Book Chapter

Differential Regulation of Microglial States by Colony Stimulating Factors

E. Richard Stanley[1]*, Fabrizio Biundo[1], Şölen Gökhan[2] and Violeta Chitu[1]

[1]Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY, United States
[2]Department of Neurology, Albert Einstein College of Medicine, Institute for Brain Disorders and Neural Regeneration, Bronx, NY, United States

***Corresponding Author:** E. Richard Stanley, [1]Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY, United States

Published **February 20, 2024**

This Book Chapter is a republication of an article published by E. Richard Stanley, et al. at Frontiers in Cellular Neuroscience in October 2023. (Stanley ER, Biundo F, Gökhan Ş and Chitu V (2023) Differential regulation of microglial states by colony stimulating factors. Front. Cell. Neurosci. 17:1275935. doi: 10.3389/fncel.2023.1275935)

How to cite this book chapter: E. Richard Stanley, Fabrizio Biundo, Şölen Gökhan, Violeta Chitu. Differential Regulation of Microglial States by Colony Stimulating Factors. In: Gesualdo M Zucco and Richard Doty, editors. Prime Archives in Neuroscience: 2nd Edition. Hyderabad, India: Vide Leaf. 2024.

Author Contributions: ES: Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. FB: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. SG: Investigation, Methodology, Writing – review & editing. VC: Formal analysis, Investigation, Methodology, Writing – review & editing.

Funding: The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by grants from the National Institutes of Health: Grant R01NS091519 (to ES) and the P30CA013330 NCI Cancer Center Grant, the Renee and Robert A. Belfer Chair in Developmental Biology (to ES) and a gift from David and Ruth Levine.

Acknowledgments: The authors thank Leslie Cummings of the Einstein Analytical Imaging Facility for help with electron microscopy and imaging. We thank Jeffrey Bajramovic for permission to use a data set from his publication [1].

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abstract

Recent studies have emphasized the role of microglia in the progression of many neurodegenerative diseases. The colony stimulating factors, CSF-1 (M-CSF), granulocyte-macrophage CSF (GM-CSF) and granulocyte CSF (G-CSF) regulate microglia through different cognate receptors. While the receptors for GM-CSF (GM-CSFR) and G-CSF (G-CSFR) are specific for their ligands, CSF-1 shares its receptor, the CSF-1 receptor-tyrosine kinase (CSF-1R), with interleukin-34 (IL-34). All four cytokines are expressed locally in the CNS. Activation of the CSF-1R in macrophages is anti-inflammatory. In contrast, the actions of GM-CSF and G-CSF elicit different activated

states. We here review the roles of each of these cytokines in the CNS and how they contribute to the development of disease in a mouse model of CSF-1R-related leukodystrophy. Understanding their roles in this model may illuminate their contribution to the development or exacerbation of other neurodegenerative diseases.

Keywords

CSF-1; CSF-2; CSF-3; CSF-1 Receptor; Microglia; Demyelinating Disease; CRL; ALSP

Regulation of Microglia by Colony Stimulating Factors

Microglia are the macrophages of the brain. They comprise ~10% of the brain cells and are distributed throughout the central nervous system (CNS) [2,3] [reviewed in Ginhoux and Prinz [4]]. Their CNS-specific roles include the maintenance of brain homeostasis and the modulation of neural circuits [5-8]. In adult healthy brain, they exhibit a ramified morphology, surveying their surrounding area [9-11]. In neuronal injury and in neurodegenerative diseases, microglia become activated and adopt a hypertrophic or ameboid shape [12-14]. Activated microglia may have opposing functions [15,16]. They can secrete neurotrophic factors to protect damaged neurons and phagocytose cellular debris, permitting tissue regeneration [17,18]. Alternatively, when excessively activated, they can secrete neurotoxic molecules, such as reactive nitrogen and oxygen species (NOS and ROS) [19-21].

CSF-1R, GM-CSFR and G-CSFR are all expressed on microglia. While their expression has been reported on some neural lineage cells [reviewed in Chitu et al. [22]], our focus here is on the actions of the CSFs mediated via their microglial receptors.

Systemic and Local Expression of CSFs

In blood, CSF-1 circulates at physiological concentrations of 4.5 ng/mL [23,24], while circulating IL-34 concentrations

(52 pg./mL) are substantially lower [25]. Circulating GM-CSF [26,27] and G-CSF [28-30] are normally barely detectable, but rise in response to inflammatory stimuli [reviewed in Dougan et al. [31], Hamilton [32]]. In humans, circulating GM-CSF gradually increases with age [33], while G-CSF decreases [29,30]. At normal physiological concentrations, recombinant CSF-1 fails to cross the blood–brain barrier (BBB) (Chitu and Stanley, unpublished). In contrast, GM-CSF can readily penetrate the BBB [34]. In rodents, G-CSF was also reported to slowly cross the BBB [35]. However, in humans its transport is limited [36,37].

In normal brain, CSF-1 and IL-34 are primarily expressed by CNS neurons, in a largely non-overlapping manner [38-40]. CSF-1 is also expressed by glia [41,42] and at low levels by microglia [43]. The regional expression of CSF-1 and IL-34 is also largely non-overlapping, with CSF-1 being found primarily in the cerebellum and white matter and IL-34 in the forebrain and grey matter [44-46]. GM-CSF (encoded by the *Csf2* gene) and G-CSF (encoded by the *Csf3* gene) are also expressed in brain at steady state, but at low levels [reviewed in Chitu et al. [22] and Biundo et al. [47]], suggesting important actions at low concentrations and/or paracrine signaling *via* local production and utilization.

CSF-1R Signaling is Required for the Development, Maintenance, and Homeostatic Functions of CNS Microglia

In the steady state, macrophage survival and proliferation are primarily regulated by the CSF-1R *via* both circulating and locally expressed CSF-1R ligands that regulate macrophage development and maintain tissue macrophage densities [23,48-50]. Consistent with these primarily trophic and anti-inflammatory roles [49,51], CSF-1 induces miRNA-21 expression in macrophages that suppresses their expression of inflammatory mediators and enhances anti-inflammatory marker expression [52].

The CSF-1R plays a central role in the development of microglia from yolk sac progenitors as well as their maintenance in adult life. In mice, erythro-myeloid progenitors (EMPs) in the yolk sac give rise to microglia and meningeal, perivascular and some choroid plexus macrophages in a CSF-1R-dependent manner [53-55] [reviewed in Prinz et al. [56]]. CSF-1R expression is first apparent at E8 in EMPs [57] and at E9 in EMP-derived A2 progenitors [58]. Following the development of the fetal circulatory system, the A2 progenitors colonize the developing brain and spinal cord at ~E9.5, where they give rise to microglia [53,58-60] and the other macrophages [61]. BBB development at ~E11.5 prevents contribution from hematopoietic stem cell-derived monocytes to the establishment of parenchymal microglia [53]. During brain development, CSF-1R signaling also promotes the postnatal colonization of the subventricular zone by microglia [62] and the establishment of microglial processes [63].

Regulation by the CSF-1R ligands differs temporally and spatially. Temporally, CSF-1 alone is required for microglial colonization and maintenance in fetal brain, while IL-34 begins to be required postnatally [38,39]. Spatially, cerebellar microglia are uniquely dependent on CSF-1 for their development and maintenance, whereas forebrain microglia mainly require IL-34 [46]. Further differential dependence on CSF-1R ligands is observed in the forebrain, where the white matter microglia are regulated by CSF-1 and the grey matter microglia by IL-34 [45,65].

Inhibition of CSF-1R signaling in the adult brain leads to massive (90–99%) microglial death [66-68]. Following cessation of treatment with CSF-1R inhibitors, the restoration of the microglial population is contributed to by the proliferation of microglia resistant to CSF-1R inhibition that share transcriptional profile similarities with microglial progenitors in the yolk sac [69,70]. It is unclear which receptors provide survival signals for these resistant cells. A pathway involving autocrine MAC2/TREM2-TYROBP has been suggested to contribute [69]. In addition, the finding that following interactions with apoptotic neurons, subsets of microglia lose their dependence on CSF-1R signaling for survival, which in

turn is maintained via the receptor tyrosine kinase Axl [71], suggests another possible mechanism.

In vitro, CSF-1 induces the proliferation of murine [72] and human [73] microglia. Two recent *ex vivo* studies utilizing human or *Macaca mulatta* microglia show that their culture in either CSF-1 or IL-34 induces identical transcriptional responses [1,43]. Pathway analysis of the latter dataset indicates that activation of CSF-1R suppresses senescence and inflammatory pathways, including the production of NOS and ROS and activates metabolic and antioxidant pathways [1] (Figure 1A). These data are consistent with a role of CSF-1R signaling in maintaining a homeostatic phenotype in microglia.

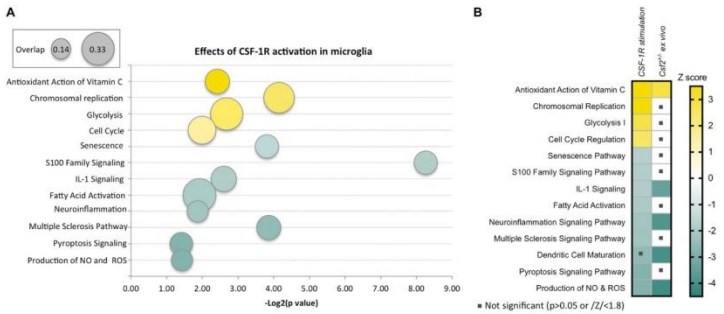

Figure 1: Effects of CSF-1R activation in microglia and overlap with the effects of reduced GM-CSF signaling. **(A)** Ingenuity Pathway Analysis-generated representation of pathways activated (yellow) or inhibited (green) in primate microglia following cultivation in CSF-1-containing medium compared to *ex vivo* microglia. The figure was generated using a published data set [1]. The ratio of number of differentially expressed genes to the total number of genes in the pathway is indicated by the size of the bubble. The sizes corresponding to the minimum (0.14) and maximum (0.33) overlap ratios are indicated in the top left box. **(B)** Decreased GM-CSF bioavailability *in vivo* induces a microglial state that partially overlaps with chronic CSF-1R stimulation *in vitro*. The heatmap shows a comparison of the pathways represented in **(A)** to pathways dysregulated in microglia isolated from *Csf2*$^{+/-}$ mice compared to wt microglia [74]. The *Z*-score scale shown on the right applies to both panels. Note that the overlapping pathways are increased antioxidant action of vitamin C and decreased IL-1 signaling, neuroinflammation signaling, dendritic cell maturation and production of NO and ROS.

Regulation of Microglial Functions by GM-CSF

Reduction of GM-CSF expression leads to decreased cognitive function [74,75], indicating that GM-CSF signaling has an important role in the functioning of the nervous system in the steady state. Intriguingly, the two subunits of GM-CSF receptor, the low affinity chain GMRα (encoded by *Csf2ra*) and the high affinity βc (encoded by *Csf2rb*), exhibit different patterns of expression in the brain, with *Csf2ra* transcripts being expressed both in microglia and neural lineage cells, and *Csf2rb* transcripts being largely restricted to microglia and having sporadic expression in the neural lineage.[1] Functional studies revealed the presence of high affinity GM-CSFRs on primary rat oligodendrocytes [76] and in microglia, while the expression of GM-CSFRs in astrocytes is controversial [reviewed in Chitu et al. [22]]. However, since the GMRα subunit alone can independently signal for glucose transport [77], the absence of βc in some cell types does not imply a lack of response to GM-CSF.

In cultured microglia, activation of GM-CSFRs induces a series of responses including rapid proliferation [72,73,78-80], development of ramified processes [81] and a series of gene expression changes. These include the upregulation of both *Csf2ra* and *Csf2rb* and the downregulation of *Csf1r* transcripts [82], suggestive of antagonistic roles of the two receptors in the control of microglia function. Indeed, while CSF-1 suppresses [41], GM-CSF induces MHC II expression in rodent microglia as well as the expression of co-stimulatory molecules B7-1 (CD80) and CD24 [82-84] and enhances their T cell stimulatory function [82,85]. However, a recent report shows that GM-CSF does not increase the expression of HLA-DR, HLA-ABC or CD80 in human microglia [86]. It remains to be established whether the priming of antigen presentation by GM-CSF is species-specific. While GM-CSF alone does not induce the secretion of the classical inflammatory cytokines IL-1β or TNFα, it has been reported to induce IL-6 production by cultured microglia [72,86]. This effect was not reproduced in organotypic hippocampal cultures [87], suggesting that *in situ* microglia might receive additional inputs that counteract the effect of GM-CSF. Nevertheless, even in the absence of

inflammatory activation, GM-CSF induced a microglial state that caused long-term disruption of neuronal network rhythms in organotypic cultures [87] and behavioral deficits consistent with schizophrenia after intra-hippocampal administration *in vivo* [88].

GM-CSF also increases the proteolytic activity of microglia through stimulation of Cathepsin S secretion [89] and upregulation of the expression of Cathepsin F, MMP-11, and MMP-12 [82]. Both myelin basic protein (MBP) and the myelin oligodendrocyte glycoprotein (MOG) are Cathepsin S substrates [90] and MMP-12 is also involved in the degradation of myelin [91]. Since GM-CSF was also reported to increase the ability of microglia to phagocytose myelin [92] it is tempting to speculate that GM-CSF-activated microglia might play a role in demyelinating diseases. Interestingly, GM-CSF was also reported to increase microglial oxidative activity *in vitro* [93,94] and to prevent the downregulation of microglial immune functions (TNFα secretion, antigen presentation) after ingestion of apoptotic cells [95]. Consistent with these observations, *ex vivo* transcriptomic profiling of microglia isolated from *Csf2*$^{+/-}$ mice reveals activation of antioxidant pathways and suppression of microglia activation, dendritic maturation and neuroinflammation (Figure 1B) [74]. Together, these data suggest that although GM-CSF does not promote an overt inflammatory reaction, it disrupts the physiological functions of microglia to prime responses associated with tissue injury.

Regulation of Microglial Functions by G-CSF

Csf3-deficient mice have impaired memory and motor deficits, decreased adult neurogenesis in the dentate gyrus area of the hippocampus, a long-term potentiation deficit and reduced dendritic complexity of hippocampal neurons [96], indicating that G-CSF signaling in the CNS also plays important roles in the steady state. G-CSF is neuronally expressed and is co-expressed with the G-CSFR in cortical neurons, where it has been suggested that its autocrine action stimulates neuronal survival [97]. However, single cell transcriptome profiling of the mouse and human brains shows that *Csf3r* transcripts encoding

the G-CSFR are found mainly in microglia (databases https://www.proteinatlas.org/, http://dropviz.org/, www.microgliasinglecell.com, https://singlecell.broadinstitute.org/single_cell/study/SCP795/a-transcriptomic-atlas-of-the-mouse-cerebellum and https://portals.broadinstitute.org/single_cell/study/aging-mouse-brain) [98-101]. Despite this, information regarding the effects of G-CSF on microglial activity is limited. While G-CSF is not a microglial mitogen [78], its administration was reported to increase microglial chemotaxis [102], to activate a Cathepsin S-CX3CR1-inducible NOS pathway and to induce the production of factors that promote neuronal excitability [103]. Together, these data suggest that many of the effects reported following G-CSF administration *in vivo* [reviewed in Chitu et al. [22]] might be mediated through regulation of microglia function.

Studies in *Csf1r*$^{+/-}$ Mice Suggest that Imbalanced Colony Stimulating Factor Signaling Leads to Dyshomeostatic Microglia

Treatment with CSF-1R inhibitors [66], or targeted inactivation of the *Csf1r* [53,67] lead to microglial death and systemically administered CSF-1 fails to cross the BBB. Thus, analysis of the role of CSF-1R in microglia function has been limited. However, *Csf1r* heterozygosity reduces the level of cell surface CSF-1Rs without causing depletion of microglia [104,105] thus offering a unique opportunity to determine how reduced CSF-1R signaling impacts microglial function. Studies to date indicate that reduced CSF-1R signaling causes a dyshomeostatic microglial phenotype [47,74,106-108] that is in part mediated by increased expression of GM-CSF and G-CSF. The following section discusses the *Csf1r*$^{+/-}$ model of CSF-1 receptor-related leukodystrophy and how it can contribute to our understanding of the role of growth factor and cytokine receptors in demyelinating disease.

Role of Colony Stimulating Factors in CSF-1 Receptor-Related Leukodystrophy

CSF-1 Receptor-Related Leukodystrophy

Rademakers and colleagues were the first to show that the adult-onset hereditary leukoencephalopathy known as Hereditary Diffuse Leukoencephalopathy with Spheroids (HDLS) was caused by dominantly inherited, mono-allelic, *CSF1R* mutations [109]. This disease, known previously by several other names [reviewed in Chitu et al. [22]] and now named CSF1R-related leukoencephalopathy (CRL) (OMIM #221820), is associated with cognitive impairment, psychiatric disorders, motor dysfunction and seizures, with an average onset at approximately 43 years of age [110]. Over 100 different *CSF1R* mutations have been reported in CRL patients, the vast majority being missense mutations within the tyrosine kinase domain. Three of these mutations cause nonsense-mediated mRNA decay (NMD) and are therefore expected to result in haploinsufficiency [111-113]. No clear genotype–phenotype correlation is apparent, apart from an earlier average disease onset in patients carrying truncating mutations or those that trigger NMD [114]. The penetrance of CRL is age-dependent, increasing from 10% at age 27 years, to 50% at age 43 years and reaching 95% at age 60 years. However, familial case reports, identifying healthy carriers of multiple different *CSF1R* mutations that are pathogenic in their close relatives [reviewed in Chitu et al. [114]], suggest incomplete penetrance even in aged individuals. Thirty-four *CSF1R* pathogenic mutations reported in CRL have been characterized in terms of expression and signaling [111,112,115-119]. Apart from the 3 NMD mutations, the remaining missense mutations, with one possible exception, are loss of function [reviewed in Chitu et al. [114]]. Although some authors have suggested that CRL mutations cause a dominant-negative, inhibitory function of the mutant chain [120], co-transfection experiments of wild type and mutant *CSF1R* constructs showed that some mutant chains do not significantly inhibit the phosphorylation of wild type CSF-1R in the response to CSF-1 [119,121], indicating that a dominant-negative mechanism is unlikely. Thus, while some of the uncharacterized CRL mutations may exhibit dominant-negative functions,

haploinsufficiency is an underlying mechanism as evidenced by the 3 NMD mutations.

The most common initial complaint of CRL patients is cognitive impairment followed by gait disorders and dyskinesia which tend to be more common in females [reviewed in Chitu et al. [114]]. These symptoms are associated with structural alterations in white matter that usually involve the corpus callosum. Histopathologically, the defining features of CRL are the presence of lipid-laden pigmented phagocytes in the affected white matter and of axonal dilations (spheroids) indicative of neurodegeneration. Interestingly, the development of spheroids is paralleled by microglial activation and there is suggestive evidence for a contribution of microglial-generated oxidative stress to the neuronal pathology and demyelination [reviewed in Chitu et al. [114]].

The Heterozygous *Csf1r* Mouse Model of CRL

Rationale for the Csf1r Heterozygous Mouse Model

The development of the *Csf1r* heterozygous C57BL6/J mouse as a model of CRL was catalyzed by the first description of *CSF1R* haploinsufficiency as a cause of CRL [121] and supported by subsequent reports of two additional CRL mutations that cause NMD [112,113]. The C57BL6/J background was chosen for two reasons: Firstly, this background caused a more severe *Csf1r* deficiency phenotype than the original FVB/NJ background and was therefore more likely to yield disease in *Csf1r* heterozygotes. Secondly, because of the availability of numerous mutant and transgenic lines on this background that would facilitate investigation of the underlying mechanisms of CRL development.

Characteristics of Disease in the Mouse Model

Csf1r$^{+/-}$ mice develop cognitive deficits, motor coordination deficits (more marked in females), depression- and anxiety- like behavior [74,104,108], that are characteristic of CRL [122] [reviewed in Chitu et al. [114]]. The penetrance of disease in 15-month-old mice is 60–70% [114,123], corresponding to a similar

degree of penetrance in humans at the equivalent age (43 years) [110,124]. The course of disease progression in mice is accelerated by a higher fat-containing diet [reviewed in Chitu et al. [114]]. Magnetic resonance imaging consistently shows callosal atrophy without calcifications. Decreased myelin staining, increases in the G-ratio of myelinated axons and in Cystatin 7 expression, indicative of active demyelination coupled with remyelination, together with axonal degeneration and the presence of axonal spheroids, are observed in the mouse model [74,108].

Csf1r$^{+/-}$ mice exhibit slightly increased microglial densities throughout the brain, even in young asymptomatic mice [104,107], that are secondary to elevation of cerebral *Csf2* expression [74]. In symptomatic *Csf1r*$^{+/-}$ mice, microglia are unevenly distributed in the white matter, with occasional clustering in the periventricular area of the corpus callosum and sporadically in the cerebellar white matter [74,108]. Clustering of white matter microglia, which is also observed in CRL [74] has recently been correlated with the active clearing of degenerated myelin [125] and might reflect active pathology. Within the corpus callosum, the microglia exhibit reduced ramification, consistent with an activated phenotype similar to that reported in early (Stage II) CRL [126]. Also similar to CRL [126-128], there is increased GFAP staining, indicative of astrocytosis, specifically within the areas of microgliosis [104,108]. *Csf1r*$^{+/-}$ mice also exhibit a decrease in the density of NeuN^{+} neurons in cortical Layer V that may be related to the callosal thinning, since some neurons within this layer project to the corpus callosum [129]. Overall, the available data indicate that symptomatic 18-month-old *Csf1r*$^{+/-}$ mice exhibit behavioral deficits similar to those found in CRL patients, accompanied by histological features similar to early stages (Stage II) of CRL [reviewed in greater detail in Chitu et al. [114]].

Evidence that CRL is a Primary Microgliopathy

Detailed pathological analysis of microglia in CRL [126] led to the concept that CRL is a primary microgliopathy in which a

genetic defect linked to microglia causes neurodegeneration [122]. Given the reported expression of the CSF-1R in neuronal cell populations [39,130-132], we addressed the contribution of *Csf1r* heterozygosity in the microglial and neuronal lineages to CRL development in the mouse model by studying disease development in $Csf1r^{fl/+}$; $Cx3cr1^{Cre/+}$ and $Csf1r^{fl/+}$; $Nestin^{Cre/+}$ mice, in which one allele of the *Csf1r* was deleted in the mononuclear phagocytes and neural lineages, respectively [108]. These studies showed that *Csf1r* heterozygosity in the mononuclear phagocytic lineage was sufficient to reproduce all aspects of the neurodegenerative disease of $Csf1r^{+/-}$ mice, with no discernable effect of deletion in the neural lineage. Furthermore, an independent study showed that there was no increased infiltration by peripheral leukocytes into the brains of symptomatic $Csf1r^{+/-}$ mice [74]. Together, these data indicate that in the mouse, CRL is a primary microgliopathy.

Roles of GM-CSF and G-CSF in CRL

Early studies of the $Csf1r^{+/-}$ mouse model revealed that despite decreased expression of the CSF-1R, microglial densities were unexpectedly slightly elevated, both prior to, and after, disease development [104,107]. This increase in microglia was not due to a compensatory increase in either of the CSF-1R ligands, CSF-1 or IL-34, as neither their mRNA, nor protein levels were altered [104]. Thus, to determine how this increased microglial density might arise on the background of decreased CSF-1R expression, mRNAs of approximately 40 inflammatory cytokines, chemokines and receptors were screened. Of these, only *Csf2* and *Csf3* mRNAs were elevated in the brains of 7-week-old pre-symptomatic $Csf1r^{+/-}$ mice and remained elevated in the older symptomatic mice [104]. These results prompted investigations with large cohorts of wt, single, and double mutant ($Csf1r^{+/-}$, $Csf1r^{+/-}$; $Csf2^{+/-}$, $Csf1r^{+/-}$; $Csf3^{+/}$, $^{-}Csf2^{+/-}$ and $Csf3^{+/-}$) male and female mice [47,74], discussed below. In the latter studies, both *CSF2* and *CSF3* mRNAs were also shown to be elevated in the brains of post-mortem CRL patients and alterations in CSF-3 signaling were also noted in an independent study [106].

Role of GM-CSF and G-CSF in the Development of Behavioral Deficits in the *Csf1r*$^{+/-}$ Model of CRL

Starting from 7 months of age, *Csf1r*$^{+/-}$ mice progressively develop cognitive and sensorimotor deficits that are associated with the loss of callosal white matter [74,104,133]. By connecting the cerebral hemispheres, the corpus callosum facilitates the integration and processing of motor, sensory, and cognitive signals. Thus, disruption of myelination could potentially contribute to both types of deficit. Consistent with the studies summarized in Section 1.3, which suggest that GM-CSF promotes a demyelinating state in microglia, examination of *Csf1r*$^{+/-}$*; Csf2*$^{+/-}$ mice revealed a substantial contribution of GM-CSF to microglia activation and demyelination in the callosal white matter [74]. Improvement of myelination in *Csf1r*$^{+/-}$*; Csf2*$^{+/-}$ mice was associated with attenuation of both cognitive and motor deficits. In contrast, targeting G-CSF did not improve myelination and had no effect on cognition. However, monoallelic disruption of *Csf3* selectively rescued the motor coordination deficits of female *Csf1r*$^{+/-}$ mice, while it also tended to worsen motor function in males [47]. This improvement might be related to attenuation of microglia dyshomeostasis in the cerebellum. Interestingly, ataxia and cerebellar involvement have been reported predominantly in female CRL patients [134-144]. Together, these data suggest that GM-CSF plays a major role in the development of CRL-like disease, while G-CSF might contribute to sex-specific phenotypes.

Role of GM-CSF and G-CSF in Microglial Dyshomeostasis in the *Csf1r*$^{+/-}$ Model of CRL

Csf1r$^{+/-}$ mice exhibit a slight early increase in microglial densities [104,107]. This increase is established during development [107] and, unless accompanied by other features such as morphological alterations or cell clustering, may not reflect a disease-related, reactive state. Consistent with the documented mitogenic effects of GM-CSF in microglia, we found that monoallelic deletion of *Csf2* normalized microglia densities in most brain regions of *Csf1r*$^{+/-}$ mice, with the

exception of the cerebellum [74]. Furthermore, the demonstration that monoallelic deletion of *Csf2rb* in microglia also prevented the increase in forebrain microglial densities, provided formal evidence that the GM-CSF-induced microgliosis is due to direct signaling in microglia [74]. In contrast, monoallelic targeting of *Csf3* in *Csf1r*$^{+/-}$ mice had limited effects on microglia densities, normalizing their levels only in the corpus callosum, cerebellum and ventral hippocampus [47]. This effect is likely indirect since G-CSF does not promote microglia proliferation [78]. Furthermore, the effects of G-CSF on microglia densities and morphology were at times dissociated, e.g., *Csf3* heterozygosity normalized the densities but not morphological alterations of microglia in the corpus callosum while having the reverse effect in the cortex of *Csf1r*$^{+/-}$ mice [47]. The factors contributing to the apparently differential effects of GM-CSF and G-CSF in different populations of brain microglia remain to be elucidated. A possible explanation resides in the differential bioavailability of ligands in the mouse brain. For example, G-CSF is selectively elevated with age in the ventral, but not dorsal hippocampus [145], a finding that could explain the dorsal region-specific response of hippocampal microglia to the monoallelic targeting of *Csf3* [47]. Another explanation is suggested by single cell transcriptomic studies which reveal the heterogeneous expression of *Csf1r*, *Csf2ra*, *Csf2rb* and *Csf3r* among mouse and human microglia (Figure 2). Irrespective of their contribution to the elevation of microglia densities and their morphological alteration, gene expression studies provide additional evidence that both GM-CSF and G-CSF promote dyshomeostatic states in *Csf1r*$^{+/-}$ microglia. Transcriptomic profiling of microglia isolated from *Csf1r*$^{+/-}$ mice suggested a maladaptive phenotype which was significantly attenuated following monoallelic inactivation of *Csf2*. The expression of gene products that mediate synapse removal, trigger cellular senescence, neurotoxicity and oxidative stress was restored with consequent reduction of the histological evidence of oxidative damage and active demyelination/remyelination [74]. Similarly, analysis of cerebellar tissue from *Csf1r*$^{+/-}$; *Csf3*$^{+/-}$ mice revealed that monoallelic targeting of *Csf3* reduced the production of C1q and its deposition on glutamatergic synapses and their consequent

excessive removal by microglia [47]. Overall, these studies show that under conditions in which the homeostatic signals provided by CSF-1R in microglia are attenuated, GM-CSF and G-CSF disrupt the normal functioning of microglia and brain homeostasis. The largely non-overlapping roles of GM-CSF and G-CSF in the development of CRL-like disease are summarized in Figure 3.

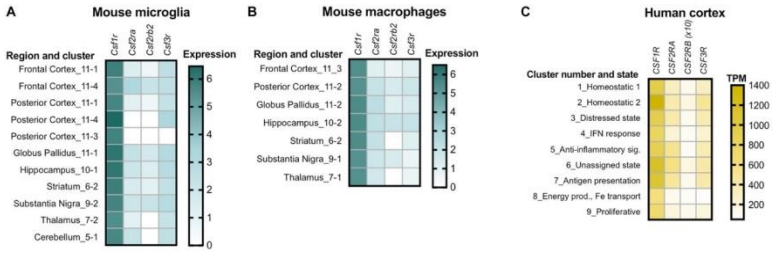

Figure 2: Variations in the expression of colony stimulating factor receptors among different populations of microglia and brain macrophages. **(A)** Variation among subclusters of microglia isolated from different regions of the adult mouse brain. Note the absence of transcripts for GM-CSFR and G-CSFR alone or in combination, in clusters of microglia isolated from the posterior cortex and the absence of transcripts for the βc high affinity subunit of GM-CSFR in thalamic and cerebellar microglia. **(B)** Mouse macrophages isolated from the same regions also show variable expression. Panels A and B were generated using data from dropviz.org [98]. **(C)** Heterogeneity of expression in human microglia isolated from the temporal and dorsolateral prefrontal cortex. The heat map was generated using data from Olah et al. [146]. To ensure visibility, the TPM values for *CSF2RB* were multiplied by a factor of 10. TPM, transcripts per million.

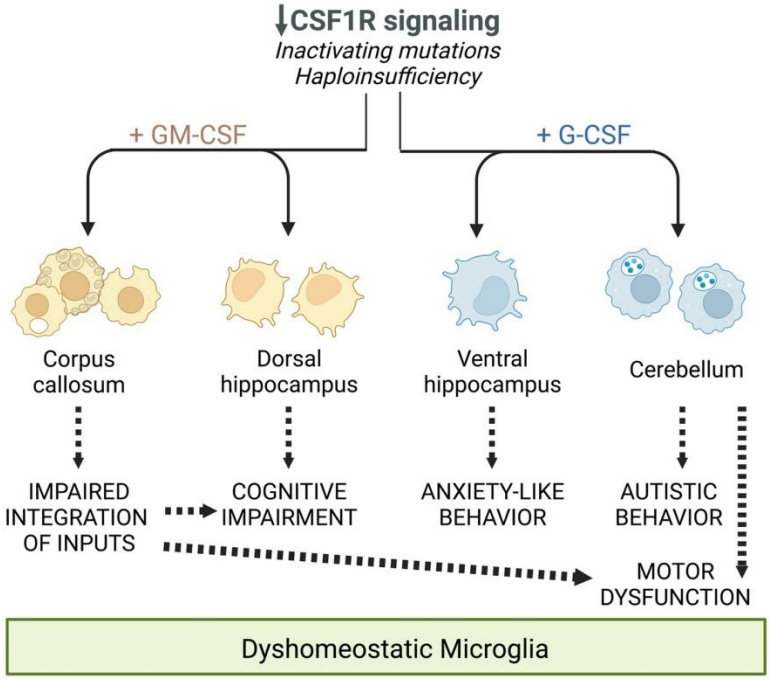

Figure 3: Schematic summarizing the differential and overlapping contributions of the increased expression of GM-CSF and G-CSF to the deficits observed in CRL mice, mediated through their regulation of microglia. Reprinted from Biundo et al. [47] with permission.

Prophylactic Potential of GM-CSF Targeting in CRL

Accumulation of evidence that CRL is a primary microgliopathy has sparked an interest in evaluating the therapeutic potential of agents known to suppress microglia activation. Recently, a one-month course of minocycline, a suppressor of the inflammatory activation of microglia, was reported to partially rescue the cognitive dysfunction in 8-month-old *Csf1r*$^{+/-}$ male mice, to reduce myelin phagocytosis by *Csf1r*$^{+/-}$ microglia to wt levels and to contribute to the preservation of callosal myelin structure and of synaptic densities in the hippocampus [133]. It is unclear if the treatment would produce the same results in *Csf1r*$^{+/-}$ female mice, who were unaffected at the same age and have not been evaluated or treated at a later time point.

Clinical studies suggested that pre-symptomatic immunosuppression with glucocorticoids is protective in patients carrying pathogenic *CSF1R* variants associated with CRL [147-149]. Further investigation in the mouse model showed that chronic prednisone administration initiated pre-symptomatically prevents the development of memory, motor coordination and social interaction deficits, as well as the associated demyelination, and neurodegeneration. Proteomic profiling *ex vivo* showed that prednisone administration suppressed a series of biological processes relevant to microglial activation and oligodendrocyte senescence [123]. These data suggest that CRL mutation carriers might benefit from prophylactic glucocorticoid treatment. The lack of improvement following steroid treatment in patients after the onset of demyelination [150] suggests that prednisone usage might be restricted to the prophylaxis of CRL. In addition, chronic glucocorticoid administration can have significant side effects both peripherally (e.g., infection, diabetes, osteoporosis) and centrally (e.g., psychosis, depression, memory decline, seizures) [151]. Thus, the exploration of downstream targets might provide therapeutic approaches with reduced side effects. Notably, glucocorticoids are known to suppress microglial activation by GM-CSF [79,81] and suppression of GM-CSF signaling through monallelic targeting of *Csf2* in the mouse model of CRL prevented the development of cognitive, motor and olfactory deficits and the loss of myelin [74]. Importantly, inhibition of GM-CSF signaling in pre-symptomatic adult *Csf1r*$^{+/-}$ mice also prevents the loss of myelin (Figure 4), indicating that GM-CSF might also be a prophylactic target in CRL. Overall, these studies indicate that CRL may be managed by using pharmaceutical approaches.

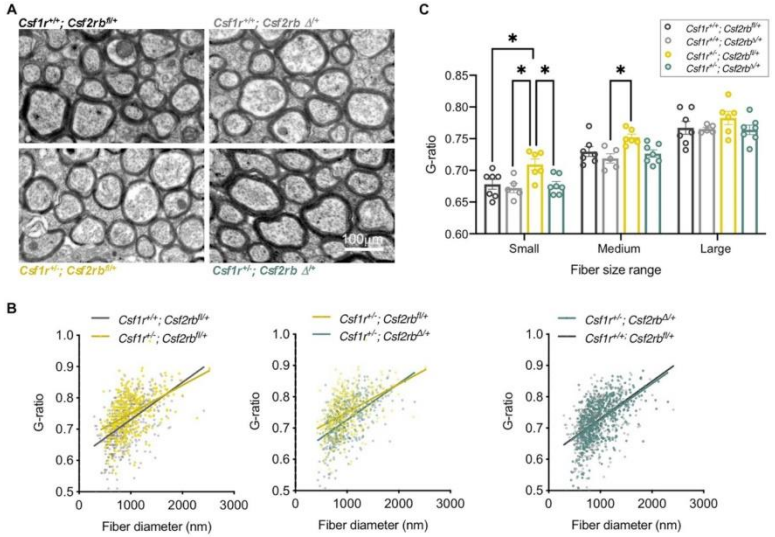

Figure 4: Inhibition of GM-CSF signaling in presymptomatic adult *Csf1r*$^{+/-}$ mice prevents demyelination. Two-month-old *Rosa26*$^{creERT2/+}$*; Csf1r*$^{+/+}$*; Csf2rb*$^{fl/+}$ and *Rosa26*$^{creERT2/+}$*; Csf1r*$^{+/-}$*; Csf2rb*$^{fl/+}$ mice [74] were injected with Tamoxifen (2.5 mg, I.P.) to induce the monoallelic deletion of *Csf2rb* (*Csf2rb*$^{\Delta/+}$), or with vehicle (corn oil). Mice were euthanized and processed for electron microscopy at 9-months of age. **(A)** Myelin ultrastructure in callosal cross-sections. **(B)** *Csf1r*$^{+/-}$ mice exhibit an increase in the ratio of the axon versus myelinated fiber (G-ratio) in low and medium diameter axons (left panel *Csf1r*$^{+/-}$*; Csf2rb*$^{fl/+}$). This reflects the limited remyelination of the fibers following the loss of myelin. This phenotype is attenuated by removal of one *Csf2rb* allele (middle panel, *Csf1r*$^{+/-}$*; Csf2rb*$^{\Delta/+}$) with *Csf1r*$^{+/-}$*; Csf2rb*$^{\Delta/+}$ becoming almost indistinguishable from wt (right panel). Individual G-ratios obtained from 5 to 7 mice/genotype are plotted against the fiber diameter. **(C)** Average G-ratios per mouse in the indicated axonal diameter ranges. Each dot on the chart represents average values from one mouse obtained as described previously [47,74]. Two-way ANOVA followed by Tukey's multiple comparison test; $*p < 0.05$.

Conclusion

Colony-stimulating factors regulate microglial development, maintenance and function in a non-overlapping and sometimes antagonistic fashion. A summary of the outcomes of their actions is presented in Figure 5. While CSF-1R ligands are constitutively expressed in the brain, transcripts for GM-CSF and G-CSF are barely detectable, but can be rapidly induced in response to

infection or injury. Consistent with the more elevated expression of its ligands in normal brain, CSF-1R is the major mediator of microglial proliferation and is required for all microglial development and maintenance [53,68]. In contrast, neither GM-CSF nor G-CSF are required for microglial development and survival. GM-CSF is also a microglial mitogen [152,153]. However, in contrast to CSF-1, GM-CSF is proinflammatory and it promotes a demyelinating phenotype [92]. G-CSF is not a microglial mitogen [78], but is also proinflammatory, inducing a pro-oxidant phenotype in microglia [103]. Studies in *Csf1r*$^{+/-}$ mice suggest that normal CNS homeostasis requires balanced signaling through all three microglial receptors [47,74]. CSF-1R signaling, in combination with low levels of GM-CSF and G-CSF, contribute to microglial homeostasis. In contrast, on the background of reduced CSF-1R signaling, elevation of either GM-CSF or G-CSF perturbs microglial homeostasis. The resulting altered states of microglia function might not be specific to CRL. Increased GM-CSF [154] and G-CSF [155,156] levels and decreased microglial *Csf1r* [157] expression have also been reported in multiple sclerosis. Furthermore, increased GM-CSF levels were also reported in Alzheimer's disease [33]. These findings suggest that imbalanced colony stimulating factor signaling might contribute to the pathogenesis of other neurodegenerative conditions.

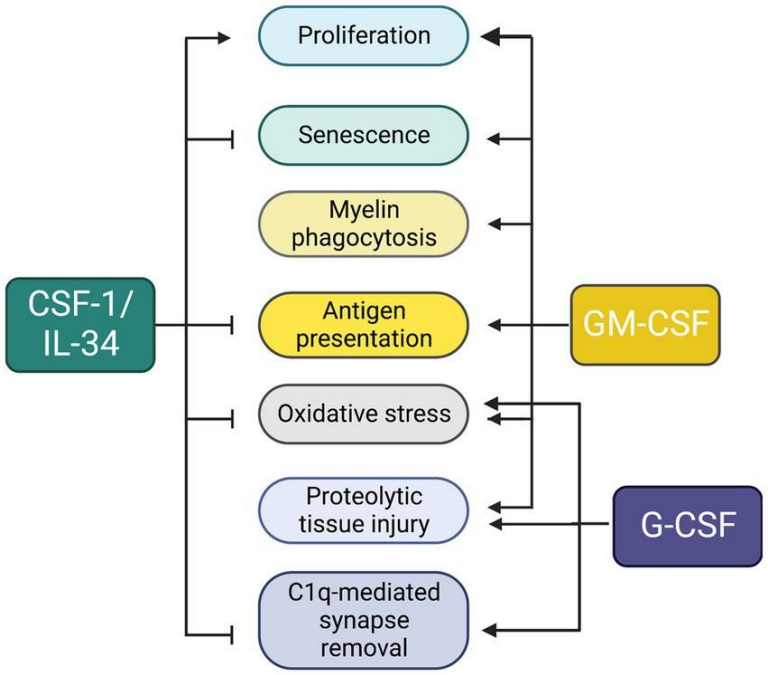

Figure 5: Unique and overlapping effects of colony stimulating factors in microglia. The diagram summarizes data obtained from studies *in vitro* (described in sections 1.2–1.4) and in *Csf1r*$^{+/-}$ mice (section 2.2.6).

Footnotes

1. ^https://singlecell.broadinstitute.org/single_cell/study/SCP263/aging-mouse-brain

References

1. Timmerman R, Zuiderwijk-Sick EA, Bajramovic JJ. P2Y6 receptor-mediated signaling amplifies Tlr-induced pro-inflammatory responses in microglia. Front. Immunol. 2022; 13: 967951.
2. Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience. 1990; 19: 151–170.
3. Yang TT, Lin C, Hsu CT, Wang TF, Ke FY, et al. Differential distribution and activation of microglia in the brain of male

C57bl/6J mice. Brain Struct. Funct. 2013; 218: 1051–1060.
4. Ginhoux F, Prinz M. Origin of microglia: current concepts and past controversies. Cold Spring Harb. Perspect. Biol. 2015; 7.
5. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, et al. Synaptic pruning by microglia is necessary for normal brain development. Science. 2011; 333: 1456–1458.
6. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR, et al. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cells. 2013; 155: 1596–1609.
7. Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, et al. Layer V cortical neurons require microglial support for survival during postnatal development. Nat. Neurosci. 2013; 16: 543–551.
8. Wake H, Moorhouse AJ, Miyamoto A, Nabekura J. Microglia: actively surveying and shaping neuronal circuit structure and function. Trends Neurosci. 2013; 36: 209–217.
9. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, et al. Atp mediates rapid microglial response to local brain injury in vivo. Nat. Neurosci. 2005; 8: 752–758.
10. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 2005; 308: 1314–1318.
11. Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat. Neurosci. 2007; 10: 1387–1394.
12. Lobsiger CS, Boillee S, Pozniak C, Khan AM, Mcalonis-Downes M, et al. C1q induction and global complement pathway activation do not contribute to Als toxicity in mutant Sod1 mice. Proc. Natl. Acad. Sci. U. S. A. 2013; 110: E4385–E4392.
13. Roth L, Van Dam D, Van Der Donckt C, Schrijvers DM, Lemmens K, et al. Impaired gait pattern as a sensitive tool to assess hypoxic brain damage in a novel mouse model of atherosclerotic plaque rupture. Physiol. Behav. 2015; 139: 397–402.
14. Fernandez-Arjona MDM, Grondona JM, Granados-Duran P, Fernandez-Llebrez P, Lopez-Avalos MD. Microglia morphological categorization in a rat model of

Neuroinflammation by hierarchical cluster and principal components analysis. Front. Cell. Neurosci. 2017; 11: 235.
15. David S, Kroner A. Repertoire of microglial and macrophage responses after spinal cord injury. Nat. Rev. Neurosci. 2011; 12: 388–399.
16. Hu X, Leak RK, Shi Y, Suenaga J, Gao Y, et al. Microglial and macrophage polarization-new prospects for brain repair. Nat. Rev. Neurol. 2015; 11: 56–64.
17. Nakajima K, Kohsaka S. Microglia: neuroprotective and neurotrophic cells in the central nervous system. Curr. Drug Targets Cardiovasc. Haematol. Disord. 2004; 4: 65–84.
18. Neumann H, Kotter MR, Franklin RJ. Debris clearance by microglia: an essential link between degeneration and regeneration. Brain. 2009; 132: 288–295.
19. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat. Rev. Neurosci. 2007; 8: 57–69.
20. Brown GC, Neher JJ. Microglial phagocytosis of live neurons. Nat. Rev. Neurosci. 2014; 15: 209–216.
21. Fu R, Shen Q, Xu P, Luo JJ, Tang Y. Phagocytosis of microglia in the central nervous system diseases. Mol. Neurobiol. 2014; 49: 1422–1434.
22. Chitu V, Biundo F, Stanley ER. Colony stimulating factors in the nervous system. Semin. Immunol. 2021; 54: 101511.
23. Bartocci A, Mastrogiannis DS, Migliorati G, Stockert RJ, Wolkoff AW, et al. Macrophages specifically regulate the concentration of their own growth factor in the circulation. Proc. Natl. Acad. Sci. U. S. A. 1987; 84: 6179–6183.
24. Janowska-Wieczorek A, Belch AR, Jacobs A, Bowen D, Padua RA, et al. Increased circulating colony-stimulating factor-1 in patients with preleukemia, leukemia, and lymphoid malignancies. Blood. 1991; 77: 1796–1803.
25. Wang H, Cao J, Lai X. Serum Interleukin-34 levels are elevated in patients with systemic lupus erythematosus. Molecules. 2016; 22: 35.
26. Kadar E, Sureda A, Mangues MA, Ingles-Esteve J, Valls A, et al. Serum levels of G-Csf, Il-3, Il-6 and gm-Csf after a single intraperitoneal dose of rhG-Csf in lethally irradiated B6D2F1 mice. Acta Haematol. 1997; 98: 119–124.

27. Frydecka D, Krzystek-Korpacka M, Lubeiro A, Stramecki F, Stanczykiewicz B, et al. Profiling inflammatory signatures of schizophrenia: a cross-sectional and meta-analysis study. Brain Behav. Immun. 2018; 71: 28–36.
28. Kiriyama R, Chichibu K, Matsuno T, Ohsawa N. Sensitive chemiluminescent immunoassay for human granulocyte colony-stimulating factor (G-Csf) in clinical applications. Clin. Chim. Acta. 1993; 220: 201–209.
29. Ishiguro A, Inoue K, Nakahata T, Nishihira H, Kojima S, et al. Reference intervals for serum granulocyte colony-stimulating factor levels in children. J. Pediatr. 1996; 128: 208–212.
30. Laske C, Stellos K, Stransky E, Leyhe T, Gawaz M. Decreased plasma levels of granulocyte-colony stimulating factor (G-Csf) in patients with early Alzheimer's disease. J. Alzheimers Dis. 2009; 17: 115–123.
31. Dougan M, Dranoff G, Dougan SK. Gm-Csf, Il-3, and Il-5 family of cytokines: regulators of inflammation. Immunity. 2019; 50: 796–811.
32. Hamilton JA. Gm-Csf in inflammation. J. Exp. Med. 2020; 217: e20190945.
33. Tarkowski E, Wallin A, Regland B, Blennow K, Tarkowski A. Local and systemic gm-Csf increase in Alzheimer's disease and vascular dementia. Acta Neurol. Scand. 2001; 103: 166–174.
34. Mclay RN, Kimura M, Banks WA, Kastin AJ. Granulocyte-macrophage colony-stimulating factor crosses the blood--brain and blood--spinal cord barriers. Brain. 1997; 120: 2083–2091.
35. Zhao LR, Navalitloha Y, Singhal S, Mehta J, Piao CS, et al. Hematopoietic growth factors pass through the blood-brain barrier in intact rats. Exp. Neurol. 2007; 204: 569–573.
36. Yuan L, Liu A, Qiao L, Sheng B, Xu M, et al. The relationship of Csf and plasma cytokine levels in Hiv infected patients with neurocognitive impairment. Biomed. Res. Int. 2015; 2015: 506872.
37. Yoshio T, Okamoto H, Kurasawa K, Dei Y, Hirohata S, et al. Il-6, Il-8, Ip-10, Mcp-1 and G-Csf are significantly increased in cerebrospinal fluid but not in sera of patients with central neuropsychiatric lupus erythematosus. Lupus. 2016; 25:

997–1003.
38. Greter M, Lelios I, Pelczar P, Hoeffel G, Price J, et al. Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia. Immunity. 2012; 37: 1050–1060.
39. Nandi S, Gokhan S, Dai XM, Wei S, Enikolopov G, et al. 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. 2012; 367: 100–113.
40. Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, et al. Il-34 is a tissue-restricted ligand of Csf1R required for the development of Langerhans cells and microglia. Nat. Immunol. 2012; 13: 753–760.
41. Lee SC, Liu W, Roth P, Dickson DW, Berman JW, et al. Macrophage colony-stimulating factor in human fetal astrocytes and microglia. Differential regulation by cytokines and lipopolysaccharide, and modulation of class ii Mhc on microglia. J. Immunol. 1993; 150: 594–604.
42. Thery C, Mallat M. Influence of interleukin-1 and tumor necrosis factor alpha on the growth of microglial cells in primary cultures of mouse cerebral cortex: involvement of colony-stimulating factor 1. Neurosci. Lett. 1993; 150: 195–199.
43. Walker DG, Tang TM, Lue LF. Studies on Colony stimulating factor Receptor-1 and ligands Colony stimulating Factor-1 and Interleukin-34 in Alzheimer's disease brains and human microglia. Front. Aging Neurosci. 2017; 9: 244.
44. Wei S, Nandi S, Chitu V, Yeung YG, Yu W, et al. Functional overlap but differential expression of Csf-1 and Il-34 in their Csf-1 receptor-mediated regulation of myeloid cells. J. Leukoc. Biol. 2010; 88: 495–505.
45. Easley-Neal C, Foreman O, Sharma N, Zarrin AA, Weimer RM. Csf1R ligands Il-34 and Csf1 are differentially required for microglia development and maintenance in White and gray matter brain regions. Front. Immunol. 2019; 10: 2199.
46. Kana V, Desland FA, Casanova-Acebes M, Ayata P, Badimon A, et al. Csf-1 controls cerebellar microglia and is required for motor function and social interaction. J. Exp.

Med. 2019; 216: 2265–2281.
47. Biundo F, Chitu V, Tindi J, Burghardt NS, Shlager GGL, et al. Elevated granulocyte colony stimulating factor (Csf) causes cerebellar deficits and anxiety in a model of Csf-1 receptor related leukodystrophy. Glia. 2023; 71: 775–794.
48. Tushinski RJ, Oliver IT, Guilbert LJ, Tynan PW, Warner JR, et al. Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells selectively destroy. Cells. 1982; 28: 71–81.
49. Cecchini MG, Dominguez MG, Mocci S, Wetterwald A, Felix R, et al. Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. Development. 1994; 120: 1357–1372.
50. Ryan GR, Dai XM, Dominguez MG, Tong W, Chuan F, et al. Rescue of the colony-stimulating factor 1 (Csf-1)-nullizygous mouse (Csf1(op)/Csf1(op)) phenotype with a Csf-1 transgene and identification of sites of local Csf-1 synthesis. Blood. 2001; 98: 74–84.
51. Chitu V, Stanley ER. Colony-stimulating factor-1 in immunity and inflammation. Curr. Opin. Immunol. 2006; 18: 39–48.
52. Caescu CI, Guo X, Tesfa L, Bhagat TD, Verma A, et al. Colony stimulating factor-1 receptor signaling networks inhibit mouse macrophage inflammatory responses by induction of microrna-21. Blood. 2015; 125: e1–e13.
53. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science. 2010; 330: 841–845.
54. Hoeffel G, Wang Y, Greter M, See P, Teo P, et al. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. J. Exp. Med. 2012; 209: 1167–1181.
55. Munro DAD, Bradford BM, Mariani SA, Hampton DW, Vink CS, et al. Cns macrophages differentially rely on an intronic Csf1r enhancer for their development. Development. 2020; 147: dev194449.
56. Prinz M, Erny D, Hagemeyer N. Ontogeny and homeostasis of Cns myeloid cells. Nat. Immunol. 2017; 18: 385–392.
57. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K,

Azzoni E, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature. 2015; 518: 547–551.
58. Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, et al. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat. Neurosci. 2013; 16: 273–280.
59. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science. 2012; 336: 86–90.
60. Hagemeyer N, Kierdorf K, Frenzel K, Xue J, Ringelhan M, et al. Transcriptome-based profiling of yolk sac-derived macrophages reveals a role for Irf8 in macrophage maturation. EMBO J. 2016; 35: 1730–1744.
61. Goldmann T, Wieghofer P, Jordao MJ, Prutek F, Hagemeyer N, et al. Origin, fate and dynamics of macrophages at central nervous system interfaces. Nat. Immunol. 2016; 17: 797–805.
62. Lelli A, Gervais A, Colin C, Cheret C, Ruiz De Almodovar C, et al. The Nadph oxidase Nox2 regulates Vegfr1/Csf-1R-mediated microglial chemotaxis and promotes early postnatal infiltration of phagocytes in the subventricular zone of the mouse cerebral cortex. Glia. 2013; 61: 1542–1555.
63. Wegiel J, Wisniewski HM, Dziewiatkowski J, Tarnawski M, Kozielski R, et al. Reduced number and altered morphology of microglial cells in colony stimulating factor-1-deficient osteopetrotic op/op mice. Brain Res. 1998; 804: 135–139.
64. Sasaki A, Yokoo H, Naito M, Kaizu C, Shultz, LD, et al. Effects of macrophage-colony-stimulating factor deficiency on the maturation of microglia and brain macrophages and on their expression of scavenger receptor. Neuropathology. 2000; 20: 134–142.
65. Badimon A, Strasburger HJ, Ayata P, Chen X, Nair A, et al. Negative feedback control of neuronal activity by microglia. Nature. 2020; 586: 417–423.
66. Elmore MR, Najafi AR, Koike MA, Dagher NN, Spangenberg EE, et al. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a

microglia progenitor cell in the adult brain. Neuron. 2014; 82: 380–397.
67. Bruttger J, Karram K, Wortge S, Regen T, Marini F, et al. Genetic cell ablation reveals clusters of local self-renewing microglia in the mammalian central nervous system. Immunity. 2015; 43: 92–106.
68. Huang Y, Xu Z, Xiong S, Sun F, Qin G, et al. Repopulated microglia are solely derived from the proliferation of residual microglia after acute depletion. Nat. Neurosci. 2018; 21: 530–540.
69. Zhan L, Fan L, Kodama L, Sohn PD, Wong MY, et al. A Mac2-positive progenitor-like microglial population is resistant to Csf1R inhibition in adult mouse brain. Elife. 2020; 9: e51796.
70. Hohsfield LA, Najafi AR, Ghorbanian Y, Soni N, Crapser J, et al. Subventricular zone/white matter microglia reconstitute the empty adult microglial niche in a dynamic wave. Elife. 2021; 10: e66738.
71. Anderson SR, Roberts JM, Ghena N, Irvin EA, Schwakopf J, et al. Neuronal apoptosis drives remodeling states of microglia and shifts in survival pathway dependence. Elife. 2022; 11: e76564.
72. Suzumura A, Sawada M, Yamamoto H, Marunouchi T. Effects of colony stimulating factors on isolated microglia in vitro. J. Neuroimmunol. 1990; 30: 111–120.
73. Lee SC, Liu W, Brosnan CF, Dickson DW. Gm-Csf promotes proliferation of human fetal and adult microglia in primary cultures. Glia. 1994; 12: 309–318.
74. Chitu V, Biundo F, Shlager GGL, Park ES, Wang P, et al. Microglial homeostasis requires balanced Csf-1/Csf-2 receptor signaling. Cell Rep. 2020; 30: 3004–3019.e5.
75. Krieger M, Both M, Kranig SA, Pitzer C, Klugmann M, et al. The hematopoietic cytokine granulocyte-macrophage colony stimulating factor is important for cognitive functions. Sci. Rep. 2012; 2: 697.
76. Baldwin GC, Benveniste EN, Chung GY, Gasson JC, Golde DW. Identification and characterization of a high-affinity granulocyte-macrophage colony-stimulating factor receptor on primary rat oligodendrocytes. Blood. 1993; 82: 3279–3282.

77. Ding DXH, Rivas CI, Heaney ML, Raines MA, Vera JC, et al. The a subunit of the human granulocyte-macrophage colony-stimulating factor receptor signals for glucose transport via a phosphorylation-independent pathway. Proc. Natl. Acad. Sci. U. S. A. 1994; 91: 2537–2541.
78. Giulian D, Ingeman JE. Colony-stimulating factors as promoters of ameboid microglia. J. Neurosci. 1998; 8: 4707–4717.
79. Ganter S, Northoff H, Mannel D, Gebicke-Harter PJ. Growth control of cultured microglia. J. Neurosci. Res. 1992; 33: 218–230.
80. Suh HS, Kim MO, Lee SC. Inhibition of granulocyte-macrophage colony-stimulating factor signaling and microglial proliferation by anti-Cd45ro: role of Hck tyrosine kinase and phosphatidylinositol 3-kinase/Akt. J. Immunol. 2005; 174: 2712–2719.
81. Tanaka J, Fujita H, Matsuda S, Toku K, Sakanaka M, et al. Glucocorticoid- and mineralocorticoid receptors in microglial cells: the two receptors mediate differential effects of corticosteroids. Glia. 1997; 20: 23–37.
82. Re F, Belyanskaya SL, Riese RJ, Cipriani B, Fischer FR, et al. Granulocyte-macrophage colony-stimulating factor induces an expression program in neonatal microglia that primes them for antigen presentation. J. Immunol. 2002; 169: 2264–2273.
83. Matyszak MK, Denis-Donini S, Citterio S, Longhi R, Granucci F, et al. Microglia induce myelin basic protein-specific T cell anergy or T cell activation, according to their state of activation. Eur. J. Immunol. 1999; 29: 3063–3076.
84. Wei R, Jonakait GM. Neurotrophins and the anti-inflammatory agents interleukin-4 (Il-4), Il-10, Il-11 and transforming growth factor-beta1 (Tgf-beta1) down-regulate T cell costimulatory molecules B7 and Cd40 on cultured rat microglia. J. Neuroimmunol. 1999; 95: 8–18.
85. Aloisi F, Ria F, Adorini L. Regulation of T-cell responses by Cns antigen-presenting cells: different roles for microglia and astrocytes. Immunol. Today. 2000; 21: 141–147.
86. Farzam-Kia N, Lemaitre F, Carmena Moratalla A, Carpentier Solorio Y, Da Cal S, et al. Granulocyte-macrophage colony-stimulating factor-stimulated human macrophages

demonstrate enhanced functions contributing to T-cell activation. Immunol. Cell Biol. 2023; 101: 65–77.
87. Dikmen HO, Hemmerich M, Lewen A, Hollnagel JO, Chausse B, et al. Gm-Csf induces noninflammatory proliferation of microglia and disturbs electrical neuronal network rhythms in situ. J. Neuroinflammation. 2020; 17: 235.
88. Zhu F, Liu Y, Zhao J, Zheng Y. Minocycline alleviates behavioral deficits and inhibits microglial activation induced by intrahippocampal administration of granulocyte-macrophage Colony-stimulating factor in adult rats. Neuroscience. 2014; 266: 275–281.
89. Liuzzo JP, Petanceska SS, Moscatelli D, Devi LA. Inflammatory mediators regulate cathepsin S in macrophages and microglia: a role in attenuating heparan sulfate interactions. Mol. Med. 1999; 5: 320–333.
90. Haves-Zburof D, Paperna T, Gour-Lavie A, Mandel I, Glass-Marmor L, et al. Cathepsins and their endogenous inhibitors cystatins: expression and modulation in multiple sclerosis. J. Cell. Mol. Med. 2011; 15: 2421–2429.
91. Hansmann F, Herder V, Kalkuhl A, Haist V, Zhang N, et al. Matrix metalloproteinase-12 deficiency ameliorates the clinical course and demyelination in Theiler's murine encephalomyelitis. Acta Neuropathol. 2012; 124: 127–142.
92. Smith ME. Phagocytosis of myelin by microglia in vitro. J. Neurosci. Res. 1993; 35: 480–487.
93. Smith ME, Van Der Maesen K, Somera FP. Macrophage and microglial responses to cytokines in vitro: phagocytic activity, proteolytic enzyme release, and free radical production. J. Neurosci. Res. 1998; 54: 68–78.
94. Tambuyzer BR, Nouwen EJ. Inhibition of microglia multinucleated giant cell formation and induction of differentiation by gm-Csf using a porcine in vitro model. Cytokine. 2005; 31: 270–279.
95. Magnus T, Korn T, Jung S. Chronically stimulated microglial cells do no longer alter their immune functions in response to the phagocytosis of apoptotic cells. J Neuroimmunol. 2004; 155: 64–72.
96. Diederich K, Sevimli S, Dorr H, Kosters E, Hoppen M, et al. The role of granulocyte-colony stimulating factor (G-Csf) in

the healthy brain: a characterization of G-Csf-deficient mice. J. Neurosci. 2009; 29: 11572–11581.
97. Schneider A, Kruger C, Steigleder T, Weber D, Pitzer C, et al. The hematopoietic factor G-Csf is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. J. Clin. Invest. 2005; 115: 2083–2098.
98. Saunders A, Macosko EZ, Wysoker A, Goldman M, Krienen FM, et al. Molecular diversity and specializations among the cells of the adult mouse brain. Cells. 2018; 174: e16.
99. Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, et al. Single-cell Rna sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. Immunity. 2019; 50: e6, 253–271.e6.
100. Ximerakis M, Lipnick SL, Innes BT, Simmons SK, Adiconis X, et al. Single-cell transcriptomic profiling of the aging mouse brain. Nat. Neurosci. 2019; 22: 1696–1708.
101. Kozareva V, Martin C, Osorno T, Rudolph S, Guo C, et al. A transcriptomic atlas of mouse cerebellar cortex comprehensively defines cell types. