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Communicating systems in the body: how microbiota and microglia cooperate

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Summary

Microglia are tissue macrophages of the central nervous system (CNS). Their key tasks are immune surveillance as well as responding to infections or other pathological states such as neurological diseases or injury. In recent years it has been discovered that microglia are additionally crucial for the maintenance of brain homeostasis during development and adulthood by adjusting the neuronal network and phagocytosing neuronal debris. Microglia persist in the CNS throughout the life of the organism and self-renew without engraftment of bone-marrow-derived cells. Until recently it remained unknown what controls their maturation and activation under homeostatic conditions. In this review we discuss new aspects of the interaction between host microbiota and brain function with special focus on the brain-resident innate immune cells, the microglia.

Keywords: gut–brain axis; macrophage; microbiota; microglia; short-chain fatty acids.

Introduction

Man and mouse are occupied by trillions of commensal bacteria that co-exist for potential mutual benefits. This host microbiota colonizes mainly the gut and the skin as well as several mucosal cavities (nasal, oral, vaginal and pulmonary). The microbiota is a necessary element for the synthesis of several vitamins (e.g. vitamins K and B), it provides energy for the host in terms of short-chain fatty acids (SCFAs) by fermentation of otherwise indigestible carbohydrates and fibres, and is involved in the metabolism of bile acids, sterols and xenobiotics.[1] In fact, there is some marked evidence that a complex composition and abundance of the gut microbiota is an essential element for the maintenance of the host's health. Altered microbial compositions have been linked to several human diseases,[2] including cardiovascular disease,[3] inflammatory bowel disease,[4] and type 1 and type 2 diabetes.[5,6] Fairly unexpectedly, multifaceted interactions between the endogenous microbiota and the hosts' central nervous system (CNS) were discovered in the past decade. The influence of the host microbiota on CNS functions have been studied using several experimental approaches, including germ-free (GF) animals[7] that have never been exposed to bacteria and viruses from embryogenesis throughout their life, manipulation or eradication of the gut bacteria with antibiotics at an adult stage,[8–10] transient colonization,[11] defined limited colonization[12] or faecal microbial transplantation.[9] Over the last few years, several new publications have defined the impact of the gut microbiota on the innate and adaptive immune system.[13]

In this review we highlight and discuss recent findings concerning the environmental factor 'host microbiota' and its influences on the CNS with special focus on its resident tissue macrophages, the microglia.

Gut–brain axis: an important connection

First, it has been described that the microbiota affects several aspects of behaviour in mice and in humans in general,[14] for example social interaction,[15] stress responsiveness,[16] depression-like behaviour,[17,18] anxiety-like behaviour[19–23] and nociceptive responses.[24] Interestingly, it was observed that the colonization of GF BALB/c mice with a strain-specific microbiota from conventionally (specific pathogen-free; SPF) raised NIH Swiss mice increased exploratory behaviour and hippocampal levels of brain-derived neurotropic factor; however, GF NIH Swiss mice with a microbiota derived from conventionally housed BALB/c mice had the opposite effect and showed reduced exploratory behaviour,[9] indicating that

microbiota-derived signals can shape host brain function in terms of behaviour. It was shown recently that the host microbiota has an effect on adult hippocampal neurogenesis leading to more immature neurons in young GF mice compared with conventionally colonized control mice, whereas the mechanisms and potential hippocampus-related behavioural changes are only poorly understood.[25,26]

