Title
To Kill a Microglia: A Case for CSF1R Inhibitors

Journal
Trends in Immunology, 41(9)

ISSN
1471-4906

Authors
Green, Kim N
Crapser, Joshua D
Hohsfield, Lindsay A

Publication Date
2020-09-01

DOI
10.1016/j.it.2020.07.001

Peer reviewed

HHS Public Access

Author manuscript

Trends Immunol. Author manuscript; available in PMC 2021 September 01.

Published in final edited form as:

Trends Immunol. 2020 September ; 41(9): 771–784. doi:10.1016/j.it.2020.07.001.

To Kill Microglia: A Case for CSF1R Inhibitors

Kim N. Green[1,*], **Joshua D. Crapser**[1], **Lindsay A. Hohsfield**[1]

[1]Department of Neurobiology and Behavior, University of California, Irvine, CA 92697, USA

Abstract

Microglia, the brain's immune sentinels, have garnered much attention in recent years. Researchers have begun to identify the manifold roles that these cells play in the central nervous system (CNS), and this work has been greatly facilitated by microglial depletion paradigms. The varying degrees of spatiotemporal manipulation afforded by such techniques allow microglial ablation before, during, and/or following insult, injury, or disease. We review the major methods of microglial depletion, including toxin-based, genetic, or pharmacological approaches, which differ in key factors including depletion onset, duration, and off-target effects. We conclude that pharmacological CSF1R inhibitors afford the most extensive versatility in manipulating microglia, making them ideal candidates for future studies investigating microglial function in health and disease.

Manipulating Microglia for Insight into Brain Function: Tools at Hand

Microglia are the primary immune cells of the brain and, together with perivascular, **choroid plexus** (see Glossary), and **meningeal macrophages**, comprise the macrophage compartment of the CNS. Under steady-state conditions, microglia are dynamic surveyors of the CNS, occupying distinct non-overlapping territories where they constantly extend and retract their processes to sample the local milieu and maintain tissue homeostasis [1–3]. Recent studies have shown that these cells are long-lived, rely on self-renewal for population maintenance, and exhibit brain region-dependent molecular and transcriptional heterogeneity [4–6]. During disease, aging, or injury, microglia undergo context-dependent transcriptional, morphological, and functional remodeling [7,8] that is generally beneficial or crucial for recovery. Variants of microglia-associated genes have also been identified as risk factors for Alzheimer's disease (AD) [9,10], frontotemporal dementia (FTD) [11], Parkinson's disease (PD) [12], and amyotrophic lateral sclerosis (ALS) [13], among others, implicating these cells in the initiation and progression of these CNS disorders. Experimental approaches to deplete resident microglia have provided unprecedented insights into the roles that these cells play in the healthy, injured, and diseased mouse brain. Several methods have been developed that allow the microglial population to be manipulated using toxin-, pharmacology-, and genetics-based approaches. Each technique has its own advantages and limitations regarding factors such as the extent and duration of microglial depletion, treatment invasiveness, physiological side effects, species, and other potential confounds, that should be discussed to correctly interpret the studies employing them. We

*Correspondence: kngreen@uci.edu (K.N. Green).

argue here that inhibitors of the colony-stimulating factor 1 (CSF1) receptor (CSF1R) – a crucial receptor for microglial survival – may offer one of the most advantageous approaches for eliminating and studying murine microglia.

Toxin-Based Models of Microglial Depletion

Early approaches to deplete microglia involved the generation of *Cd11b–HSVTK* transgenic mice that overexpress the herpes simplex virus-derived thymidine kinase (HSVTK) under the control of the *Cd11b* promoter (i.e., in cells of myeloid origin). In the presence of ganciclovir, HSVTK is activated and induces apoptosis in $CD11b^+$ mitotic cells [14,15]. However, systemic and extended administration of ganciclovir leads to fatal anemia owing to loss of $CD11b^+$ cells that are necessary for normal hematopoiesis [15] and enucleation of red blood cells [16], among other side effects [17]. To circumvent this myelotoxicity, investigators have relied on either wild-type (WT) bone marrow (BM) transfer [15] or intracerebroventricular (i.c.v.) administration of ganciclovir [18], which requires surgical implantation of an osmotic pump [19,20] and provides more selective treatment for microglial elimination.

An alternative genetic approach to deplete myeloid cell populations, including microglia, uses diphtheria toxin (DT)-based models. These combine myeloid promoter-driven Cre recombinase mouse lines with transgenic mice harboring genes for diphtheria toxin receptor (*DTR*) or diphtheria toxin A (*DTA*) downstream of *loxP*-flanked STOP sequences, which are removed upon Cre recombination to drive gene expression in myeloid cells [21]. Subsequent administration of DT causes acute cell death in myeloid cells expressing *DTR*, an effect that can be similarly achieved by direct cellular expression of *DTA* [21]. Studies generally utilize either $Cx3cr1^{cre/+}$ or $Cx3cr1^{creER/+}$ mouse lines to achieve constitutive or inducible DTR/DTA expression and cell depletion, respectively, although the availability of newer microglial Cre lines (e.g., *Tmem119*) may provide more specific interventions [22–24]. Similar DTR-based methods for myeloid tissue depletion, including microglia, have recently been extended to rat models [25]. Of relevance, inducible $Cx3cr1^{creER/+}$ depends upon tamoxifen administration for recombination and expression of DTR/DTA in $Cx3cr1^+$ cells, providing greater temporal control than constitutive Cre lines [23,26,27]. An additional advantage of this method includes the ability to selectively target microglia over circulating monocytes and other short-lived myeloid populations by exploiting the higher turnover rate of the latter [23].

However, DTR models only offer short-lived microglial depletion (<5 days [23]), induce a cytokine storm [23], rely on Cre recombinase which itself is toxic, and utilize tamoxifen (for inducible Cre lines) that binds to and is biologically active on macrophages and other estrogen receptor-expressing cells [1]. There are additional confounds related to the genetics involved, such as haploinsufficiency of the Cre driver gene, reported leakiness [28,29], and broad cell death of $Cx3cr1^+$ myeloid cell populations. The development of destabilized Cre recombinase (DD-Cre)-based depletion systems will be able to address some, but not all, of these issues (e.g., gene induction via the antibiotic trimethoprim instead of tamoxifen); nevertheless, these approaches have their own unique technical challenges [30]. Alternatively, **clodronate liposomes** provide for a toxin-based method to target microglia

and other macrophages without genetic modification by virtue of the liposome-encapsulated drug's specific uptake and lysosomal processing by phagocytic cells, thereby triggering apoptosis [31]. However, clodronate liposomes do not cross the blood–brain barrier (BBB), and thus require surgical infusion directly into the CNS in animal models, along with several other caveats (e.g., rapid but short-lived, inflammatory cytokine induction), summarized in Table 1 (Key Table) [31–33].

Colony-Stimulating Factor 1 (CSF1) Receptor Signaling and the Evolution of Genetic Microglial Depletion Models

Extensive research over the past 50 years has demonstrated that signaling through CSF1 and its cognate receptor (CSF1R) is essential for microglial cell survival, and this pathway has played in a fundamental role in the development of genetic models for microglial depletion. CSF1, also known as macrophage colony-stimulating factor (M-CSF), is a hematopoietic growth factor/cytokine that is involved in promoting macrophage proliferation, differentiation, and survival [34]. The sole receptor for CSF1 (CSF1R) is a membrane-spanning tyrosine kinase receptor, the product of the *c-Fms* proto-oncogene [34]. *Csf1r* is expressed in, and mostly restricted to, macrophage and microglia populations, as evidenced by *in situ* hybridization [35] and *Csf1r* promoter-driven GFP expression in transgenic MacGreen mice [36,37]. The central role of CSF1/CSF1R in myeloid biology was discovered upon characterization of osteopetrotic *op*/*op* mice ($Csf1^{op}/Csf1^{op}$), which harbor a spontaneous mutation in the *Csf1* gene with consequent loss of CSF1 [38,39]. Research on these mice revealed diverse phenotypic changes including osteoporosis, brain abnormalities (e.g., slowed neuronal process outgrowth, abnormal auditory and visual evoked potentials, aberrant cortical circuitry) [40], substantially reduced macrophage/monocyte populations in blood, bone, and BM [41,42], and significantly reduced microglial densities in white matter tracts, as well as modestly reduced microglial densities in the grey matter/cerebral cortex relative to WT mice [43].

