang WK, et al. Minocycline attenuates experimental colitis in mice by blocking expression of inducible nitric oxide synthase and matrix metalloproteinases. Toxicol Appl Pharmacol 2009; 237: 69–82.
Jäger S, Stange EF, Wehkamp J. Inflammatory bowel disease: an impaired barrier disease. Langenbecks Arch Surg 2013; 398: 1–12.
Jangi S, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, et al. Alterations of the human gut microbiome in multiple sclerosis. Nat Commun 2016; 7: 12015.
Kadowaki A, Miyake S, Saga R, Chiba A, Mochizuki H, Yamamura T. Gut environment-induced intraepithelial autoreactive CD4(+) T cells suppress central nervous system autoimmunity via LAG-3. Nat Commun 2016; 7: 11639.
Kamphuis WW, Derada Troletti C, Reijerkerk A, Romero IA, de Vries HE. The blood-brain barrier in multiple sclerosis: microRNAs as key regulators. CNS Neurol Disord Drug Targets 2015; 14: 157–67.
Katlinskaya YV, Katlinski KV, Lasri A, Li N, Beiting DP, Durham AC, et al. Type I interferons control proliferation and function of the intestinalepithelium. Mol Cell Biol 2016; 36: 1124–35.
Katsanos AH, Katsanos KH. Inflammatory bowel disease and demyelination: more than just a coincidence? Expert Rev Clin Immunol 2014; 10: 363–73.
Kelly CP, Green PH, Murray JA. Larazotide acetate in patients with coeliac disease undergoing a gluten challenge: a randomised placebo-controlled study. Aliment Pharmacol Ther 2013; 37: 252–62.
Khaleghi S, Ju JM, Lamba A, Murray JA. The potential utility of tight junction regulation in celiac disease: focus on larazotide acetate. Therap Adv Gastroenterol 2016; 9: 37–49.
Kiela PR, Ghishan FK. Physiology of intestinal absorption and secretion. Best Pract Res Clin Gastroenterol 2016; 30: 145–59.
Kim HH, Jeong IH, Hyun JS, Kong BS, Kim HJ, Park SJ. Metabolomic profiling of CSF in multiple sclerosis and neuromyelitis optica spectrum disorder by nuclear magnetic resonance. PLoS One 2017; 12: e0181758.
Kimura K, Nakamura M, Sato W, Okamoto T, Araki M, Lin Y, et al. Disrupted balance of T cells under natalizumab treatment in multiple sclerosis. Neurol Neuroimmunol Neuroinflamm 2016; 3: e210.
Kirchner M, Mafura M, Hunt T, Abu-Oun M, Nunez-Garcia J, Hu Y, et al. Antimicrobial resistance characteristics and fitness of Gram-negative fecal bacteria from volunteers treated with minocycline or amoxicillin. Front Microbiol 2014; 5: 722.
König J, Wells J, Cani PD, García-Ródenas CL, MacDonald T, Mercenier A, Whyte J, et al. Human intestinal barrier function in health and disease. Clin Transl Gastroenterol 2016; 7: e196.
Kosmidou M, Katsanos AH, Katsanos KH, Kyritsis AP, Tsivgoulis G, Christodoulou D, et al. Multiple sclerosis and inflammatory bowel diseases: a systematic review and meta-analysis. J Neurol 2017; 264: 254–9.
Kouchaki E, Tamtaji OR, Salami M, Bahmani F, Daneshvar Kakhaki R, Akbari E, et al. Clinical and metabolic response to probiotic supplementation in patients with multiple sclerosis: a randomized, double-blind, placebo-controlled trial. Clin Nutr 2017; 36: 1245–8.
Kraus J, Ling AK, Hamm S, Voigt K, Oschmann P, Engelhardt B. Interferon-beta stabilizes barrier characteristics of brain endothelial cells *in vitro*. Ann Neurol 2004; 56: 192–205.
Krom H, Sprangers F, van den Berg R, Benninga MA, Kindermann A. Transverse myelitis as manifestation of celiac disease in a toddler. Pediatrics 2017; 139: e20161381.
Kwon HK, Kim GC, Kim Y, Hwang W, Jash A, Sahoo A, et al. Amelioration of experimental autoimmune encephalomyelitis by probiotic mixture is mediated by a shift in T helper cell immune response. Clin Immunol 2013; 146: 217–27.
Lange LS, Shiner M. Small-bowel abnormalities in multiple sclerosis. Lancet 1976; 2: 1319–22.
Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci USA 2011; 108 (Suppl 1): 4615–22.
Leffler DA, Kelly CP, Abdallah HZ. A randomized, double-blind study of larazotide acetate to prevent the activation of celiac disease during gluten challenge. Am J Gastroenterol 2012; 107: 1554–62.
Leffler DA, Kelly CP, Green PH. Larazotide acetate for persistent symptoms of celiac disease despite a gluten-free diet: a randomized controlled trial. Gastroenterology 2015; 148: 1311–19.
LeMessurier KS, Häcker H, Chi L, Tuomanen E, Redecke V. Type I interferon protects against pneumococcal invasive disease by inhibiting bacterial transmigration across the lung. PLoS Pathog 2013; 9: e1003727.
Li H, Sun J, Wang F, Ding G, Chen W, Fang R, et al. Sodium butyrate exerts neuroprotective effects by restoring the blood-brain barrier in traumatic brain injury mice. Brain Res 2016; 1642: 70–8.
Li Q, Wang C, Tang C, He Q, Li J. Lymphocyte depletion after alemtuzumab induction disrupts intestinal fungal microbiota in cynomolgus monkeys. Transplantation 2014; 98: 951–9.
Li Q, Zhang Q, Wang C, Jiang S, Li N, Li J. The response of intestinal stem cells and epithelium after alemtuzumab administration. Cell Mol Immunol 2011; 8: 325–32.
Li Q, Zhang Q, Wang C, Tang C, Zhang Y, Jiang S, et al. Influence of alemtuzumab on the intestinal Paneth cells and microflora in macaques. Clin Immunol 2010; 136: 375–86.
Li QR, Wang CY, Tang C, He Q, Li N, Li JS. Reciprocal interaction between intestinal microbiota and mucosal lymphocyte in cynomolgus monkeys after alemtuzumab treatment. Am J Transplant 2013; 13: 899–910.
Li YC, Chen Y, Du J. Critical roles of intestinal epithelial vitamin D receptor signaling in controlling gut mucosal inflammation. J Steroid Biochem Mol Biol 2015; 148: 179–83.
Lin L, Zhang J. Role of intestinal microbiota and metabolites on gut homeostasis and human diseases. BMC Immunol 2017; 18: 2.

Liu W, Chen Y, Golan MA, Annunziata ML, Du J, Dougherty U, et al. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. J Clin Invest 2013; 123: 3983–96.

Long TM, Nisa S, Donnenberg MS, Hassel BA. Enteropathogenic Escherichia coli inhibits type I interferon- and RNase L-mediated host defense to disrupt intestinalepithelial cell barrier function. Infect Immun 2014; 82: 2802–14.

Lundblad C, Axelberg H, Grände PO. Treatment with the sphingosine-1-phosphate analogue FTY 720 reduces loss of plasma volume during experimental sepsis in the rat. Acta Anaesthesiol Scand 2013; 57: 713–18.

Ma N, Wu Y, Xie F, Du K, Wang Y, Shi L, et al. Dimethyl fumarate reduces the risk of mycotoxins via improving intestinal barrier and microbiota. Oncotarget 2017; 8: 44625–38.

Mangalam A, Shahi SK, Luckey D, Karau M, Marietta E, Luo N, et al. Human gut-derived commensal bacteria suppress CNS inflammatory and demyelinating disease. Cell Rep 2017; 20: 1269–77.

Martini E, Krug SM, Siegmund B, Neurath MF, Becker C. Mend your fences: the epithelial barrier and its relationship with mucosal immunity in inflammatory bowel disease. Cell Mol Gastroenterol Hepatol 2017; 4: 33–46.

Melton AC, Bailey-Bucktrout SL, Travis MA, Fife BT, Bluestone JA, Sheppard D. Expression of αvβ8 integrin on dendritic cells regulates Th17 cell development and experimental autoimmune encephalomyelitis in mice. J Clin Invest 2010; 120: 4436–44.

Metz LM, Li DKB, Traboulsee AL, Duquette P, Eliasziw M, Cerchiaro G, et al. Trial of minocycline in a clinically isolated syndrome of multiple sclerosis. N Engl J Med 2017; 376: 2122–33.

Miller AE. Teriflunomide: a once-daily oral medication for the treatment of relapsing forms of multiple sclerosis. Clin Ther 2015; 37: 2366–80.

Mirabella M, Cianfoni A, Bucci M, Nociti V, Sancricca C, Patanella AK, et al. Coeliac disease presenting with acute disseminated encephalomyelitis. Eur J Neurol 2006; 13: 202–3.

