



# Strategies for Manipulating Microglia to Determine Their Role in the Healthy and Diseased Brain

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## Abstract

Microglia are the specialized macrophages of the central nervous system and play an important role in neural circuit development, modulating neurotransmission, and maintaining brain homeostasis. Microglia in normal brain is quiescent and show ramified morphology with numerous branching processes. They constantly survey their surrounding microenvironment through the extension and retraction of their processes and interact with neurons, astrocytes, and blood vessels using these processes. Microglia respond quickly to any pathological event in the brain by assuming amoeboid morphology devoid of branching processes and restore homeostasis. However, when there is chronic inflammation, microglia may lose their homeostatic functions and secrete various proinflammatory cytokines and mediators that initiate neural dysfunction and neurodegeneration. In this article, we review the role of microglia in the normal brain and in various pathological brain conditions, such as Alzheimer's disease and multiple sclerosis. We describe strategies to manipulate microglia, focusing on depletion, repopulation, and replacement, and we discuss their therapeutic potential.

**Keywords** Microglia depletion · Microglia repopulation · Microglia replacement · Microglia transplantation

## Introduction

Microglia are the tissue-resident immune cells of the central nervous system (CNS) and represent approximately 10% of the total glial cell population. They derive from the primitive yolk sac and proliferate in the brain rudiment of the developing embryo from embryonic day 8 (E8). The embryonic microglial precursors are distinct from those for the monocyte/macrophage system [1–5]. Once established in the CNS parenchyma, microglia are sustained by the proliferation of resident progenitor cells, rather than by blood-borne cells [6, 7]. They have a remarkable capacity to adapt to the CNS environment by undergoing functional and morphological changes. They play a key role in the CNS immune defense, but their functions extend well beyond this. For instance, they are involved in the development of the CNS, in

maintaining brain homeostasis, and in responding to nearly all CNS disturbances. They also provide a link between the neurological and immunological activity of the CNS.

In the resting state, microglia in the adult brain have a small cell body and a highly ramified morphology with a large number of processes. The cells constantly survey their surrounding microenvironment through the extension and retraction of these highly motile processes, and they also interact with neurons, astrocytes, and blood vessels via these processes [8–12]. In the healthy CNS, various receptors are expressed on microglia, such as CD200R, CX3CR1, and siglecs, which respond to ligands produced by neurons, such as CD200, CX3CL1, and sialic acid [13–15]. This enables microglia-neuron interactions to take place that can maintain the microglia in a quiescent state. This is achieved through activation of immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which suppress the intracellular immune signaling initiated by immunoreceptor tyrosine-based activation motifs (ITAM), as found on certain microglial receptors such as TREM2 and CR3 (CD11b/CD18) [16]. In this way, the microglia-neuron interactions serve to suppress and strictly regulate inflammatory responses that can otherwise damage the surrounding neurons. This is important, as the latter have a limited capacity for regeneration.

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However, microglia-neuron interactions can be disrupted by the loss of CNS homeostasis or following an injury, infection, or other CNS pathology. This induces microglial activation, which leads to significant morphological changes, most notably the retraction and thickening of the ramified processes and cell body enlargement [17, 18]. In the case of chronic neurodegenerative diseases, the microglia acquire an amoeboid shape that is associated with increased phagocytosis, which serves to clear the disease-related debris. Along with these morphological changes, the microglia upregulate various inflammatory proteins.

As microglia play an important role in various brain processes, their dysfunction can have a marked effect on the normal brain as well as on neurological disease progression. It is therefore important to carry out studies to investigate their role in more detail in both the normal and the diseased brain. In the following sections, we discuss the use of various pharmacological agents to deplete microglia in order to study their effects on the brain.

## Microglia in Normal Brain Development

At the early stages of development, microglia display an amoeboid morphology with no or few processes. The cells express matrix metalloproteinases (MMPs), such as MMP-8 and -9, that play a key role in the migration of the microglia. When MMP is inhibited, microglial migration is found to be disrupted [4, 19, 20]. In the postnatal CNS, other factors are thought to be involved in guiding microglia to their final destination in the CNS parenchyma, such as CX3CR1, a chemokine receptor [21]. In support of this, postnatal CX3CR1 knockout mice have been shown to have delayed recruitment of microglia to the developing hippocampus and somatosensory cortex, with a delay of several days [21, 22]. This finding could be attributed to a chemoattractant effect caused by fractalkine (CX3CL1) or to altered microglial motility in the knockout mice. Purinergic receptors, P2Y12 receptor and P2Y6 receptor, may also be involved in microglial migration during development, as there is evidence that they can facilitate migration as well as functional maturation [23–25]. Once established in the brain, the microglia play a critical role in the development and maintenance of the CNS throughout life, as described below.