In general, the mechanisms by which the microbiota influences the host are manifold and complex (Fig. 1).[27] Commensal microbiota can either have a direct impact on the production of metabolic precursors like tryptophan and neurotransmitters (e.g. serotonin, noradrenaline or dopamine) or produce active mediators like SCFAs, which are commensal anaerobic bacteria-derived fermentation products.[14,21,28] SCFAs are also known to inhibit histone deacetylases and thereby result in epigenetic changes.[29] Further, the gut is connected to the CNS through the vagus nerve, enabling a direct communication through a neurochemical pathway.[30] A third pathway linking the microbiota and the CNS is the release of microbial-associated molecular patterns (MAMPs) in the gut.[14] MAMPs such as bacterial lipoproteins, double-stranded RNA, lipopolysaccharide and many others are recognized by different receptors, mostly belonging to the Toll-like receptors (TLRs) in association with the myeloid differentiation primary response gene, MyD88, signalling pathway, which is known to be involved in several aspects of the host immune response.[31] A recently proposed mechanism as to how the host may control the microbiome is host epithelial derived miRNA, which is proposed to control bacterial gene expression.[32]

It was suggested by Braniste and colleagues that blood–brain barrier (BBB) integrity in the frontal cortex, hippocampus and striatum is influenced by gut microbiota.[33]