Miranda-Hernandez S, Baxter AG. Role of toll-like receptors in multiple sclerosis. Am J Clin Exp Immunol 2013; 2: 75–93.

Miura S, Tsuzuki Y, Kurose I, Suematsu M, Shigematsu T, Kimura H, et al. Endotoxin stimulates lymphocyte-endothelial interactions in rat intestinal Peyer's patches and villus mucosa. Am J Physiol 1996; 271: G282–92.

Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, et al. 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 2015; 10: e0137429.

Mizuno M, Noto D, Kaga N, Chiba A, Miyake S. The dual role of short fatty acid chains in the pathogenesis of autoimmune disease models. PLoS One 2017; 12: e0173032.

Morís G. Inflammatory bowel disease: an increased risk factor for neurologic complications. World J Gastroenterol 2014; 20: 1228–37.

Moubareck C, Gavini F, Vaugien L, Butel MJ, Doucet-Populaire F. Antimicrobial susceptibility of bifidobacteria. J Antimicrob Chemother 2005; 55: 38–44.

Mu Q, Kirby J, Reilly CM, Luo XM. Leaky gut as a danger signal for autoimmune diseases. Front Immunol 2017; 8: 598.

Nakahashi-Oda C, Udayanga KG, Nakamura Y, Nakazawa Y, Totsuka N, Miki H, et al. Apoptotic epithelial cells control the abundance of Treg cells at barrier surfaces. Nat Immunol 2016; 17: 441–50.

Nalle SC, Turner JR. Intestinal barrier loss as a critical pathogenic link between inflammatory bowel disease and graft-versus-host disease. Mucosal Immunol 2015; 8: 720–30.

Nouri M, Bredberg A, Weström B, Lavasani S. 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 2014; 9: e106335.

Ochoa-Repáraz J, Colpitts SL, Kircher C, Kasper EJ, Telesford KM, Begum-Haque S, et al. Induction of gut regulatory CD39+ T cells by teriflunomide protects against EAE. Neurol Neuroimmunol Neuroinflamm 2016; 3: e291.

Ochoa-Repáraz J, Magori K, Kasper LH. The chicken or the egg dilemma: intestinal dysbiosis in multiple sclerosis. Ann Transl Med 2017; 5: 145.

Ochoa-Repáraz J, Mielcarz DW, Ditrio LE, Burroughs AR, Begum-Haque S, Dasgupta S, et al. Central nervous system demyelinating disease protection by the human commensal Bacteroides fragilis depends on polysaccharide A expression. J Immunol 2010a; 185: 4101–8.

Ochoa-Repáraz J, Mielcarz DW, Wang Y, Begum-Haque S, Dasgupta S, Kasper DL, et al. A polysaccharide from the human commensal Bacteroides fragilis protects against CNS demyelinating disease. Mucosal Immunol 2010b; 3: 487–95.

Odenwald MA, Turner JR. The intestinal epithelial barrier: a therapeutic target? Nat Rev Gastroenterol Hepatol 2017; 14: 9–21.

Olivares-Villagómez D, Van Kaer L. Intestinal intraepithelial lymphocytes: sentinels of the mucosal barrier. Trends Immunol 2018; 39: 264–75.

Ortiz GG, Pacheco-Moisés FP, Macías-Islas MÁ, Flores-Alvarado LJ, Mireles-Ramírez MA, González-Renovato ED, et al. Role of the blood-brain barrier in multiple sclerosis. Arch Med Res 2014; 45: 687–97.

Pászti-Gere E, Jerzsele Á, Balla P, Ujhelyi G, Székács A. Reinforced epithelial barrier integrity via matriptase induction with sphingosine-1-phosphate did not result in disturbances in physiological redox status. Oxid Med Cell Longev 2016; 2016: 9674272.

Paterson BM, Lammers KM, Arrieta MC. The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: a proof of concept study. Aliment Pharmacol Ther 2007; 26: 757–66.

Patten DA, Collett A. Exploring the immunomodulatory potential of microbial-associated molecular patterns derived from the enteric bacterial microbiota. Microbiology 2013; 159: 1535–44.

Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J Nutr 2009; 139: 1619–25.

Persson EK, Scott CL, Mowat AM, Agace WW. Dendritic cell subsets in the intestinal lamina propria: ontogeny and function. Eur J Immunol 2013; 43: 3098–107.

