Regulation of microglial survival and proliferation in health and diseases

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Abstract

Microglia play an important role in the development and maintenance of the central nervous system (CNS) under homeostatic conditions as well as during neurodegenerative diseases. Recent observations in human genomics and advances in genetic mouse models have provided insights into signaling pathways that control development, survival, proliferation and function of microglia. Alteration of these pathways contributes to the pathogenesis of CNS diseases. Here we review the current literature regarding the roles of these microglial pathways in both the normal and diseased brain and discuss areas that require further investigation.

Keywords

1. Overview: signals that govern microglial survival and proliferation

Microglia are central nervous system (CNS) resident macrophages that perform many important functions during development and in maintaining homeostasis as well as in countering inflammatory conditions within the CNS [1,2]. Like other tissue macrophages, microglia develop during embryogenesis [3,4]. Primitive macrophages derived from early *erythro*-myeloid precursors in the embryonic yolk sac seed the developing brain as early as embryonic day 9.5 [5]. These primitive macrophages then proliferate, differentiate, and mature into microglia [3,4]. In steady state conditions, microglia are maintained in an autonomous fashion throughout life, with very little contribution from bone marrow-derived cells [3,4]. Several signals govern the development and maintenance of microglia (Fig. 1). First, like other macrophages, microglia require signals for survival and proliferation from colony stimulating factor-1 receptor (CSF-1R), which is triggered by either CSF-1 or interleukin-34 (IL-34), both of which are differentially expressed in specific regions of the prenatal and postnatal CNS [6–9]. Mice that are deficient in IL-34 or CSF-1 have fewer microglia in various regions of the brain [6,9,10]. Engagement of CSF-1R by CSF-1 or IL-34 results in oligomerization and *trans*-phosphorylation of CSF-1R, internalization of the ligand-receptor complex, as well as phosphorylation and activation of downstream cytoplasmic mediators that promote survival and proliferation [11,12]. Optimal CSF-1R

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signaling also requires additional signals, particularly from the DNAX activating protein of 12 kDa (DAP12), an adapter protein that contains cytosolic tyrosine-based activation motifs (ITAMs) [11,13,14]. Activation of DAP12 is achieved through association with an Ig superfamily cell surface receptor, triggering receptor expressed on myeloid cells 2 (TREM2), which detects phospholipids and lipoproteins [15]. Activation of the TREM2/ DAP12 complex leads to the phosphorylation of the kinase Syk, which synergizes with CSF-1R signaling. Macrophages lacking DAP12 proliferate poorly and do not survive [14]. In addition, recent transcriptional profiling and epigenetic analyses revealed a requirement for TGF-β signaling [16,17]; TGF-β imprinting, combined with activation of the transcription factor PU.1, drives the expression of many microglial specific genes [16,17]. Interestingly, TGF-β deficiency results in a gradual decline in microglia numbers in the brain [16]. In this review, we discuss the impact of these survival signals on microglial function during normal homeostasis and their potential role during neurodegenerative diseases.

2. Rare hereditary neurodegenerative diseases resulting from loss of function in either the CSF-1R or TREM2/DAP12 signaling pathways

Recently, it has been appreciated that microglia play an essential role in the normal function of the CNS. Impaired microglia function has been reported to result from hemizygous loss of a functional CSF-1R or through homozygous loss of function mutations in either TREM2 or its signaling partner DAP12 [18–28]. Hemizygous loss of CSF-1R results in adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), whereas total loss of TREM2/DAP12 signaling causes polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy, which is also known as Nasu-Hakola disease (NHD) [20– 22,24–26,28].

In the CNS, CSF-1R signaling is important for the survival and maintenance of both neurons and microglia [29]. CSF-1R haploinsufficiency results in the autosomal dominant development of ALSP, which is characterized by adult-onset dementia with a progressive decline in motor skills accompanied by epilepsy [24,28]. In order to better understand the underlying biology of ALSP, a mouse model of ALSP was generated by deleting one copy of the $Csf1r$ gene [29]. Aged $Csf1r^{+/-}$ mice have pronounced cognitive defects [29]. Histological examination revealed increased demyelination and neurodegeneration as well as fewer neurons in the brains of $Csf1r^{+/-}$ mice than in those of $Csf1r^{+/+}$ mice [29]. Interestingly, although CSF-1R signaling is important for the survival of microglia, $Csf1r^{+/-}$ mice have elevated numbers of microglia throughout the brain [9,29]. Chitu et al. hypothesized that the additional microglia result from increased microglia activation/ proliferation, which is promoted by inflammation triggered by the excess myelin debris in $Csf1r^{+/-}$ mice [29]. Further characterization of the $Csf1r^{+/-}$ mice will provide additional insight into ALSP.