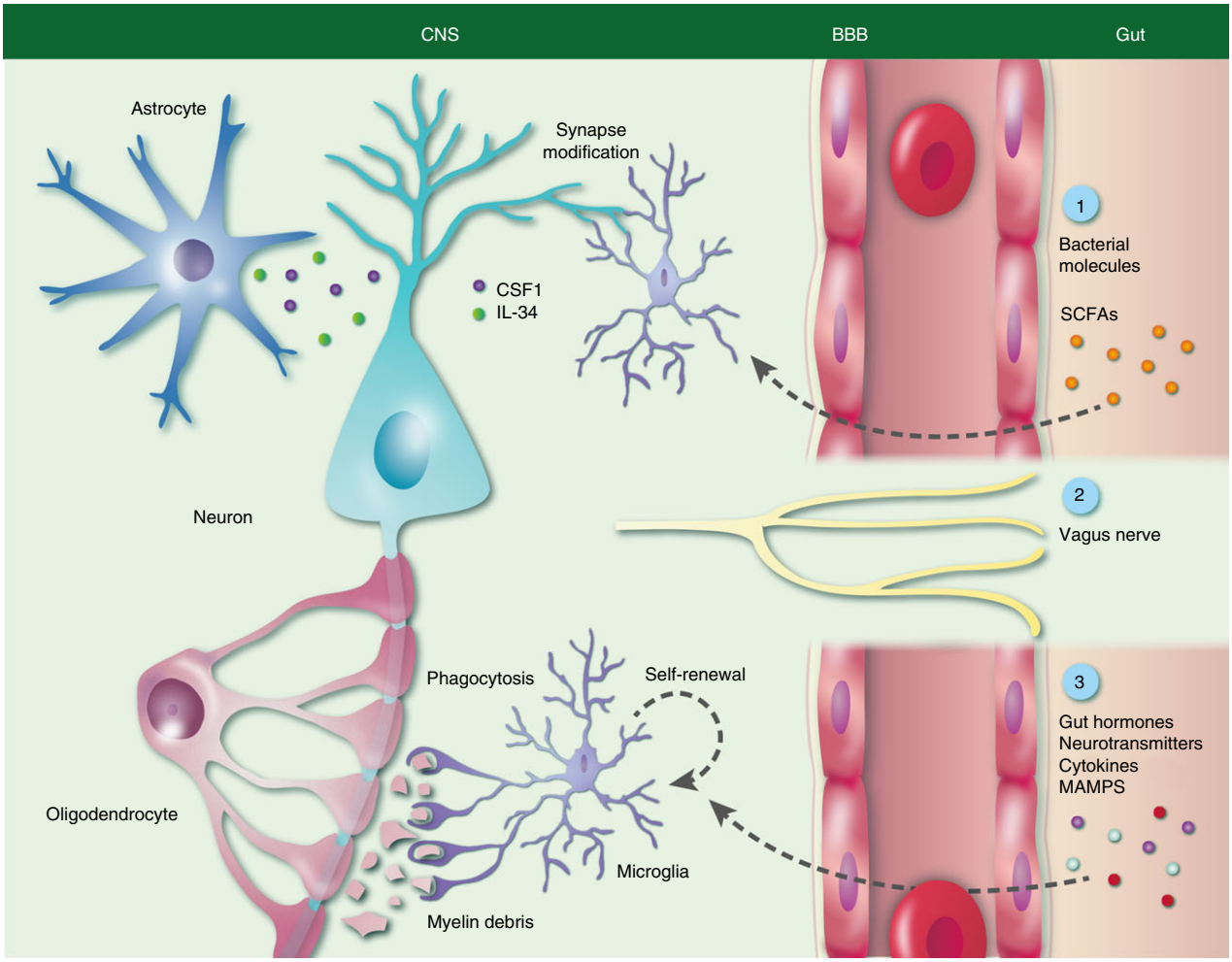

Figure 1. Microglia functions under homeostatic conditions and its modulation by host microbiota. During adulthood cortical microglia survey mature neurons and are involved in learning-induced dendritic spine formation and nourish neurons. Furthermore, colony-stimulating factor 1 (CSF-1), interleukin-34 (IL-34) and short-chain fatty acids (SCFAs) are important components for microglia function and maturation. Direct signalling via the vagus nerve and other molecules like gut hormones, neurotransmitters, cytokines or microbial-associated molecular patterns (MAMPs) may also affect microglia.

They provided evidence that BBB permeability is increased in GF mice already during embryonal development – visible from E16.5 until adulthood – caused by reduced tight junction protein expression. Further, the impaired BBB integrity in GF mice could be rescued by recolonization with a complex intestinal microbiota or the application of butyrate. Pericyte numbers seem not to be altered but a more detailed analysis of all components of the BBB, namely endothelial cells, pericytes and astrocytes, including their function and mechanism by which SCFAs mediate BBB integrity remain to be investigated. Intriguingly, antibiotic-induced alteration of the gut microbiota in adult mice also leads to decreased tight junction expression in the hippocampus, whereas in the amygdala an increased expression of tight junction proteins was observed,[34] indicating potential region-specific differences. Similar findings could be determined regarding the blood–testis barrier, as GF mice feature reduced expression of adherens and tight junctions proteins and increased blood–testis barrier permeability,[35] suggesting the general involvement of the gut microbiota in the regulation of the blood–tissue barriers.[36]

Recent evidence demonstrated myelination being influenced by the microbiota under steady-state conditions in some specific anatomical brain regions like the prefrontal cortex, but the (patho-)physiological consequences are not yet examined.[37]

The impact of microbiota on neuropsychiatric disorders

The findings described above implicate that under homeostatic conditions brain function and architecture are highly influenced by gut microbiota. In addition, it has only recently been appreciated that commensals obviously influence CNS diseases as well.

A crucial role of the gut microbiota could be demonstrated in a relapsing–remitting (RR) mouse model (transgenic SJL/J anti-MOG TCR transgenic RR mice) whereby mice spontaneously develop features similar to those of human multiple sclerosis.[38] Mice that are kept under normal SPF conditions spontaneously develop disease hallmarks mediated by triggering peripheral immune processes driven by myelin-specific $CD4^+$ T cells, whereas mice housed in a sterile environment are nearly protected.[38] It remains open, whether the microbiota influences directly the pathological process or the generally impaired immune system of GF mice is contributing to this observation.