During both embryonic and postnatal development, a large number of neurons die in a process known as programmed cell death (PCD) [26]. The microglia are thought to clear these apoptotic cells by phagocytosis [27]. This clearance is necessary for tissue homeostasis and the proper development of synaptic circuits. In the developing zebrafish brain, it has been shown that apoptotic neurons attract microglia via nucleotide signaling [28], which suggests that these

processes play a role in microglial colonization during development.

There is evidence that microglia can also promote neuronal cell death in a process that is unrelated to PCD. For instance, in proliferative zones of the developing forebrain of monkeys and rats, microglia have been shown to engulf non-apoptotic neural precursors [29]. In addition, it has been found that microglial depletion increases the number of neural precursors in the subventricular zone (SVZ); in these studies, the microglia were eliminated by injecting clodronate liposomes into the fetal cerebral cortex or deactivated in utero using tetracyclines [29]. Experiments using cerebellar slice cultures have also shown that the elimination of microglia strongly reduces Purkinje cell apoptosis, which is normally promoted by superoxide ions that are produced by microglial respiratory bursts [30]. Microglia have also been reported to eliminate cells other than neurons by phagocytosis, specifically astrocytes in the developing amygdala and retina [31, 32]. Taken together, these findings indicate that microglia play a key role in restraining the number of neural and glial cells during development.

However, there is also evidence that microglia support the production of neural precursors in the SVZ and that they promote the survival of layer 5 pyramidal neurons at the early postnatal stages [33, 34]. The reasons for this discrepancy remain unclear but may relate to the specific methods used to eliminate the microglia or to manipulate their phenotype. Nevertheless, the different approaches, whether genetic or pharmacological, can help us to better understand the role of microglia in brain development.

## Microglial Regulation of Synapses and Neuronal Functions

During CNS development, synaptic contacts are overproduced. Mature synaptic networks are formed by the functional maturation of appropriate synaptic connections, while the others are eliminated [35]. There is evidence that microglia contribute to the formation of these mature synaptic networks. For instance, in postnatal development, microglia have been found to contain both presynaptic and postsynaptic proteins, specifically SNAP25, a presynaptic vesicle-associated protein at the excitatory synapse, and PSD95, a postsynaptic density protein. This synaptic protein uptake, due to phagocytosis, is thought to relate to CX3CL1-CX3CR1 signaling [21]. In support of this, CX3CR1 knockout mice have been found to have a higher frequency and amplitude of excitatory postsynaptic currents in pyramidal cells, which suggests that microglia are involved in synapse elimination during development through CX3CL1-CX3CR1 signaling [21, 36].

The microglial phagocytosis of presynapses has been shown to occur in an activity-dependent manner, which

involves the C3 and C1q complement proteins; this has been shown for retinal ganglion cells in the dorsal lateral geniculate nucleus during eye-specific segregation [37]. In line with this, C1q knockout mice have been shown to have increased functional connectivity of excitatory synapses, leading to the development of epileptiform activity later in life, thus indicating that synapse elimination was impaired during development [38]. It has also been reported that phosphatidylserine needs to be exposed on synapses to enable microglia-mediated pruning of newly-formed olfactory bulb neurons in adult mice, a process that involves C1q or C3 binding to the phosphatidylserine [39, 40]. These results imply that C1q and C3 may bind to less active synapses, thus flagging them for removal by microglia. The microglia would need to continuously monitor and respond to the activity-induced changes in neuronal synapses.

