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The Gut Microbiome as a Regulator of the Neuroimmune Landscape

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Abstract

The gut microbiome influences many host physiologies, spanning gastrointestinal function, metabolism, immune homeostasis, neuroactivity, and behavior. Many microbial effects on the host are orchestrated by bidirectional interactions between the microbiome and immune system. Imbalances in this dialogue can lead to immune dysfunction and immune-mediated conditions in distal organs including the brain. Dysbiosis of the gut microbiome and dysregulated neuroimmune responses are common comorbidities of neurodevelopmental, neuropsychiatric, and neurological disorders, highlighting the importance of the gut microbiome–neuroimmune axis as a regulator of central nervous system homeostasis. In this review, we discuss recent evidence supporting a role for the gut microbiome in regulating the neuroimmune landscape in health and disease.

1. INTRODUCTION

The mammalian gastrointestinal tract harbors trillions of diverse microorganisms, including fungi, viruses, and bacteria, that are collectively known as the gut microbiota. The gut microbiota is juxtaposed against a vast intestinal barrier fortified by immune cells that interact bidirectionally with luminal and mucosal microbes and microbial products. Through direct and indirect signaling with the enteric immune system, the gut microbiome serves as a key regulator of immune homeostasis and immune responses that extend beyond the intestine to distal organs. Accumulating evidence reveals that the gut microbiome impacts the development and function of the neuroimmune system. The ability of the gut microbiome to regulate the neuroimmune system represents a major pathway by which microbes impact brain homeostasis and symptoms of neurological disease. In this review, we focus on how the gut microbiome can regulate major compartments of the neuroimmune system, including brain-resident immune cells and barrier tissues surrounding the central nervous system (CNS) (**Figure 1**). We further discuss how alterations in microbiome-neuroimmune interactions can contribute to risk for neurological disorders such as Alzheimer disease (AD), Parkinson disease (PD), and multiple sclerosis (MS).

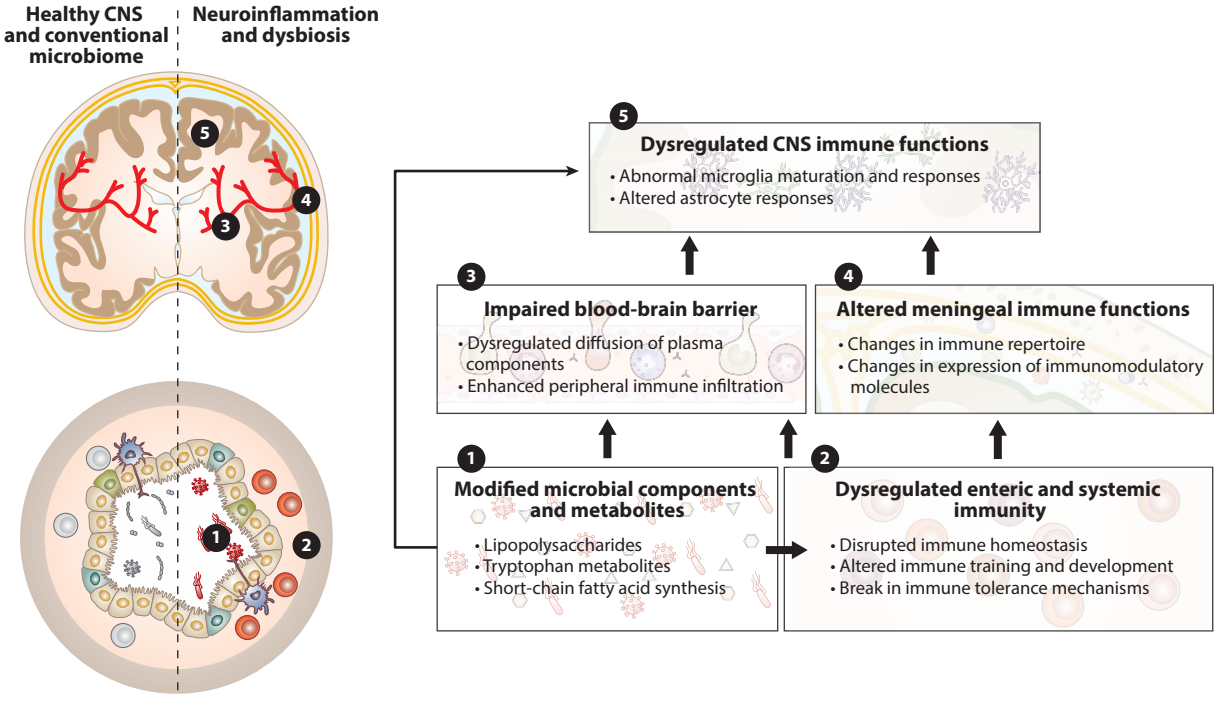


Figure 1

The gut-neuroimmune axis is a regulator of central nervous system (CNS) homeostasis. The gut microbiome is essential for host immune functions and actively regulates peripheral and CNS immune homeostasis through microbiota-derived components and metabolites such as lipopolysaccharides, derivatives of tryptophan metabolism, and short-chain fatty acids. In neuroinflammatory disorders, aberrations in gut microbiota homeostasis, or dysbiosis, are a common comorbidity that can lead to changes in microbiota-derived compounds (❶), resulting in altered systemic homeostasis (❷), impaired blood-brain barrier function (❸), and dysregulated meningeal (❹) and CNS immune homeostasis (❺). Collectively, these changes orchestrate the neuroinflammatory milieu observed in gut-neuroimmune interactions during CNS disease.

2. MICROGLIA

Microglia are resident myeloid cells of the CNS that are essential for homeostatic functions in the brain including neurogenesis, neurotransmission, synaptic remodeling, neuroinflammation, and injury repair (1, 2). Although microglia share many transcriptional programs with tissue-resident macrophages, they are distinguishable by their developmental origins and capacity to self-renew (3). Additionally, microglia exhibit developmental and regional heterogeneity (3). While they can respond to local CNS-derived cues and factors of peripheral origin, the full repertoire of signals and transduction cascades that determine microglial function remains to be fully elucidated. Because microglial dysfunction is implicated in a variety of neurological disorders, uncovering key functional regulators and underlying cellular programs may reveal new molecular targets for combating symptoms of disease.

Recent preclinical research suggests that the gut microbiome plays an essential role in regulating microglial physiology (**Figure 2*a***) and that targeting enteric dysfunction observed in many neurological disorders could restore microglial function. In an early comparative study, microglia from germ-free (GF) mice exhibited an immature phenotype characterized by transcriptional programs associated with diminished immune function (type 1 IFN signaling, pathogen recognition, and antigen presentation) and enhanced proliferation (*Ki67* and *Ddit4*) and survival (*Csf1r* and *Pu.1*) (4), relative to controls from conventionally colonized [specific-pathogen-free (SPF)] mice. Interestingly, the key transcription factor *Mafb*, which is responsible for guiding the microglial transition from early to adult phenotype, was upregulated in microglia from GF mice despite their immature state (4, 5), further suggesting that the absence of the gut microbiota leads to abnormalities in microglial development. Consistent with this, treating adult SPF mice with broad-spectrum antibiotics (cefoxitin, gentamicin, metronidazole, and vancomycin) for four weeks recapitulated several characteristics of immature microglia observed in GF mice, indicating that active signaling from the gut microbiome is required for maintaining microglial homeostasis (4). Notably, some differences in transcriptional signatures were detected in prenatal microglia from fetuses of GF dams compared to SPF controls, suggesting a role for the maternal gut microbiome in modulating microglial development even before microbial colonization of offspring at birth (5–7). Colonization of neonatal GF mice with complex microbiota or a consortium of bifidobacteria representing the infant microbiota, but not a limited consortium of altered Schaedler flora (ASF), partially restored microglial phenotypes, further indicating that specific bacterial members in early-life gut microbiota play a critical role in priming microglial maturation programs (4, 8, 9).