Autism spectrum disorder (ASD) is a frequent neuropsychiatric disorder that is supposed to be caused by multifactorial aetiology.[39] Affected people suffer from impaired sociability and communication, as well as frequent repetitive and stereotyped behaviours. Some potential risk factors for ASD are linked to the gut microbiota, such as perinatal infection, hospitalization or early antibiotic exposure.[40] It was observed that SCFAs may trigger ASD in rodents[41] and, supporting this finding, it has been described that children with ASD display increased faecal SCFA concentrations;[42] however, the precise mechanism is not fully understood.[40] A probiotic treatment with the human commensal *Bacteroides fragilis* was shown to ameliorate ASD-linked behaviour in mice by normalizing 4-ethylphenylsulphate levels.[43]

The presence of gut microbiota is also pathophysiologically relevant for Parkinson's disease as patients displayed an altered composition of the gut microbiota including reduced abundance of *Prevotellaceae* and increased abundance of *Enterobacteriaceae* compared with matched controls.[44] *Prevotellaceae* are known to provide potential beneficial SCFAs, thiamine and folate, which are found to be decreased in patients with Parkinson's disease.[44] The relative abundance of *Enterobacteriaceae* was associated with the severity of motor symptoms.[44] Beyond these correlative data it is proposed that various α-synuclein forms can spread from the gut to the brain by microtubule-associated transport via the vagus nerve, suggesting that this pathway might be used for the transport of misfolded proteins as well. However, the detailed mechanism has yet to be determined.

Further, the gut microbiota may play a crucial role in the pathogenesis of other neurodegenerative diseases, such as Alzheimer's disease and amyotrophic lateral sclerosis[45,46] because it is known that neurons,[21] astrocytes[47] and microglia[48] are shaped by the host microbiota and/or their metabolites, and microglial activation accompanied by the production of potential neurotoxic factors are common features of these diseases.[49] Hence, it is likely that gut bacteria affect a wide range of neurological disorders, whereby it should be considered generally whether an observed altered composition of the microbiome is cause or consequence. A functional link between ascertained bacterial species and findings should be determined.

Factors modulating fate and function of tissue macrophages in the CNS

Different types of macrophages in the CNS are related to separate compartments. The non-parenchymal macrophages comprise meningeal, perivascular and choroid plexus macrophages.[49,50] Under steady-state conditions, microglia are the only myeloid cell type distributed throughout the CNS parenchyma with region-specific varying numbers and with a wide range of morphologies.[51,52] These CNS immune cells belong to the large family of mononuclear phagocytes, which further includes peripheral tissue-specific macrophages, different subsets of dendritic cells and circulating monocytes.[53,54] In mice all mentioned CNS macrophages – except choroid plexus

macrophages – are derived from prenatal sources such as the yolk sac and self-renew throughout life.[55–59] Specifically, microglia originate in a c-MYB-independent but Runt-related transcription factor 1 (RUNX1), interferon regulatory factor (IRF8) and PU.1-dependent manner from $CD45^-$ $Tie2^+$ $c\text{-}KIT^+$ $F4/80^-CX_3CR1^-$ erythromyeloid progenitors of the yolk sac during primitive haematopoiesis.[55,56,60,61] It is convincingly assumed that during embryonal development the forming CNS is uncoupled by the closing BBB starting from E14.5,[62,63] leading to a defined and restricted environment presumably excluding substantial immigration of peripheral myeloid cells from the definitive haematopoiesis.[50,55,56,60,61] However, in a zebra fish model it was recently described by using high temporal–spatial resolution fate mapping that multiple sources may give rise to adult microglia.[64]

It is well known that under steady-state conditions, microglia as innate immune cells actively survey their surrounding environment with their highly motile processes, as shown using *in vivo* time lapse two-photon-imaging of *Cx3cr1*^{GFP} mice.[65,66] In addition, several studies revealed that microglia are critical for maintaining tissue homeostasis during development and adulthood.[49,50,67] In particular, microglia carry out distinct region-specific tasks such as neuronal circuit development and modification of synapses in the cortex or phagocytosis of myelin debris in the white matter (Fig. 1).[68–73] Various pathological stimuli (such as bacterial or viral infection) or diseases (neuroinflammatory, neurodegenerative, neuro-oncological or neuropsychiatric) can cause rapid recruitment of microglia to sites of injury, resulting in a resident innate immune response.[50,74,75] In general, these microglial responses include characteristic macrophage functions such as phagocytosis, antigen presentation and the production and release of immunomodulatory factors.