The elimination of synapses by microglia has been suggested to be involved not only in neural circuit formation during development but also in synaptic modification during adulthood. In the adult brain, microglia have frequent, brief direct contact with neuronal synapses, which is modified by the amount of neuronal activity. Ischemic brain injury has been found to induce an increase in the duration of microglial contact, which is associated with the disappearance of the presynaptic bouton, thus indicating that the microglia eliminate the dysfunctional synaptic structures [9]. Similarly, the elimination of microglia has been found to increase both excitatory and inhibitory connections to the visual cortex in adult mice, implying that microglia regulate synaptic connections throughout adulthood [41]. There is also evidence that forgetting is regulated by microglia-mediated synaptic elimination in the hippocampus, as shown in healthy adult mice. This process involves an activity- and complement-dependent pathway, so that complement inhibition or microglial depletion prevents the forgetting of contextual fear memories [42].

As well as synapse elimination, there is evidence that microglia may also induce synapse formation in certain cases. For instance, experiments in mice have shown that microglial contact with dendritic shafts increases the rate of filopodium formation in the somatosensory cortex [43], which relates to the formation of synapses. Also, the transient partial ablation of microglia in postnatal mice has been found to decrease the frequency of the miniature excitatory postsynaptic current (mEPSC) in sensory cortical pyramidal cells as well as the functional connectivity, thus indicating that microglia are required for spine formation [43].

There is evidence that microglia are also involved in controlling neuronal activity, in addition to their role in synaptic regulation. Microglia have been reported to provide negative feedback for the control of neuronal activity by converting ATP to AMP/ADO. This is essential for protecting the brain from excessive activation in both healthy and diseased states

[44]. Microglial interaction with synapses has also been found to increase neuronal activity in the mature, healthy brain, which helps to synchronize local populations of neurons [45]. This shows that microglia can affect neuronal activity through their contact with synapses.

Microglia have also been found to modulate synaptic function through soluble mediators, such as brain-derived neurotrophic factor (BDNF). A microglia-specific knockout of BDNF in CX3CR1-CreER mice has shown that microglial BDNF facilitates synapse formation and controls the expression of AMPA and NMDA receptor subunits. This influences performance on certain tasks, such as motor learning and fear conditioning in adult mice [46]. Microglia can also control synaptic maturation indirectly through astrocytes. For instance, in the juvenile hippocampus and in certain diseases, microglia are mobilized by activating TLR4. This induces a rapid release of microglial ATP that activates the purinergic receptor P2Y1 on astrocytes. This in turn induces the release of astrocytic glutamate that regulates the synaptic strength at Schaffer collateral-CA1 synapses [11].

## Microglia in Neuropathological Conditions

### Rett Syndrome

Rett syndrome is an X-linked neurodevelopmental disorder that primarily occurs in girls. It is most commonly caused by a mutation in the *MECP2* gene [47]. *MECP2* is a transcriptional repressor that binds to methylated DNA via its methyl binding domain and transcriptional repression domain [48]. This promotes the recruitment of histone deacetylases that repress transcription. The pattern of *MECP2* expression in the CNS during the embryonic and early postnatal periods is crucial for normal brain development. *MECP2* is expressed in neurons and all glial cells, including neural stem cells, astrocytes, and microglia [49–52]. The latter are believed to play a key role in the pathogenesis of Rett syndrome by releasing an abnormally high level of glutamate, which causes excitotoxicity, and by excessively eliminating synapses by engulfing presynaptic axon terminals in the late stages of the disease [53, 54]. It has been shown that transplantation of wild-type bone marrow into *MECP2*-null mice can halt the disease progression [55]. This therapeutic improvement was linked to myeloid chimerism in the CNS, induced by whole-body irradiation, and involved the migration of bone-marrow-derived microglia into the brain parenchyma. However, another study reported almost no effect of re-introducing wild-type myeloid cells in three different mouse models of Rett syndrome [56]. The reasons for this difference remain unclear.

## Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the excessive accumulation and sustained deposition of amyloid beta ( $A\beta$ ). Neurofibrillary tangles are also found to form inside neurons resulting from hyperphosphorylation of the tau protein [57–59]. These pathological changes induce a progressive cognitive decline. Although the cause of the neurodegeneration remains unknown, microglia are believed to play a key role in the pathogenesis of AD. In support of this, microglia have been found cluster around  $A\beta$  deposits in the brains of patients with AD, and they form a cellular component of senile plaques.