Pröbstel AK, Baranzini SE. The role of the gut microbiome in multiple sclerosis risk and progression: towards characterization of the "MS Microbiome". Neurotherapeutics 2018; 15: 126–34.

Qu L, Li Q, Jiang H, Gu L, Zhang Q, Wang C, et al. Effect of anti-mouse CD52 monoclonal antibody on mouse intestinal intraepithelial lymphocytes. Transplantation 2009; 88: 766–72.

Qu LL, Lyu YQ, Jiang HT, Shan T, Zhang JB, Li QR, et al. Effect of alemtuzumab on intestinal intraepithelial lymphocytes and intestinal barrier function in cynomolgus model. Chin Med J 2015; 128: 680–6.

Raftery T, Martineau AR, Greiller CL, Ghosh S, McNamara D, Bennett K, et al. Effects of vitamin D supplementation on intestinal permeability, cathelicidin and disease markers in Crohn's disease: results from a randomised double-blind placebo-controlled study. United European Gastroenterol J 2015; 3: 294–302.

Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 2004; 118: 229–41.

Reinhold AK, Rittner HL. Barrier function in the peripheral and central nervous system-a review. Pflugers Arch 2017; 469: 123–34.

Reynolds JM, Martinez GJ, Nallaparaju KC, Chang SH, Wang YH, Dong C. Cutting edge: regulation of intestinal inflammation and barrier function by IL-17C. J Immunol 2012; 189: 4226–30.

Rochereau N, Verrier B, Pin JJ, Genin C, Paul S. Phenotypic localization of distinct DC subsets in mouse Peyer Patch. Vaccine 2011; 29: 3655–61.

Rodrigo L, Hernández-Lahoz C, Fuentes D, Alvarez N, López-Vázquez A, González S. Prevalence of celiac disease in multiple sclerosis. BMC Neurol 2011; 11: 31.

Ronchetti AM, Henry B, Ambert-Balay K, Pothier P, Decroocq J, Leblond V, et al. Norovirus-related chronic diarrhea in a patient treated with alemtuzumab for chronic lymphocytic leukemia. BMC Infect Dis 2014; 14: 239.

Rothhammer V, Mascanfroni ID, Bunse L, Takenaka MC, Kenison JE, Mayo L, et al. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. Nat Med 2016; 22: 586–97.

Rumah KR, Linden J, Fischetti VA, Vartanian T. Isolation of Clostridium perfringens type B in an individual at first clinical presentation of multiplesclerosis provides clues for environmental triggers of the disease. PLoS One 2013; 8: e76359.

Rumah KR, Vartanian TK, Fischetti VA. Oral multiple sclerosis drugs inhibit the in vitro growth of epsilon toxin producing gut bacterium, clostridium perfringens. Front Cell Infect Microbiol 2017; 7: 11.

Salvatore S, Finazzi S, Ghezzi A, Tosi A, Barassi A, Luini C, et al. Multiple sclerosis and celiac disease: is there an increased risk? Mult Scler 2004; 10: 711–12.

Sánchez-Navarro M, Garcia J, Giralt E, Teixidó M. Using peptides to increase transport across the intestinal barrier. Adv Drug Deliv Rev 2016; 106: 355–66.

Schumann M, Siegmund B, Schulzke JD, Fromm M. Celiac disease: role of the epithelial barrier. Cell Mol Gastroenterol Hepatol 2017; 3: 150–62.

Secher T, Kassem S, Benamar M, Bernard I, Boury M, Barreau F, et al. Oral Administration of the probiotic strain escherichia coli nissle 1917 reduces susceptibility to neuroinflammation and repairs experimental autoimmune encephalomyelitis-induced intestinal barrier dysfunction. Front Immunol 2017; 8: 1096.

Shen B, Yu H, Hao X, Qu L, Cai X, Li N. Impact of antimouse CD52 monoclonal antibody on graft's $\gamma\delta$ intraepithelial lymphocytes after orthotopic small bowel transplantation in mice. Transplantation 2013; 95: 663–70.

Shen B, Yu H, Yu T, Shen J, Meng N, Cai X. A CD52 antibody impairs mouse-transplanted intestinal tight junctions. J Surg Res 2015; 196: 278–84.

Shi N, Li N, Duan X, Niu H. Interaction between the gut microbiome and mucosal immune system. Mil Med Res 2017; 4: 14.

Simone IL, Federico F, Trojano M, Tortorella C, Liguori M, Giannini P, et al. High resolution proton MR spectroscopy of cerebrospinal fluid in MS patients. Comparison with biochemical changes in demyelinating plaques. J Neurol Sci 1996; 144: 182–90.