For improved understanding of CSF1R signaling, $Csf1r^{-/-}$ mice were generated that display an exacerbated version of the $Csf1^{op}/Csf1^{op}$ phenotype, including skeletal deformities, shortened lifespan, and neurodevelopmental abnormalities [37,44]. Both $Csf1r^{-/-}$ and $Csf1^{op}/Csf1^{op}$ mice exhibit reduced peripheral tissue-resident macrophage populations relative to WT mice; however, although only partial microglial depletion is evident in some brain regions of $Csf1^{op}/Csf1^{op}$ mice, $Csf1r^{-/-}$ mice display a brain-wide absence of microglia [37,45]. The discrepancy between $Csf1^{op}/Csf1^{op}$ and $Csf1r^{-/-}$ microglial numbers was explained by the discovery of a second ligand for CSF1R, IL-34 [46], which when absent also results in substantial microglial reductions, for example in $Il34^{lacZ/lacZ}$ mice [47] because *Il34* expression is highly enriched in adult mouse brain compared to *Csf1* [48]. Despite similar biological activities, *Il34* and *Csf1* exhibit distinct spatial and temporal expression patterns [49] that differentially drive regional microglial identity and function [50]. *Il34* expression is detected in the embryonic brain at embryonic (E) day E11.5, earlier than *Csf1* expression at E13.5; although both ligands are broadly expressed in the adult, *Il34* expression at both postnatal (P) days P8 and P60 is significantly elevated in the cerebral cortex, olfactory bulb, striatum, and hippocampus relative to *Csf1* mRNA expression [49]. A

recent study also showed that CSF1, independently of IL-34, is also an essential factor for human and mouse cerebellar microglia [50]. In addition, microglial reductions and impaired survival have been observed in mice with disrupted expression of other microglia-associated gene products, including transcription factors *Spi1* (*Pu.1*) [51] and *Irf8* [52], as well as *Tgfb1* [53] (summarized in Table 1). However, conditional knockout mice harboring a loxP-flanked exon within the *Csf1r* gene (i.e., $Csf1r^{flox/flox}$) have allowed spatial and temporal control of microglia upon combination with the appropriate Cre lines [54]. For example, microglia-specific ablation of *Csf1r* can be achieved by driving Cre expression under a promoter for a microglia-specific transcription factor, for example $Sall1^{creER/+}Csf1r^{flox/flox}$ mice, resulting in transient elimination of microglia with tamoxifen [55].

Recently, a constitutive genetic model was generated, the $Csf1r^{\Delta FIRE/\Delta FIRE}$ mouse, in which a portion of intron 2 of *Csf1r* was knocked out – a region containing a highly conserved super-enhancing *Fms* intronic regulatory element (FIRE) that has binding sites for many macrophage transcription factors. Although completely lacking microglia, $Csf1r^{\Delta FIRE/\Delta FIRE}$ mice appear to be developmentally normal, healthy, and fertile [56], providing a novel model to explore microglial roles in both development and adulthood. Similarly, $Csf1r^{-/-}$ rats have recently been described which, unlike mice, survive into adulthood despite a dramatic loss of microglia, with little overt neurological phenotype other than reduced myelination (Table 1) [57]. A spectrum of autosomal dominant and recessive neurological skeletal disorders have been reported in humans with mutations in *CSF1R*, and, although outside the scope of this article, these warrant further investigation to answer crucial questions regarding CSF1R function, haploinsufficiency, convergence with animal models, and loss of CSF1R in different myeloid cell populations. Nevertheless, accumulating evidence indicates that disease severity in humans and animal models is dependent on residual CSF1R function [58]. Specifically, individuals homozygous for CSF1R loss-of-function mutations exhibit early lethality, complete absence of microglia, and several structural brain anomalies including **agenesis of the corpus callosum**, enlarged lateral and fourth ventricles, and impaired cerebellar development [59], recapitulating many of the phenotypes observed in *Csf1r*-deficient mice [44,60]. Although CSF1R expression is restricted to cells of the myeloid lineage in the adult vertebrate brain, expression in non-myeloid cells remains controversial [58]. *Csf1r* is reportedly expressed by some neurons during development in the mouse brain [48], and loss of CSF1 signaling in these cells and $CSF1R^{+}$ osteoclasts is thought to elicit the gross abnormalities observed in murine $Csf1^{-/-}$ and $Csf1r^{-/-}$ brains, in addition to defects in craniofacial structure [48,61]. However, further research will be necessary to reconcile the discrepancies in the effects of impaired CSF1R signaling on developmental survival and brain structure across species and across genetic mutations, as well as to what extent microglia are involved in this process.

Pharmacology-Based Microglial Depletion: CSF1R Inhibitors Allow Investigations into Adult Microglial Homeostasis

Although the first generation of CSF1R-associated knockout mice (e.g., $Csf1^{-/-}$, $Csf1r^{-/-}$, $Csf1^{op}/Csf1^{op}$) revealed that CSF1R signaling is essential for the development of microglia, studies into the role of this axis in the adult mouse CNS were limited. Our research group

initially identified two CSF1R inhibitors (CSF1Ri), PLX3397 and PLX647, that cross the BBB, and tested their ability to prevent microglial proliferation in mouse models of lipopolysaccharide (LPS)-induced neuroinflammation. However, instead of inhibiting proliferation, we observed robust reductions in microglial numbers throughout the brain relative to vehicle-treated mice, demonstrating microglial dependence on CSF1R signaling in adult mouse CNS tissue [62]. Subsequent formulation of PLX3397 in rodent chow at a dosage of 290 ppm afforded a non-invasive method for CSF1R inhibition, and CSF1Ri concentrations in the low micromolar range resulted in 50% depletion of microglia within 3 days of treatment, and up to 99% depletion by 3 weeks in adult WT mice. Increasing the dose of PLX3397 in chow to 600 ppm induced ~99% depletion of microglia within 7 days [63] (Figure 1). Of clinical relevance, PLX3397, or pexidartinib, has been granted US FDA approval as a drug treatment for tenosynovial giant cell tumors, making it an ideal candidate for its application in other disorders involving myeloid dysfunction [64]. However, limitations of this compound include its relatively poor CNS penetrance (~5% [62]) and potential off-target effects on related receptor tyrosine kinases c-Kit and Fms-like tyrosine kinase 3 (FLT3) [64]. Additional inhibitor screening led to the identification of PLX5622, which exhibited both a higher specificity for CSF1R and improved brain penetrance (20%) than PLX3397, and delivery in chow at 1200 ppm induced the depletion of N80% of microglia within 3 days in adult mice [65]. Research utilizing these inhibitors has reported microglial loss throughout the CNS, including the murine brain parenchyma, spinal cord, and retina [66–68], owing to microglial cell death [62] rather than to downregulation of microglial markers [69].

Increasingly widespread use of PLX3397 and PLX5622 has shown that this method of microglial depletion can be fast-acting and versatile, further confirmed in various mouse models of CNS disease and/or injury [65,66,69–74], as well as in other species including non-human primates [75], and humans [76]; specifically, microglial densities were quantified in resected glioblastoma tissue from patients treated orally with 1000 mg/day PLX3397 for 7 days, and compared to historical samples from the same patients [76]. Notably, CSF1Ri induced microglial death without subsequent inflammation, cytokine storm, or BBB damage, and had no detectable negative effects on behavior, cognition, or general health when tested on mouse models and non-human primates [62,75]. Furthermore, the regulation of CNS CSF1Ri through the formulation of different inhibitor concentrations in chow allows titration of microglial depletion (i.e., 20%, 50%, or 99% elimination depending on the dose), thus permitting comprehensive manipulation of the microglial population [62,63,65,77]. Microglial depletion is sustained for as long as treatment is continued – for example, we recently attained 6 months of uninterrupted microglial depletion (~99%) with PLX5622, including in the **5×FAD mouse model** of AD [65] (Table 1).