Compared with their tissue relatives in peripheral organs, microglia exhibit a specific gene expression profile and a distinct chromatin state.[76–80] In two large-scale studies, Lavin *et al.*[77] and Gosselin *et al.*[78] demonstrated that gene expression patterns and epigenetic identities of macrophages are only partly shaped during development, but rather are shaped also by the local microenvironment, which is capable of reprogramming the genetic imprints, suggesting a remaining plasticity. In fact, Gosselin *et al.*[78] could show that freshly isolated microglia and large peritoneal macrophages lose their specific gene expression patterns to a great extent *in vitro*. Furthermore, when large peritoneal macrophages were incubated with transforming growth factor-β_1 (TGF-β_1), which is important for microglial development and homeostasis,[79] approximately 50% of genes usually expressed by microglia *in vivo* could be induced in these macrophages, indicating that environmental factors in the CNS have a determining influence to induce a microglia phenotype.[78] Environmental signals that further shape microglia properties are certainly not limited to TGF-β_1 and growth factors like colony-stimulating factor 1 (CSF-1) and interleukin-34 (IL-34) but rather include other factors that are essential for maintaining homeostasis of these unique CNS-resident tissue macrophages.[81]

How host microbiota controls microglia maturation and immune function

It was recently shown that host microbiota is also an essential environmental factor shaping the brain innate immune system, in particular the maturation and function of microglia.[48] Non-colonized young adult GF mice exhibit stunted microglia under homeostatic conditions compared with microglia from mice bred under common SPF settings (Fig. 2).[48] RNA-sequencing analysis of FACS-sorted $CD11b^+$ $CD45^{lo}$ microglia from GF and SPF animals revealed marked differences regarding their cell differentiation, transcription factor binding and proliferation. Microglia expressed reduced mRNA levels for several activation markers under GF conditions (e.g. *Il1α*, *Stat1*, *Jak3*, *B2m*), whereas transcripts either for inhibitors of transcription, such as *Nfkbiα* (encodes IκBα), or the essential microglia transcription and survival factor *Sfpi1*, encoding PU.1, were up-regulated. Further, the c-*fms* gene, encoding for the integral tyrosine kinase transmembrane receptor CSF-1 receptor (CSF-1R), which is elementary for microglial proliferation, maturation, function and survival,[82–85] was found to be increased in GF microglia in addition to other genes controlling proliferation, cell cycle and apoptosis (e.g. *Cdk9*, *Ccnd3*, *Bcl2*). There is also evidence that microglia from CSF-$1R^{op/op}$ mice display reduced numbers of processes.[86] Notably, it is known that DNA damage-inducible transcript 4 (DDIT4, also known as REDD1) is induced by energy stress and essentially influencing cell growth, proliferation and survival via inhibition of the activity of the mammalian target of rapamycin complex (mTORC) 1 and activation of mTORC2.[87–89] Germ-free microglia express higher levels of *Ddit4* compared with SPF mice. Accordingly, a salient hyper-ramified morphology as well as an increased microglial density were observed in different brain regions in GF mice accompanied by an increased proliferation rate. In contrast, cell numbers of neuroectodermal cells, such as astrocytes, oligodendrocytes and neurons, were not altered. Although it was recently discovered that some brain regions show a leaky BBB,[33] no lymphocytic infiltrates were detectable in GF brains.[48]

It is known that microglial numbers increase in the first 2 weeks after birth and then start to decline in the third postnatal week.[68,90] A higher microglia density therefore indicates an immature microglial attribute in GF mice. Remarkably, these findings contrast data from bone-marrow-derived haematopoietic cells, such as peripheral tissue macrophages, neutrophils and