In the CNS, TREM2 expression is limited to microglia [23,26,30,31]. Mutations resulting in the loss of TREM2 signaling, involving either TREM2 or its signaling adaptor DAP12, result in NHD, which is characterized by severe, early-onset, progressive dementia with

bone involvement [20–22,25,26]. NHD patients typically succumb to disease before the 5th decade of life [20–22,26]. A hallmark of NHD is the demyelination of subcortical white matter [32]. Using a cuprizone-induced mouse model of oligodendrocyte toxicity and demyelination that recapitulates some of the pathological findings in individuals with NHD, we recently demonstrated that TREM2 promotes the microglial response to myelin debris [33]. Mice lacking TREM2 were unable to clear myelin debris and had more axonal dystrophy, fewer oligodendrocytes, and prolonged demyelination [33]. Additionally, microglia in *Trem2*-deficient mice did not expand as quickly as those in wild-type mice in response to cuprizone challenge [33]. However, the microglia population in *Trem2*-deficient mice was sustained for an extended period of time following cuprizone challenge, which correlated with the prolonged demyelination observed in these mice [33]. These results offer a mechanistic explanation for the role of microglia in a mouse model of NHD and provide insight into the function of microglia in the CNS.

3. Microglia play an important role in the development and maintenance of the CNS

Most functional studies of microglia have focused on the role of activated microglia in the inflamed or diseased brain. Until recently, the function of microglia in the normal resting CNS was unknown. However, two studies using two-photon microscopy revealed that microglia actively survey their surroundings in a healthy, resting CNS [34,35]. Microglia were observed extending and retracting their processes and interacting with astrocytes and neurons, which suggests that they are dynamic cells in the unperturbed CNS [34,35]. These studies supported the long-standing hypothesis that microglia may play an active role in maintaining CNS functions in steady-state.

The use of mouse models in recent studies has aided in elucidating the variety of roles that microglia play in the developing as well as the adult brain. In the developing brain, neurons make many productive and non-productive connections with surrounding cells. The "pruning" of non-productive connections is an important step in the development of a normally functioning brain. Recent evidence has indicated that microglia survey synaptic connections and engulf synapses during postnatal brain development [36–38]. The engulfment of synapses by microglia appears to depend on several signals. Following engagement with microglia, neurons transiently upregulate expression of CX3CL1, which interacts with CX3CR1 expressed on microglia [37]. A recent study found that microglial engulfment of synapses is dependent on expression of CX3CL1 by neurons [37]. A separate study showed that expression of the complement receptor 3 (CD11b/CD18) complex on microglia, along with deposition of complement component 3 on synapses, is also required for microglial engulfment of synapses [38]. Synaptic engulfment by microglia also appears to be influenced by the productivity of the synapse, as synapses that are active are less likely to be engulfed by microglia [38]. Finally, microglia do not appear to be important for brain development in the embryonic mouse, as *Csf1r*-deficient mice, which have significantly reduced numbers of microglia, appear to have normal brains at birth [36]. However, following birth, the brains of *Csf1r*-deficient mice develop enlarged ventricles and have more neurons and astrocytes but fewer oligodendrocytes than do brains of wild-type mice

[36]. *Csf1r*-deficient mice generally do not survive until adulthood [36]. Taken together, these data indicate that unimpaired development and survival of microglia are essential for the development of normal neuronal function.

While these studies indicate that microglia are important for development of the CNS during a short period after birth, a potential role for microglia in maintaining the CNS throughout adulthood has also been explored. Using a *CX3CR1*^{CreER} mouse, which allows for tamoxifen-inducible depletion of microglia in the adult brain, Parkhurst et al. examined the role of microglia in the adult CNS [39]. Microglia, through the production of brain-derived neurotrophic factor, were found to be important for maintaining synaptic plasticity, which has been linked to learning [39]. In addition, microglia have been implicated in synaptic transmission; several studies demonstrated that microglia regulate basal glutamatergic as well as GABAergic synaptic transmission [39–42]. As a result of microglia depletion later in life, these microglia-dependent signaling events do not occur and normal synaptic functions are compromised.

