Gut reactions: How the blood–brain barrier connects the microbiome and the brain

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Impact statement

The connection between the gut microbiome and central nervous system (CNS) disease is not fully understood. Host immune systems are influenced by changes to the microbiota and offers new treatment strategies for CNS disease. Preclinical studies provide evidence of changes to the blood–brain barrier when animals are subject to experimental gut infection or when the animals lack a normal gut microbiome. The intestine also contains a barrier, and bacterial factors can translocate to the blood and interact with host immune cells. These metastatic bacterial factors can signal T-cells to become more CNS penetrant, thus providing a novel intervention for treating CNS disease. Studies in humans show the therapeutic effects of T-cell engineering for the treatment of leukemia, so perhaps a similar approach for CNS disease could prove effective. Future research should begin to define the bacterial species that can cause immune cells to differentiate and how these interactions vary amongst CNS disease models.

Abstract

A growing body of evidence indicates that the microbiome interacts with the central nervous system (CNS) and can regulate many of its functions. One mechanism for this interaction is at the level of the blood–brain barriers (BBBs). In this minireview, we examine the several ways the microbiome is known to interact with the CNS barriers. Bacteria can directly release factors into the systemic circulation or can translocate into blood. Once in the blood, the microbiome and its factors can alter peripheral immune cells to promote interactions with the BBB and ultimately with other elements of the neurovascular unit. Bacteria and their factors or cytokines and other immune-active substances released from peripheral sites under the influence of the microbiome can cross the BBB, alter BBB integrity, change BBB transport rates, or induce release of neuroimmune substances from the barrier cells. Metabolic products produced by the microbiome, such as short-chain fatty acids, can cross the BBB to affect brain function. Through these and other mechanisms, microbiome–BBB interactions can influence the course of diseases as illustrated by multiple sclerosis.

Keywords: Microbiome, blood–brain barrier, immune system, T-cell, multiple sclerosis

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Introduction

Evidence shows that the gut microbiome communicates with the central nervous system (CNS).[1] Early research on the communication between the gut and the CNS predominantly focused on gastrointestinal hormones and enteric nerves as routes for cross talk.[2–6] A growing body of evidence supports that these routes of gut–brain communication are influenced by the microbiome, as has recently been reviewed elsewhere.[7–10] In this review, we will discuss how gut microbiome interactions with the peripheral immune and neuroimmune systems involve the blood–brain barrier (BBB).

Gut microbes may affect their host by reprogramming immune cells,[11] promoting cytokine secretion,[12] manufacturing bacteriophages,[13] translocating into the systemic circulation,[14] and in some cases moving across the

BBB.[15] Gut bacteria release factors and metabolites into the blood that can readily cross the BBB or otherwise interact with barrier cells and so affect the CNS.[9,16] A nearly 40-year-old example of this are muramyl peptides which are derived from bacteria and appear in both urine and brain and promote sleep.[17,18] Bidirectional communication between gut and brain may alter cognitive functions[8,10,14,19,20] and can also play a vital role in mediating stress-related behaviors, such as anxiety and depression.[19,21,22] The complex communication between the gut and the brain has recently become a popular topic for the development of effective therapeutics for CNS disease. To better treat CNS diseases, more focus should be given to the interactions between the gut microbiome, the peripheral immune system, and CNS barriers.

Gut–immune interactions and their contributions to host immunity

The distal gut of mammals hosts an enriched, highly diverse bacterial ecosystem which comprises a major portion of the microbiome. Bacteria have symbiotic relationships with their hosts and begin to populate the gut soon after birth,[23] perhaps even during gestation.[24] Approximately 70% of the immune system also resides in the gut; therefore, gut microbial-host interactions are important in the development and maintenance of host immunity.[25,26]

The majority of gut microbes reside within the lumen of the intestines, which is lined by epithelial cells that form a barrier to and an interface for host–microbial interactions. Disruption of the gut epithelial barrier may permit the unregulated translocation of gut microbes into the lamina propria, which is where most of the immune cells within the gut reside (Figure 1(i)). Immune cells reside in organized structures of the gut, which include Peyer's patches, isolated lymphoid follicles, and mesenteric lymph nodes within the lamina propria which are collectively referred to as gut-associated lymphoid tissues (GALT).[27] The GALT is highly adaptive and can tolerate, and regulate, colonization by commensal bacteria.