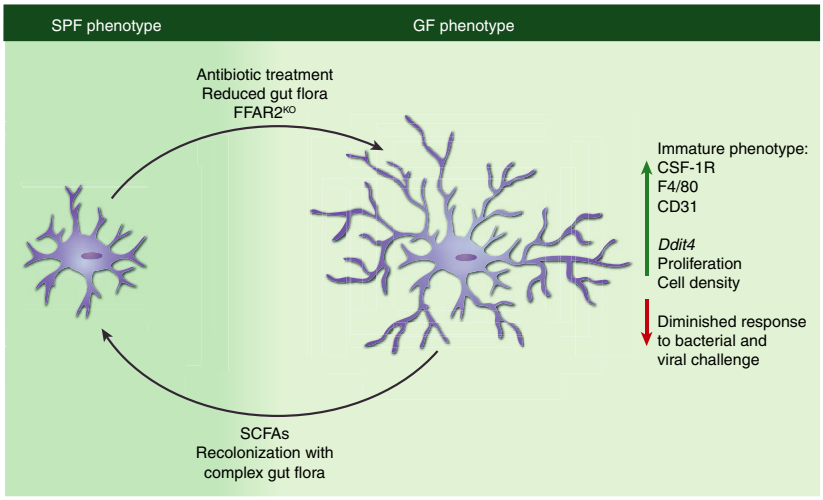

Figure 2. Immature microglia phenotype under germ-free (GF) conditions. Mice raised under conventional (specific pathogen-free; SPF) conditions exhibit mature microglia with ramified morphology that respect territorial boundaries (SPF; left). Microglia from GF mice (right) display a hyper-ramified morphology, increased cell density accompanied by increased proliferation rate, up-regulation of colony-stimulating factor 1 receptor (CSF-1R), F4/80 and CD31 and a changed gene expression profile more related to that of immature cells. Eradication of host microbiota with antibiotics, the presence of a gut flora with strongly limited diversity or FFAR2-deficiency in the host are causing a microglial phenotype similar to that observed in GF mice, whereas recolonization with a highly diverse gut flora or the application of SCFA is able to re-establish a mature microglial phenotype.

monocytes, which are most likely found to be diminished in GF mice (Table 1).[10,91,92] Notably, reduced cell numbers for granulocytes, monocytes, granulocyte–monocyte progenitors and haematopoietic stem/progenitor cells (lineage − Sca1$^+$ c-kit^{hi} cells) corresponds to host microbiota complexity.[93] F4/80^{hi} and CD11b$^+$ Gr1$^-$ F4/80^{lo} splenic and bone marrow macrophages are described to be reduced in GF mice and in broad-spectrum antibiotic (ABX)-treated SPF mice.[91] Moreover, it was shown that SCFAs and MAMPs were not sufficient to restore splenic macrophage numbers. Instead, only recolonization with a complex flora was able to rescue macrophage numbers and reduce bacterial burden upon *Listeria monocytogenes* infection in the spleen, indicating a restored proper innate immune response against infection.[91] Further, Kupffer cells, the tissue macrophages of the liver, were reduced in GF and ABX-treated Swiss Webster male mice[94] and C57BL/6 mice, respectively.[91] Interestingly, Kupffer cells exhibited a reduced MHC class II expression and an increased phagocytic capacity in GF animals.[89] These ABX-treated mice displayed mitigated liver damage in an ischaemia–reperfusion injury model.[94] Contradictory data were recently published by Zhang *et al*. that did not find changed Kupffer cell numbers in the liver of ABX-treated C57BL/6 mice.[92] In addition, dermal macrophage populations were found to be decreased in GF mice[95].