Smith RJ, Rao-Bhatia A, Kim TH. Signaling and epigenetic mechanisms of intestinal stem cells and progenitors: insight into crypt homeostasis, plasticity, and niches. Wiley Interdiscip Rev Dev Biol 2017; 6: e281.

Smits LP, Bouter KE, de Vos WM, Borody TJ, Nieuwdorp M. Therapeutic potential of fecal microbiota transplantation. Gastroenterology 2013; 145: 946–53.

Spirin NN, Rumiantseva TA, Kiselev DV, Spirina1 NN. Cellular composition of Peyer's patches and peripheral lymph nodes in experimental allergic encephalomyelitis during fingolimod therapy. Zh Nevrol Psikhiatr Im S S Korsakova 2014; 114: 71–6.

Stange EF. Improvement of a 'Leaky' intestinal barrier. Dig Dis 2017; 35: 21–4.

Stio M, Retico L, Annese V, Bonanomi AG. Vitamin D regulates the tight-junction protein expression in active ulcerative colitis. Scand J Gastroenterol 2016; 51: 1193–9.

Stremmel W, Gauss A. Lecithin as a therapeutic agent in ulcerative colitis. Dig Dis 2013; 31: 388–90.

Sturgeon C, Fasano A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. Tissue Barriers 2016; 4: e1251384.

Sugito K, Koshinaga T, Inoue M, Ikeda T, Hagiwara N, Fukuzawa M. Effect of FTY720 in rat small bowel transplantation: expression of mucosal addressin cell adhesion molecule-1. Transplant Proc 2005; 37: 4472–4.

Swidsinski A, Dörffel Y, Loening-Baucke V, Gille C, Göktas Ö, Reißhauer A, et al. Reduced mass and diversity of the colonic microbiome in patients with multiple sclerosis and their improvement with ketogenic diet. Front Microbiol 2017; 8: 1141.

Takata K, Kinoshita M, Okuno T, Moriya M, Kohda T, Honorat JA, et al. The lactic acid bacterium Pediococcus acidilactici suppresses autoimmune encephalomyelitis by inducing IL-10-producing regulatory T cells. PLoS One 2011; 6: e27644.

Tamtaji OR, Kouchaki E, Salami M, Aghadavod E, Akbari E, Tajabadi-Ebrahimi M, et al. The effects of probiotic supplementation on gene expression related to inflammation, insulin, and lipids in patients with multiple sclerosis: a randomized, double-blind, placebo-controlled trial. J Am Coll Nutr 2017; 36: 660–5.

Tanaka T, Ohtsuka Y, Yagita H, Shiratori Y, Omata M, Okumura K. Involvement of alpha 1 and alpha 4 integrins in gut mucosal injury of graft-versus-host disease. Int Immunol 1995; 7: 1183–9.

Tang X, Maricic I, Kumar V. Anti-TCR antibody treatment activates a novel population of nonintestinal CD8 alpha alpha+ TCR alpha beta+ regulatory T cells and prevents experimental autoimmune encephalomyelitis. J Immunol 2007; 178: 6043–50.

Teixeira B, Bittencourt VC, Ferreira TB, Kasahara TM, Barros PO, Alvarenga R, et al. Low sensitivity to glucocorticoid inhibition of in vitro Th17-related cytokine production in multiple sclerosis patients is related to elevated plasma lipopolysaccharide levels. Clin Immunol 2013; 148: 209–18.

Tremlett H, Fadrosh DW, Faruqi AA, Hart J, Roalstad S, Graves J, et al. Gut microbiota composition and relapse risk in pediatric MS: a pilot study. J Neurol Sci 2016b; 363: 153–7.

Tremlett H, Fadrosh DW, Faruqi AA, Zhu F, Hart J, Roalstad S, et al. Gut microbiota in early pediatric multiple sclerosis: a case-control study. Eur J Neurol 2016a; 23: 1308–21.

Tschurtschenthaler M, Wang J, Fricke C, Fritz TM, Niederreiter L, Adolph TE, et al. Type I interferon signalling in the intestinal epithelium affects Paneth cells, microbial ecology and epithelialregeneration. Gut 2014; 63: 1921–31.

Venkatesan A. Multiple sclerosis and infections. Neurodegener Dis Manag 2015; 5 (Suppl 6): 11–14.