The immune system within the gut includes lymphocytes and resident macrophages that are dispersed throughout the lamina propria and basal epithelium. Resident macrophages in the gut are largely unresponsive to commensal bacteria and their components, as they lack the lipopolysaccharide (LPS) co-receptor and pro-inflammatory responses are suppressed by anti-inflammatory cytokines produced by the GALT.[28] The GALT is a site where T-cells have been shown to respond to glycoprotein autoantigen presentation, or become influenced by the direct communication with the innate immune system (Figure 1(ii)).[7] Translocated microbial antigens may encounter dendritic cells, which present the antigen to T-cells and B-cells to stimulate their differentiation and maturation.[27] The gut is constantly exposed to a wide

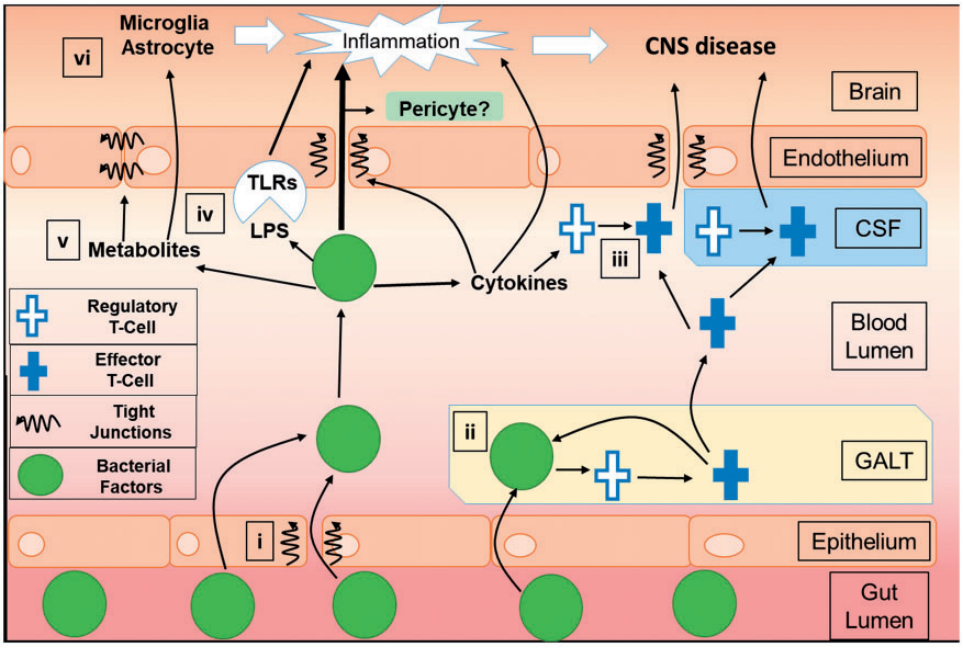

Figure 1. The microbiome communicates with immune cells throughout the body and can affect the blood–brain barrier (BBB) and CNS function. The gut lumen is constantly exposed to bacterial factors from the outside environment. Disruption of the gut epithelial barrier may permit the unregulated translocation of gut microbes into the lamina propria (i). Bacterial factors can infiltrate the GALT, and the blood lumen, where they interact with various immune cells, including T-cells (ii). Certain bacterial factors can stimulate effector-type T-cell differentiation. Regulatory T-cells survey the GALT, blood, and CSF and changes to the local microbiome can promote T-cell brain infiltration (iii). Circulating bacterial factors can upregulate inflammatory cytokine levels, affect BBB integrity and promote neuroinflammation. LPSs are produced by bacterial factors and can act on endothelial TLRs to promote neuroinflammation and CNS disease (iv). Bacterial metabolites can upregulate tight junction proteins and improve BBB integrity (v). Metabolites can also cross the BBB to impact glial cells and neuroinflammation. The role of the microbiome on pericytes remains unclear (vi).
CSF: cerebral spinal fluid; CNS: central nervous system; GALT: gut-associated lymphoid tissues; LPS: lipopolysaccharide; TLRs: toll-like receptors.

repertoire of antigens derived from food and microbes; therefore the resident immune system must be highly specialized.