The often observed decrease in cell numbers might be in some cases caused by the fact that growth factors like CSF-1 or CSF-2 are reduced in the respective compartments or organs of GF animals (Table 1).[10,96,97] However, brains of GF animals showed slightly increased *Csf1* mRNA levels in the cortex and cerebellum whereas the second known ligand for the CSF-1R *Il34* was not affected by gut microbiota.[98,99]

One common feature of microglia is that each cell covers its own defined territory.[100] In contrast, GF microglia frequently crossed the neighbouring microglial territories and touched adjacent microglia, indicating a disturbed microglial network. In FACS analysis microglia from GF mice showed an up-regulation of the surface markers CSF-1R, F4/80 and CD31, which are known to be down-regulated in mature adult microglia.[55] In addition, the expression of the maturation marker *Apoe* was increased in GF microglia, usually declining during microglial maturation.[79] Another crucial factor for the homeostasis of microglia is TGF-β as *Tgfβ1*-deficient mice show reduced microglia numbers in the CNS.[79] Interestingly, we detected – in line with the already mentioned augmented microglial density – an increased *Tgfβ1* expression in microglia from GF-housed mice.[79]

To further investigate the bacterial factors mediating the maturation of microglia, mice harbouring a strongly reduced microbiota with a defined subset of known bacterial species, so-called tri-colonized altered Schaedler flora (ASF) mice, were analysed. These mice harboured only three bacterial strains, namely *Bacteroides distasonis* (strain ASF 519), *Lactobacillus salivarius* (strain ASF 361) and *Clostridium* cluster XIV (ASF 356),[101] instead of the 400–1000 strains usually found in SPF mice.[101] Although SPF controls and tri-colonized ASF mice exhibited the same bacterial loads, microglia from the tri-colonized

Table 1. Effect of host microbiota on cell density in different innate immune cell populations

Immune cell type	Cell density (GF)	Cell density (ABX)	Change of growth factors	References
Microglia	↑	No change	CSF-1: Slightly ↑ IL-34: not changed	Erny *et al.* (2015)[48]
Neutrophils	↓	↓	CSF-2↓	Zhang *et al.* (2015)[92] Khosravi *et al.* (2014)[91] Balmer *et al.* (2014)[93] Deshmukh *et al.* (2014)[10]
Monocytes	↓ (spleen) ↓ (bone marrow) ↓ (blood)	↓ Not changed Not changed	Suggested CSF-1 ↓ (data not shown)	Khosravi *et al.* (2014)[91] Zhang *et al.* (2015)[92]
Kupffer cells	↓ Not changed	↓	Not changed	Corbitt *et al.* (2013)[94] Khosravi *et al.* (2014)[91] Zhang *et al.* (2015)[92]
Gut macrophages		↓	CSF-1 ↓	Muller *et al.* (2014)[97]
Splenic macrophages	↓	↓	Suggested CSF-1 ↓ (data not shown)	Khosravi *et al.* (2014)[91] Zhang *et al.* (2015)[92]
Bone marrow macrophages	↓		Suggested CSF-1 ↓ (data not shown)	Khosravi *et al.* (2014)[91]
Skin macrophages	↓		Unknown	Tamoutounour *et al.* (2013)[95]

ASF mice had still markedly altered microglia morphology, function and maturation, which indicates that bacterial complexity is essential for microglia prosperity.[48] Importantly, recolonization with a diverse microbiota through SPF donors largely rescued the immature microglial phenotype, which could have major therapeutic implications as the full maturation of these cells can also be attained at later stages during adulthood. As GF mice have never been exposed to microbiota and therefore microglia development might also be influenced in those mice, the microbiota of adult SPF mice was eradicated by ABX. Microglia from these ABX-treated mice displayed an immature phenotype of microglia reminiscent of the malformed microglia observed in GF animals, suggesting that continuous input signals from host microbiota are required for microglia properties postnatally, independent from developmental imprints. It was proposed that minocycline, a second-generation tetracycline, inhibits microglial activation and therefore affects neurodegenerative, neuropsychiatric and inflammatory diseases.[102,103] However, it is not yet clarified how this 'inhibition' is generated. Orally administered minocycline can certainly affect the composition of the gut microbiota but is also well-absorbed and is able to penetrate the brain parenchyma and could inhibit microglia independent of the abundance of the microbiota.[104] There is some evidence that minocycline attenuates the nuclear factor-κB pathway in microglia *in vitro* but the exact mechanism is not understood.[105] The potential interaction of minocycline and microglia is further discussed elsewhere.[106] Interestingly, in control experiments intraperitoneal administration of a mixture of non-absorbable antibiotics (namely neomycin, bacitracin and pimaricin) to SPF mice or oral administration to GF mice did not influence behaviour.[9] The antibiotic cocktail used in the study[48] was composed of three antibiotics (cefoxitine, gentamicin and vancomycin) that are described not to reach the brain tissue,[107] but the fourth substance metronidazole is considered to penetrate at least as poorly the CNS.[107] Therefore, further experiments are necessary to clarify whether metronidazole is able to affect microglia directly.

The pathways mediating this gut–microglia connection are not yet known but in contrast to other myeloid cells[92,108] this is most likely independent of TLR-signalling as microglia from mice deficient for TLR-2, -3, -4, -7and -9[109] have similar features to the respective wild-type animals.[48] Instead, SCFAs rather than TLR ligands were found to mediate signals to microglia *in vivo*.[48] The oral application of a mixture of the three major SCFAs acetate, propionate and butyrate was sufficient to drive maturation of microglia. It is known that SCFAs are able to cross the BBB[110,111] and may therefore affect microglia directly. In general, SCFAs can also be recognized by specific receptors, such as free fatty acid receptor 2 (FFAR2) [also known as G protein-coupled receptor (GPR) 43)] or FFAR3 (GPR41).[112,113] In general, GPRs are a large class of seven transmembrane spanning proteins that regulate a wide range of signalling events, whereas activation of FFAR2 by SCFAs is mediated through a dual coupling through Gi/o and Gq signalling.[113] It is known that FFAR2 regulates immune system properties.[114] Interestingly, FFAR2-deficient mice displayed microglia reminiscent of those found in GF mice, although FFAR2 is not expressed in any adult brain cell including microglia and endothelial cells.[76,113] This indicates that an indirect signalling of SCFAs through peripheral myeloid or lymphocytic cells expressing FFAR2, such as splenic or enteric macrophages, to the adult brain is conceivable. Despite this possible connection, it has not been investigated whether FFAR2 is

expressed at earlier stages during development. Other possible routes linking the gut and microglia such as direct signalling via the vagus nerve or other mediators like gut hormones, neurotransmitters, cytokines or MAMPs, which may also affect microglia, have not been investigated (Fig. 1).[27]

What are the functional consequences of microglial malformation and immaturity discovered in GF mice? Upon either bacterial (lipopolysaccharide) or viral (lymphocytic choriomeningitis virus) challenge, microglia from GF mice exhibited a restricted or disturbed innate immune response compared with microglia from SPF mice. Similar impaired immune responses are also described for other innate immune cells in ABX-treated SPF or GF mice, such as peritoneal macrophages[115] alveolar macrophages[116] or natural killer cells.[108]

Concluding remarks

In the past few years, the link between the host microbiota and human health and disease has become apparent. An unbalanced composition of host microbiota is becoming recognized as a crucial environmental factor that strongly impacts the host immune system, metabolism and has an important role in many diseases, such as systemic diseases (obesity, diabetes), gut-related irritable bowel syndrome and inflammatory bowel disease.[117] A recent study by Tillisch and colleagues described how ingestion of probiotic bacteria alters brain function in humans.[118] It can be assumed that numerous interactions between host microbiota and the CNS immune systems exist, which can essentially shape the outcome of a plethora of neuroinflammatory, neuro-oncological and neurodegenerative CNS diseases. The future treatment of CNS disorders in man can certainly take advantage of the intimate and mutual interactions of the gut inhabitants with the brain that can be considered as an 'axis of the good'.

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Disclosures

The authors declare no competing financial interests.

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