Microglia in Physiology and Disease

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Abstract

As the immune-competent cells of the brain, microglia play an increasingly important role in maintaining normal brain function. They invade the brain early in development, transform into a highly ramified phenotype, and constantly screen their environment. Microglia are activated by any type of pathologic event or change in brain homeostasis. This activation process is highly diverse and depends on the context and type of the stressor or pathology. Microglia can strongly influence the pathologic outcome or response to a stressor due to the release of a plethora of substances, including cytokines, chemokines, and growth factors. They are the professional phagocytes of the brain and help orchestrate the immunological response by interacting with infiltrating immune cells. We describe here the diversity of microglia phenotypes and their responses in health, aging, and disease. We also review the current literature about the impact of lifestyle on microglia responses and discuss treatment options that modulate microglial phenotypes.

1. INTRODUCTION

In preparation for this review, we analyzed the recently published literature on microglia. In a previous review written five years ago, more than 1,000 papers were cited (1, 2). Since then, the number of publications, including reviews, on microglia has increased every year. Therefore, we have chosen to provide an overview on the concepts and trends on microglial research in this meta-review. It has become evident that microglial cells are involved in essentially all brain diseases ranging from neurodenerative disorders such as Alzheimer's disease (AD), traumatic brain injury such as spinal cord lesions, and psychiatric diseases such as schizophrenia. For several diseases, microglia may in fact be initial elements at the onset of disease. As representatives of the innate immune system of the brain, microglia are the local interaction partners of infiltrating immune cells. Moreover, it became evident that they also play a role during the development and proper functioning of the healthy brain. Their ability to remove synapses is a way of interfering with the neuronal network.

2. THE DISCOVERY AND DEFINITION OF MICROGLIA

In 1856, Rudolf Virchow defined glial cells as a cell population in the brain that is distinct from neurons (3). In the following decades, this field of research showed little progress. At the end of the nineteenth century, there was a debate about the concept of neuroglia: Whereas the group around Carl Weigert (4) argued that glial cells simply form a matrix of processes in which independent nuclei are embedded, others viewed glial cells as distinct cell types. To manifest their cellular entity, von Lenhossek gave them the new name, astrocytes (5). At the beginning of the twentieth century, Ramón y Cajal postulated a third cellular element in addition to neurons and astrocytes by describing a population of apolar cells (6). At that time, pathologists recognized particular cell types in the diseased brain. These cells were described as rod cells (Stäbchenzellen) or as granular cells by Alois Alzheimer and Franz Nissl (7, 8). It was evident that these cells were found in all types of brain diseases, such as dementia and multiple sclerosis (MS), as well as in brain injury.

In 1919, Pio del Río Hortega introduced the modern terminology of how we describe glial cells today, namely by distinguishing between astrocytes, microglia, and oligodendrocytes in four papers originally published in Spanish, but now available as a commented English translation (9). For decades, microglial cells were no longer the focus of neuroscience research until the pioneering work of Georg Kreutzberg's group. He developed a new preparation, the facial nerve lesion, and analyzed the resulting response in the facial nucleus (10). This allowed Kreutzberg's group to study the process of microglial activation, which in turn stimulated the evolution of research in the field and resulted in numerous articles over the last decade.

3. MICROGLIA PHYSIOLOGY

3.1. The Developmental Origin of Microglia

Literature on the origin of microglial cells was recently reviewed (11–15). Río Hortega already noted that cells enter the brain early in development, which he named microgliocytes. He argued that these cells were of mesodermal origin. The exact origin of microglia was only recently resolved. They originate from a pool of primitive macrophages from the yolk sac that appear in the mouse at embryonic (E) day 8.5. Also around that developmental time period, the first ameboid cells appear in the neuroepithelium. At approximately E13, microglial precursors can be detected at the base of the fourth ventricle. The origin of this cell population was established by using mouse models in which these early precursors can be fate mapped within a very short time window during embryonic

development. These cells constitute an independent lineage distinct from other hematopoetic stem cells. In humans, microglial-like cells can already be detected at 13 weeks of gestation, whereas ramified microglia are detected at week 21. Close to term, a well-differentiated population of microglial cells is present. A factor that is important for the development of microglia is CSF-1 signaling, because in mice lacking the receptor, the number of tissue macrophages including microglia is strongly reduced. Although CSF-1 may not be a ligand for the receptor, interleukin-34 (IL-34) is. A deficiency of IL-34 results in a reduction of microglial density. Moreover, the adaptor protein DAP12 for the CSF-1 receptor seems to be important, as microglia density is decreased in mice deficient in DAP12. Interestingly, mutations in the *DAP12* gene are a cause of the Nasu-Hakola disease in humans, characterized by psychotic symptoms, neurodegeneration, and encephalopathy. The interferon regulatory factor (IRF)-8 is also essential for the development of microglial cells. As a result, IRF-8-deficient mice show a significantly reduced microglial density in adults.

There was a long debate about the replenishment of microglia from the blood system in the adult and under pathological conditions. Recent evidence, however, indicates that the intrinsic pool of microglia replaces the microglia population if depleted. The best evidence comes from experiments with parabiotic mice, in which the blood systems of two individuals are interconnected. Another study showed that adult mouse brains depleted of microglia by selective CSF-1R inhibitors completely repopulated with new microglia through the proliferation of nestin-positive cells that then differentiate into microglia. These approaches demonstrated that the intrinsic adult microglial population does not originate from blood cells but only from an intrinsic source.

3.2. The Role of Microglia in the Development of the Neuronal Network

A number of recent studies provide evidence that microglial cells play an important role during development (16–20). Microglial cells are the professional phagocytes of the brain and eliminate entire cells or cellular substructures, especially synapses. It is well established that approximately half of the neurons or glial cells such as oligodendrocytes that are generated during development are subsequently eliminated. Microglial cells obviously recognize cells that undergo programmed cell death, and they migrate to different regions of the central nervous system (CNS) usually right before or during the peak of programmed cell death. One signaling cascade by which microglial cells interact with motor neurons to induce cell death is mediated by tumor necrosis factor alpha (TNF-α). Another signaling cascade of this interaction is the production of superoxide 1 during the respiratory burst. Microglial cells are also described as influencing development in a positive way by promoting neural precursor cell proliferation and survival. Targeting microglia with the drug minocycline during development results in an increased neuronal apoptosis in the cerebral cortex, indicating that microglia promote neuronal survival.

One well-known process during development is synaptic pruning, referring to the elimination of excess synaptic connections. In the visual system, microglia eliminate the presynaptic inputs from the retinal ganglion cells into the dorsal lateral geniculate nucleus. This correlates with the peak of pruning during the development of this pathway. Molecular signals for this interaction involve elements known from the innate immune system, namely complement factors C1q and C3. It is assumed that these complement factors tag synapses for elimination. Another molecule that may be important for promoting the role of microglial cells during development is fractalkine or CX3CR1, a chemokine produced by neurons. Microglial cells are the only intrinsic brain cells that express receptors for CX3CR1. Although microglia deficient in CX3CR1 still eliminated apoptotic neurons, they did not offer neurotrophic support to surrounding neurons. CX3CR1-deficient mice showed a reduced connectivity between cortex and hippocampus, which also has an impact on mouse behavior.