Because microglia play roles in synaptic pruning, maintenance of synaptic plasticity, as well as signaling between neurons, it was reasonable to suggest that they may also have an impact on higher functions, such as olfactory sensing and learning. Indeed, further examination of *Csf1r*-deficient mice revealed that they have a significantly impaired ability to find food, which is characteristic of compromised olfactory capacity [36]. Additionally, mice transiently depleted of microglia (*CX3CR1*^{CreER}:*R26*^{iDTR/+} *mice* after tamoxifen administration) failed to adequately perform a number of tasks testing both motor skills and learning, including RotaRod training, freezing in response to a conditioned stimulus, and time spent interacting with a new object [39]. Taken together these data demonstrate that development and survival of microglia are essential for the development and maintenance of the CNS.

4. TREM2 variants in neurodegenerative diseases

The innate immune response to damage-associated signals is paramount for the early host response to both pathogens and sterile insults. The innate immune system relies primarily on both cell surface and intracellular receptors that recognize common motifs to trigger responses to insults. The TREM2/DAP12 heterodimer represents one example of a crucial innate immune receptor expressed by microglia [18,19,23,26,27,30,31].

Because of the dramatic early onset dementia and unique pathology observed in individuals with total loss of TREM2 signaling, it was surprising that recent genome wide association studies (GWAS) and more conventional sequencing studies found that heterozygousity for the rare TREM2 variant R47H is associated with a significantly increased odds ratio for the development of late-onset Alzheimer's disease (AD) [43–46]. Unlike the pathology in NHD, partial loss of TREM2 signaling resulted in individuals that are heterozygous for TREM2 R47H developing AD, which is characterized by deposition of amyloid-β (Aβ) plaques and hyper-phosphorylated tau tangles, which leads to the development of dementia [43–46]. We have recently demonstrated that, in a mouse model of AD, significantly more amyloid-β (Aβ) accumulated in the brains of TREM2-deficient than in those of TREM2-sufficient mice

[27]. Accumulation of Aβ was strongly associated with an increase in neuronal loss [27]. We also determined that TREM2-deficient microglia were less responsive to amyloid plaques [27]. Furthermore, the microglia from TREM2-deficient animals were also much more dystrophic and more likely to undergo apoptosis than microglia from wild-type animals [27]. Using a reporter cell assay we determined that the R47H variant of TREM2 is less responsive to TREM2 activating phospholipid ligands than is wild-type TREM2 [27].

Interestingly, the rare TREM2 variant R47H does not contribute to an increased odds ratio for the development of AD in all populations that have been examined. GWAS studies performed on individuals of East Asian origin did not detect an increase in the risk of developing AD for those carrying the TREM2 R47H variant [47–51]. These findings most likely indicate that the development of AD is multifactorial and certain populations may possess factors that compensate for the partial loss of TREM2 function. Further investigation into the apparent lack of a role for the R47H TREM2 variant in the development of AD in these populations will improve our understanding of both TREM2 biology and the progression of AD.

Because the R47H variant of TREM2 was associated with a significantly increased chance for developing AD in certain populations, it was hypothesized that this TREM2-variant might also contribute to the development of other neurodegenerative diseases. A recent effort to quantify the contribution of the R47H variant to the development of Parkinson's disease (PD), frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), ischemic stroke, and progressive supranuclear palsy revealed that the R47H variant of TREM2 is associated with a statistically significant increase in the odds ratio for the development of both PD and FTD [52]. In contrast, another GWAS study found no correlation between the R47H variant of TREM2 and the development of FTD, PD, or ALS [53]. Finally, yet another study found a statistically significant increase in the odds ratio that R47H individuals are at risk for developing spontaneous ALS [54]. Therefore, it is currently unclear what, if any, role the R47H variant of TREM2 plays in the development of PD, FTD, and ALS [52–54]. Further characterization of the development of PD, FTD, or ALS in individuals harboring the R47H variant of TREM2 as well as the use of animal models to examine the role of TREM2 in models of PD, FTD, and ALS, will improve our understanding of the role of TREM2 in these neurodegenerative diseases.

5. Innate immune receptor in AD

Beyond TREM2, several other proteins that are expressed by microglia have also been implicated in the development and progression of AD including CD33 and complement receptor 1 (CR1). CD33 was identified as playing a role in the development of late-onset AD in GWAS studies [55,56]. Mechanistic studies into the role of CD33 in AD development were performed by analyzing samples obtained from individuals bearing either the common CD33 variant or one of the minor CD33 allele variants (rs3865444). Individuals with the CD33 rs3865444^T variant had less CD33 expression on microglia and less detectable Aβ throughout their brains [57]. Additionally, CD33 expression inhibited the uptake of Aβ by microglia *in vitro*, while CD33-deficiency reduced brain levels of insoluble Aβ42 and amyloid plaque burden in APP(Swe)/PS1(ΔE9) mice [57]. Another study investigating the

role of a different CD33 variant, rs3865444[C], found that this variant is associated with increased CD33 expression [58]. Increased CD33 was associated with diminished Aβ internalization and more neuronal cell damage surrounding plaques as well as an increase in the overall number of activated microglia [58]. Taken together these studies indicate that CD33 expression inhibits phagocytosis of Aβ and that overex-pression of CD33 is detrimental while decreased CD33 is beneficial during AD development and progression [57,58].

The role of CR1 in microglia appears to be neuroprotective but in some respects can enhance neurodegeneration [59]. A copy number variant (CNV) of CR1 that results in the overproduction of a larger CR1 variant known as CR1-s has been found to be strongly associated with the development of AD [60]. The mechanism by which the production of CR1-s is linked to neurodegeneration in AD is still unclear. It has been proposed that because CR1-s has additional binding sites for C3b/C4b the production of CR1-s is directly linked to enhanced complement activation and inflammation which in turn drives the development of AD [60,61]. Additional work to determine the mechanisms by which both CD33 and CR1 contribute to the development of AD is warranted as an understanding of these mechanisms may directly lead to more effective therapeutic approaches for AD.

6. CSF-1R blockade in glioblastoma multiforme tumors

Glioblastoma multiforme (GBM) tumors are the most common primary brain tumor. GBMs represent approximately 45% of all malignant primary brain tumors and are one of the most aggressive and difficult to treat, with a mean 5 year survival rate of ~3.3% [62,63]. Current treatment for GBM is limited to resection of the tumor mass when possible and irradiation; in most cases treatment is not efficacious [62]. Recent characterization of GBM tumors has determined that several cell populations that do not originate in the tumor are present within the tumor mass [64]. The primary immune cells that have been identified as part of GBM tumors are tumor-associated macrophages/microglia (TAMs) [65]. The origin of the TAMs is currently controversial. A study by Badie and Schartner suggested that the origin of the TAMs was mixed with microglia representing the TAMs on the outside border of the tumor while the tumor infiltrating TAMs originated from the monocyte pool [66]. However, recent studies have suggested that resident microglia are the primary contributor to the TAM pool with little contribution from monocytes [67]. In GBMs, TAMs comprise up to 1% of the total cells in the tumor mass [65]. TAMs are known to have severely compromised immune function, with a decreased capacity for cytokine secretion and a hypoactive TLR response [65]. As TAMs have been implicated in the maintenance and progression of several different types of tumors, including GBMs, and TAMs have been associated with higher tumor grade and poor prognosis, the regulation and perturbation of TAMs represents an interesting therapeutic approach for the treatment of GBMs [67–71].

A role for CSF-1R signaling in the survival of TAMs during the development of GBMs has recently been appreciated; Pyonteck et al. demonstrated that blockade of CSF-1R signaling significantly reduced the number of TAMs in a mouse model of GBM [70]. They also found that, in the context of CSF-1R blockade, TAM survival became dependent on the secretion of granulocyte-macrophage colony stimulating factor (GM-CSF) and interferon-γ (IFN-γ)

by the glioma [70]. GM-CSF and IFN-γ signaling not only promoted TAM survival but also influenced the polarization of the TAMs [70]. TAMs stimulated with GM-CSF and IFN-γ in the absence of CSF-1R signaling developed a less M2-like phenotype and were less efficient at promoting the growth and survival of the tumor [70]. Further insight into the effect of CSF-1R blockade on TAMs can be gained from examination of CSF-1R blockade in other tumor models. CSF-1R blockade in combination with radiotherapy was found to significantly improve the efficacy of radiotherapy treatment in a prostate cancer model [71]. Taken together, these data indicate that the disruption of normal CSF-1R signaling, either alone or in combination with radiotherapy, may dramatically affect TAMs and may be a more effective therapeutic approach for the treatment of GBMs in the future.