A number of susceptibility genes have been identified for AD, including the genes encoding amyloid precursor protein (APP), apolipoprotein E, presenilin 1 and 2, TREM2, and CD33 [59]. Several of these genes are expressed by microglia. For instance, TREM2 is a microglial  $A\beta$  receptor. Inhibiting the expression of TREM2 in microglia has been found to disrupt phagocytosis and increase the expression of proinflammatory mediators, which accelerates the accumulation of  $A\beta$  and increases neurotoxicity [60]. CD33 is a receptor that has been found to have high levels of expression in the microglia of patients with AD. This has been associated with reduced phagocytosis of  $A\beta$  and an impaired clearance of  $A\beta$  [61].

Scavenger receptors are also expressed by microglia and several of these have been shown to interact with  $A\beta$ , including CD36, class A1 scavenger receptors (Scara1), and the receptor for advanced glycation endproducts (RAGE) [62–64]. In addition, the pattern recognition receptors TLR2 and TLR4 are expressed by microglia, and functional deficiency in these is associated with increased  $A\beta$  deposits. In a murine model of AD, activating TLR4 signaling through the systemic administration of a TLR4 agonist has been found to increase the microglial phagocytosis of  $A\beta$  and enhance cognitive functioning [65–67]. These findings suggest that phagocytosis in the CNS is either impaired in AD or becomes overwhelmed by the continuous deposition of  $A\beta$ .

During the progression of AD, proinflammatory cytokines are produced by the microglia, which are thought to accelerate the disease progression and cognitive decline [68, 69]. In support of this,  $A\beta$  has been shown to induce the microglial production of proinflammatory cytokines, such as IL-1 $\beta$  and TNF- $\alpha$ , which can induce neuronal cell death [70, 71]. It has also been shown that mice lacking Nlrp3 or Casp1, which activate proinflammatory cytokines, are protected from spatial memory loss in a mouse model of AD [72].

## Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative condition characterized by the accumulation of  $\alpha$ -synuclein in neurons and the loss of dopaminergic neurons in the substantia nigra pars compacta [73]. Mutations in several different genes, including *SNCA* and *PRKN*, are known to cause the disease [74]. The most commonly studied animal models of PD are produced by injecting 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine, two neurotoxic agents that induce the death of dopaminergic neurons in the substantia nigra, resulting in PD-like symptoms [75].

Although the precise contribution of microglia to PD is not entirely understood, it is believed that proinflammatory cytokines produced by microglia promote the development of the pathology. *LRRK2*, which encodes leucine-rich repeat kinase 2, is one of the most commonly mutated genes in both idiopathic and familial PD; it is highly expressed in microglia and induces microglial activation in response to inflammation through p38 MAPK and NF- $\kappa$ B signaling pathways [76, 77]. The involvement of microglia has also been implicated in a MPTP-induced macaque model of PD. Here, IFN- $\gamma$  and TNF- $\alpha$  have been found to be elevated in the serum and CNS during the development of the disease, and deletion of the corresponding genes in rodents shows that both of these cytokines are required to fully activate microglia [78]. Similarly, microglia have been shown to produce proinflammatory cytokines when exposed to  $\alpha$ -synuclein, which may affect the disease progression [79].

Microglia have also been implicated in other processes in PD. For instance, there is evidence that microglia phagocytose and degrade  $\alpha$ -synuclein released by neurons, a process that requires TLR4, thereby protecting neurons [80]. It has also been shown that  $\alpha$ -synuclein is transferred between microglia through tunneling nanotubes, which may be the mechanism that induces the propagation of  $\alpha$ -synuclein [81]. These studies suggest that microglia can promote neuronal survival or exacerbate the disease, depending on their activation profile, and are thus key players in influencing disease progression.

## Microglia in MS and EAE

Multiple sclerosis (MS) is a chronic inflammatory disease with a significant autoimmune component that leads to demyelination and the progressive loss of functional neurons. MS is characterized by multifocal white matter lesions, and active lesions are always associated with the presence of activated macrophages and/or microglia. This indicates that these cells play an important role in MS [82].

Experimental autoimmune encephalomyelitis (EAE) is used as an animal model of demyelinating diseases such as MS. It has been found that microglia are involved in the

onset of EAE. Specifically, in the acute stages of EAE, T cells have been found to initiate contact with resting microglia in the brain parenchyma [83]. It has also been shown that the expansion of microglia is required for EAE disease progression; when microglial expansion is prevented, mice do not develop severe EAE [84, 85]. Studies have found that the microglia-mediated disease progression involves the NF- $\kappa$ B pathway. Specifically, microglia-specific TAK1 or NIK1 depletion has been shown to affect the activation of proinflammatory pathways through the NF- $\kappa$ B pathway, and result in animals resistant to EAE. However, appropriate T cell responses were found in the periphery [85, 86]. The TAK1 deletion also attenuated CCL2 expression leading to decreased infiltration by inflammatory monocytes, while the NIK1 deletion attenuated the infiltration by T cells. These results indicate that microglia play an essential role in the pathogenesis of EAE.

### Stroke or Ischemic Brain Damages

Ischemic brain injuries mainly contain ischemic stroke, cerebral white-matter ischemia, and neonatal hypoxia-ischemic brain damage, which are the third-leading cause of human disability [87]. In middle cerebral artery occlusion (MCAO), animal model of ischemic stroke, activation of microglia can be detected at the boundary of ischemic lesions within 30 min [88]. Activated microglia are important for post-ischemic inflammation, which has long been considered a negative factor contributing to stroke outcomes [89]. However, during the acute phase of stroke, it is reported that proliferating resident microglial cells protect neurons by producing neurotrophic factors such as IGF1 and selective ablation of proliferating microglia after transient MCAO decrease IGF1 increasing lesion size and number of apoptotic neurons [90]. Also, transplantation of exogenous microglia after or during focal ischemic stroke improves behavioral recovery [91, 92]. In contrast to acute phase, during the chronic phase, activated microglia located in the peri-infarct and distal regions can induce delayed selective neuronal loss. Activated microglia release a large variety of proinflammatory mediators, including IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , which can lead to acute inflammatory reactions [93, 94]. The excessive production of inflammatory cytokines can exacerbate damage to neighboring neurons and result in delayed deterioration of ischemic tissue. Microglia also induce delayed neuronal cell loss by phagoptosis whereby microglia phagocytose viable neurons [95, 96]. Genetic deletion of MFG-E8 or phagocytic receptor MerTK, attenuate delayed neuronal loss and long-term functional deficits [96]. Inhibition of phosphatidylserine exposure on neurons by the knockdown of TMEM16F, after ischemia, decrease microglial phagocytosis of stressed neurons and reduced motor deficits after transient MCAO in rats [97]. Additionally,

inhibition of the P2Y<sub>12</sub> receptor by clopidogrel also prevented this microglial recruitment and neuronal loss [98]. These results indicate that microglia play both beneficial and harmful role in ischemia depending with the disease phase.

## Strategies for Manipulating Microglia

### Microglial Depletion for Studying the Role of Microglia in the Normal and Diseased Brain

Various methods have been developed for depleting microglia, including the use of pharmacological and genetic approaches [46, 99–102]. In this review, we focus on the pharmacological approach, and in particular the use of colony-stimulating factor 1 receptor (CSF1R) inhibitors.

CSF1R is required for the trophic support of microglia to ensure their viability. Blockade of this receptor through CSF1R inhibitors has been found to lead to a 90% depletion of microglia [99]. This method can therefore be used to study the role of microglia in the normal brain. Although it was initially thought that microglial depletion had no short- or long-term effects on normal brain functions, recent studies using CSF1R inhibitors have shown that it prevents the forgetting of contextual fear memories. This therefore indicates the essential role of microglia in normal brain functions [42].

CSF1R inhibitors have also been used to investigate the role of microglia in brain pathology. For instance, they have been administered in mouse models of intracerebral hemorrhage and shown that microglial depletion reduces the inflammation, promotes brain recovery, and prevents disruption to the blood–brain barrier, leading to a reduced infiltration of leukocytes [103]. CSF1R inhibitors have also been used to study microglial depletion in mouse models of AD. This has also revealed beneficial outcomes including reduced neuronal loss, improved memory functions, and a partial prevention of disease progression. However, there was little effect on the amyloid levels and plaque loads [104]. Microglial depletion has also been found to have beneficial effects in other disease models. For instance, in a mouse model of status epilepticus, it was found that microglia induced the activation of astrocytes; depleting the microglia led to a decrease in astrogliosis and reduced the seizure susceptibility [105]. In a mouse model of glutamate-induced glaucoma, microglia were found to mediate retinal excitotoxicity by secreting TNF- $\alpha$ , and microglial depletion protected the neurons [106]. Similarly, in EAE, microglial depletion was found to improve both the physical symptoms and physiological characteristics of the disease [107].

However, microglial depletion has not always been found to yield beneficial outcomes. In a mouse model of ischemic stroke, the depletion of microglia was found to increase the

size of the infarct with increased cell death, inflammatory mediator levels, and leukocyte infiltration into the CNS [108]. Similarly, in a mouse model of traumatic brain injury (TBI), microglial depletion was found to increase the core area of the injury. The microglia were found to be required to convert astrocytes to their protective phenotype, by down-regulating the astrocytic P2Y1 receptor [109]. In a mouse model of secondary progressive MS, the depletion of microglia was found to exacerbate the secondary progression of EAE and increase the mortality rates by promoting inflammation, demyelination, and axonal degeneration [110]. Similarly, in a mouse model of neurotropic coronavirus (JHMV), microglial depletion was found to impair myelin repair which prolonged the disease. However, the kinetics of virus clearance was not affected. These results indicate that microglia-mediated debris clearance is required for remyelination [111].

Together, these findings support the view that microglial depletion has context-dependent effects. It is possible that the exact timing of the depletion may be important to obtain beneficial effects in different diseases.

### Microglial Depletion and Repopulation as a Therapeutic Tool for CNS Diseases

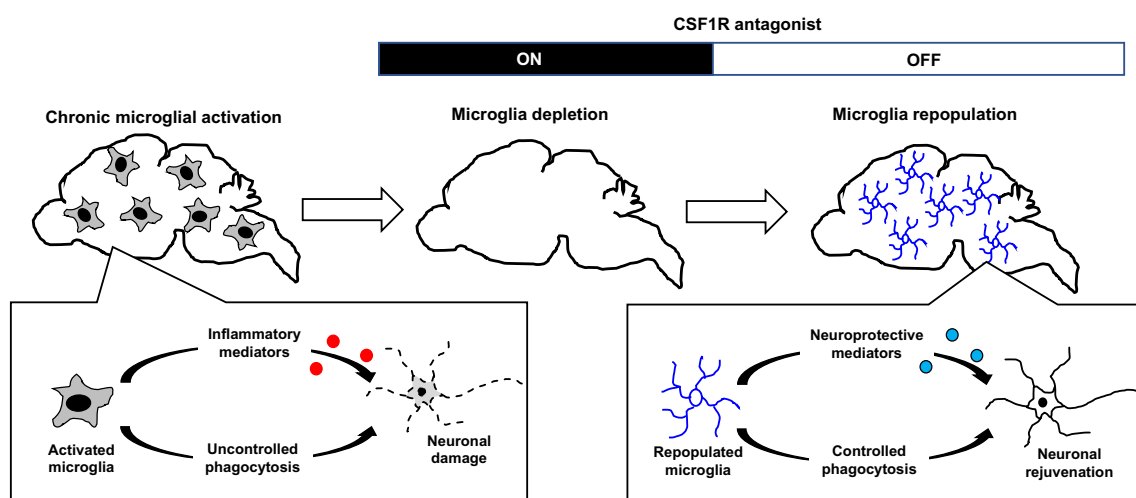
As described above, microglia can be depleted using pharmacological as well as genetic approaches. It has been found that upon cessation of the depleting agent, microglia rapidly recover, reaching normal levels within a week [99]. Recent studies have shown that this results from the proliferation of a residual pool of microglia [112]. In the healthy brain, these repopulated microglia have been found to have transcriptomic profiles indicating the resting state phenotype

[99]. This suggests that repopulation may be a promising strategy for the treatment of neuropathology in diseases with a prolonged proinflammatory response, such as TBI (Fig. 1).

In a recent study, microglial depletion and repopulation was induced during the chronic phase of TBI in rats. This was found to attenuate the neuroinflammation and related neurodegeneration, and to improve motor and cognitive functions [113]. Microglial depletion and repopulation have also been studied in the aged brain. Here, microglia have been found to have a more proinflammatory phenotype with older age, which is believed to play an important role in age-related cognitive decline. It has been found that microglial depletion and repopulation in aged mouse brains partially restores the transcriptomic profile seen in younger brains, with a decrease in the age-related upregulation of genes to control levels [114]. There was also a restoration of long-term potentiation and a reversal of age-related deficits in spatial learning. These results indicate that microglial depletion and repopulation could potentially be used therapeutically for diseases with microglial dysfunction or senescence [114]. However, prior to clinical translation, further preclinical studies are required to determine the optimal timing between depletion and repopulation, the long-term effects on CNS homeostasis, and the long-term therapeutic efficacy.