Together, this work demonstrates that CSF1Ri in mice have given researchers an unprecedented ability to study both (i) the importance of CSF1R signaling in adult microglia, and (ii) the effects of microglial depletion for any duration on CNS development, homeostasis, and disease. Other putative specific CSF1Ri (e.g., JNJ-40347527 and GW2580) are available, and studies have shown that low concentrations of these CSF1Ri in

the CNS can alter microglial phenotype (i.e., cell proliferation and self-renewal) without extensive cell loss, in turn offering therapeutic benefits in mouse models of neurological disorders [78–81]. In these studies, administration of low-dose CSF1Ri could attenuate synaptic and neuronal degeneration, reduce neuroinflammation, slow disease progression, and improve cognitive and behavioral function in mouse models of AD [78,81], PD [82], ALS [83], prion disease [80], lupus [84], and spinal cord injury [85]. The lack of observed microglial cell death when using these inhibitors has been attributed to their poor BBB penetrance and thus low CNS exposure of CSF1R, as seen with antibodies that do not readily cross the BBB [86,87]. In line with this, reports in mice show that higher doses or longer exposures (i.e., months vs days of treatment) to these CSF1Ri (i.e., GW2580 and BLZ945) do indeed lead to microglial cell loss instead of reduced proliferation [88,89]. In adult *Cx3cr1*$^{GFP/+}$ mice, 7 days of treatment with BLZ945 (200 mg/kg/day) resulted in a significant reduction of parenchymal microglial cells in areas of white matter [89]. Furthermore, recent studies show that peripheral administration of function-blocking antibodies against the cognate ligands CSF1 or IL-34 induce brain region-specific microglial ablation in a dose-dependent manner [74]. In adult mice, anti-CSF1 antibodies effectively deplete white matter microglia, whereas anti-IL-34 antibodies effectively deplete grey matter microglia, consistent with regional ligand expression [74]. Moving forward, these antibodies might allow even more nuanced investigations of microglial populations.

Caveats and Controls for CSF1Ri-Mediated Microglial Depletion

CSF1Ri is a highly effective and versatile method to achieve microglial elimination; however, investigators should be aware of the associated caveats (summarized in Table 1). Although this method is surgically noninvasive, treatment is dependent on peripheral administration of a small molecule which, like any exogenously administered agent (e.g., tamoxifen or ganciclovir), may have off-target effects. Despite the robust dependency of microglia on CSF1R signaling, CSF1R is expressed on all myeloid cells throughout the body, and thus CSF1Ri can also interfere with signaling through this receptor in peripheral myeloid cell populations. In both humans and mice, PLX3397 (at 1000 mg in humans and 400 mg/kg in mice) has been reported to decrease a specific subset of circulating monocytes [76,90]. We and others have not observed significant alterations in blood and spleen myeloid cell populations (including circulating monocytes and tissue macrophages) utilizing either PLX3397 (at a dose of 290 mg/kg or lower) or the more selective inhibitor PLX5622 in mice; however, these studies are limited, and require further examination of circulating and other peripheral tissue-resident macrophage populations [65,71,91–95]. Furthermore, any effects on peripheral myeloid cell populations can be controlled by including treatment groups with classes or dosages of CSF1Ri that attain little to no BBB penetrance in healthy adult mice, such as PLX73086 [66], Ki20227 [96], or PLX3397 at 75 ppm [97]. It should be noted that the use of CSF1Ri in development shares similar limitations to studies utilizing global constitutive *Csf1r*$^{-/-}$ mice, resulting in abnormal phenotypes such as bone malformations and altered hypothalamic-related processes [98]. An important recent discovery showed that intra-peritoneal postnatal BM transplant can rescue ~50% of *Csf1r*$^{-/-}$ mouse pups, which again typically do not survive past weaning age, and can partially rescue many *Csf1r*$^{-/-}$-associated phenotypes, including loss of microglia [44,60,99]. In theory, such

implementation could have similar effects in developmental CSF1Ri studies, and thus warrants further research as a complementary technique.

What Have We Learned from Microglial Depletion Models?

Although microglia have well-established roles in immunity and immunosurveillance, other homeostatic functions have come to light, including essential roles in synaptic refinement and sculpting, as well as in **neurogenesis** [3,100,101]. Studies in mice indicate that microglia are involved in regulating learning-induced synaptic modifications (in early adulthood) [27] and in phagocytosing apoptotic neuronal progenitors in CNS neurogenic niches [102,103]. Microglial depletion models have been widely utilized to enhance our knowledge of microglial homeostasis, and recent studies using CSF1Ri have shown that microglial depletion in the healthy adult mouse brain does not induce behavioral or cognitive impairments, nor does it exact specific pathologies or overt phenotypes on the CNS [24,62]. Thus, it appears that microglia are in this sense dispensable in the healthy adult brain, outside their pivotal roles in immunity [104]. Microglia do, however, appear to be essential for proper brain development, and have a spectrum of unique phenotypic and functional roles [3]. During embryonic and postnatal development, depletion studies in mice have shown that microglia promote the survival of developing neurons through the release of neurotrophic factors [105]; moreover, imaging and genetic studies have demonstrated that microglia remove apoptotic neurons and debris [106,107], and engage in synaptic pruning and neuronal circuit maturation [108–110]. Any disturbance in the ability to carry out these roles can lead to lifelong impairments in cognition and behavior [100,111].

Despite the lack of an overt phenotype produced by chronic microglial elimination, more nuanced effects have been identified that indicate microglial homeostatic function. Several **oligodendrocyte** precursor cell (OPC)-related genes (e.g., *Cspg4*, *Pdgfra*) are downregulated with PLX3397-mediated microglial depletion, accompanied by increases in mature oligodendrocyte-expressed genes (e.g., *Cldn11*, *Cnp*, *Mag*) [63], consistent with previous reports utilizing the inhibitors PLX3397, PLX5622, and BLZ945 in mice [89,112]. A recent report showed that PLX5622-resistant microglia in $Cnp^{-/-}$ mice (a mouse model for white matter inflammation and catatonia) display a highly inflammatory phenotype leading to OPC phagocytosis and reduced OPC cell numbers relative to WT mice [113]. However, studies indicate that these OPC-related effects are dose-and CSF1Ri specificity-dependent, and that long-term oral administration of CSF1Ri has no effect on mature oligodendrocytes or myelin protein expression in adult mice [112]. Although microglial regulation of astrocyte reactivity is apparent [114], neuronal gene expression is largely unaffected by CSF1Ri in the healthy mouse brain [65]. Together with the absence of microglial gene expression with chronic CSF1Ri treatment, this suggests that microglial absence does not inherently elicit pathologic or immune mechanisms in other cells [65].

Despite the lack of clear changes at the transcriptional level, however, cellular alterations have been documented after CSF1Ri treatment, including increases in neuronal dendritic spine densities, synaptic markers, and neurogenesis compared to untreated controls [70,101,115,116]. One of the largest and most consistent effects we observe with microglial elimination via PLX3397 and PLX5622 in mice is a brain-wide increase in **perineuronal**

nets (PNNs) [117] – specialized extracellular matrix (ECM) structures that form primarily around **GABAergic interneurons** to effectively 'lock' synapses in place and provide synaptic stability [118]. PNN structural modification regulates interneuron firing rate [119], synaptic transmission [120], and the molecular composition of synapses [121]; thus, homeostatic and disease-related interactions between microglia and the ECM may have implications for related neuronal and synaptic physiology. This is underscored by recent work in mice showing that ECM clearance by microglia in response to neuronal IL-33 can promote synaptic remodeling and plasticity because genetic deletion of neuronal IL-33 ($Il33^{flox/flox}$:$Syn1^{cre}$), or inducible myeloid-specific deletion of cognate microglial receptor IL1RL1 ($Il1rl1^{flox/flox}$:$Cx3cr1^{cre/+}$) resulted in reduced hippocampal dendritic spine densities and impaired remote fear memory recall precision relative to controls [122].

Microglial elimination studies have also provided insight into microglial population dynamics. We have shown that, upon cessation of CSF1Ri treatment (i.e., PLX3397 or PLX5622) in adult WT mice, the microglial compartment has a remarkable capacity to regenerate and repopulate the brain with new microglial cells without a contribution from peripheral cells [62]. These repopulating cells eventually become indistinguishable from their original counterparts in regards to density, morphology, tiling pattern, gene expression, and immune response [123]. Furthermore, CSF1Ri-treated (i.e., PLX3397 and PLX5622) mice can undergo multiple depletion–repopulation cycles in the CNS, given adequate time between treatments [63]. In our original study characterizing microglial elimination–repopulation, two proliferating cell populations were identified: surviving microglia and an unknown non-myeloid cell [62]. Subsequent studies using lineage-tracing approaches via $Cx3cr1^{creER/+}$ mice have shown that microglial self-renewal is driven solely by the proliferation of surviving microglia [5]. In addition, although microglial repopulation occurs in virtually every depletion model, it displays differential sources and kinetics based on the mode of depletion. For example, repopulating microglia are found to proliferate in clusters following DT-mediated ablation [23], whereas brain-wide, uniform proliferation is observed after CSF1Ri treatment in mice [62]. Of note, researchers have found that perturbations in BBB integrity or immune activation (e.g., cytokine storm) can allow engraftment of peripheral myeloid cells in place of endogenously repopulating microglia. This occurs either when irradiation is combined with CSF1Ri treatment [54] or toxin depletion in $Cx3cr1^{creER/+}$–*iDTR* mice [23], or in the absence of irradiation upon tamoxifen or ganciclovir delivery in $Cx3cr1^{creER/+}$–*DTA* mice [26] or *CD11b–HSVTK* mice [19], respectively. Together, these tools provide remarkable control of the brain myeloid population and may enable more comprehensive neuroimmunological inquiries.