The gut microbiota plays an important role in Th17 cell differentiation, an important class of $CD4^+$ helper T-cells, and their infiltration into the brain (Figure 1(iii)).[29,30] Further, the impact of the gut microbiota on immune cells is not limited to T-cells. In a study by Möhle *et al*., it was found that antibiotic-induced dysbiosis increased levels of $Ly6C^{hi}$ monocytes in the brain, and this increase was associated with improved neurogenesis and memory retention.[31]

Microbiome dysbiosis may occur in response to colonization by pathogenic microorganisms and/or as a result of altered immune function. A prototypic example of gut dysbiosis is *Clostridium difcile* infection; risks of contracting such an infection are increased with the use of antibiotics that may deplete the healthy microbiome, as well as attenuated immunity, such as that which occurs in the elderly.[32] Depletion of $CD4^+$ T-cells in the gut lamina propria is an early event in acute HIV infection and persists even after onset of antiretroviral therapy.[33] T-cell depletion in HIV has been associated with dysbiosis, disruption of the epithelial barrier, intestinal inflammation, and escape of immunogenic bacterial components into the circulation.[34,35] The BBB provides the CNS with additional protection against the systemic circulation. In the next section, we will focus on how changes to the microbiome can influence the structure and function of the BBB.

Microbiota and the BBB

The vascular BBB is comprised of specialized brain endothelial cells that prevent the unrestricted leakage of plasma proteins into the CNS, and act as a regulatory interface between brain and blood, performing nutritive, homeostatic, and communication roles. Tight junction proteins, including claudins, tricellulin, occludin, and zona occludens, are specialized features of brain endothelial cells and restrict paracellular diffusion of substances between the blood and brain. Disruption of tight junctions can lead to a leaky BBB and expose the CNS to harmful substances in the circulation. BBB structural and functional disruption has been implicated in many CNS disorders, contributing to CNS diseases.

For decades, it has been understood that bacteria and their cell wall constituents can cause BBB dysfunction. Many of the studies that support our current knowledge have been in the context of life-threatening infections that involve or model sepsis, meningitis, and/or the systemic inflammatory response syndrome. In the case of sepsis, the gut microbiome plays a clear causal role when infections arise from intestinal perforations. Sepsis is routinely modeled in rodents using cecal ligation and puncture, and BBB changes that have been reported in this model include increased permeability, upregulated expression of cell adhesion molecules, leukocyte and platelet attachment to brain endothelium, and increased brain uptake of neutral amino acids and tumor necrosis factor (TNF)-α.[36–39]

Bacteria known to cause meningitis can cross the BBB through interactions of bacterial pilli or cell wall components such as lipoteichoic acid (LTA) with the brain endothelium, inducing transcytosis.[40] Some CNS-tropic bacteria may cross the BBB in the absence of disruption,[40] whereas others require disruption and/or engagement of peripheral immune cells with the brain endothelium for CNS dissemination.[41] Brain endothelial cells express Toll-like receptors (TLRs), which enable their direct responses to bacterial cell wall components such as LPS from Gram-negative bacteria and LTA from Gram-positive bacteria through TLR4[42] and TLR2, respectively (Figure 1(iv)).[42,43] LPS and LTA can also induce the production and release of pro-inflammatory mediators from other cell types, which then modulates BBB function.[44] BBB functions altered directly or indirectly by LPS include tight junction expression and BBB integrity, immune cell trafficking, permeabilities to HIV-1 and insulin, efflux of amyloid beta peptide, and secretions of cytokines, chemokines, nitric oxide, and prostaglandins by the barrier cells.[45–52]

A recent study by Braniste *et al*. compared germ-free mice (those that have never encountered a live bacterium) to pathogen-free mice (those raised in an environment free of monitored mouse pathogens), and demonstrated that germ-free mice exhibited decreased expression in tight junction proteins, occludin and claudin-5, as well as associated evidence of tight junction deficits.[53] These changes occurred in the absence of changes in vascular density or pericyte coverage of the brain endothelium. Moreover, colonization of germ-free mice with flora from pathogen-free mice restored BBB function. Leclercq *et al*. found that treatment with low-dose penicillin early in life led to long-term changes in the gut microbiota and upregulated expression of tight junction proteins.[54] These data suggest certain live bacteria can positively influence the BBB, helping to regulate the interaction between the periphery and the CNS. Although germ-free mouse models are useful in establishing causality in gut microbiota–brain interactions, they are not the most clinically relevant models and care should be taken when interpreting results.