One study reported that the influence of the gut microbiome on microglial development and function occurs in a sex-dependent manner. In GF dams, microglia from prenatal males (embryonic day 18.5) exhibited downregulation of transcriptional programs involved in metabolism, cell and protein organization, and adaptive immune responses while prenatal females did not display major perturbations. In contrast, microglia from adult GF females exhibited dysregulation of cell morphogenesis, transcription, chemotaxis, and adaptive immune responses whereas microglia from adult GF males did not show striking differences (6). Antibiotic treatment (ampicillin, streptomycin, colistin, and amphotericin) for one week in adult SPF mice led to differential regulation of pathways related to signal transduction and stress response in both sexes. But these mice also displayed some sexually dimorphic effects, as demonstrated by changes in immune response genes, particularly in males, and transcription-related pathways, particularly in females (6). This is consistent with a mouse model of AD (APPPS1–21) where long-term antibiotic treatment (kanamycin, gentamicin, colistin, metronidazole, and vancomycin) beginning at postnatal day 14 resulted in sex-specific changes in gut microbiota composition, circulating inflammatory

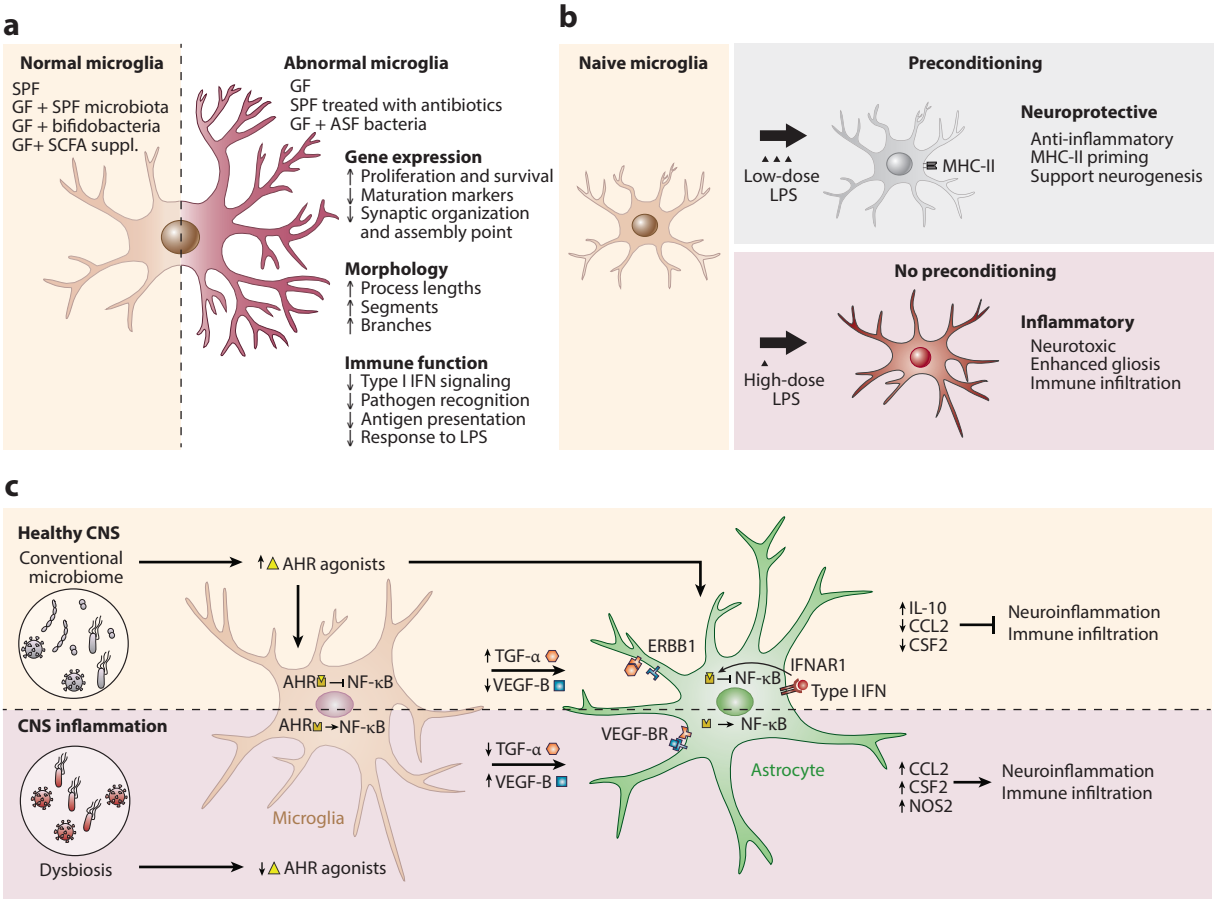


Figure 2

Microbiome-dependent effects on microglia and astrocyte physiology. (*a*) In the absence of a complex microbiota, microglia fail to develop properly and exhibit an immature phenotype. GF mice reared in the absence of microbial colonization or treated with antibiotics have microglia with altered gene and protein expression that are markers related to proliferation, survival, synaptic organization, and maturation and exhibit abnormal morphology and attenuated immune responses. These alterations are restored in GF mice by colonization with a conventional microbiota (SPF), selective colonization with a consortium of bifidobacteria, or supplementation with SCFAs, but not by colonization with a limited altered Schaedler flora. (*b*) LPSs are the main components of the outer membrane of gram-negative bacteria and are potent activators of innate immune responses. Microglial responses to LPS are dependent on the dose and frequency, which can skew microglia to neuroprotective or neuroinflammatory phenotypes. Repeated exposure to low-dose LPS preconditions microglia to an anti-inflammatory and neuroprotective phenotype and primes antigen-presentation pathways that augment neuroprotective responses. In contrast, a single high dose of LPS elevates neuroinflammatory responses that promote gliosis and neuronal damage, which can be further exacerbated by peripheral immune infiltration. (*c*) Gut microbiome–mediated dietary metabolism of tryptophan to indole is required for host AHR ligand synthesis. The AHR is expressed by microglia and astrocytes, and its activation inhibits the master regulator of proinflammatory responses, NF-κB. In astrocytes, AHR expression is enhanced by type I interferon signaling and leads to reduced neuroinflammatory responses. Enhanced microglial AHR signaling induces TGF-α, which binds to astrocytic epidermal growth factor receptor (ERBB1), and reduces expression of VEGF-B, which binds to VEGF-BR, resulting in anti-inflammatory pathways by inhibiting CCL2 and CSF2 while promoting IL-10. In the absence of AHR signaling, microglia increase the ratio of VEGF-B:TGF-α signals that augment CCL2, CSF2, and NOS2 production by astrocytes, leading to enhanced immune infiltration and neuroinflammation. Abbreviations: AHR, aryl hydrocarbon receptor; ASF, altered Schaedler flora; CCL2, chemokine ligand 2; CNS, central nervous system; CSF2, colony-stimulating factor 2; GF, germ-free; IFNAR, interferon-α/β receptor; LPS, lipopolysaccharide; NOS2, nitric oxide synthase 2; SCFA, short-chain fatty acid; SPF, specific-pathogen-free; TGF-α, transforming growth factor alpha; VEGF-BR, vascular endothelial growth factor B receptor.

mediators, microglial morphology, and plaque burden (10). It will be interesting to evaluate the reproducibility of these sex-dependent effects of the gut microbiome on microglial transcriptional signatures and to further determine their influences on cellular function. Examining molecular regulators of sex-dependent changes in microglial activity could reveal novel insights into the pathogenesis of many neurodevelopmental, neuropsychiatric, and neurodegenerative disorders and prevalence in gender bias.