3.3. Microglia-Synapse Interaction in the Adult Brain

There is increasing evidence that microglial cells are responsible for the removal of synapses not only during development, but also in the normal healthy brain in the framework of neuronal plasticity (21–24). More than a decade ago, it was noted that under homeostatic conditions, microglial cells scan the brain environment by constantly moving their processes. Microglial cells have a defined territory and scan their environment within several hours. Some of the processes rest for several minutes and make direct contact with neuronal synapses at a frequency of about one per hour. In the visual cortex, it was demonstrated that microglia interact with axonal terminals and dendritic spines, and this interaction depends on changes in neuronal activity. Ultrastructural studies have revealed that microglial cells contain remnants of axonal terminals and dendritic spines both in cell bodies and in processes, as shown in visual and auditory cortices. Neuronal activity regulates the microglial engulfment of synaptic structures, whereas microglial cells preferentially remove less active inputs.

3.4. Interaction with Neuronal Precursor Cells

It is becoming clear that microglial cells interact with neural precursor cells (NPCs) during development and in adulthood, as summarized in two publications (3, 25). In the monkey, it was noted that early in development, the interface between the ventricular and the subventricular zone is populated by microglia. Later in development, microglia are also found in the subventricular zone. These observations were confirmed in humans and rodents. These microglial cells phagocytose neural precursor cells characterized by expression of *Pax6* and *Tbr2*. In the embryo, microglial activation [by lipopolysaccharide (LPS)] or suppression of activation (by doxycycline) had no apparent effect on the number of precursor cells. Interference with microglial activation caused by treatment with minocycline inhibited embryonal neurogenesis and oligodendrogenesis.

In contrast, in the adult animal, inflammation by LPS injection was found to reduce the production of new neurons in the neurogenic zone of the adult hippocampus. The involvement of microglia is inferred by the observation of a negative correlation between newborn neurons and activated microglia. In vitro experiments indicate that IL-6 released from microglia can result in the apoptosis of neuroblasts.

Microglial cells are present in the subgranular and subventricular zone in the mature brain. It is assumed that they phagocytose the excess newborn cells that undergo apoptosis in these neurogenic regions. Indeed, evidence shows that blocking microglial phagocytosis increased the number of apoptotic NPCs. A number of factors are considered to be involved in microglia-neural precursor interactions. These signaling substances include transforming growth factor β (TGF-β), TNF-α, vascular endothelial growth factor, fractalkine, insulin-like growth factor 1 (IGF1), and Toll-like receptor (TLR) 9.

3.5. Gender Differences

Recent data from rodents indicate significant differences in microglia between males and females (26, 27). Early in development, testosterone produced by the testis gets access to the brain and is aromatized to estradiol, which is the dominant masculinizing hormone in the rodent brain. The preoptic area is an essential brain region that shows characteristic neuroanatomical differences between males and females. In the male preoptic area, astrocytes are more ramified, and the density of dendritic spines is increased. Males have a higher density of microglia that show an activated morphological phenotype characterized by increased body size and reduced branching pattern

and process length. Treating females with estradiol after birth leads to a masculine microglial phenotype. Minocycline, which was shown to interfere with microglial activation, reduced the masculinized phenotype. When applied early in development, minocycline has a long-term impact in preventing masculinization, and it eventually leads to changes in adult sexual behavior, an effect that mimics the treatment with estradiol. It is speculated that these long-term changes of the microglia phenotype may explain gender differences in disorders such as autism.

3.6. Differences Between Rodent and Human Microglia

The vast majority of the studies on microglial cells in health and disease rely on rodents as experimental models. Several observations indicate that rodent and human microglial are distinct (28, 29); for example, in vitro rodent microglia proliferate much more compared with human microglia. This difference was also observed in AD, in which microglia do not proliferate in humans but do in mouse models of this disease. There are differences in mediators of neuroinflammation, such as TGF-β1, which is important in mice but less so in adult human microglia. The LPS receptor TLR4 is also a major way to activate mouse microglial cells where it is highly expressed, but it seems much less important in humans. Another example relates to the new molecule family involved in innate immune responses, the siglecs. Despite a similar gene signature in human and mouse microglia (30), there are twice as many siglec genes in humans compared to mice. This indicates that the variety of siglecs in humans is much higher, which may have an impact on several diseases, including AD (31). Another lead substance of microglial activation is the production of nitric oxide (NO) through the activity of inducible NO synthase (iNOS). Whereas mice respond with high production of NO upon inflammatory stimuli, this induction is much lower in human microglia. This also has implications on drug development, as NO production is used in many screening models as a tool to monitor microglial activation. Consequently, there is a strong need to confirm data obtained from rodents in human microglia. There are essentially three strategies to obtain human microglia. One is from surgery using the resection material from glioma or epilepsy patients. The cells of monocytic origin in the brains of glioma patients are, however, a mix of intrinsic microglia and invaded monocytes. The epilepsy tissue itself does not represent a normal environment. Another source of microglia is postmortem tissue, but its availability depends on the country of origin and its unique national legal regulations. However, this source is also variable depending on the cause of death and the time span between microglia isolation after death. Another recently followed strategy is the generation of brain cells from induced pluripotent stem cells. There are now protocols developed for the generation of microglia from mouse embryonic stem cells (32) and from human pluripotent stem cells (33), but it remains unclear how well they represent the properties of naive microglia.

3.7. Microglial Heterogeneity

Microglial diversity by responses and responders was recently reviewed (34). In his original description of microglia, Río Hortega already distinguished between different subtypes of microglia. He described microglia as having no apparent contact with other cell types, but he also discussed microglia associated with neurons that he termed neuronal satellites. Indeed, this subtype was recently rediscovered (35, 36). Río Hortega also described vascular satellites and astroglial satellites. Recent research showed that a subtype of microglia only found in the cortex contacts the axon initial segment with a single process (36).

A distinct type of microglia residing in the subventricular zone was characterized by the low expression of purinoceptors and a lack of adenosine 5′-triphosphate (ATP)-inducible chemotaxis

(37). One form of heterogeneity is due to a high diversity in the expression of functional neurotransmitter receptors found in cell culture, in freshly isolated cells, and in situ (38–40). There is high diversity in the expression of receptors for neurotensin 2, dopamine, endothelin, galanine, histamine, neurotensin, nicotine, serotonin, somatostatin, substance P, GABA, and vasopressin.