A potential mechanism by which live bacteria influence the BBB is by producing metabolites that can alter CNS function (Figure 1(v)). Short-chain fatty acids (SCFAs), such as butyrate, acetate, and propionate, are produced through the fermentation of dietary fibers by the gut microbiota.[55] Bacteria such as *Clostridium tyrobutyricum*, which produce high levels of butyrate, have been shown to improve BBB integrity in germ-free mice, which was associated with an upregulation of tight junction protein expression.[53] Moreover, SCFAs were able to improve a dysfunctional BBB in germ-free mice[53] and benefit the intestinal barrier as well.[56]

Microbiome and specific cell-types of the neurovascular unit

Brain endothelial cells interact with other closely apposed cell types, including pericytes and astrocytes, as well as neurons, microglia, peripherally derived immune cells, and the basement membrane and glycocalyx. These

components are collectively termed the neurovascular unit (NVU), and their interactions support and regulate BBB functions.[57] In this section, we will discuss the effects of gut microbiota on the NVU, as well as how perturbations of the microbiome affect BBB integrity and CNS function (Figure 1(vi)).

In addition to endothelial cells, the microbiota may affect other CNS cell types of the NVU. Pericytes are important contributors to BBB induction and maintenance,[58] and pericyte loss has been associated with CNS disease including Alzheimer's[55] and diabetes.[59,60] To date, one study has examined pericytes in the context of changes in the microbiome. In this study, pericyte numbers in the CNS were unaffected in germ-free mice, indicating that BBB disruption in this model is not due to pericyte loss.[53] Although it is presently unclear how the microbiome could affect brain pericyte functions, it was recently shown that tumor-related pericytes are influenced by changes in the microbiome in a mouse colon cancer model.[61] Further, brain pericytes are responsive to immune stimuli such as LPS.[62] As such, more studies are warranted to determine how changes in microbiota may affect pericytes.

Astrocytes are also closely associated with the brain endothelium and contribute to BBB integrity and function.[63] In germ-free mice, there are no overt changes in the numbers or localization of $GFAP^+$ astrocytes in the brain.[16] However, gut metabolites may protect the BBB under inflammatory conditions. Bacterial metabolites of dietary tryptophan exert anti-inflammatory effects in astrocytes via activation of the aryl hydrocarbon receptor (AHR), which synergizes with type 1 interferon signaling through AHR upregulation.[64] In the same study, tryptophan metabolites were found to lessen the disease severity in a mouse model of multiple sclerosis (MS). As BBB dysfunction is highly implicated in MS (see below), an important future direction to this work is to determine whether gut metabolites of tryptophan are BBB-protective in MS, which could be mediated by limiting immune responses in astrocytes.

Erny *et al*. demonstrated that the microbiota influences microglial function and morphology. They found that germ-free mice had an immature phenotype, with increased proliferative markers and morphological alterations such as increased segment lengths, branching, and contacts with adjacent microglia. Further, microglia from germ-free mice have attenuated responses to inflammatory stimuli.[16] In the same study, evidence of immune cell trafficking and perivascular inflammation was absent in germ-free mice. These data suggest that the microbiome affects the development and maturation of microglia; however, it is presently unclear how microglia function in germ-free mice or how conditions of dysbiosis affects the BBB.

A particularly interesting, yet unanswered question is how the signals of the microbiota are transmitted across the BBB. Many modes of communication are conceivable, which may include (1) microbes or their components entering the brain to act on non-endothelial cells of the NVU directly, (2) microbes or their components interacting with brain endothelial cells to alter their functions or secretions, or (3) microbes altering the production of peripheral components which may directly enter the brain or influence brain endothelial cell functions or secretions. Development of a better understanding of these mechanisms is critical to the global understanding of how the microbiome affects the CNS.

Immune cell contribution to CNS disease

For many years, the CNS was considered an immune privileged site protected by a tightly regulated BBB. The CNS demands oxygen and nutrients and thus requires a large supply of blood; however, the vessel walls must also be protective against peripheral toxins and bacteria that could disrupt normal brain function. The idea of an "immune privileged brain" has evolved recently into an understanding that the immune privilege of the brain is relative and not absolute.