PSD-95: postsynaptic density protein 95

BDNF: brain-derived neurotrophic factor

VGLUT2: vesicular glutamate transporter 2

2.1. Microbial Effects on Microglia-Dependent Synaptic Pruning

Downstream of their effects on gene expression programs in microglia, the gut microbiome is implicated in regulating key microglial functions, such as synaptic pruning. During neurogenesis and synaptogenesis, excess synaptic connections between neurons are generated and are then removed by activity-dependent synaptic pruning. Mice treated with antibiotics (ampicillin, gentamicin, metronidazole, neomycin, and vancomycin) for two weeks exhibited microglia with enriched expression of pathways related to synapse organization and assembly, suggesting that the gut microbiome can modulate microglia-mediated synaptic pruning (11). In a fear-conditioning and extinction-learning paradigm, antibiotic-treated mice had higher baseline spine elimination and failed to form spines during extinction learning, indicating defects in synaptic remodeling possibly due to dysregulated pruning by microglia. In line with this, defects in synaptic transmission were further demonstrated in GF and antibiotic-treated mice when examining synaptic plasticity-related proteins PSD-95, synaptophysin, and BDNF (11, 12). Neonatal colonization of GF mice with a consortium of human-derived bifidobacteria normalized synaptic pruning, as assessed by the levels of the presynaptic marker VGLUT2, as well as firing rates, as measured by electrophysiological recordings in Purkinje cell climbing fibers. This suggests that select members of the gut microbiota are responsible for microbial effects on microglia and synaptic plasticity (8). However, the pathways and biomolecules that mediate microbial effects on microglia-mediated synaptic pruning remain unclear.

Synaptic pruning by microglia is dependent on the complement system, an innate immune process (13, 14). In the CNS, microglia express high levels of the complement proteins C1qa and C3 and exclusively express the C3 receptor, CD11b (14, 15). In GF mice, microglia isolated from neonates express higher levels of C1qa and lower levels of CD11b compared to control animals, while the converse is observed in adult microglia, suggesting a link between the gut microbiome and the complement system in microglia (5, 16). Indeed, the gut microbiome can regulate peripheral complement protein expression and activity in the intestines (17) and skin (18), but its effects on the CNS complement system remain uncertain. In the event of CNS injury and neurodegenerative disease, the brain upregulates complement proteins that can lead to microglia-mediated synaptic loss (19–21). In addition, CNS injury and intestinal dysbiosis can promote blood-brain barrier (BBB) breakdown and encourage the influx of plasma proteins such as complement into the brain (22). However, it remains undetermined whether peripheral complement can modulate synaptic pruning mechanisms by microglia. Furthermore, the complement system plays a role in glia-mediated amyloid β clearance in the brain, and mice deficient in C3 protein are protected from synaptic loss and neurodegeneration despite elevated plaque volume (19, 23, 24). Early studies using transgenic mice deficient in C3 and CD11b to uncover the link between complement and synaptic pruning did not address the potential impact of reduced complement-mediated immunity on gut microbiota composition. Indeed, adult C3-deficient mice exhibited diminished levels of bacteria belonging to the phylum *Bacteroidetes* and enhanced levels of *Firmicutes* (25). *Bacteroidetes* and *Firmicutes* are major producers of specific short-chain fatty acids (SCFAs) that are implicated in microglia maturation and function as well as BBB integrity (26). However, how these shifts in microbiota composition during C3 deficiency may impact microglia function remains unclear.

AP-1: activator protein 1

Peripheral immune cells modulate microglia maturation and synaptic pruning by acting as intermediate sensors of microbial metabolites and establishing transient residency in the brain parenchyma (27), cerebral spinal fluid (CSF), and meninges (28–30). In a recent study, $CD4^+$ T cells in the brain were essential for microglial maturation, as assessed by transcriptional state (*AP-1*, *Klf4*, *Egr1*) and morphology (more dendrites with shorter processes and earlier branching) (27). These alterations to microglial maturation disturbed synaptic pruning transcriptional programs (*AP-1*, *Egr1*, and *Lamp1*) and resulted in elevated synaptic density and retention of immature long thin spines in cortical pyramidal neurons (27). However, the microbial molecules and identity of T cell–derived cytokines, as well as the associated cellular mechanisms, remain to be determined. Ultimately, targeting the gut microbiome to treat microglial dysfunction during synaptopathies could offer a novel approach to normalize synaptic density (31). However, additional research is required to uncover the influence of the human gut microbiome on human microglial function to assess the potential for clinical translation.

2.2. Microbe-Derived Immunomodulators: Short-Chain Fatty Acids

SCFAs are products of microbial fermentation of dietary fibers and are potent regulators of host physiology. In the absence of the gut microbiota, levels of SCFAs are substantially reduced in the host, which contributes to defects in microglial maturation seen in GF and antibiotic-treated mice (4). Oral supplementation with SCFAs reversed immaturity-related microglial phenotypes, such as density and processes morphology (numbers of segments, branching points, and terminal points and cell volumes) in GF mice, but failed to reverse *Ddit4* expression and protein markers (CD31 and F4/80) of maturation. Furthermore, mice deficient in the SCFA receptor FFAR2/GPR43 exhibited immature microglial phenotypes, similar to those seen in GF mice, suggesting that receptor-mediated signaling of SCFAs promotes microglial maturation (4). Surprisingly, FFAR2 is not expressed by CNS cells (4), including microglia, indicating that a yet unknown intermediate responder may be required to sense SCFAs and transduce them into signals that regulate microglial development. Indeed, SCFAs can also modulate myeloid cell function in vitro in the presence of FFAR inhibitors (32), indicating that direct ligand-receptor signaling is not the only mechanism of action for SCFAs.

SCFAs can alter microglial function through modulation of epigenetic programming. The SCFA butyrate is a histone deacetylase inhibitor (HDACi) and can augment antimicrobial activity of macrophages by inhibiting HDAC3 (33, 34). Additionally, supplementing primary microglia cultures with butyrate significantly inhibited lipopolysaccharide (LPS)-induced inflammation by triggering the AKT and Rho family of GTPase pathways associated with microglial process elongation (35). Acetate, the most abundant SCFA in the brain (36), can also inhibit HDAC activity and expression and has the added ability to promote histone acetylation by serving as a substrate for histone acetyltransferases (37). Supplementing cultured microglia with acetate reduced inflammatory signals by reversing LPS-induced H3K9 hypoacetylation and nonhistone protein acetylation (38). In a rat model of neuroinflammation, acetate supplementation via a single oral dose of glyceryl triacetate increased brain acetyl-CoA levels by almost 2.2-fold and reduced glial activation by 40–50% (39). This is consistent with long-term acetate supplementation, which reversed H3K9 hypoacetylation and reduced proinflammatory cytokine IL-1β production (40). Moreover, acetyl-CoA is a central metabolic intermediate used in the citric acid cycle and oxidative phosphorylation, which have been linked to macrophage polarization that contributes to neuroprotective effects (41). Therefore, SCFAs can potentially regulate microglial function through epigenetic intermediates, though more research is needed to evaluate the direct and indirect effects of SCFAs on microglia in vivo and in the context of disease.

While the exact signaling mechanisms remain unclear, several studies demonstrate that SCFAs modify microglial activation and neuroinflammation, as well as symptoms of neurodegenerative diseases across different mouse models. In the α-synuclein overexpression and MPTP models for PD, oral supplementation with SCFAs or fecal microbiota transplantation from SPF mice worsened endophenotypes of disease, which were correlated with enhanced microglia activation, as measured by increased ameboid morphology and TNF-α (42–44). These studies suggest a negative impact of the gut microbiome on PD symptoms. In contrast, clinical data suggest that PD patients exhibit dysbiosis that reduces SCFA signaling (45), which aligns with studies reporting that enhanced butyrate by supplementation or treatment with *Clostridium butyricum* or a cocktail of *Lactobacillus rhamnosus* GG, *Bifidobacterium animalis lactis*, and *Lactobacillus acidophilus* improved motor function and symptom outcome in the MPTP model (46, 47). This is consistent with another study where propionate supplementation improved motor function and reduced dopaminergic neuron loss in the 6-hydroxydopamine model of PD (48). In the 5xFAD model of AD, butyrate supplementation improved synaptic plasticity, and this improvement was associated with reduced protein expression of myeloid-associated proinflammatory cytokines (TNF-α, IL-6, IL-1β) in the hippocampus and cortex (49). This is consistent with findings in the APP/PS1 AD model, where treatment with *C. butyricum* led to enhanced butyrate production in the gut, reduced levels of TNF-α and IL-1β throughout the brain, and improved cognitive function (50). However, treatment with a combination of SCFAs (butyrate, acetate, and propionate) in the APPPS1–21 model increased microglial recruitment to amyloid β plaques, yet plaque clearance was reduced, ultimately leading to worsened burden (51). Overall, interventions that augment SCFA-related pathways in mouse models of neurodegeneration yielded inconsistent results, and these early studies suggest that SCFA dysregulation can modulate microglia function and risk for neurotoxicity versus neuroprotection.