Another type of diversity relates to TLR4 signaling. The activation of TLR4 receptors triggers the release of TNF-α in only a subset of cells. Further diversity was found with respect to the release of macrophage inflammatory protein 1 alpha (MIP-1α). This was not related to differential TLR4 expression but obviously relies on distinct pathways within microglial cells. Moreover, only a subset of microglial cells expresses detectable amounts of major histocompatibility complex class II (MHC-II), and this diversity also refers to costimulatory molecules CD80 and CD86. An initial genome-wide analysis of microglia in different brain regions at different ages described distinct region-specific microglia phenotypes (41). The authors found differences in pathways related to immune regulation and heterogeneity in different brain regions and among individuals of different age.

3.8. Heterogeneity of the Microglial Activation Process

Microglial activation occurs under any kind of pathologic insult in the brain. On the basis of classical neuropathological studies, activated microglia are characterized by a change in the morphologic phenotype, from a highly ramified cell to an amoeboid cell. Derived from the macrophage field, two types of activation states are postulated, including the classical activation, the M1 state as the proinflammatory state, and the alternative activation, the M2 state related to repair. For microglia, the concept of M1/M2 polarization is questioned (42). This general concept was recently replaced by a new proposal for classification. Today, we know that microglial activation is a highly dynamic process that can be quite different in diverse pathologic situations; it also changes during the pathologic process. Sequencing data from defined pathological states indicates more heterogeneity than initially considered (see Section 4). This issue has also been addressed in several reviews (43–48).

3.9. Microglial Involvement in Neuroprotection and Neurotoxicity

Microglial activation was typically considered to be a negative event. For example, in vitro experiments showed that the supernatants of microglial cells exhibit neurotoxic activity. More recent in situ data, however, indicate that microglial cells can also exhibit important neuroprotective activity (49, 50).

Microglial cells remove synaptic input that is no longer functional (10). This phenomenon of synaptic stripping was observed in the facial nucleus and is considered neuroprotective. In the model of mild microglial activation it was observed that microglia encapsulate neuronal cell bodies and thereby displace synapses. This results in an increase in neuronal activity and stimulation of NMDA receptors, which results in calcium influx into neurons and post-translational activation of antiapoptotic and neuroprotective factors. This involves the transcription factor NFI-A that promotes neuronal survival. It is currently unknown how microglial cells selectively remove axosomatic inhibitory synapses while leaving excitatory synapses intact. Microglial cells express a variety of neurotransmitter receptors, including those for GABA and glutamate, and they might selectively sense GABAergic activity and thereby modulate their behavior.

There is increasing evidence that microglial cells can suppress neuroinflammation and thereby protect nerve tissue by the release of anti-inflammatory mediators. In experimental meningitis, microglial cells initially produce the proinflammatory IL-6, but subsequently, they produce

anti-inflammatory IL-10 as a negative feedback loop. Another factor produced by microglia is TGF-β, which downregulates inflammation. Using a mouse model in which microglial cells can be depleted, a neuronal loss in layer V neurons was observed, indicating that microglia provide trophic support for these cells; EDF1 is considered the mediating factor. In a mouse model of stroke, a reduction of microglial proliferation led to a larger stroke lesion and increased neuronal death. Although most experimental paradigms indicate that microglial cells are neuroprotective, there is one contrasting example: In a mouse model of MS, microglial paralysis resulted in a delayed onset and reduced clinical score. But in general, most evidence suggests that microglial cells play more of a neuroprotective role.

3.10. The Microglial Toolbox

Most recent studies on microglia used the mouse as an experimental model. This was strongly facilitated by the availability of mouse strains in which microglial cells are labeled by fluorescent protein, the availability of microglia-specific Cre-lox systems that allow depletion of defined genes, and tamoxifen-induced Cre lines that permit lineage tracing of cells (for an overview, see 51, 52). Mouse lines were also generated in which microglia can be selectively ablated. One is the microglia-specific expression of diphtheria toxic receptor, and another mouse model involves the expression of herpes simplex-derived thymidine kinase, which processes ganciclovir to induce strand breaks in newly synthesized DNA. Several microglia-specific promoters can be employed to distinguish microglia from other brain cells. These promoters drive gene expression of integrin CD11b, CD11c, LysM, the F4/80, M-CSF receptor, Iba1, or the fractalkine receptor CX3CR1. The recent advent of CRISPR-Cas9 technology will undoubtedly broaden the available genetic manipulations with respect to microglia.

The mitochondrial 18 kDa translocator protein (TSPO), previously known as peripheral benzodiazepine receptor, has become a prominent biomarker used in positron emission tomography (PET) studies. Increased binding to the TSPO ligand might be associated with microglia activation. Several radio ligands are available and have been used extensively in clinical trials and animal studies (reviewed in 53). Because we have highlighted the heterogeneity of the microglia activation process in different diseases and during the course of the disease, correlating TSPO binding with microglia activation should be reviewed critically. We emphasize that TSPO expression should be analyzed within specific disease contexts rather than merely equated with the reified concept of neuroinflammation, as TSPO is also found in reactive astrocytes and endothelial cells.

4. MICROGLIA MOLECULAR PROFILING

4.1. Microglia Transcriptome Profiles

Microglia functions are clearly reflected by transcriptome profiles (54–57). Comparing tissue macrophage transcriptomes, including microglia, shows that these cells actively adjust to their environment (58). Several studies have identified genes that are specifically expressed by microglia, and these transcripts show significant overlap (see **Table 1**). A total of 106 transcripts were identified as specific for mouse microglia compared to other myeloid cells and neural cells (30). A subset of these transcripts was also found enriched in human microglia. Hickman et al. (59) identified a microglia-specific signature comprising approximately 100 genes, which they delineated as the sensome, indicative of active surveillance functions by microglia of their environment. Interestingly, *Tyrobp* (see Section 7) was a key gene in the sensome network. Zhang et al. (60) were the

Table 1 Selectively expressed genes in mouse microglia in comparison to macrophages and neural cells

Gene symbol	Reference(s)
Crybb1	58, 30, 59, 60
Cx3cr1	58, 30, 59, 60
Fcrls	58, 30, 59, 60
Hexb	58, 30, 59, 60
Itgb5	58, 30, 59, 60
Olfml3	58, 30, 59, 60
P2ry12	58, 30, 59, 60
P2ry13	58, 30, 59, 60
Rnase4	58, 30, 59, 60
Slc2a5	58, 30, 59, 60
Tmem119	58, 30, 59, 60
Trem2	58, 30, 59, 60
Gpr34	58, 30, 59, 60
Siglech	58, 30, 59, 60
Gpr84	58, 59, 60
Socs3	58, 30, 60
Csfr1	30, 59, 60
Tyrobp	59, 60

first to provide quantitative microglia transcriptome data. These data compare well with previous semiquantitative data sets that were produced with microarrays.

Recently, an open access database (**http://www.goad.education**) was launched, aimed at facilitating access to glia-derived transcriptome data (61). The GOAD database contains most published transcriptomes of microglia, astrocytes, and oligodendrocytes, with both healthy and pathological conditions. Currently, most of the microglia transcriptomes are derived from mice, whereas transcriptomes of other species including humans are only sparsely available. A recent study indicates regional differences of the microglia transcriptome (41), and another study reports on single-cell microglia transcriptomes (62).

4.2. Epigenetics

Epigenetics describes the regulation of transcriptional activity of cells as determined by the cellular environment. It involves modifications of the chromatin structure not only at promoters and genes but also at enhancer and repressor sites. For myeloid cell lineages, PU.1 is the master regulator that primes the enhancers inherited in the development of myeloid cells. PU.1 cooperates with stimulus-dependent transcription factors, which are induced by the environment; thus, PU.1 determines the tissue macrophage phenotype.

The genome-wide enhancer and promoter landscapes of mouse macrophages, including microglia, were recently described (63, 64). The tissue macrophage landscape profiles include sites that underlie the general macrophage phenotype, but there are also unique promoter and enhancer profiles specific for microglia (64). Factors from the tissue environment activate signaling cascades that result in cooperative binding of signaling-derived transcription factors with PU.1. The microglia-specific open chromatin and enhancer regions were enriched for Mef2c

transcription factor binding sites, and as such, Mef2c is a likely candidate that determines the microglia epigenetic profile. PU.1 binding sites in microglia were also enriched for *Smad*- and *Ctcf*-type transcription factors (63). This suggests that these transcription factors are coregulators of PU.1 that mediate a microglia-specific enhancer profile. Interestingly, SMAD2 is a transcription factor that signals downstream of TGF-β, a factor previously shown to be important for maintenance of the microglia profile (30). The microglia phenotype is modulated by miRNAs that regulate protein expression by silencing mRNA translation or degrading gene transcripts. Several miRNAs, including miR-424, miR-222, and miR-155, are involved in the differentiation of the monocyte lineage (57). miR-124 is involved in maintenance of the resting state of microglia by targeting the CEBPα/PU.1 pathway. Both CEBPα and PU.1 are transcription factors involved in the differentiation of myeloid cells. The expression of miR-124 was highest in CD45 low-resident microglia, and miR-124 was downregulated after exposure of microglia to proinflammatory cytokines. During development in mice, the expression level of miR-124 was low. Expression of other miRNAs, including miR-21, miR-146a/b, and miR-155, was associated with the proinflammatory activation of microglia. Thus, miR-155 was shown to be upregulated in response to proinflammatory stimuli and induces expression of IL-6. MiR-146 is regulated by nuclear factor-kappa B (NF-κB) and upregulated by proinflammatory signals. In AD mouse models, miR-146 targets several proinflammatory cytokines, including complement factor H (CFH) and the β-amyloid precursor protein TSPAN12 (65). MiR-146 is also upregulated in HIV-infected microglia and induces upregulation of MCP-2. In contrast, it was also reported that a lack of PS2 function mediates a downregulation in this expression of miR-146a and thereby enhances expression of the transcription factor IL-1 receptor associated kinase-1 (IRAK-1) and NF-κB transcriptional activity (65). MiRNA-99a, −125b-5p, and −342-3p expressed by mouse microglia are not found in other tissue macrophages (30).

5. GLIOMA

Brain tumors are strongly infiltrated by intrinsic microglia and peripheral monocytes (66, 67). This brain macrophage fraction can amount to up to 50% of the cells in the tumor. In a mouse model of neurofibromatosis, type 1 optic glioma silencing of microglial cells resulted in reduced tumor proliferation. In high-grade gliomas brain macrophages also accumulate in the tumor tissue and can be isolated both in mouse models of glioma as well as in human resection material. Using RNA microarray analysis, approximately 1,000 transcripts were increased in glioma-associated microglia/macrophages relative to control microglial cells, and these genes show little overlap with reported genes for M1 or M2 phenotypes. A similar approach analyzing the transcriptome of human microglia/macrophages revealed that genes related to immune activation in mice are not found in human cells, probably due to the much slower progression of the tumor in humans (68).

Several factors are released from glioma cells and attract microglia/macrophages to the tumor tissue. Recently identified chemoattractant factors include MCP-1, CXCL12, GDNF, and CSF-1. In general, microglia/macrophages promote tumor growth. One path is via the epidermal growth factor that stimulates glioblastoma cell proliferation. CSF-1 is both a chemoattractant for microglia and converts microglia into a protumorigenic phenotype. MMP2 is released from glioma cells in an inactive longer form that needs to be cleaved. Microglial cells/brain macrophages express the enzyme responsible for MMP2 cleavage, MT1-MMP. Under normal conditions, microglial cells do not express this enzyme, but its expression is induced by glioma cells. The signaling factor of this glioma–brain macrophage interaction is versican, which activates TLR2, triggering the expression of MT1-MMP. Thus, many complex interactions occur between glioma and microglia/brain macrophages. For instance, glioma stem cells reside in the perivascular niche and are

highly resistant to radiation and chemotherapy-released periostin, which attracts microglia/brain macrophages and promotes tumor growth. Microglia/brain macrophages are also considered as a target for therapy. Minocycline interferes with the activation process of microglia and is currently being tested in a clinical trial.

6. MICROGLIA IN INFLAMMATORY DISEASES

6.1. Multiple Sclerosis

Microglia and macrophages are considered as the damaging elements in MS, as revealed by animal experiments on experimental autoimmune encephalomyelitis (EAE) (69–71). Using a mouse model in which the microglial cells can be depleted and inactivated, a delay in EAE onset and reduced severity of clinical symptoms were observed in combination with reduced inflammation, indicating that microglial cells can be detrimental. Human PET studies indicate that the abundance of activated microglial cells correlates with clinical disability. These studies also support the hypothesis that microglial activation is an early event preceding demyelination and lesion formation. Fibrinogen may be an activation signal for microglia infiltrating the brain due to mild damage of the blood-brain barrier. It is also suggested that axonal degeneration triggers microglial activation. Microglial clusters form before the onset of clinical symptoms, indicating that microglial cells are very early elements in the development of this disease. One of these early steps involves microglial proliferation and upregulation of MHC-II. Therefore, microglial cells act as antigen-presenting cells for the invading T cells, leading to their expansion and disease progression.

One interesting molecule involved in microglial inactivation is the E3 ubiquitin ligase Peli1. Its effect is mediated by TLR and IL-1 signaling, which leads to microglial activation and progression of MS. Similarly, macrophage migratory inhibitory factor, which is correlated with the clinical decline in MS patients, can activate microglia. Although most of these studies indicate a role of microglial cells in promoting disease progression, there are indications that microglia might also exert beneficial functions. One of those mechanisms is the removal of apoptotic cells and myelin debris, which supports tissue regeneration and affects the maturation of oligodendrocyte progenitor cells. Thus, stimulation of phagocytosis and thus the removal of debris has a marked amelioration on the clinical outcome.