### Microglial Replacement as a Potential Therapeutic Approach

Microglial depletion and repopulation is a potential therapeutic tool for microglia that are not genetically dysfunctional. However, there are certain diseases that are caused by pathogenic mutations that result in genetically dysfunctional microglia, such as primary microglia-associated



**Fig. 1** Potential scheme of microglial repopulation therapy. Chronically activated microglia secrete inflammatory cytokines as well as uncontrolled phagocytosis of neurons which can lead to neuronal loss

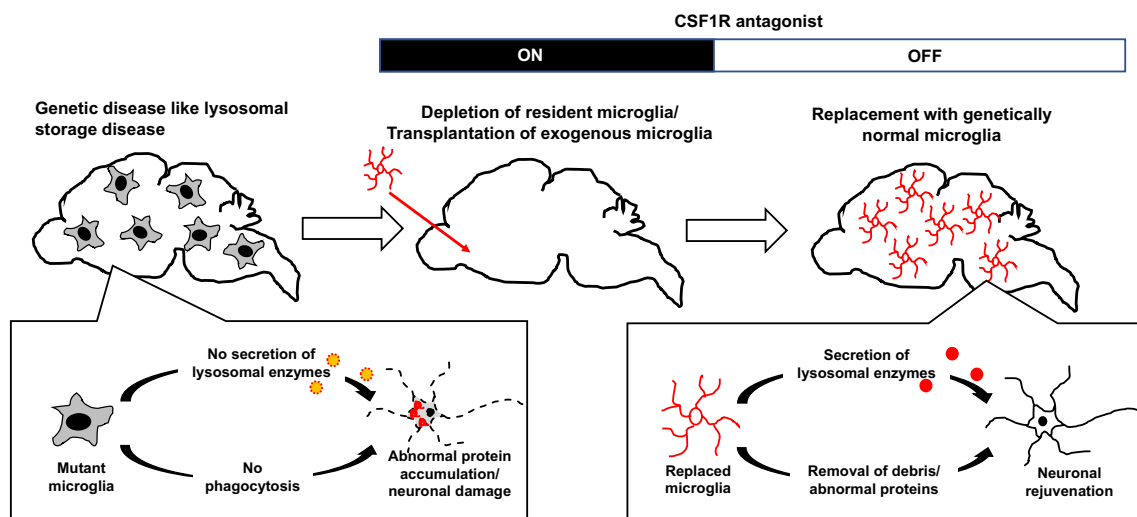
(left panel). Selective ablation of microglia with repopulation will decrease their activation. The newly derived microglia can perform the normal functions and maintain tissue integrity (right panel)

leukodystrophies, where there are mutations in genes such as TREM2, TYROBP, CSF1R, and USP18 [115, 116]. In these diseases, microglial repopulation following depletion would not be an effective therapeutic strategy. Instead, it would be desirable to replace the dysfunctional cells with normal-functioning microglia. For this, the total depletion of mutation-carrying microglia would be required, followed by replacement through the transplantation of normal microglia. This could have great potential as a therapeutic intervention (Fig. 2).

Apart from microglia-associated leukodystrophies, microglial replacement could be an effective intervention for lysosomal storage diseases (LSDs). These diseases are a group of inherited metabolic disorders caused by mutations in genes encoding lysosomal hydrolases, integral membrane proteins, and transporters [117]. There are a broad spectrum of systemic and CNS manifestations, but CNS involvement is observed in the majority of LSDs, with neurodegeneration and demyelination [118]. The severity of the CNS symptoms can vary greatly, ranging from mild mental retardation to severe and rapidly-progressing motor and intellectual disability. Although enzyme replacement therapy has been developed for patients with LSDs, involving injection of a recombinant proenzyme, this cannot be used to treat the CNS involvement, as the systemically-delivered enzyme cannot enter the CNS because of the blood–brain barrier [119].

Microglial replacement could therefore be used to enable treatment of the CNS in the disorder. Here, normal microglia would replace the dysfunctional microglia and would provide critical enzymes that could restore brain function.

Currently, hematopoietic stem cell transplantation (HSCT) is used to treat LSDs, and through this, microglia are replaced with bone marrow-derived cells [120–122], as the preclinical studies focused on bone marrow transplants. Although microglia are not normally replaced with bone marrow-derived myeloid precursors, treatment using whole-body irradiation and busulfan allows the bone marrow-derived cells to colonize the brain by inducing microglial senescence [123, 124]. However, the busulfan-mediated myeloablation prior to HSCT is associated with the rapid, complete, and permanent loss of adult neurogenesis, which may account for the patients' cognitive deficits [124, 125]. It is therefore desirable to develop an alternative method for replacing microglia, without the need for myeloablation. This has currently been successfully achieved in adult mice, through microglial depletion and replacement through transplantation [126]. However, further studies are required to determine the optimal timing between depletion and replacement, and to determine the critical number of cells that need to be replaced to obtain a therapeutic benefit. In addition, the long-term effects on brain function and the long-term therapeutic efficacy will also need to be investigated in preclinical studies.



**Fig. 2** Transplantation of microglia to treat CNS diseases. In diseases like lysosomal storage disease, endogenous dysfunctional microglia cannot degrade and phagocytose the abnormally accumulated proteins. The accumulated proteins can cause neuronal damage (left panel). Human or mouse iPS cells can be differentiated into microglia. Differentiated microglia cells are then transplanted into the

brain after the depletion of endogenous dysfunctional microglia. The normal enzyme secreted by transplanted microglia will degrade the abnormally accumulated proteins in neurons and glial cells with therapeutic effect. Also, the transplanted normal microglia will also clear abnormally accumulated proteins by phagocytosis (right panel)

## Microglial Replacement for Studying the Characteristics of Human Microglia

The characteristic features of microglia differ between mice and humans [127–129]. As a result, experiments performed on murine microglia do not always accurately reflect the responses of human cells. To date, relatively few studies have been conducted on human microglia because of the low availability of cells and the need for an experimental system to study the cells *in vivo*.

Recently, however, various methods have been developed that enable human induced pluripotent stem cells to differentiate into microglia (iPSMG) [130–136]. These methods can provide an ample supply of cells to study the characteristics of human microglia. Furthermore, studies have now succeeded in investigating the *in vivo* characteristics of iPSMG by injecting the cells into the brains of immunodeficient adult or neonatal mice. For instance, we recently depleted the endogenous microglia in immunocompetent mice using a CSF1R antagonist, and then transplanted human microglia into the mouse brain via a non-invasive, transnasal route [137]. We were able to show that the iPSMG proliferated and matured morphologically in the mouse brain. Additionally, the iPSMG were found to have a more complex *in vivo* morphology than endogenous mouse microglia [137, 138]. Although it remains unclear how this morphological complexity could affect different brain functions, we speculate that the longer and more complex processes may enable the iPSMG to actively survey and interact with more synapses, thus influencing neurons. Experiments using iPSMG-transplanted mice have also shown that human microglia respond differently to A $\beta$  compared with mouse microglia, thus revealing novel, human-specific aspects of the microglial response [133, 134].

To conclude, the transplantation of either normal, pre-activated, or genetically-modified iPSMG has the potential to improve our understanding of human microglia in both the healthy and the diseased brain. By using iPSMGs in conjunction with animal disease models, new insight could be gained into human pathological brain conditions.

### Future Directions

Microglia have multiple roles in the normal brain and in CNS diseases. As microglia can be depleted, repopulated, and replaced, they can be effectively studied *in vivo*, and they represent a promising target for therapeutic interventions. However, the short- and long-term effects of microglial depletion, repopulation, and replacement need

to be investigated further and at different levels, from cellular interactions to overall brain function. In addition, the optimal timing of depletion and repopulation needs to be determined for different CNS diseases so that the beneficial effects can be maximized and any adverse effects minimized. In conclusion, the short-term depletion and subsequent repopulation or replacement of microglia holds great promise for studying their role in both health and disease.

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### Declarations

**Competing interests** The authors declare no competing interests.

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