Vijiaratnam N, Rath L, Xu SS, Skibina O. Pancolitis a novel early complication of Alemtuzumab for MS treatment. Mult Scler Relat Disord 2016; 7: 83–4.

Volynets V, Rings A, Bárdos G, Ostaff MJ, Wehkamp J, Bischoff SC. Intestinal barrier analysis by assessment of mucins, tight junctions, and α-defensins in healthy C57BL/6J and BALB/cJ mice. Tissue Barriers 2016; 4: e1208468.

Wan Saudi WS, Sjöblom M. Short-chain fatty acids augment rat duodenal mucosal barrier function. Exp Physiol 2017; 102: 791–803.

Wang Y, Begum-Haque S, Telesford KM, Ochoa-Repáraz J, Christy M, Kasper EJ, et al. A commensal bacterial product elicits and modulates migratory capacity of CD39(+) CD4 T regulatory subsets in the suppression of neuroinflammation. Gut Microbes 2014; 5: 552–61.

Wekerle H. Brain autoimmunity and intestinal microbiota: 100 trillion game changers. Trends Immunol 2017; 38: 483–97.

Wells JM, Brummer RJ, Derrien M, MacDonald TT, Troost F, Cani PD, et al. Homeostasis of the gut barrier and potential biomarkers. Am J Physiol Gastrointest Liver Physiol 2017; 312: G171–93.

Wells JM, Rossi O, Meijerink M, van Baarlen P. Epithelial crosstalk at the microbiota-mucosal interface. Proc Natl Acad Sci USA 2011; 108 (Suppl 1): 4607–14.

Westall FC. Molecular mimicry revisited: gut bacteria and multiple sclerosis. J Clin Microbiol 2006; 44: 2099–104.

Willemsen LE, Koetsier MA, van Deventer SJ, van Tol EA. Short chain fatty acids stimulate epithelial mucin 2 expression through

differential effects on prostaglandin E(1) and E(2) production by intestinal myofibroblasts. Gut 2003; 52: 1442–7.

Yablecovitch D, Shabat-Simon M, Aharoni R, Eilam R, Brenner O, Arnon R. Beneficial effect of glatiramer acetate treatment on syndecan-1 expression in dextran sodium sulfate colitis. J Pharmacol Exp Ther 2011; 337: 391–9.

Yacyshyn B, Meddings J, Sadowski D, Bowen-Yacyshyn MB. Multiple sclerosis patients have peripheral blood CD45RO + B cells and increased intestinal permeability. Dig Dis Sci 1996; 41: 2493–8.

Yanagawa Y, Masubuchi Y, Chiba K. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats, III. Increase in frequency of CD62L-positive T cells in Peyer's patches by FTY720-induced lymphocyte homing. Immunology 1998; 95: 591–4.

Yang S, Yu M, Sun L, Xiao W, Yang X, Sun L, et al. Interferon-γ-induced intestinal epithelial barrier dysfunction by NF-κB/HIF-1α pathway. J Interferon Cytokine Res 2014; 34: 195–203.

Yi H, Jiang D, Zhang L, Xiong H, Han F, Wang Y. Developmental expression of STATs, nuclear factor-κB and inflammatory genes in the jejunum of piglets during weaning. Int Immunopharmacol 2016; 36: 199–204.

Yong VW, Wells J, Giuliani F, Casha S, Power C, Metz LM. The promise of minocycline in neurology. Lancet Neurol 2004; 3: 744–51.

Yoseph BP, Klingensmith NJ, Liang Z, Breed ER, Burd EM, Mittal R, et al. Mechanisms of intestinal barrier dysfunction in sepsis. Shock 2016; 46: 52–9.

Yu T, Yang HS, Lu XJ, Xia ZS, Ouyang H, Shan TD, et al. Association of bactericidal dysfunction of paneth cells in streptozocin-induced diabetic mice with insulin deficiency. Med Sci Monit 2016; 22: 3062–72.

Zaura E, Brandt BW, Teixeira de Mattos MJ, Buijs MJ, Caspers MP, Rashid MU, et al. Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. MBio 2015; 6: e01693–15.

Zikou AK, Kosmidou M, Astrakas LG, Tzarouchi LC, Tsianos E, Argyropoulou MI. Brain involvement in patients with inflammatory bowel disease: a voxel-based morphometry and diffusion tensor imaging study. Eur Radiol 2014; 24: 2499–506.

Zundler S, Becker E, Weidinger C, Siegmund B. Anti-adhesion therapies in inflammatory bowel disease-molecular and clinical aspects. Front Immunol 2017; 8: 891.