7. Concluding remarks

The roles of microglia in normal health and disease pathogenesis are increasingly appreciated. It has become clear that microglia play an essential part in the development and maintenance of the CNS and that perturbation of normal microglia functions can contribute to the development of neurodegenerative diseases *i.e.* NHD and AD. Future work focused on signaling pathways that modulate microglial activation, survival and proliferation will allow for further insights into the pathogenesis of disease of the CNS and may provide new and more effective therapeutics for the treatment of diseases of the CNS including AD and GBM.

Acknowledgments

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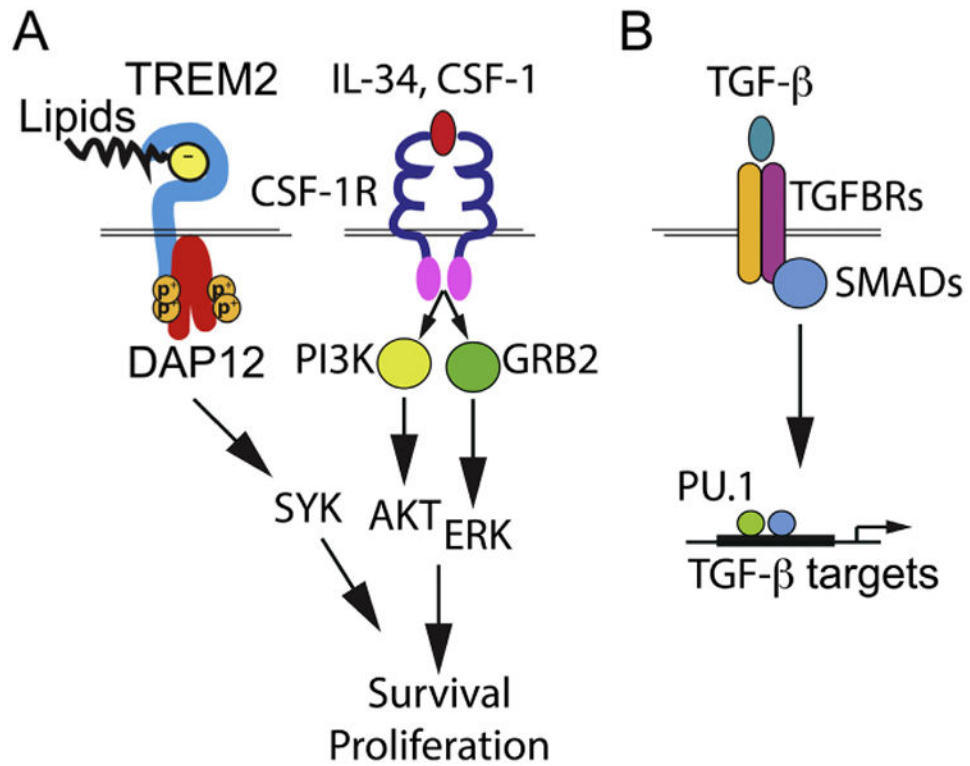
Fig. 1.
Signals that govern the development and maintenance of microglia.
The development and maintenance of microglia require several signals. (**A**) CSF-1 and its analog IL-34 trigger the activation of CSF-1R and the phosphorylation of AKT and ERK that promote the survival and proliferation of microglia. Optimized CSF-1R signaling requires TREM2, a lipid sensor. Through its adapter DAP12, activation of TREM2 leads to the phosphorylation of Syk. (B) In addition, recent transcriptional profiling and epigenetic analyses revealed a requirement for TGF-β signaling in microglia, resulting in the activation of SMADs. Activation and translocation of SMADs into the nuclei, combined with the transcription factor PU.1, drives the expression of many microglial specific genes.