An interesting question is whether microglial cells are also directly affected by drugs that are currently used in clinics. Interferon 1 was shown to inhibit the antigen-presenting function of microglia. It also suppresses the neurotoxic production of superoxide and thus prevents microglia-induced neuronal cell death. Glatiramer acetate indirectly affects microglia via factors released from T cells, leading to an inhibition of TNF-α release and a reduction in microglial proliferation. In human PET studies, the levels of activated microglia were reduced after treatment. The newly introduced drug fingolimod is associated with an inhibition of microglial activation and thus the reduced release of TNF-α and IL-1β. Lastly, oral dimethyl fumarate inhibits in vitro activation of microglia and the subsequent release of proinflammatory signals. In conclusion, microglia are very early elements in the onset of MS. They play an ambivalent role and are targets for therapeutic interventions.

6.2. Stroke

The literature on stroke was recently reviewed (72–75). Cerebral ischemia leads to neuronal depletion, activation of microglia, and infiltration of blood-borne immune cells. Several factors,

such as cytokines and reactive oxygen species (ROS), induce the breakdown of the blood-brain barrier, which facilitates the infiltration of circulating monocytes, neutrophils, and lymphocytes. Several approaches can be used to distinguish mouse microglia from bone marrow–derived monocytes to determine whether they have a distinct impact. One is the use of $CD11b^{+}CD45^{high}$ and $CD11b^{+}CD45^{low}$ as markers for peripheral monocytes/macrophages versus microglia cells, respectively. Another approach employs an animal model with fluorescent bone marrow transplants after irradiation. Parabiosis was also employed in ischemia research, indicating that amoeboid microglia proliferate in the periphery of the ischemic tissue. Infiltrating cells started to populate the brain five days after the ischemic onset; however, these cells are less numerous than intrinsic microglia and did not proliferate. These studies show that microglial cells are the first to be activated to clear cell debris by phagocytosis and contribute to the resolution of inflammation. Histological analyses of human autopsy material indicate a similar chronology of events, as observed in mouse models, but it occurs with delay and is spread out in time. At present, the only successful treatment for ischemic stroke is thrombolysis with tissue plasminogen activator. All strategies targeting immune cells have failed in human trials despite their success in mouse models. Reasons for this failure are extensively debated; they might be due to a difference between the rodent and the human immune system, timing of the intervention, and the general lack of comprehensive understanding of immune cell responses after stroke.

7. MICROGLIA PRIMING

Aged microglia become more responsive to proinflammatory stimuli, a process that is referred to as microglia priming (76, 77). This is defined as an enhanced and prolonged response to homeostatic disturbance that is much stronger compared to naive microglia, and primed microglia have an enlarged soma and shorter dendritic arbors (76, 77). The enhanced primed microglial response includes proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, but also IL-10 and TGF-β. In addition, primed microglia express markers that include MHC-II, AXL, Lgals3, and CD11C (78).

Microglia priming is viewed as a cellular response by which a relatively small disturbance in CNS homeostasis can contribute to the progression of neurodegeneration, for instance, during normal aging. Priming of microglia is observed in a variety of neuropathological conditions and is proposed to be the result of persistent neuroinflammation and/or exposure of microglia to misfolded proteins or neuronal debris that occurs during aging or neuropathological disease. In addition, downregulation of neuronal ligands that activated inhibitors, such as fractalkine, CD47, and CD200, as well as siglecs, may contribute to microglia priming. Primed microglia also mount an enhanced response in reaction to peripheral stimuli, such as peripheral inflammation, injury, and stress. Interestingly, enhanced LPS-induced proinflammatory activity in aged brain is not suppressed by IL-10, TGF-β, or IL-4, suggesting that microglia priming is insensitive to anti-inflammatory regulation. Clearly, microglia priming leads to increased neuronal loss and the enhanced progression of neurodegenerative diseases.

8. MICROGLIA AND NEURODEGENERATION

Neurodegenerative disorders are hallmarked by age-related deposition of debris and aggregated and misfolded proteins (79–81). Under neurodegenerative conditions, microglia react to misfolded proteins, aggregates, and cellular debris. Microglia express dedicated pattern recognition receptors (PRRs) that sense microbial molecules, i.e., highly conserved pathogen-associated molecular patterns (PAMPs). Other PRRs in microglia detect damage-associated molecular patterns (DAMPs). DAMPs can be released by damaged cells, including misfolded proteins, aggregated

peptides, and nucleic acids, and are found under neurodegenerative conditions (82). Thus, various endogenous molecules, associated with neurodegeneration, such as aggregated Aβ, α-synuclein, mutant huntingtin, the superoxide dismutase 1 (SOD1), and chromogranin, act as DAMPs and activate PRRs. Stimulation of PRRs leads to the sustained release of neuroinflammatory factors, which causes cell death, promotes pathology, and contributes to neurodegeneration and disease progression.

In most neurodegenerative diseases, microglia are stimulated by high levels of immune factors. Activation often occurs through TLRs, (including TLR-4 and TLR-6) and their coreceptors CD36, CD14, and CD47 (83, 84). The inflammasomes constitute another group of PRRs. Inflammosomes are large protein complexes that contain a sensor molecule belonging to the NOD-like receptor (NLR) family or a pyrin and HIN domain-containing protein, the adaptor protein ASC, and caspase 1 (79, 84). For neurodegenerative conditions, the NOD-, LRR-, and pyrin domain–containing 3 (NLRP3) are particularly relevant because they sense a broad range of misfolded/aggregated proteins related to neurodegenerative diseases. Other inflammasomes, including NLRP1 and NLRP2, may be involved in neurodegeneration related to brain trauma. Caspase-1 of NLRP3 cleaves proinflammatory cytokines of the interleukin-1β (IL-1β) family, such as IL-1β and IL-18, which leads to the release of these cytokines. Accordingly, NLRP3 inflammasome activity is held largely responsible for the considerable production and release of these proinflammatory cytokines upon neurodegeneration.

In addition to PRRs, microglia express a variety of purinergic receptors that are activated by extracellular nucleotides, such as ATP and UTP, and nucleosides such as adenosine that are released by injured cells. Activation of microglia under neurodegenerative conditions induces the release of ROS and NO through activation of NADPH oxidase, myeloperoxidase, and iNOS.

Overlap clearly exists between the signaling pathways induced by PAMPs and DAMPs. In general, the inflammatory response often results in the activation of transcription factors, including NF-κB, activator protein-1 (AP-1), cAMP response element binding protein, CCAAT/enhancer binding protein, and IRF, leading to the complex transcriptional regulation of cytokine and chemokine networks. Activation of innate immune signaling under neurodegenerative conditions also has an impact on the phagocytic capacity of microglia. Thus, activation of the complement cascade, purinergic receptors, and the TREM2/TYROBP receptor complex regulates microglial phagocytosis during neurodegenerative disease.