Indeed, blood-borne immune cells have been shown to cross the vascular BBB and choroid plexus (Figure 1(iii)), entering the healthy brain at very low levels,[65] and T-cells have been shown to survey the choroid plexus and meninges.[50] T-cells in these compartments contribute to the host response to stress and injury. Around 80% of immune cells in the cerebrospinal fluid (CSF) are $CD4^+$ T-cells,[66] which are known to mediate CNS homeostasis.[67,68] Interestingly, mice without T-cells exhibit impaired learning and social behavior.[67] From these studies, we can infer that T-cells secrete soluble factors that are carried by the CSF to brain regions associated with rodent behavior. Interferon gamma is one factor secreted by T-cells that has been shown to play a vital role in rodent prefrontal cortex neurophysiology and social behavior.[69]

The immune response to gut dysbiosis has been linked to a number of CNS diseases in which BBB dysfunction is also implicated. The gut microbiome mediates the release of inflammatory cytokines which can activate endothelial cells or be actively transported across the brain endothelial cell layer and deposited within the CNS.[12] The microbiota can also manipulate T-cells in the gut, which may promote heightened CSF surveillance and contribute to CNS inflammation and aberrant behavior. Such interactions could be especially relevant in MS, a disease in which T-cells are thought to substantially drive CNS pathology. Known contributions of the gut microbiome to MS that implicate the BBB are discussed below.

Multiple sclerosis: Impact of gut microbiota on BBB

MS is a chronic demyelinating neuroinflammatory disease that affects the brain and spinal cord. Immune cell infiltration causes CNS lesions that are hallmarks of MS, and is believed to occur as a result of autoimmunity against CNS antigens. BBB dysfunction is evident in MS and in an animal model of MS, experimental autoimmune encephalomyelitis (EAE).[70]

Alterations in BBB functions are important in the development and progression of MS.[71,72] Such alterations include BBB leakiness, loss of tight junctions, and activation of proteolytic enzymes, which can facilitate degradation of the extracellular matrix and BBB disruption.[71,73] T-cell homing to peripheral lymphoid tissues was also shown to

be important to the development of EAE, as T-cells that are re-isolated after homing and injected into naïve animals cross the BBB and initiate disease more rapidly.[74]

The gut microbiome has been implicated in the development of MS. Berer *et al*. showed that germ-free mice were resistant to developing EAE, however the introduction of non-pathogenic commensal bacteria in germ-free mice permits EAE development.[11,75] In addition, BBB transport of TNF-α is doubled in EAE,[76] which could further augment neuroinflammation. These results suggest the gut microbiome contributes to inflammatory responses that influence the course of MS.

Additionally, dietary factors may contribute to dysbiosis and MS progression. Cao *et al*. demonstrated that caffeine-free high-sucrose cola beverages enhanced EAE pathogenesis by worsening demyelination and increasing CNS infiltration of Th17 cells.[77] Interestingly, caffeinated high-sucrose cola beverages counteracted the effect of the high-sucrose on disease pathogenesis. EAE mice given high-sucrose cola consumption exhibited numerous enriched taxa, which have been implicated in pathogenic T-cell responses.[78–81]

In addition to T-cell lymphocytes, the gut microbiome also influences peripheral myeloid cells and more recently has been shown to affect CNS myeloid cells. Gut microbiota and their metabolites have been implicated in the pathogenesis of other autoimmune diseases, including rheumatoid arthritis and type I diabetes, which also involve T-cell-mediated inflammatory pathology and BBB disruption.[60,73,82–84] These studies clearly suggest that changes to the microbiome can influence CNS disease pathogenesis through changes to the immune system.

Conclusion

A growing amount of evidence supports that the gut microbiome contributes to CNS function and that gut dysbiosis may be a causal factor in a wide range of CNS diseases. The BBB, being a predominant interface for communication between the CNS and periphery, is a palpable site at which signals from the microbiome may be transmitted to the CNS. In this review, we have highlighted potential mechanisms for such communication, as well as recent literature that has demonstrated that the BBB and NVU can be altered under conditions of dysbiosis.

Cross-talk between the gut microbiome and the immune system is crucial for gut–brain communication. The interactions between the microbiota and immune cells can occur at many biological sites throughout the body (Figure 1) and can subsequently affect the brain endothelium, or even cells within the CNS, including those of the NVU. It is now evident in pre-clinical models of MS that the gut microbiome affects disease progression by regulating T-cell trafficking across the BBB. The BBB and microbiome are independently implicated in a number of other conditions that affect the CNS, including Alzheimer's disease[12,85] and diabetes.[60,86]

We anticipate that future work will illuminate novel relationships of the BBB and the microbiome that drive our understanding of how the interactions of these two components in physiological states promote brain health and how dysbiosis contributes to CNS disease. By developing a more comprehensive mechanistic view of gut microbial effects on CNS function including those incorporating the CNS barriers, we open up possibilities for many new therapeutic strategies to combat some of the most devastating CNS diseases faced by society today.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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