Microglial depletion paradigms have afforded much knowledge on the roles these cells play in disease pathogenesis, injury, and recovery. Investigating microglial function via their removal or replacement in animal models of brain disorders has provided insights into the evolving spectrum of their context-dependent phenotypes, which may be beneficial or detrimental [24,124]. The timing of CSF1Ri treatment and microglial depletion relative to disease/injury onset, as well as the type of disease model itself (e.g., acute, neurodegenerative), should be duly considered when interpreting and designing such studies. For example, on the one hand, pharmacological elimination of microglia following brain

injury has been shown to be protective [70,77]. In these studies, microglial elimination has improved behavioral-, cognitive-, inflammation-, and synaptic-related outcomes in mouse models of neuronal injury and AD [70,77]. Moreover, CSF1Ri treatment before and/or during symptom/pathology onset is widely beneficial in several progressive neurodegenerative models [65,81,117,125]. Indeed, recent studies have shown that microglial depletion using PLX3397 and/or PLX5622 fully prevents neuronal loss and atrophy in mouse models of AD [69], **tauopathy** [90], and Huntington's disease [117], suggesting that the microglial response to extracellular plaques or intraneuronal aggregates might primarily drive neurodegeneration. However, on the other hand, increased neuronal damage can accompany microglial elimination when it occurs during the acute stages of injury [70] or before ischemic stroke [71,126], whereas the opposite occurs in hemorrhagic stroke [73] and traumatic brain injury [127]. Whether microglial repopulation/inhibitor cessation occurs before experimental readout also factors into the interpretation of the results; for example, microglial repopulation – but not depletion – can improve recovery following neuronal injury [115] and traumatic brain injury [128,129], as well as reversing age-induced long-term potentiation (LTP) and cognitive deficits in aged mice relative to controls [101]. These results thus emphasize the fundamental roles microglia may play in health and disease, and suggest clinical contexts in which targeting microglia might have beneficial outcomes.

Recently, a report elucidated the functional role of microglia in mediating the loss of **contextual fear memory** via **complement**-dependent removal and lysosomal degradation of synaptic elements (i.e., **PSD95**) in healthy adult mice [130]. This study parallels our previous work showing enhanced spatial memory in healthy mice following microglial depletion via PLX3397 or PLX5622 [62,65,70]. Indeed, some of the primary effects of microglial elimination in mice that we have consistently found are increases in PSD95 and **synaptophysin** synaptic staining [70], enhanced dendritic spine densities [70,115], and unimpaired or improved performance in memory-related tasks relative to controls, even after 6 months of chronic microglial depletion [65]. Similarly, microglial depletion consistently induces increased neurogenesis [101] and upregulation of PNNs [117], suggesting multiple mechanisms by which microglia might impact on cognition and brain function.

Concluding Remarks

Pharmacological CSF1Ri allow unprecedented control of microglial population dynamics in animal models, attaining rapid and sustained depletion through oral administration in chow, without inducing notable compensatory mechanisms or debilitating health effects. Indeed, perhaps one of the most interesting findings from CSF1Ri research is that microglial loss does not induce any overt phenotypic or cognitive abnormalities in otherwise healthy adult mice. We and others [104] hypothesize that changes in microglial phenotype associated with canonical 'activation' are far more detrimental to parenchymal homeostasis, and thus cognition, than the absence of unstimulated microglia expressing homeostatic phenotypes – or, as we say, 'bad' microglia are worse than no microglia. It is fairly clear that this is not entirely the case in development because early microglial loss results in brain abnormalities [37,40,48,59,98,116] and/or premature death [53] across numerous preclinical models and species, an observation mirrored by human case reports [59]. In models where discrepancies

arise in this regard, such as the *Csf1r*$^{\Delta FIRE/\Delta FIRE}$ mice which reportedly do not present overt neurological disruption despite a lack of microglia from birth [56], we predict that more nuanced measures of synaptic connectivity (e.g., dendritic spine complexity or LTP) will reveal consistent deficits. If not, it is possible that compensatory mechanisms have been activated [56]. Either outcome would underscore the necessity of microglial function for normal development.

Although brain-wide elimination of microglia is clinically unlikely, it is an effective approach to elucidate the roles of these cells in any given biological process. These studies, together with the recently expanding analysis of microglia at single-cell resolution [6,7], have given greater insight into brain myeloid biology than was previously possible in neuroimmunology research. Future preclinical studies combining both microglial depletion and single-cell profiling may elucidate the differential effects of CSF1Ri on specific microglial subtypes (see Outstanding Questions). Furthermore, CSF1Ri at concentrations that do not induce microglial death but modulate the phenotype may – depending on the microglial response and pathological context – confer benefits in disease models, offering more clinically relevant indications (see Outstanding Questions). The ability to replace microglia through cessation of CSF1Ri treatment [123] also has obvious clinical merit, bolstered by restorative effects in aging [101] and injury [115] mouse models, and warrants continued investigation. Because CSF1Ri effectively depletes microglia in humans [76] and nonhuman primates [75], this approach is translationally relevant and, together with the recent FDA approval of PLX3397, it is likely that CSF1Ri will remain a mainstay of basic and biomedical microglial research for some time to come.

Acknowledgments

This work was supported by the National Institutes of Health under awards R01NS083801 (NINDS), R01AG056768 (NIA), and P50AG016573 (NIA) to K.N.G., and F31NS108611 (NINDS) to J.D.C. L.A.H. was supported by an Alzheimer's Association Research Fellowship (AARF-16-442762).

Glossary

5×FAD mouse model
a transgenic mouse model of Alzheimer's disease (AD), in which mice express human amyloid precursor protein (APP) and presenilin 1 (PSEN1) transgenes harboring five familial AD (FAD)-causing mutations

Agenesis of the corpus callosum
a rare brain disorder in which the corpus callosum, a white matter tract that connects the two brain hemispheres, is partially or completely absent

Choroid plexus
a structure located in the ventricles that plays an important role in regulating immune cell entry into the CNS and producing cerebrospinal fluid

Clodronate liposomes

clodronate-encapsulated liposomes are phagocytosed by macrophages and, upon engulfment, result in the release of clodronate into the phagocytic cell, effectively inducing apoptosis and macrophage ablation

Complement
the complement system or cascade plays a major part in the innate immune system. Complement proteins are soluble plasma proteins that opsonize pathogens and other pathological targets for clearance, effectively serving as molecular danger or ‘eat me’ signals to phagocytes

Contextual fear memory
subjects are exposed to a specific neutral condition or context (e.g., room, tone, or light) that is associated with an aversive stimulus (e.g., shock), resulting in a fear response to the neutral context. This model allows the study of contextual fear learning and memory. Refers to the ability of subjects to recall the context that predicts danger or a fearful stimulus

GABAergic interneurons
neurons that produce γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter of the adult brain

Meningeal macrophages
the population of macrophages that reside in the meninges that cover and protect the brain and spinal cord

Neurogenesis
the process by which new functional neurons are generated from adult neural precursor cells

Oligodendrocyte
a distinct type of glial cell in the CNS that plays a central role in myelination to support and insulate neuronal axons

Perineuronal nets
specialized extracellular matrix structures that surround some types of neuronal cell bodies and dendrites in the mammalian CNS to regulate synaptic plasticity and stabilization

PSD95
a major component of excitatory synapses that acts as a crucial scaffolding protein localized at the postsynaptic density (PSD) of excitatory synapses to regulate many receptors, channels, and signaling molecules. PSD95 is commonly utilized as a marker for labeling postsynaptic synapses

Synaptophysin
also known as major synaptic vesicle protein p38, synaptophysin is an integral membrane glycoprotein that is localized to presynaptic vesicles and ubiquitously expressed in all neurons. It is commonly utilized as a marker for labeling synaptic terminals

Tauopathy

a class of neurodegenerative disease caused by or associated with the aggregation or misfolding of tau protein in the brain

References

1. Hume DA et al. (2019) The mononuclear phagocyte system: the relationship between monocytes and macrophages. Trends Immunol. 40, 98–112 [PubMed: 30579704]
2. Nimmerjahn A et al. (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. Science 308, 1314–1318 [PubMed: 15831717]
3. Kierdorf K and Prinz M (2017) Microglia in steady state. J. Clin. Invest 127, 3201–3209 [PubMed: 28714861]
4. Reu P et al. (2017) The lifespan and turnover of microglia in the human brain. Cell Rep. 20, 779–784 [PubMed: 28746864]
5. Zhan L et al. (2019) Proximal recolonization by self-renewing microglia re-establishes microglial homeostasis in the adult mouse brain. PLoS Biol. 17, e3000134 [PubMed: 30735499]
6. Masuda T et al. (2019) Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. Nature 566, 388–392 [PubMed: 30760929]
7. Hammond TR et al. (2019) Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. Immunity 50, 253–271 [PubMed: 30471926]
8. Mathys H et al. (2019) Single-cell transcriptomic analysis of Alzheimer's disease. Nature 570, 332–337 [PubMed: 31042697]
9. Lambert JC et al. (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat. Genet 45, 1452–1458 [PubMed: 24162737]
10. Nott A et al. (2019) Brain cell type-specific enhancer–promoter interactome maps and disease-risk association. Science 366, 1134–1139 [PubMed: 31727856]
11. Cuyvers E et al. (2014) Investigating the role of rare heterozygous TREM2 variants in Alzheimer's disease and frontotemporal dementia. Neurobiol. Aging 35, 726.e11–726.e19
12. Rayaprolu S et al. (2013) TREM2 in neurodegeneration: evidence for association of the p.R47H variant with frontotemporal dementia and Parkinson's disease. Mol. Neurodegener 8, 19 [PubMed: 23800361]
13. Cady J et al. (2014) TREM2 variant p.R47H as a risk factor for sporadic amyotrophic lateral sclerosis. JAMA Neurol. 71, 449–453 [PubMed: 24535663]
14. Gowing G et al. (2006) Mouse model for ablation of proliferating microglia in acute CNS injuries. Glia 53, 331–337 [PubMed: 16276506]
15. Heppner FL et al. (2005) Experimental autoimmune encephalomyelitis repressed by microglial paralysis. Nat. Med 11, 146–152 [PubMed: 15665833]
16. Simard AR et al. (2006) Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. Neuron 49, 489–502 [PubMed: 16476660]
17. Ding Z et al. (2014) Antiviral drug ganciclovir is a potent inhibitor of microglial proliferation and neuroinflammation. J. Exp. Med 211, 189–198 [PubMed: 24493798]
18. Bennett RE and Brody DL (2014) Acute reduction of microglia does not alter axonal injury in a mouse model of repetitive concussive traumatic brain injury. J. Neurotrauma 31, 1647–1663 [PubMed: 24797413]
19. Varvel NH et al. (2012) Microglial repopulation model reveals a robust homeostatic process for replacing CNS myeloid cells. Proc. Natl. Acad. Sci. U. S. A 109, 18150–18155 [PubMed: 23071306]
20. Prokop S et al. (2015) Impact of peripheral myeloid cells on amyloid-beta pathology in Alzheimer's disease-like mice. J. Exp. Med 212, 1811–1818 [PubMed: 26458768]
21. Buch T et al. (2005) A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. Nat. Methods 2, 419–426 [PubMed: 15908920]
22. Kaiser T and Feng G (2019) Tmem119–EGFP and Tmem119–CreERT2 transgenic mice for labeling and manipulating microglia. eNeuro 6 0448–18.2019

23. Bruttger J et al. (2015) Genetic cell ablation reveals clusters of local self-renewing microglia in the mammalian central nervous system. Immunity 43, 92–106 [PubMed: 26163371]
24. Waisman A et al. (2015) Homeostasis of microglia in the adult brain: review of novel microglia depletion systems. Trends Immunol. 36, 625–636 [PubMed: 26431940]
25. De Luca SN et al. (2019) Conditional microglial depletion in rats leads to reversible anorexia and weight loss by disrupting gustatory circuitry. Brain Behav. Immun 77, 77–91 [PubMed: 30578932]
26. Lund H et al. (2018) Competitive repopulation of an empty microglial niche yields functionally distinct subsets of microglia-like cells. Nat. Commun 9, 4845 [PubMed: 30451869]
27. Parkhurst CN et al. (2013) Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 155, 1596–1609 [PubMed: 24360280]
28. Fonseca MI et al. (2017) Cell-specific deletion of C1qa identifies microglia as the dominant source of C1q in mouse brain. J. Neuroinflammation 14, 48–48 [PubMed: 28264694]
29. Chappell-Maor L et al. (2019) Comparative analysis of CreER transgenic mice for the study of brain macrophages: a case study. Eur. J. Immunol 50, 353–362 [PubMed: 31762013]
30. Sando III R et al. (2013) Inducible control of gene expression with destabilized Cre. Nat. Methods 10, 1085–1088 [PubMed: 24056874]
31. Van Rooijen N and Sanders A (1994) Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. J. Immunol. Methods 174, 83–93 [PubMed: 8083541]
32. Han X et al. (2019) Microglial depletion with clodronate liposomes increases proinflammatory cytokine levels, induces astrocyte activation, and damages blood vessel integrity. Mol. Neurobiol 56, 6184–6196 [PubMed: 30734229]
33. Serrats J et al. (2010) Dual roles for perivascular macrophages in immune-to-brain signaling. Neuron 65, 94–106 [PubMed: 20152116]
34. Sherr CJ et al. (1985) The c-fms proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. Cell 41, 665–676 [PubMed: 2408759]
35. Hume DA et al. (1995) Detection of c-fms protooncogene in early mouse embryos by whole mount in situ hybridization indicates roles for macrophages in tissue remodelling. Br. J. Haematol 90, 939–942 [PubMed: 7669676]
36. Sasmono RT et al. (2003) A macrophage colony-stimulating factor receptor-green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. Blood 101, 1155–1163 [PubMed: 12393599]
37. Erblich B et al. (2011) Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. PLoS One 6, e26317 [PubMed: 22046273]
38. Wiktor-Jedrzejczak W et al. (1990) Total absence of colony-stimulating factor 1 in the macrophage-deficient osteopetrotic (op/op) mouse. Proc. Natl. Acad. Sci. U. S. A 87, 4828–4832 [PubMed: 2191302]
39. Yoshida H et al. (1990) The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. Nature 345, 442–444 [PubMed: 2188141]
40. Michaelson MD et al. (1996) CSF-1 deficiency in mice results in abnormal brain development. Development 122, 2661–2672 [PubMed: 8787741]
41. Wiktor-Jedrzejczak W et al. (1992) CSF-1 deficiency in the op/op mouse has differential effects on macrophage populations and differentiation stages. Exp. Hematol 20, 1004–1010 [PubMed: 1505635]
42. Felix R et al. (1990) Impairment of macrophage colony-stimulating factor production and lack of resident bone marrow macrophages in the osteopetrotic op/op mouse. J. Bone Miner. Res 5, 781–789 [PubMed: 2204254]
43. Kondo Y and Duncan ID (2009) Selective reduction in microglia density and function in the white matter of colony-stimulating factor-1-deficient mice. J. Neurosci. Res 87, 2686–2695 [PubMed: 19396881]
44. Dai XM et al. (2002) Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. Blood 99, 111–120 [PubMed: 11756160]