8.1. Microglia in Alzheimer's Disease

Ample experimental evidence has shown the fundamental role of neuroinflammation in AD pathology (85–88). Early reports revealed that proinflammatory microglia surround Aβ plaques. However, the implications of these pathological hallmarks and the therapeutic relevance remain elusive. Recent large genome-wide association studies have revealed single nucleotide polymorphisms (SNPs) that are risk factors for AD. Several genes associated with these SNPs encode proteins that are related to functions of microglia, including TREM2, CD33, CR1, ABCA7, SHIP1, and APOE. Interestingly, the proteins are all involved in signaling pathways leading to phagocytosis and cytokine production. Possible involvement of microglia phagocytosis and immune responses in AD was corroborated by a recent large transcriptome study by Zhang et al. (89), who identified transcriptome networks in postmortem brain tissues from late-onset AD patients. The study revealed an immune- and microglia-specific module strongly associated with AD pathology, which contained the TREM2-associated protein TYROBP as a key regulator.

In addition to AD-related polymorphisms of microglia-related genes, specific changes in microglia functions are associated with AD. On a critical note, their association with late-onset AD is evident. Therefore, it is difficult to distinguish changes in microglia function related to aging from those associated with AD, as there is likely some overlap.

Recent publications on transcriptome (78, 89) and identification of the relationship between AD and mutations in microglial genes encoding TREM2 and CD33 have established a firm link between immune alterations and AD pathogenesis. Association of reactive microglia with Aβ deposits as well as their secretion of proinflammatory cytokines, chemokines, ROS, and acute phase proteins was described extensively. In addition, fibrillary forms of Aβ induce synthesis and secretion of proinflammatory cytokines, including IL-1β, IL-6, TNF-α, TGF-β, ROS, and other proinflammatory proteins. Furthermore, it was shown that Aβ induces NLRP3 inflammasome activity (90). The NLRP3 inflammasome seems to play an important role in the pathogenesis and progression of AD and may provide an interesting target therapy for AD.

Microglial extensions are very motile and are involved in the surveillance of the CNS parenchyma. Clearly, immune surveillance decreases with age. The environment around Aβ plaques is highly chemotactic and contains increased levels of chemoattractants for microglia, including MCP-1, MIP-1α, MIP-1β, IL-8, and MCSF, leading to increased numbers of plaque-associated microglia. Cells around Aβ plaques in APP transgenic mice are less motile compared to wild-type mice, suggesting a decreased function of microglia.

Microglia express a variety of genes, including TLR2, TLR4, CD14, RAGE, the scavenging receptors CD36 and SCA, and formyl peptide receptor FPRL-1, which indicate that microglia can phagocytose Aβ. Additional clearance capacity may come from macropinocytosis of soluble forms of Aβ and through secretion of Aβ degrading enzymes, including insulin degrading enzyme, neprilysin, and MMP9. In line with microglia motility, clearance capacity decreases with age, and it was recently shown that declining phagocytic activity of microglia correlates with Aβ plaque deposition (91). Furthermore, the aforementioned mutations in TREM2 and in CD33 corroborate involvement with the decreased phagocytic activity in AD pathology. In addition, the loss of protein homeostasis, a prominent feature in neurodegenerative diseases, may lead to insufficient degradation of phagocytosed Aβ protein, resulting in a decrease in microglia Aβ clearance capacity.

8.2. Microglia in Amyotrophic Lateral Sclerosis and Parkinson's Disease

Microglia are involved in amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) (92–96). In addition to data derived from ALS patients, much of the information about the role of microglia in ALS is obtained from transgenic SOD–ALS mice. It was proposed that at the early stage of ALS, microglia exert protective activity, showing increased expression levels of brain-derived neurotrophic factor (BDNF). However, microglia at a later stage also enhance motor neuron death due to secretion of neurotoxic factors, e.g., high-mobility group box 1 (HMGB1), which promotes transcription of a wide range of proinflammatory genes. Furthermore, it was proposed that at the final stage of ALS, impaired microglial function may accelerate ALS disease progression.

Phagocytosis by microglia is an important factor in neurological disease, and altered phagocytic activity plays a role in ALS. Missense variants of TREM2, granulin, and profilin 1 are acknowledged as risk factors for ALS, resulting in decreased microglial phagocytosis and altered neuroimmune responses. The intracellular degradation of phagocytized material is also hampered in ALS and may lead to increased proinflammatory activity.

PD is characterized by microglia activation. PET imaging studies show that this occurs widely throughout the brain. Lewy bodies are the primary pathological feature of PD, and α-synuclein

is the primary component of these protein aggregates. Neurons secrete α-synuclein through an unknown mechanism. Microglia are the primary scavengers of α-synuclein and likely take the burden of α-synuclein from neurons. This has a proinflammatory effect on microglia, which to a large extent is mediated via leucine-rich repeat kinase.

IL-1 and IL-18 are important cytokines in the pathology of PD. Involvement of the Nlrp3 inflammasome in PD was recently described (97). This finding is corroborated by the fact that *Nlrp3*$^{-/-}$ mice are resistant to the toxic effect of MPTP on dopamine neurons with concomitant low levels of IL-1β and IL-18.

9. MICROGLIA AND AGING

Several microglia phenotypes are associated with aging (98, 99). Brain aging and neurodegenerative disease are associated with neuroinflammation. Senescent proinflammatory microglia display specific morphological features, including an increased soma volume and thicker processes. In addition, senescent proinflammatory microglia express proinflammatory markers, including MHC-II, IL-1β, IL-6, and TNF-α, and contain lipofuscin granules. Oxidative damage of mitochondrial DNA in aged microglia causes increased production of ROS and enhances oxidative stress and neuroinflammation by the activation of NF-κB.

Expression profiles of senescent microglia show a wide range of proinflammatory transcripts, such as TLRs, inflammasome components, complement factors, and phagocytic receptors. The transcriptional networks of upregulated genes of the mouse models for aging and neurodegeneration were conserved and differed fundamentally from the acute inflammatory gene expression network induced by LPS in young mice (78). Whereas acute inflammatory networks were strongly enriched for NF-κB signaling factors, the profile of microglia from aged and neurodegenerative mice contained gene expression patterns related to phagosome, lysosome, antigen presentation, and AD signaling. This suggests that a shared pattern of conserved signaling pathways contributes to the phenotype of senescent microglia. Microglia motility changes with age in that young microglia enhance motility and stretch their ramifications readily upon administration of ATP, whereas aged microglia are much less motile (100).

Dystrophic microglia were reported in human brain tissue characterized by deramification, loss of fine cytoplasmic processes, cytoplasmic beading, and cytoplasmic fragmentation (98). Remarkably, dystrophic microglia are associated with tau-positive degenerative neurons in postmortem AD patient brain tissue, suggesting a relationship of tau pathology with microglia dystrophy. It is thus concluded that a lack of microglial support, rather than neuroinflammation, is responsible for neurodegeneration.

It was also suggested that microglial senescence may result from telomere shortening. Telomeres, the endpoints of eukaryotic chromosomes, shorten with age, leading to the replicative senescence of microglia that are normally capable of self-renewal (99). Telomere shortening was observed in rat microglia (29). Because microglia have an extensive capacity for cell division and proliferate upon tissue damage and inflammation (101), decreased telomere length could reduce their capacity to mount proper responses against CNS damage or infection and consequently lead to the loss of tissue homeostasis. Interestingly, in mTERC$^{-/-}$ telomerase-deficient mice, telomere shortening occurs and is associated with decreased microglia proliferation in vitro. However, under physiological conditions in vivo, gene expression and functionality of mTERC$^{-/-}$ microglia are comparable to wild-type mice even at old age, suggesting that under homeostatic conditions telomere shortening is not a relevant issue for microglia aging, at least in mice. This issue clearly needs to be addressed further in human microglia.