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

5xFAD: 5 familial Alzheimer disease

APP/PS1: amyloid precursor protein and presenilin 1

2.3. Microbe-Derived Immunomodulators: Lipopolysaccharide

Toll-like receptors (TLRs) are a group of widely distributed receptors across many cell types and are best known for their role in innate immune responses. In the CNS, TLRs are expressed by neural stem cells, neurons, oligodendrocytes, astrocytes, and microglia (52), and they regulate processes involved in neurodevelopment (16), neuroplasticity (53), and neurodegeneration (54). However, only microglia express the full repertoire of TLR1–9 (54), which enables them to detect a variety of gut microbe–associated molecular patterns, such as double-stranded RNA (TLR3), LPS (TLR4), lipoproteins (TLR2/6), peptidoglycans (TLR2/6), single-stranded RNA (TLR7), and CpG-DNA (TLR9) (55). Surprisingly, SPF mice with global deficiency in TLR 2/3/4/7/9 displayed normal appearance, density, morphology, and maturation status of parenchymal microglia, suggesting that the gut microbiome does not regulate microglial development and maintenance through TLR signaling (4). Nevertheless, mice reared without gut microbes displayed functional defects in microglia that impaired responses to TLR signaling. GF mice that were injected systemically or intracerebrally with LPS exhibited microglia with reduced innate immune responses compared to SPF mice, as indicated by decreases in cytokine and chemokine production (4).

Although LPS is widely used to induce microglial proinflammatory responses and exacerbate CNS disease, we must also consider how LPS responses can vary (priming versus tolerance) due to the heterogeneity of LPS moieties, dosage, timing, and site of injection as well as context-dependent effects of gene-environment interactions, injury, and disease (56, 57) (**Figure 2*b***). Indeed, systemic LPS preconditioning can elicit CNS tolerance mechanisms and dampen subsequent brain injury and neuroinflammation (58–60). In an early study using reciprocal bone marrow transplantation of wild-type and TLR4-deficient mice to selectively deplete TLR4

expression from the CNS or hematopoietic system, consecutive low-dose systemic LPS exposure skewed microglia toward an anti-inflammatory phenotype independent of hematogenous TLR4. Furthermore, microglial TLR4 activation by LPS was required for protection from cryogenic brain injury, suggesting that LPS-induced tolerance can occur within the CNS (61). This is consistent with an in vitro study where repeated low-dose exposure to LPS induced neuroprotective phenotypes in the microglia cell line C8-B4 and primary peritoneal macrophages (62, 63), perhaps through a process mediated by TRIF signaling and epigenetic programming (64, 65). However, it remains unclear whether gut-derived LPS and the variety of LPS moieties from gram-negative commensal bacteria can elicit immunomodulatory effects in the CNS. In humans, gram-negative bacteria account for 9–47% of the stool, with the phylum *Bacteroidetes* a majority of the population (66, 67). Interestingly, LPS produced by *Bacteroides* spp. (LPS-BS) exhibits much lower endotoxicity compared to the LPS produced by *Escherichia coli* (LPS-E) and may be the most abundant form of LPS in the human gut (68). However, LPS-BS is implicated in both protective (69, 70) and neurotoxic (71) mechanisms and thus warrants more investigation in the context of neuroimmune interactions.

Nevertheless, several studies utilizing oral supplementation to recapitulate exposure to gut-derived LPS have demonstrated that dietary LPS-E can modulate neurological function, including anxiety-like behaviors (72) and taste response (73). Dietary LPS from *Pantoea agglomerans* ameliorated impaired memory function associated with high-fat-diet-induced accumulation of amyloid β peptides in vivo, which may be due to enhanced microglial phagocytosis, as demonstrated in vitro (74, 75). LPS-E supplementation for two weeks in GF mice sufficiently primed microglia antigen-presentation machinery and protected them from pathological features associated with infection by the neurotropic JHM strain of the mouse hepatitis virus (JHMV) (76). These effects were mediated by microglial TLR4-dependent signaling, as rearing mice with microglia-specific deficiency in TLR4 reduced LPS-induced priming and worsened JHMV infection. Consistent with a role for TLR4 particularly in microglia, transplantation of bone marrow from TLR4 knockout mice into wild-type mice had no significant effect on microglial response to orally administered LPS and sustained protection from JHMV infection (76). Taken together, these studies suggest that the gut microbiome can regulate microglial function through direct LPS-TLR4 interactions.

Although intestinal LPS-induced priming of microglial functional responses can protect the host from infection, abnormal exposure to microbiota-derived antigens may be detrimental during chronic neuroinflammatory disorders. Elevated levels of endotoxin in the blood can lead to systemic inflammation and decreases in BBB integrity that expose microglia to peripheral proinflammatory factors and exacerbate neuroinflammation (77). Indeed, elevated levels of LPS are observed in amyotrophic lateral sclerosis (78), AD (79), severe autism (80), and metabolic disorders (81). Additionally, intravenous injection of LPS into healthy human volunteers enhanced systemic inflammation, activated microglia, and induced sickness behavior (82). However, whether there is a role for gut microbiota–derived endotoxins in activating microglia and promoting neuroinflammation remains unclear.

2.4. Microbe-Derived Immunomodulators: AHR Ligands

Tryptophan is an essential amino acid that is primarily acquired through dietary intake in animals. The gut microbiome plays an essential role in intestinal tryptophan metabolism and regulates major pathways for producing tryptophan derivatives, including kynurenine, serotonin, and indole precursors, and ligands of aryl hydrocarbon receptor (AHR) (83). Enteric tryptophanase-expressing bacteria catalyze the conversion of dietary tryptophan to indole, which the host uses as a precursor for the synthesis of AHR agonists, such as indoxyl sulfate and indole-3-propionic acid

(IPA) (84). As such, indoxyl sulfate and IPA were undetectable in GF mice, indicating that their bioavailability is dependent on the gut microbiome (84).

AHR is expressed by a variety of CNS-resident cells and peripheral immune cells and has been the subject of many recent reviews (83, 85, 86). In mouse models of experimental autoimmune encephalitis (EAE) (86), ischemic stroke (87), intracerebral hemorrhage (88), and LPS-induced neuroinflammation (89), AHR expression is upregulated in various brain-resident cells including microglia, but the exact contribution of microglial AHR signaling to the manifestation of disease symptoms remains undescribed. In globally AHR-deficient mice, microglial activation was enhanced in experimental models of autoimmune uveitis (90) and retinal degeneration (91), which is consistent with studies utilizing microbiota-associated AHR ligands (urolithin A and indoxyl sulfate) to attenuate proinflammatory markers associated with microglial activation (92, 93). However, recent reports demonstrate that microglial AHR signaling may have dichotomic pro- and anti-inflammatory effects. In one study, both genetic silencing of AHR and activation of AHR by AHR ligands (formylindolo[3,2-b]carbazole or 3-methylcholanthrene) dampened LPS-induced microglial activation (89). This aligns with reports demonstrating that AHR antagonism (CH223191 and 6,2′,4′-trimethoxyflavone) is protective during ischemic stroke by blocking microglial activation and preserving neurogenesis (87, 94), as well as reports suggesting that indoxyl sulfate can promote a neurotoxic environment in glial mixed cultures (95, 96). Moreover, conditional deletion of AHR in neural stem or progenitor cells during ischemic stroke was protective and dampened astrogliosis and microgliosis, whereas conditional deletion of AHR in microglia (93) and astrocytes (97) exacerbated EAE pathology. Together, these studies suggest that AHR signaling represents a pathway for microbiome-neuroimmune interactions that is dependent on the type of neuroinflammatory event, availability of different microbial AHR agonists, and potential contribution of other central and peripheral cell types that express AHR. Furthermore, the complexity of AHR signaling in the CNS demonstrates the importance of CNS-resident cell cross talk during neuroinflammation, of which microglia-astrocyte interactions play a prominent role.

3. ASTROCYTES

Astrocytes are tissue-resident stromal cells of the CNS that promote proper development and activity of the brain by providing structural support, synthesizing metabolites, modulating neurotransmission, and contributing immune-related functions (98). Although astrocytes are not the primary immune cells of the CNS, they can respond to immunomodulatory cytokines and regulate microglial activity by secreting additional cytokines that promote pro- and anti-inflammatory programs (99, 100). Under inflammatory conditions, reactive astrocytes coupled with microglial activation are major contributors to the overall neuroinflammatory milieu observed in many neurological disorders (101, 102). Additionally, astrocytes are important for the assembly of the BBB neurovascular unit and glia limitans of the glymphatic system. Therefore, astrocytes are poised to respond to altered central and systemic processes regulated by the microbiome such as AHR signaling, microglia function, and peripheral immunity (98).

In astrocytes, AHR signaling works in concert with the microbiome and neuroimmune landscape to regulate neuroinflammatory responses (**Figure 2*c***). Ampicillin treatment to eliminate AHR-producing microbes reduced AHR signaling in astrocytes and exacerbated EAE pathology, and this was associated with enhanced NF-κB activity and downstream transcription of proinflammatory response genes, which resulted in microglial activation (97). Through a similar mechanism, microglia-specific AHR signaling blocked astrocyte reactivity by increasing the ratio of transforming growth factor alpha (TGF-α) to vascular endothelial growth factor B (VEGF-B) engagement on astrocytic receptors ERBB1 and FLT1, respectively, further demonstrating the integral role

LysM: lysozyme M

of microglia-astrocyte interactions during CNS autoimmunity (93). Importantly, chronic lesions in MS patients exhibited a lower TGF-α:VEGF-B ratio, suggesting that dysregulated tryptophan metabolism and AHR signaling participate in MS pathology. This is consistent with primary human astrocyte cultures, where IPA inhibited LPS-induced inflammation (103) and TGF-α and VEGF-B suppressed and boosted, respectively, proinflammatory gene expression (93). Furthermore, the clinical use of potent small molecules that activate AHR, such as laquinimod, illustrates the therapeutic potential for harnessing microbial tryptophan metabolism to promote AHR signaling. In preclinical studies, laquinimod attenuated EAE symptoms and neuroinflammation by modulating systemic and central immunity (104, 105). In one study, wild-type bone marrow transplantation into AHR-deficient mice partially restored laquinimod efficacy (104) but was independent of LysM-expressing immune cells (105). Additionally, conditional deletion of AHR from astrocytes significantly, but not completely, blocked laquinimod effectiveness (105). Taken together, these studies indicate that global AHR signaling is required for optimal anti-inflammatory effects of AHR ligands during CNS autoimmunity. However, sequestering AHR signaling to the CNS, where it most potently inhibits EAE, may be necessary to avoid potential off-target effects.

Surprisingly, AHR expression is regulated by immunomodulators such as type I interferons, suggesting a role for AHR signaling during immune responses. Notably, IFN-β, which is the first-line therapy for MS, suppresses CNS inflammation by inducing AHR expression in astrocytes. In the absence of astrocytic AHR, IFN-β loses its therapeutic effects in EAE (97). In addition to type I interferons, IFN-γ (type II interferon) can also regulate astrocyte anti-inflammatory function in a microbiota-dependent manner. In one study, GF and antibiotic-treated mice displayed reduced IFN-γ expression by gut-derived meningeal natural killer (NK) cells, which subsequently reduced TNF-related apoptosis–inducing ligand (TRAIL) expression on astrocytes (28). Since TRAIL-DR5 (death receptor 5) interactions promote apoptosis of activated immune cells to prevent tissue damage in the periphery (106), it was suggested that $TRAIL^+$ astrocytes were responsible for eliminating $DR5^+CD4^+$ T cells during homeostasis and thereby limiting CNS-recruited effector T cells during EAE. As such, genetic silencing of IFN-γ receptor on astrocytes using lentiviral delivery exacerbated EAE pathology (28). However, this contrasts with a previous report of a study using a similar approach to silence the IFN-γ receptor on astrocytes, which attenuated EAE (107). Interestingly, the authors also reported that microglial IFN-γ signaling may be protective during EAE (107), suggesting an alternate mechanism for gut-derived $IFN\text{-}\gamma^+$ meningeal NK cells to mediate effects in the CNS.

While these initial studies highlight the ability of the gut microbiome to influence astrocyte biology through direct and indirect pathways, additional research is needed to test the reproducibility of these findings and to resolve the molecular and cellular mechanisms involved. This is particularly the case on the microbial end, as the identities of specific tryptophanase-expressing bacterial taxa responsible for promoting indole derivatives that impact brain AHR signaling remain unclear. In addition, exactly how the gut microbiome controls transcriptional programs in meningeal NK cells, which are originally derived from the gut, is yet unknown. Elucidating the molecular underpinnings of microbiome-astrocyte interactions will inform additional efforts to evaluate the ability of microbiome-based interventions to dampen astrocyte reactivity in the context of neurological disease.

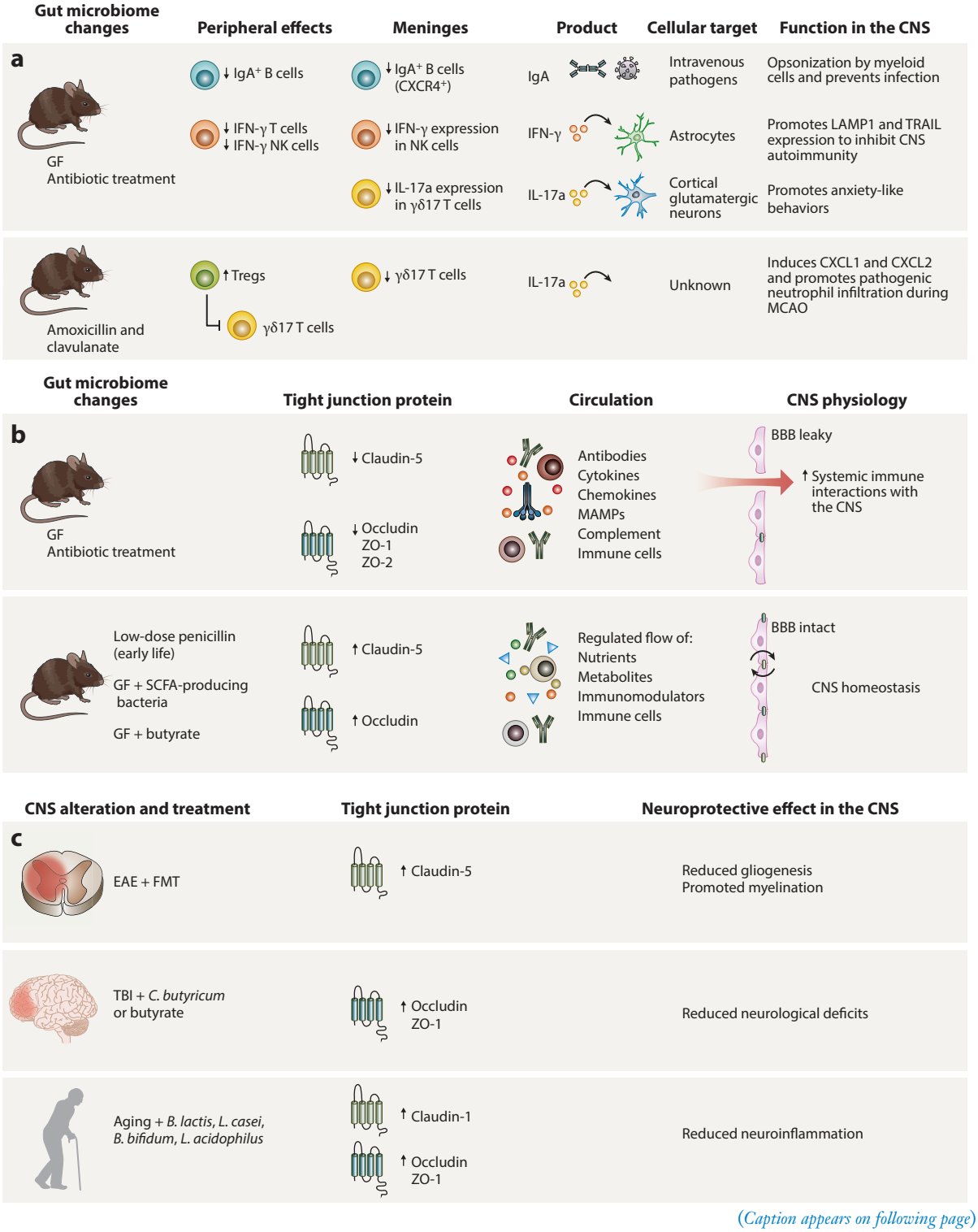
4. MENINGEAL IMMUNITY

The meninges are immunologically active barrier tissues that line the CNS and contribute key functions in immune surveillance, neuroinflammatory responses, and recovery from injury (108). Using high-dimensional cytometry on microdissected border regions (dural and subdural), recent

studies have characterized the vast heterogeneity of immune cells that exist in the meningeal compartment (109, 110). While it consists largely of macrophage subtypes, innate and adaptive immune cells such as dendritic cells (DCs), neutrophils, NK cells, T cells, B cells, and innate lymphoid cells (ILCs) are also observed. Residing within close proximity to the brain parenchyma, meningeal immune cells are capable of secreting a variety of pro- and anti-inflammatory cytokines that bind to receptors expressed on neurons and glia (108). For example, accumulation of T cell–derived IL-4 in the meninges is critical for skewing meningeal myeloid cells away from proinflammatory programs to promote cognitive function such as learning and memory (111). Furthermore, blocking immune cell trafficking in the periphery reduces overall cellular frequencies in the meninges, suggesting that peripheral immune cells replenish the meningeal immune compartment (108). Interestingly, one study employed confocal imaging and 3D reconstruction in healthy mouse and human samples to demonstrate that a small proportion of $CD4^+$ T cells cross beyond the glia limitans and leptomeninges to reside in close proximity to microglia and astrocytes (27). This suggests a mechanism for immune infiltration through the meninges. Taken together, resident and transient meningeal immune cells that respond to peripheral influences can directly impact the function of brain parenchymal cells.

Growing evidence implicates the gut microbiome as a key determinant of peripheral immune functions that impact the meningeal immune system (**Figure 3*a***). As previously mentioned, a recent study revealed that meningeal IFN-γ-producing NK cells are derived from the gastrointestinal tract, where they are programmed by microbiota-derived signals (28). These gut-licensed meningeal IFN-γ^+ NK cells promoted anti-inflammatory effects of astrocytes and limited CNS inflammation by inducing T cell apoptosis during EAE. In another study, microbiota deficiency reduced transcription of *Il17a* by meningeal γδ17 T cells, which modulated anxiety-like behaviors in mice (30). Consistent with this, antibiotic (amoxicillin and clavulanate) treatment to deplete select gut microbes shifted enteric and meningeal immune profiles, which led to protection from ischemic injury induced by middle cerebral artery occlusion (112). These effects were due at least in part to microbial induction of intestinal T regulatory cells (Tregs) and their downstream immunosuppression of peripheral γδ17 T cells that infiltrate the leptomeninges to induce neuroinflammation.

In addition to microbial modulation of cell-mediated immune responses in the meninges, there is some evidence that gut microbes impact meningeal humoral immune responses. Like in the intestine, where the microbiome guides the development of resident IgA^+ plasma cells (113), the dura mater adjacent to dural venous sinuses of the meninges harbors IgA^+ plasma cells that protect the CNS from intravenous pathogens and are also influenced by the gut microbiome (29). Compared to SPF controls, GF mice exhibit reduced meningeal IgA^+ plasma cells by ∼90%, but the level of meningeal IgA^+ plasma cells is restored by conventionalization with SPF microbiota or monocolonization with *Citrobacter rodentium* or segmented filamentous bacteria (SFB). Additionally, colonization of GF mice with healthy human microbiota restored meningeal IgA^+ plasma cells in a manner that was dependent upon the microbial diversity of the human donor. Sequencing the B cell receptors (BCRs) of intestinal and meningeal B cells and comparing them to germline sequences to test for BCR clonality and diversity revealed that over 20% of dural B cell clones arose from gut-educated B cells (29). Interestingly, IgA^+ plasma cells were found within the CNS parenchyma under chronic neuroinflammatory conditions such as MS, and gut-trained IL-10-producing IgA^+ plasma cells dampened neuroinflammation and reduced EAE pathology (114). Overall, the meninges and brain borders are emerging as critical sites for neuroimmune communication across the gut-brain axis, consisting of a diverse immune repertoire for which key functions are understudied. Technological advancements that enable targeted manipulation and tracking of meningeal immune cell subtypes are needed to selectively probe their influences on neurological



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

The microbiome regulates the CNS barrier tissues. (*a*) The meninges are immunologically active barrier tissues that line the CNS and are replenished by circulating immune cells that are trained by the gut microbiome. In GF and antibiotic-treated mice, the meninges exhibit limited capacity to elicit immune-related functions due to reduced cellular frequencies or inhibited expression of secreted products. Reduced frequencies of meningeal IgA^+ B cells can enhance susceptibility to intravenous pathogens that invade the CNS. Inhibited expression of IFN-γ by meningeal NK cells can lead to a reduced capacity for astrocytes to limit CNS autoimmunity through LAMP1- and TRAIL-mediated apoptosis of T cells. Reduced expression of IL-17a by meningeal γδ17 T cells can improve anxiety-like behaviors by limiting IL-17a signaling to cortical glutamatergic neurons. In addition, selective antibiotic treatment using amoxicillin and clavulanate to shift the composition of the microbiota can also impact meningeal γδ17 T cells. By enhancing peripheral Tregs that limit γδ17 T cell development, reduced IL-17a signaling and downstream CXCL1 and CXCL2 expression can improve symptoms associated with the experimental model of stroke (middle cerebral artery occlusion) by blocking pathogenic neutrophil infiltration into the CNS. (*b*) Tight junction proteins are essential for barrier tissues such as the BBB to function properly. GF mice reared without gut microbiota or treated with antibiotics exhibit increased BBB leakiness due to decreased expression of tight junction proteins (claudin-5, occludin, ZO-1, and ZO-2). Increased BBB permeability can boost the transfer of immunomodulatory blood components and immune cells, resulting in enhanced systemic immune interactions with the CNS. By supplementing GF mice with butyrate, or by enhancing SCFA-producing bacteria using low-dose penicillin early in life, or by colonizing GF mice with *Clostridium tyrobutyricum* or *Bacteroides thetaiotaomicron*, expression of claudin-5 and occludin was restored and resulted in increased BBB integrity that supported a healthy CNS. (*c*) Experimental models of CNS alterations such as EAE, TBI, and aging are often coupled with decreased expression of tight junction proteins and reduced BBB function. In the EAE model, transplantation with the SPF microbiota (fecal microbiota transplantation) increased claudin-5 expression and improved clinical symptoms by reducing gliosis and promoting myelination. In a mouse model for TBI, colonization with *Clostridium butyricum* or supplementation with butyrate increased occludin and ZO-1 expression and reduced neurological deficits associated with TBI. In a mouse model of aging, treatment with a consortium of *Bifidobacterium lactis*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus acidophilus* increased claudin-1, occludin, and ZO-1 expression, resulting in reduced neuroinflammation. Abbreviations: BBB, blood-brain barrier; CNS, central nervous system; CXCL1, chemokine ligand 1; EAE, experimental autoimmune encephalitis; FMT, fecal microbiota transplantation; GF, germ-free; LAMP1, lysosome-associated membrane protein 1; MAMP, microbe-associated molecular pattern; MCAO, middle cerebral artery occlusion; NK, natural killer; SCFA, short-chain fatty acid; SPF, specific-pathogen-free; TBI, traumatic brain injury; TRAIL, TNF-related apoptosis–inducing ligand; Treg, T regulatory cell; ZO-1, zonula occludens-1.

function. Further examination of the gut microbiome as a key regulator of meningeal immune function will inform efforts to target meningeal neuroimmune interactions to treat symptoms of neurological disease.

5. BLOOD-BRAIN BARRIER

Another barrier tissue that regulates neuroimmune interactions is the BBB. The BBB is a dynamic and highly regulated interface consisting of the cerebral microvascular endothelium, pericytes, neurons, astrocytes, and extracellular matrix (115). To limit paracellular diffusion of water-soluble substances while facilitating selective transport of nutrients and metabolites from the blood into the brain, the endothelium expresses a variety of tight junction proteins, solute carriers, and receptors (116). Under homeostatic conditions, the BBB plays an essential role in limiting neuroimmune interactions between the periphery and the brain by acting as a physical barrier against the influx of peripheral immune cells and components. Under pathological conditions where the BBB is compromised, enhanced barrier permeability can lead to neuroinflammation in response to infiltrating immune cells and proinflammatory signaling molecules. However, acute neuroinflammation may also be essential for tissue repair and recovery (117). Thus, the need to actively regulate BBB integrity and function in a context-dependent manner highlights the importance of identifying molecular regulators of BBB-mediated neuroimmune interactions.

The gut microbiome regulates the development and function of the intestinal epithelial barrier, raising the question of whether it also impacts other mucosal and interorgan barriers including the BBB (118, 119) (**Figure 3*b***). In one study that compared BBB permeability in GF versus SPF mice, elevated levels of intravenously injected probes were observed in the brain parenchyma of

GF mice, which correlated with diminished expression of the tight junction proteins occludin and claudin-5 (120). These markers of increased BBB permeability in GF mice were prevented by conventionalization with an SPF microbiota, colonization with the SCFA-producing bacteria (*Clostridium tyrobutyricum* or *Bacteroides thetaiotaomicron*), or supplementation with butyrate. Similarly, antibiotic treatment in mice (bacitracin, neomycin, natamycin, meropenem, and vancomycin) and rhesus monkeys (amoxicillin and clavulanic acid) also resulted in reduced BBB integrity, which was correlated with increases of the phylum *Proteobacteria* and decreases in SCFA-producing bacteria (*Phascolarctobacterium*, *Subdoligranulum*, *Faecalibacterium*, *Blautia*, *Roseburia*, *Ruminococcus*, *Coprococcus*, *Dorea*, and *Anaerostipes*) of the phylum *Firmicutes* (121, 122). This aligns with another study where low-dose penicillin early in life increased *Firmicutes* and *Proteobacteria* in juvenile mice and also enhanced BBB integrity by promoting expression of occludin and claudin-5 (123). These effects may be mediated by direct signaling of SCFAs onto endothelial cells, as treating the human brain endothelial cell line (hCMEC/D3) with propionate inhibited inflammatory and oxidative stress pathways while enhancing efflux transporter expression (124). However, more research is needed to test this hypothesis in vivo.

In addition to studies examining microbial regulation of BBB permeability during homeostasis, several studies have evaluated microbial effects on BBB integrity in models of neurological disease (**Figure 3*c***). In the EAE model of MS, transplantation with SPF microbiota rectified shifts in microbiota composition and reduced disease severity. These changes correlated with improved BBB function as measured by decreases in peripheral dye diffusion into the brain parenchyma and enhanced expression of claudin-5 (125). In a genetic model for hypertensive stroke in rats, cross fostering with normotensive controls revealed that passive gut microbiota transfer from controls can promote BBB integrity, as measured by reduced IgG diffusion, and reverse stroke-related phenotypes in susceptible mice (126). In an experimental model of traumatic brain injury, treatment with *C. butyricum* and butyrate promoted BBB integrity by reversing decline in occludin and zonula occludens-1 (127, 128). Moreover, a few studies have reported that treatment with a cocktail of *B. animalis lactis*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *L. acidophilus* or a cocktail of *Bifidobacterium longum*, *Lactobacillus bulgaricus*, and *Streptococcus thermophiles* resulted in reduced inflammation, improved BBB integrity, and increased memory behavior in mouse models of aging and postoperative cognitive dysfunction (129, 130). Although the molecular mechanisms remain unclear, improved BBB integrity is thought to limit systemic interaction of peripherally derived soluble molecules such as antibodies, cytokines, chemokines, microbe-associated molecular patterns, microbial metabolites, and complement that can augment neuroinflammatory responses. In addition, immune cell trafficking is also regulated by the BBB and is a major pathway for peripheral immune interactions with the CNS.

A continued debate within the field of neuroimmunology is whether peripheral immune cells infiltrate into the brain parenchyma in steady state. Resolution of this question is made challenging due at least in part to a lack of definitive surface markers that can differentiate between brain parenchymal and peripheral immune cells, as well as the potential contamination of brain preparations by immune-rich tissues such as the blood, meninges, and choroid plexus. However, recent studies using high-dimensional cytometry coupled with intravenous labeling of peripheral immune cells suggest that nonmicroglial immune cells are present in the brain parenchyma at very low frequencies (27, 131). Importantly, elevated levels of $CD4^+$ T cells are observed in the CNS tissue during gestation and aging, suggesting their involvement during neurodevelopment and age-related neurodegeneration (27). Nevertheless, more studies are needed to confirm the presence of different immune subsets within the brain parenchyma as well as the full dynamics of immune infiltration through different barrier tissues. What is widely accepted is that under pathological conditions with a perturbed BBB, immune cells actively infiltrate the CNS (132, 133),

though the consequence of immune infiltration is dependent on the immune subset present and the associated CNS state. Since the microbiome can regulate both peripheral immune cell physiology and BBB function, the ability of the microbiome to impact CNS immune cell infiltration could be due to altering immune cell function and/or BBB permeability.

In a recent study describing CNS immune cell dynamics after a three-week course of antibiotics (metronidazole, vancomycin, neomycin, and ampicillin) administered by daily gavage to induce dysbiosis in young adult mice, only B cell frequencies were slightly increased (from 2.6% to 4.3%) in the antibiotic-treated group (134). However, aged mice treated with antibiotics were enriched for $CD8^+$ T cells and $Ly6C^{hi}$ monocytes, while exhibiting decreases in ILCs and $Ly6C^{lo}$ monocytes. Since aging is associated with altered BBB function (135), this study suggests that antibiotic-induced dysbiosis can alter immune composition in the brain, which is further augmented in the context of CNS disease. In another study where a more intensive course of antibiotics was used (ampicillin + sulbactam, vancomycin, ciprofloxacin, imipenem + cilastatin, and metronidazole, seven weeks ad libitum), the authors observed that $Ly6C^{hi}$ monocytes in the brain were significantly reduced, which was associated with reduced hippocampal neurogenesis. These effects were reversed after restoring the gut microbiota by fecal microbiota transplantation, treatment with the probiotic VSL#3, or adoptive transfer of $Ly6C^{hi}$ monocytes (136). This aligns with a recent report on traumatic brain injury demonstrating that antibiotic pretreatment exacerbated neuronal death, microgliosis, and fear memory behavior, with no differences in markers of BBB integrity despite reduced brain infiltration of $Ly6C^{hi}$ monocyte and T cells (137). These reports suggest a BBB-independent pathway for neuroprotective immune infiltration regulated by the gut microbiome. However, this contrasts with another study where dysbiosis induced by antibiotics (bacitracin, neomycin, natamycin, meropenem, and vancomycin) in mice enhanced BBB breakdown characterized by reduction in zonula occludens-1/2 and occludin, and simultaneously augmented monocyte infiltration in a CCR2-dependent manner (121). This aligns with another study where probiotic treatment (VSL#3) dampened monocyte recruitment in a mouse model of inflammation-associated sickness behavior (138). While these studies provide initial evidence that altering the gut microbiome may impact features of the BBB, further experiments that apply more rigorous methods of investigating BBB integrity and immune infiltration are needed to assess how perturbations in the gut microbiome affect BBB structure and function under homeostasis and disease.

VSL#3: mix of *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus delbrueckii* subsp. *Bulgaricus*

CCR: C-C chemokine receptor

6. MICROBIOTA-DEPENDENT T CELL INTERACTIONS IN MULTIPLE SCLEROSIS

MS is a classic example wherein loss of BBB integrity, peripheral immune activation and infiltration, gliosis, and T cell–dependent demyelination are pathological hallmarks of disease. MS is a heterogeneous disorder, associated with more than 100 predisposing genetic variants as well as environmental factors such as vitamin D deficiency, circadian disruption, viral infections, and more recently, dysbiosis of the intestinal microbiota (139). In particular, lower relative abundances of *Prevotella*, *Faecalibacterium prausnitzii*, *Bacteroides coprophilus*, and *Bacteroides fragilis* and higher relative abundances of *Methanobrevibacter* and *Akkermansia muciniphila* were observed in MS patients compared to healthy controls (140). Transplantation of the fecal microbiota from MS patients into GF mice exacerbated MS-associated pathology in response to EAE, which suggests a role for the gut microbiome in the manifestation of symptoms of MS (141). These detrimental effects of the microbiome were correlated with reduction in IL-10 production by Tregs (142), implicating dysfunctional Tregs with reduced capacity to inhibit pathogenic neuroinflammation induced by proinflammatory T helper type 1 (Th1) and Th17 cells. Indeed, MS patients exhibit

CXCR3: C-X-C motif chemokine receptor 3

elevated levels of Th17 cells in the CSF and intestines (143), as well as IL-17a in the CSF and serum (144). Interestingly, transplantation of SPF microbiota into the EAE mouse model attenuated EAE symptoms (125), suggesting that chronic dysbiosis may drive clinical symptoms and that restoring the composition of the gut microbiota may alleviate MS-related symptoms.

Several studies also suggest that the gut microbiome is necessary to trigger myelin autoantigen recognition to exacerbate autoimmune demyelination (145, 146). Induction of EAE in GF or antibiotic-pretreated mice led to significantly delayed disease onset and reduced disease severity, which were correlated with reduced Th17 cells and IL-17a signaling relative to SPF controls (145, 147–149). However, antibiotic treatment for two weeks during active disease did not alleviate clinical symptoms (150), suggesting a critical role of the gut microbiome during disease onset. Th17 cells develop primarily in the small intestinal lamina propria and require continuous microbiota-derived signals for maintenance (151, 152). One of these signals can be derived from SFB. Monocolonization with SFB restored Th17 levels through classical MHC-II antigen-presentation mechanisms by intestinal DCs (152, 153). Accordingly, restoring Th17 development in GF mice by gut colonization with SFB enhanced susceptibility to EAE (148).

Conversely, restoring Treg development by gut colonization with *B. fragilis* expanded protection against EAE (154). The capacity for *B. fragilis* to drive Treg development involves the expression of polysaccharide A (PSA), as gut colonization with PSA-deficient *B. fragilis* failed to induce Tregs (155) and reestablished EAE susceptibility (154). Consistent with this, administration of purified PSA promoted Treg development and protected mice from EAE (154). Subsequent mechanistic studies suggest that PSA signals to TLR2 (155) expressed on enteric $CD103^+$ DCs (156) and induces $CD39^+$ Treg development in the mesenteric lymph nodes. Interestingly, CD39 is a marker coregulated by receptors of enhanced migratory potential (CCR5, CCR6, and CXCR3) to CNS tissues, which results in anti-inflammatory pathways in the CNS and draining cervical lymph nodes (157, 158). This aligns with studies that employed oral treatment with *Prevotella histicola* (another underrepresented microbe in MS patients) every other day, which restored microbiota composition to pre-EAE conditions and skewed $CD4^+$ T cell maturation programs toward Tregs and away from Th1 and Th17 cells (159, 160). Surprisingly, expansion of *A. muciniphila* (an overrepresented microbe in MS) after microRNA-30d regulation of β-galactosidase also conferred protection by promoting Tregs (161). Although *A. muciniphila* can promote Th1 programs in vitro (142), multiple reports indicate that this microbe can also exert anti-inflammatory properties including Treg differentiation in vivo (162, 163).

SCFAs are implicated in neuroinflammatory processes such as BBB permeability and microglia-mediated inflammation, which are key characteristics of MS pathology. In MS patients, serum levels of SCFAs are decreased, suggesting a role for SCFAs in MS pathogenesis (164–166). Several types of clostridia are known to promote Treg development in the colon (167) through SCFAs, and gut colonization with 17 human gut–derived clostridia strains or *C. butyricum* alone improved the clinical outcome of EAE (168, 169). Interestingly, supplementation with SCFAs such as butyrate and propionate promoted Treg differentiation in vivo (170, 171); however, an in vitro study suggests that SCFA supplementation can also skew naive T cell differentiation toward Th1 and Th17 cells depending on the overall immunological milieu (172). T cells are not known to express SCFA receptors, but they can be modulated by SCFAs directly through epigenetic and immunometabolic reprogramming (172), as well as broad indirect immunomodulatory effects associated with SCFAs. In a recent study, MS patients displayed reduced propionic acid (PA) in serum and stool samples, which was associated with depleted SCFA-producing *Butyricimonas*. Supplementation with PA for 14 days shifted the Treg and Th17 balance to a more regulatory phenotype in the blood and enhanced the suppressive capacity of isolated Tregs in vitro. Importantly, long-term PA supplementation reduced overall disease progression as assessed by relapse

rate and subcortical gray matter volume in MS patients (173). Altogether, these findings implicate the gut microbiome and microbiome-derived metabolites in regulating neuroimmune processes that impact risk for MS.

7. CONCLUDING REMARKS

The gut-neuroimmune axis represents a major pathway for the gut microbiome to influence the CNS. The microbiome is critical for the maturation and function of the resident immune cells and glia in the brain, which help to regulate core processes, such as neurogenesis and neurotransmission, that influence a variety of host behaviors. The gut microbiome also regulates brain barrier tissues. For example, it regulates the integrity of the BBB and the repertoire of leukocytes in the meningeal immune compartment, which together serve as the interface between the CNS and systemic immunity. In the periphery, the gut microbiome is crucial for immune development and function, not only locally in the intestine but systemically as well. Collectively, these findings establish the gut microbiome as an important modifier of neuroimmune interactions within the brain itself and between the brain and periphery. While recent studies have been foundational in identifying roles for the gut microbiome in neuroimmune modulation, molecular signaling mechanisms by which the gut microbiome impacts host neuroimmune homeostasis and responses to challenge remain unclear. A few studies highlight the ability of microbiome-dependent metabolites such as SCFAs in guiding microglial development and peripheral T cell differentiation in ways that influence the severity of neuroinflammation in the brain. Additional investigation is needed to clearly define the select microbes, microbial factors, their host cell targets, and their signal transduction cascades that ultimately determine neuroimmune functions that are integral to brain activity and behavior. Ultimately, studying microbiome-neuroimmune interactions will reveal fundamental relationships between the gut and brain and inform the development of novel therapeutics for the treatment of a variety of neurological diseases.

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