45. Ginhoux F et al. (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330, 841–845 [PubMed: 20966214]
46. Lin H et al. (2008) Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. Science 320, 807–811 [PubMed: 18467591]
47. Wang Y et al. (2012) IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. Nat. Immunol 13, 753–760 [PubMed: 22729249]
48. Nandi S et al. (2012) The CSF-1 receptor ligands IL-34 and CSF-1 exhibit distinct developmental brain expression patterns and regulate neural progenitor cell maintenance and maturation. Dev. Biol 367, 100–113 [PubMed: 22542597]
49. Wei S et al. (2010) Functional overlap but differential expression of CSF-1 and IL-34 in their CSF-1 receptor-mediated regulation of myeloid cells. J. Leukoc. Biol 88, 495–505 [PubMed: 20504948]
50. Kana V et al. (2019) CSF-1 controls cerebellar microglia and is required for motor function and social interaction. J. Exp. Med 216, 2265–2281 [PubMed: 31350310]
51. Beers DR et al. (2006) Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. Proc. Natl. Acad. Sci. U. S. A 103, 16021–16026 [PubMed: 17043238]
52. Kierdorf K et al. (2013) Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat. Neurosci 16, 273–280 [PubMed: 23334579]
53. Butovsky O et al. (2014) Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. Nat. Neurosci 17, 131–143 [PubMed: 24316888]
54. Cronk JC et al. (2018) Peripherally derived macrophages can engraft the brain independent of irradiation and maintain an identity distinct from microglia. J. Exp. Med 215, 1627–1647 [PubMed: 29643186]
55. Buttgereit A et al. (2016) Sall1 is a transcriptional regulator defining microglia identity and function. Nat. Immunol 17, 1397–1406 [PubMed: 27776109]
56. Rojo R et al. (2019) Deletion of a Csf1r enhancer selectively impacts CSF1R expression and development of tissue macrophage populations. Nat. Commun 10, 3215 [PubMed: 31324781]
57. Pridans C et al. (2018) Pleiotropic impacts of macrophage and microglial deficiency on development in rats with targeted mutation of the Csf1r Locus. J. Immunol 201, 2683–2699 [PubMed: 30249809]
58. Hume DA et al. (2020) Phenotypic impacts of CSF1R deficiencies in humans and model organisms. J. Leukoc. Biol 107, 205–219 [PubMed: 31330095]
59. Oosterhof N et al. (2019) Homozygous mutations in CSF1R cause a pediatric-onset leukoencephalopathy and can result in congenital absence of microglia. Am. J. Hum. Genet 104, 936–947 [PubMed: 30982608]
60. Li J et al. (2006) Conditional deletion of the colony stimulating factor-1 receptor (c-fms proto-oncogene) in mice. Genesis 44, 328–335 [PubMed: 16823860]
61. Wiktor-Jedrzejczak WW et al. (1982) Hematological characterization of congenital osteopetrosis in op/op mouse. Possible mechanism for abnormal macrophage differentiation. J. Exp. Med 156, 1516–1527 [PubMed: 7130905]
62. Elmore MR et al. (2014) Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. Neuron 82, 380–397 [PubMed: 24742461]
63. Najafi AR et al. (2018) A limited capacity for microglial repopulation in the adult brain. Glia 66, 2385–2396 [PubMed: 30370589]
64. Tap WD et al. (2015) Structure-guided blockade of CSF1R kinase in tenosynovial giant-cell tumor. N. Engl. J. Med 373, 428–437 [PubMed: 26222558]
65. Spangenberg E et al. (2019) Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer's disease model. Nat. Commun 10, 3758 [PubMed: 31434879]
66. Bellver-Landete V et al. (2019) Microglia are an essential component of the neuroprotective scar that forms after spinal cord injury. Nat. Commun 10, 518 [PubMed: 30705270]

67. Paschalis EI et al. (2018) Permanent neuroglial remodeling of the retina following infiltration of CSF1R inhibition-resistant peripheral monocytes. Proc. Natl. Acad. Sci. U. S. A 115, E11359–E11368 [PubMed: 30442669]
68. Okunuki Y et al. (2018) Microglia inhibit photoreceptor cell death and regulate immune cell infiltration in response to retinal detachment. Proc. Natl. Acad. Sci. U. S. A 115, E6264–E6273 [PubMed: 29915052]
69. Spangenberg EE et al. (2016) Eliminating microglia in Alzheimer's mice prevents neuronal loss without modulating amyloid-beta pathology. Brain 139, 1265–1281 [PubMed: 26921617]
70. Rice RA et al. (2015) Elimination of microglia improves functional outcomes following extensive neuronal loss in the hippo-campus. J. Neurosci 35, 9977–9989 [PubMed: 26156998]
71. Szalay G et al. (2016) Microglia protect against brain injury and their selective elimination dysregulates neuronal network activity after stroke. Nat. Commun 7, 11499 [PubMed: 27139776]
72. Qu W et al. (2017) Inhibition of colony-stimulating factor 1 receptor early in disease ameliorates motor deficits in SCA1 mice. J. Neuroinflammation 14, 107 [PubMed: 28545543]
73. Li M et al. (2017) Colony stimulating factor 1 receptor inhibition eliminates microglia and attenuates brain injury after intracerebral hemorrhage. J. Cereb. Blood Flow Metab 37, 2383–2395 [PubMed: 27596835]
74. Easley-Neal C et al. (2019) CSF1R ligands IL-34 and CSF1 are differentially required for microglia development and maintenance in white and gray matter brain regions. Front. Immunol 10, 2199 [PubMed: 31616414]
75. Hillmer AT et al. (2017) Microglial depletion and activation: A [^{11}C]PBR28 PET study in nonhuman primates. EJNMMI Res. 7, 59 [PubMed: 28741281]
76. Butowski N et al. (2015) Orally administered colony stimulating factor 1 receptor inhibitor PLX3397 in recurrent glioblastoma: an Ivy Foundation Early Phase Clinical Trials Consortium phase II study. Neuro-Oncology 18, 557–564 [PubMed: 26449250]
77. Dagher NN et al. (2015) Colony-stimulating factor 1 receptor inhibition prevents microglial plaque association and improves cognition in 3xTg-AD mice. J. Neuroinflammation 12, 139 [PubMed: 26232154]
78. Mancuso R et al. (2019) CSF1R inhibitor JNJ-40346527 attenuates microglial proliferation and neurodegeneration in P301S mice. Brain 142, 3243–3264 [PubMed: 31504240]
79. De Lucia C et al. (2016) Microglia regulate hippocampal neurogenesis during chronic neurodegeneration. Brain Behav. Immun 55, 179–190 [PubMed: 26541819]
80. Gomez-Nicola D et al. (2013) Regulation of microglial proliferation during chronic neurodegeneration. J. Neurosci 33, 2481–2493 [PubMed: 23392676]
81. Olmos-Alonso A et al. (2016) Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer's-like pathology. Brain 139, 891–907 [PubMed: 26747862]
82. Neal ML et al. (2020) Pharmacological inhibition of CSF1R by GW2580 reduces microglial proliferation and is protective against neuroinflammation and dopaminergic neurodegeneration. FASEB J. 34, 1679–1694 [PubMed: 31914683]
83. Martinez-Muriana A et al. (2016) CSF1R blockade slows the progression of amyotrophic lateral sclerosis by reducing microgliosis and invasion of macrophages into peripheral nerves. Sci. Rep 6, 25663 [PubMed: 27174644]
84. Chalmers SA et al. (2017) CSF-1R inhibition attenuates renal and neuropsychiatric disease in murine lupus. Clin. Immunol 185, 100–108 [PubMed: 27570219]
85. Gerber YN et al. (2018) CSF1R inhibition reduces microglia proliferation, promotes tissue preservation and improves motor recovery after spinal cord injury. Front. Cell. Neurosci 12, 368 [PubMed: 30386212]
86. MacDonald KPA et al. (2010) An antibody against the colony-stimulating factor 1 receptor depletes the resident subset of monocytes and tissue- and tumor-associated macrophages but does not inhibit inflammation. Blood 116, 3955–3963 [PubMed: 20682855]
87. Obst J et al. (2020) Inhibition of IL34 unveils tissue-selectivity and is sufficient to reduce microglial proliferation in chronic neurodegeneration. BioRxiv. Published online March 13, 2020. 10.1101/2020.03.09.976118

88. Askew K et al. (2017) Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. Cell Rep. 18, 391–405 [PubMed: 28076784]

89. Hagemeyer N et al. (2017) Microglia contribute to normal myelinogenesis and to oligodendrocyte progenitor maintenance during adulthood. Acta Neuropathol. 134, 441–458 [PubMed: 28685323]

90. Shi Y et al. (2019) Microglia drive APOE-dependent neurodegeneration in a tauopathy mouse model. J. Exp. Med 216, 2546–2561 [PubMed: 31601677]

91. Valdearcos M et al. (2014) Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function. Cell Rep. 9, 2124–2138 [PubMed: 25497089]

92. Hilla AM et al. (2017) Microglia are irrelevant for neuronal degeneration and axon regeneration after acute injury. J. Neurosci 37, 6113–6124 [PubMed: 28539419]

93. Mok S et al. (2014) Inhibition of CSF-1 receptor improves the antitumor efficacy of adoptive cell transfer immunotherapy. Cancer Res. 74, 153–161 [PubMed: 24247719]

94. Wheeler DL et al. (2018) Microglia are required for protection against lethal coronavirus encephalitis in mice. J. Clin. Invest 128, 931–943 [PubMed: 29376888]

95. Merry TL et al. (2020) The CSF1 receptor inhibitor pexidartinib (PLX3397) reduces tissue macrophage levels without affecting glucose homeostasis in mice. Int. J. Obes 44, 245–253

96. Ohno H et al. (2006) A c-fms tyrosine kinase inhibitor, Ki20227, suppresses osteoclast differentiation and osteolytic bone destruction in a bone metastasis model. Mol. Cancer Ther 5, 2634–2643 [PubMed: 17121910]

97. Lee S et al. (2018) Targeting macrophage and microglia activation with colony stimulating factor 1 receptor inhibitor is an effective strategy to treat injury-triggered neuropathic pain. Mol. Pain 14, 1744806918764979

98. Rosin JM et al. (2018) Depletion of embryonic microglia using the CSF1R inhibitor PLX5622 has adverse sex-specific effects on mice, including accelerated weight gain, hyperactivity and anxiolytic-like behaviour. Brain Behav. Immun 73, 682–697 [PubMed: 30056204]

99. Bennett FC et al. (2018) A combination of ontogeny and CNS environment establishes microglial identity. Neuron 98, 1170–1183 [PubMed: 29861285]

100. Hammond TR et al. (2018) Microglia and the brain: complementary partners in development and disease. Annu. Rev. Cell Dev. Biol 34, 523–544 [PubMed: 30089221]

101. Elmore MRP et al. (2018) Replacement of microglia in the aged brain reverses cognitive, synaptic, and neuronal deficits in mice. Aging Cell 17, e12832 [PubMed: 30276955]

102. Sierra A et al. (2010) Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. Cell Stem Cell 7, 483–495 [PubMed: 20887954]

103. Fourgeaud L et al. (2016) TAM receptors regulate multiple features of microglial physiology. Nature 532, 240–244 [PubMed: 27049947]

104. Norris GT and Kipnis J (2019) Immune cells and CNS physiology: microglia and beyond. J. Exp. Med 216, 60–70 [PubMed: 30504438]

105. Ueno M et al. (2013) Layer V cortical neurons require microglial support for survival during postnatal development. Nat. Neurosci 16, 543–551 [PubMed: 23525041]

106. Takahashi K et al. (2005) Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J. Exp. Med 201, 647–657 [PubMed: 15728241]

107. Brockhaus J et al. (1996) Phagocytozing ameboid microglial cells studied in a mouse corpus callosum slice preparation. Glia 16, 81–90 [PubMed: 8787776]

108. Tremblay M et al. (2010) Microglial interactions with synapses are modulated by visual experience. PLoS Biol. 8, e1000527 [PubMed: 21072242]

109. Paolicelli RC et al. (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333, 1456–1458 [PubMed: 21778362]

110. Schafer DP et al. (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74, 691–705 [PubMed: 22632727]

111. Thion MS et al. (2018) Microglia and early brain development: an intimate journey. Science 362, 185–189 [PubMed: 30309946]

112. Liu Y et al. (2019) Concentration-dependent effects of CSF1R inhibitors on oligodendrocyte progenitor cells *ex vivo* and *in vivo*. Exp. Neurol 318, 32–41 [PubMed: 31029597]
113. Garcia-Agudo LF et al. (2019) Genetically induced brain inflammation by Cnp deletion transiently benefits from microglia depletion. FASEB J. 33, 8634–8647 [PubMed: 31090455]
114. Liddelow SA et al. (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541, 481–487 [PubMed: 28099414]
115. Rice RA et al. (2017) Microglial repopulation resolves inflammation and promotes brain recovery after injury. Glia 65, 931–944 [PubMed: 28251674]
116. Milinkeviciute G et al. (2019) Microglia regulate pruning of specialized synapses in the auditory brainstem. Front. Neural Circuits 13, 55–55 [PubMed: 31555101]
117. Crapser JD et al. (2020) Microglial depletion prevents extracellular matrix changes and striatal volume reduction in a model of Huntington's disease. Brain 143, 266–288 [PubMed: 31848580]
118. Fawcett JW et al. (2019) The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. Nat. Rev. Neurosci 20, 451–465 [PubMed: 31263252]
119. Tewari BP et al. (2018) Perineuronal nets decrease membrane capacitance of peritumoral fast spiking interneurons in a model of epilepsy. Nat. Commun 9, 4724 [PubMed: 30413686]
120. Blosa M et al. (2015) The extracellular matrix molecule brevican is an integral component of the machinery mediating fast synaptic transmission at the calyx of Held. J. Physiol 593, 4341–4360 [PubMed: 26223835]
121. Favuzzi E et al. (2017) Activity-dependent gating of parvalbumin interneuron function by the perineuronal net protein brevican. Neuron 95, 639–655 [PubMed: 28712654]
122. Nguyen PT et al. (2020) Microglial remodeling of the extracellular matrix promotes synapse plasticity. Cell. Published online June 26, 2020. 10.1016/j.cell.2020.05.050
123. Elmore MR et al. (2015) Characterizing newly repopulated microglia in the adult mouse: impacts on animal behavior, cell morphology, and neuroinflammation. PLoS One 10, e0122912 [PubMed: 25849463]
124. Han J et al. (2017) An updated assessment of microglia depletion: current concepts and future directions. Mol. Brain 10, 25 [PubMed: 28629387]
125. Asai H et al. (2015) Depletion of microglia and inhibition of exosome synthesis halt tau propagation. Nat. Neurosci 18, 1584–1593 [PubMed: 26436904]
126. Otxoa-de-Amezaga A et al. (2019) Microglial cell loss after ischemic stroke favors brain neutrophil accumulation. Acta Neuropathol. 137, 321–341 [PubMed: 30580383]
127. Wang C-F et al. (2019) Depletion of microglia attenuates dendritic spine loss and neuronal apoptosis in the acute stage of moderate traumatic brain injury in mice. J. Neurotrauma 37, 43–54 [PubMed: 31397209]
128. Willis EF et al. (2020) Repopulating microglia promote brain repair in an IL-6-dependent manner. Cell 180, 833–846 [PubMed: 32142677]
129. Henry RJ et al. (2020) Microglial depletion with CSF1R inhibitor during chronic phase of experimental traumatic brain injury reduces neurodegeneration and neurological deficits. J. Neurosci 40, 2960–2974 [PubMed: 32094203]
130. Wang C et al. (2020) Microglia mediate forgetting via complement-dependent synaptic elimination. Science 367, 688–694 [PubMed: 32029629]

Highlights

Microglial depletion animal models provide insight into microglial cell function in the healthy and diseased brain.

Microglia are dependent on colony-stimulating factor 1 receptor (CSF1R) signaling for survival in the vertebrate brain.

CSF1R inhibition allows rapid and titratable myeloid cell population manipulation in the rodent CNS.

We posit that optimal microglial depletion models (e.g., CSF1R inhibitors) should ideally enable the elimination of these cells for any duration of time in different cell subsets. These models might also stimulate microglial repopulation in any model or species, and act in a clinically relevant fashion.

Outstanding Questions

Can we formulate CSF1Ri to selectively target other CNS myeloid cell populations (i.e., meningeal, perivascular, or choroid plexus macrophages)?

What are the phenotypic and therapeutic effects of modulating microglia with CSF1Ri doses that do not result in cell depletion?

Can CNS delivery of CSF1Ri be improved?

What is the relationship between loss of *Csf1r* function (owing to haploinsufficiency or mutation in *Csf1r*) and chronic CSF1R inhibition in the vertebrate brain?

Can we combine microglial elimination with targeted genetic approaches to better understand the mechanisms underlying microglial cell biology versus the effects of their global loss in the brain?

Are the beneficial effects of CSF1R modulation seen in rodent models of disease translatable to humans?

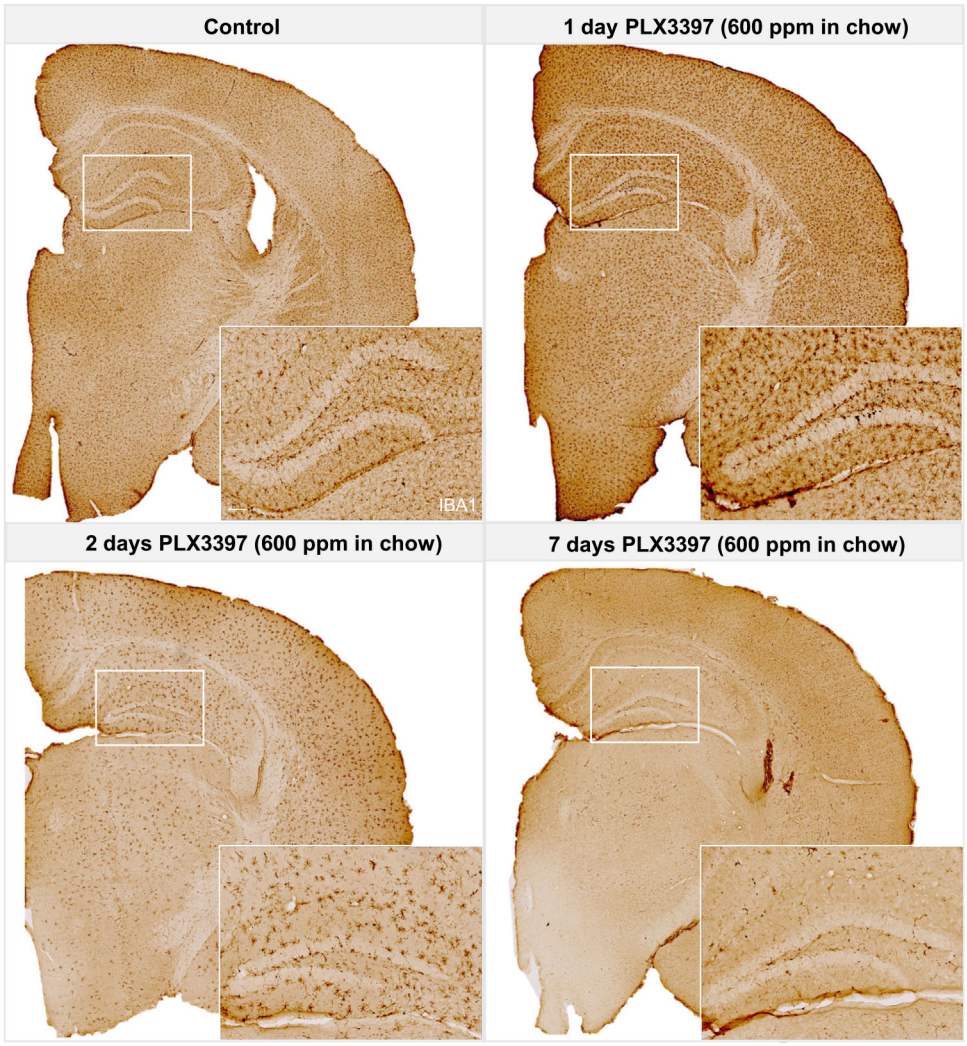

Figure 1.

Widespread Depletion of Murine Microglia with Orally Administered Colony-Stimulating Factor 1 Receptor (CSF1R) Inhibitors. For illustrative purposes, wild-type mice aged 2 months were treated with the CSF1R inhibitor PLX3397 (600 ppm in chow) for 1, 2, or 7 days. Controls were treated for 7 days with vehicle chow. To visualize microglial depletion, representative micrographs of brain sections stained via chromogenic immunohistochemistry using the common microglial marker ionized calcium binding adaptor molecule 1 (IBA1) are shown. Higher-resolution images are provided as insets. Scale bar, 120 μm.

(Key Table) Table 1.

Common Models of Microglial Depletion[a]

Approach	Duration	Extent of Depletion	Cytokine Storm	Requires Invasive Surgery	Requires genetic crosses	Potential gene haploinsufficiency	Will affect peripheral cells	Dependent on exogenous agent	Developmental Defects	Species	References
Advantages						**Disadvantages**					
CSF1R inhibitors	Indefinite	0–100%	No	No	No	No	Yes[$]	Yes	No*	Any	[62, 63, 65, 75, 77, 98]
• No cognitive/behavioral impairments. • No BBB damage. • Orally bioavailable. • FDA approved. • Elimination sustainable virtually indefinitely. • Inhibitor cessation induces cell repopulation.						• Potential off-target effects of PLX3397 on related tyrosine kinase receptors (e.g. c-Kit, FLT3). • Not suitable for some developmental studies due to potential neuronal and/or osteoclast CSF1R expression.					
Diphtheria toxin-mediated	<5 days	80–90%	Yes	No	Yes	Yes	Yes[&]	Yes	No*	Mouse/ Rat	[21, 23, 25–27]
• Inducible depletion allows selective targeting of microglia (and other long-lived myeloid cells) over short-lived BDMC. • Availability of novel microglial-specific Cre lines allow more selective depletion (e.g Tmemll9).						• Short-lived depletion. • Cognitive impairment. • Variable Cre recombination efficiency. • Cre/loxP leakiness. • TAM-associated side effects. • TAM non-specific activation on estrogen receptor cells. • Cre recombinase toxicity.					
Cdllb-HSVTK	<28 days	90–97%	?	Yes	Yes	Yes	Yes	Yes	No	Mouse	[14–20]
• Treatment withdrawal causes microglia replacement by peripheral cells.						• BBB compromise. • Ganciclovir-associated side effects. • Requires BM transfer (i.e. irradiation) or surgical implantation of osmotic pump for selective microglial depletion.					
Clodronate Liposomes	<5 days	Variable	Yes	Yes	No	No	No	Yes	No	Any	[31–33]
• Can target specific subsets of macrophages (e.g. i.c.v. administration of mannosylated liposomes targets perivascular macrophages).						• Do not cross BBB: surgery required to target microglia. • Short-lived depletion. • Induces cytokine release, astrogliosis, and blood vessel damage.					
***Csf1r*$^{-/-}$ / *Csf1r*$^{fl/f}$**	Lifelong / indefinite	100%	No	No	Yes	KO	Yes	No	Yes	Mouse	[37, 44, 54]
• Can be used to transplant exogenous myeloid cells into the brain. • Conditional knockout (using loxP sites and Cre lines) allows spatial and temporal control of microglial depletion.						• Shortened lifespan, unless carefully nurtured. • Exhibit skeletal and neurodevelopmental abnormalities. • Reduced tissue resident macrophages. • Discrepancies in observed abnormalities between different genetic backgrounds in mice.					
***Csf1r*$^{-/-}$**	Lifelong	100%	No	No	Yes	KO	Yes	No	Yes	Rat	[57]
• No microglia. • Survive into adulthood. • No overt brain abnormalities.						• Reduced myelination. • Reduced tissue resident macrophages.					
***Csf1*$^{-/-}$(*Csf1*$^{op/op}$)**	Lifelong	30%	No	No	Yes	KO	Yes	No	Yes	Mouse	[37–44, 61]
						• Majority of macrophages are absent. • Olfactory bulb and fertility deficits.					

Approach	Duration	Extent of Depletion	Cytokine Storm	Requires Invasive Surgery	Requires genetic crosses	Potential gene haploinsufficiency	Will affect peripheral cells	Dependent on exogenous agent	Developmental Defects	Species	References
						• Abnormal brain development.					
IL34$^{-/-}$	Lifelong	70%	No	No	Yes	KO	No	No	Yes	Mouse	[47]
• Little effect on other myeloid cells (e.g. blood monocytes and tissue macrophages).						• Depleted Langerhans cells resulting in reduced contact hypersensitivity. • Reduced lung dendritic cells.					
CSF1R$^{\Delta FIRE/\Delta FIRE}$	Lifelong	100%	No	No	Yes	KO	Yes	No	Yes	Mouse	[56]
• No microglia. • Developmentally normal, healthy, and fertile. • Survive into adulthood.											
TGF-β1$^{-/-}$	Lifelong	100%	No	No	Yes	KO	Yes	No	Yes	Mouse	[53]
						• Peripheral delivery of TGF-β required for mouse viability. • Motor deficits/mortality reported from 4–6 months of age. • Neurotransmitter/synapse deficits. • Increased infiltration of peripheral monocytes.					
PU.1$^{-/-}$	Lifelong	100%	No	No	Yes	KO	Yes	No	Yes	Mouse	[51–52]
						• Fail to develop microglia, resulting in shortened lifespan (unless provided with BM transplant from wild-type mice).					

[a] Abbreviations: BBB, blood–brain barrier; BM, bone marrow; BMDMCs, BM-derived myeloid cells; CSF1R, colony-stimulating factor 1 receptor; i.c.v., intracerebroventricular; KO, knockout; TAM, tamoxifen.

[b] Can be controlled for with peripheral inhibitors.

[c] Unless administered during development.

[d] The extent of depletion and the cells affected depend on the Cre driver line.