10. MICROGLIA IN PSYCHIATRIC DISORDERS

10.1. Autism Spectrum Disorder

With respect to microglia, immune regulation, and psychiatric disorders, most reviews focus on autism spectrum disorder (ASD) (102–107). In a mouse model of Rett syndrome, a condition that shares some symptoms with regressive autism, transplantation of wild-type microglia alleviates behavioral and physiological symptoms (108). However, another recent study failed to find any benefit from wild-type microglia transplantation in three rodent models of Rett syndrome, including the model used in the original study (109). It is worth highlighting the differences between Rett syndrome and ASD with respect to microglia: Rett syndrome lacks microglia phagocytosis and shows decreased levels of BDNF, whereas there is microglia activation and/or proliferation and possibly defective BDNF signaling in ASD. Rett syndrome occurs almost exclusively in females, but ASD affects males more often; Rett syndrome is also characterized by neurologic disabilities, unlike ASD. A recent study observed that genes expressed at higher levels in males are significantly enriched for genes upregulated in postmortem autistic brain, including astrocyte and microglia markers (110). Sexual dimorphism in cortical microglia may put males at a greater risk for ASD.

Comparing the expression of genes in postmortem autistic and control brains revealed two distinct networks that are disrupted in the brains of individuals with ASD. Genes associated with synaptic function are downregulated, whereas immune-related genes are upregulated. Postmortem studies of brains from ASD patients revealed changes in microglia density and morphology in distinct brain regions. A conservative estimate suggests that at least 69% of individuals with an ASD diagnosis have microglial activation or neuroinflammation, as measured by PET with the TSPO ligand. Although many diverse environmental factors contribute to ASD, most converge on alterations in immune responses during prenatal or early postnatal development. It is hypothesized that the rise in ASD over the past decade reflects the exposure to an increasing number of environmental stressors during critical periods of development that results in disease expression in individuals with a vulnerable genetic background.

10.2. Microglia and Affective Disorders

Postmortem brain analyses and PET studies indicate that microglial abnormalities are present in patients with schizophrenia (111, 112), similar to ASD. The current consensus is that alterations in the immune system as well as neuroinflammation lead to progressive brain changes in schizophrenia: Activated microglia promote the expression of inflammatory cytokines that stimulate indoleamine 2, 3-dioxygenase (IDO) activity and deplete CNS tryptophan, ultimately leading to lower levels of serotonin and alterations in glutamate, dopamine, and downstream ROS. Compared to antipsychotic monotherapy, minocycline, when added to an antipsychotic, showed a significant reduction in symptoms and additive cognitive benefit in double-blind, randomized trials of patients with recent-onset schizophrenia. Antipsychotic drugs counteract the inflammatory effects of cytokine networks, although the exact mechanism is unknown.

Recent studies using the TSPO radio ligand and cerebrospinal fluid analysis have shown that bipolar disorder also involves microglial activation along with alterations in peripheral cytokines. This points to the efficacy of adjunctive anti-inflammatory therapies in bipolar disorder and major depression (113–115). Microglia may provide a physiological link between the serotonin system and the GSK-3β/Wnt pathway, which is dysregulated in bipolar disorder. Postmortem studies in bipolar disorder and major depression suggest microgliosis and increased levels of iNOS, IL-1β, and NF-κB components. It is hypothesized that peripheral inflammation could lead to a

disintegration of the blood-brain barrier and an increased influx of blood monocytes that in turn activate microglia.

Taken together, it is clear that in psychiatric disorders a peripheral dysbalance of the immune system is accompanied by neuroinflammation that involves microglia activation. It was recently shown that the strongest genetic association in schizophrenia at a population level in the major histocompatibility complex arises in part from many structurally diverse alleles of the complement component 4 genes (116). We have observed an increase in the complement 4 receptor CD18 expression in microglia derived from a mouse model of schizophrenia (S.A. Wolf, H. Boddeke, H. Kettenman, unpublished data). Together with the human data, this shows the importance of the interplay between the neuronal and glia networks via the complement system in psychiatric disorders.

It seems likely that microglia can be affected by environmental factors (e.g., inflammation) during pregnancy and prenatal development. This in itself could be the basis of aberrant synaptogenesis and synaptic pruning, which lead to neuronal dysfunction and disrupted neuronal-microglia interaction in later life. At early stages of the disease, the microglia are activated, which is characterized by increased soma size, ROS, and proinflammatory cytokine production. Chronic activation of microglia might drive them into cellular exhaustion (see Section 9) and cause a loss of function such as phagocytosis, a microglial phenotype that shares several features with microglia in AD. Thus, microglia not only have a distinct profile in different (psychiatric) diseases but also acquire different phenotypes over the course of the disease.

11. MICROGLIA AND LIFESTYLE

A rather recent line of research started to investigate the relationship between lifestyle and microglia function. It is crucial to understand how lifestyle choices affect the communication between microglia, other brain cells, and peripheral organs to maintain brain function and prevent maladaptive responses that emerge during diseases or environmental factors throughout life.

11.1. Neuroinflammation Induced by Stress

Stress is known to affect the bidirectional communication between the nervous and immune systems, leading to elevated levels of stress mediators, including glucocorticoids (GCs) and catecholamines. GCs may promote brain inflammation, including the proinflammatory activation of microglia. For example, IL-1β and prostaglandin PGE2 are released from microglia activated by repeated stress. Under chronic mild stress, IL-1β activates the hypothalamic-pituitary-adrenal (HPA) axis, thereby stimulating GC release, which in turn decreases motivation to obtain reward. PGE2 and its receptor EP1 mediate elevated anxiety and social avoidance induced by repeated social defeat. Therefore, stress, GCs, and microglial cytokine and chemokine release seem to form a vicious cycle.

Microglia modulation is associated with early-life/prenatal stress as well as with stressors in adulthood. This supports the two-hit hypothesis, which proposes that early life stress primes microglia, leading to a potentiated response to subsequent stress. Moreover, activation of the sympathetic nervous system and HPA axis stimulates the trafficking of GC-insensitive monocytes to the brain. Indeed, emerging evidence implicates a novel neuroimmune circuit involving microglia activation and sympathetic outflow to the peripheral immune system that further reinforces stress-related behaviors by facilitating the recruitment of inflammatory monocytes to the brain. Sensitization of microglia and redistribution of primed monocytes are implicated in the reestablishment of anxiety-like behavior in a manner dependent on IL-1RI and multiple chemokines.

Recent reviews conclude that rather than being epiphenomena, the aforementioned stress-induced microglial alterations have broader behavioral implications, with the available evidence implicating microglia in directly regulating certain aspects of cognitive function and emotional regulation (117–122). Stress is a risk factor for psychiatric disorders. The development and exacerbation of depression and anxiety are associated with exposure to repeated psychosocial stress. An overactive HPA axis might also be partially responsible for an overactive immune response in obese individuals. This is in line with a documented comorbidity of lower stress resistance and obesity in depression.

11.2. Neuroinflammation Induced by Diet

Microglia express receptors for a variety of metabolic factors and hormones, suggesting that they actively participate in metabolic function. One recent review coined the complex neural-glia network in the hypothalamus the metabolic sensor, as it receives information directly from blood-borne factors (123–129). Microglia and astrocytes participate in the hypothalamic inflammatory response to high-fat diet (HFD)-induced obesity, contributing to inflammatory-related insulin and leptin resistance. Microglia and astrocytes also enter a reactive state following a hypercaloric challenge. Hypercholesterolemia, which is often seen in obesity, is associated with increased oxidative stress and the development of neurotoxicity orchestrated by microglia in the hypothalamus, hippocampus, and frontal cortex with prolonged HFD exposure. In a small cohort of obese patients, an increased binding to a TSPO ligand hinted at a neuroinflammatory stage in obesity. Similar to psychosocial stress, under- or overnutrition during pregnancy and early neonatal life might prime the immune system, including microglia.

Any diffusible substance whose expression or presence in fetal tissues is altered by maternal nutrition would be a candidate for mediating changes associated with disease programming. Factors that are gaining attention for their importance in disease risk programming by maternal nutritional status include fatty acids, which can signal through TLRs to initiate an inflammatory response. Recently, the role of the gut microbiome in health and disease has received significant attention. In regard to early life programming, parental consumption of a Western diet was shown to alter concurrently the offspring immunity and gut microbiome in mouse and nonhuman primate models. Interestingly, in a mouse model with no gut bacteria (germ free), the microglia could not fight a viral brain infection sufficiently. Supplementation of these mice with short-chain fatty acids (SCFAs) allowed them to restore microglia function. This shows the close association between the gut and the brain, as SCFAs are produced when dietary fiber is fermented in the colon by microbiota.

11.3. Neuroinflammation Induced by Alcohol

The neurotoxic effects of alcohol are well studied in vitro and in animal models (130–133). Clinical studies have found increased microglial activation in the postmortem brains of alcoholics. Alcohol-induced activation of microglia results in the microglial release of proinflammatory factors, specifically TNF-α and ROS, which augment neurotoxicity and an increased neuronal release of TGF-β. In vivo binge-like alcohol exposure in rodents also results in neurotoxicity, but in contrast to chronic exposure to alcohol, microglia show partial activation and proliferation but do not release proinflammatory factors. Based on similar observations during abstinence, microglia might play an additional role in homeostatic regenerative mechanisms. Repeated stimulation of the innate immune system during chronic or heavy alcohol consumption might lead to decreased inhibition of the mesolimbic reward system, and thus, increased drinking. Treatment with

minocycline reduced voluntary alcohol consumption in adult mice. These studies suggest that microglia might mediate alcohol preference and contribute to the development of alcohol use disorder. A possible mechanism for microglia activation is through the alcohol-induced release of the danger signal alarmin, HMGB1, that activates microglial TLR4, primes the NLRP3 inflammasome, and leads to a neuronal hyperexcitability and excitotoxicity neuronal death. Neuroinflammation in the amygdala contributes directly to withdrawal behavior and symptoms. In the context of alcohol exposure and withdrawal, gut dysbiosis contributes to neuroinflammation in the amygdala. Alcohol consumption increases intestinal permeability and results in increased levels of PAMPs, such as endotoxin in the systemic circulation that triggers inflammation (134).

12. METHODS OF BALANCING NEUROINFLAMMATION

Minocycline modulates microglial properties and was shown to be beneficial in a plethora of pathologies, both in animal models and in clinical trials. These pathologies include schizophrenia, ischemia, AD, Huntington's disease, depression, PD, MS, ALS, alcoholism, traumatic brain injury, neuropathic brain, HIV/AIDS, and cancer, as critically reviewed recently (135). Because microglia in these pathologies have different phenotypes, it is unlikely that the mode of action of minocycline is directed to one specific target or pathway. Moreover, minocycline can target the peripheral immune system, the gut microbiota, or other cells. In addition, natural products or bioactive compounds along with some enrichment strategies are used to target neuroinflammation and thus microglia (136–138).

One of the most studied natural products with regard to brain function is ginseng. Among others, it was shown to have an effect on microglia function (139). The metabolites of ginseng (ginsenosides) inhibit iNOS. The ginsenosides Rg3 and Rg5 modulated an increase in TNF-α, IL-1β, and IL-6 mRNA after LPS injection in mice. Protective effects of treatment occurred via the phospho-p38, iNOS, and cyclooxygenase-2 (COX-2) signaling pathways in LPS-stimulated microglial cells. Another good albeit less-studied natural product is raw honey, as it possesses memory-enhancing effects as well as anxiolytic, antinociceptive, anticonvulsant, and antidepressant properties. Research suggests that the polyphenol constituents of honey can quench biological ROS and counter oxidative stress while restoring the cellular antioxidant defense system. Honey polyphenols are also directly involved in apoptotic activities while attenuating microglia-induced neuroinflammation. In addition, food-derived flavonoids, such as luteolin, apigenin, and quercetin, have anti-inflammatory properties. Other notable examples of food-derived components include resveratrol found in grapes, which acts through SIRT1 (a member of the sirtuins superfamily) to deacetylate several transcription factors, including NF-κB. Curcumin is found in turmeric and inhibits NF-κB- and AP-1 DNA–binding activity. Additional evidence suggests that the polyunsaturated fatty acid (PUFA) DHA, a major component of fish oil, may play a role in microglia-mediated synaptic pruning (140, 141). It was proposed that the intake of flavonoids, n-PUFAs, and antioxidant nutrients reduces both systemic inflammation and neuroinflammation and thus reduces cognitive decline during aging. Another option to target neuroinflammation is eating less: "Dietary restriction attenuates the age-related activation of astrocytes and microglia with concomitant beneficial effects on neurodegeneration and cognition" (142, p. 83).

There is one additional natural method to pamper microglia homeostasis and thus brain health: voluntary physical exercise. From preclinical studies, the positive effects of exercise are related to increased levels of neurotrophic factors, elevated expression of anti-inflammatory cytokines, and reduced levels of proinflammatory cytokines and activated microglia (143, 144). Exercise also has a global anti-inflammatory effect in the organism, such as an increase of IL-10 and glucocorticoids.

In addition, the metabolome is changed after exercise. Microglia might sense the changes in cytokines, metabolites, and hormones and initiate a response to exercise by producing growth factors, such as BDNF and IGF1, which in turn keeps the neuronal network intact and maintains the generation of new neurons.

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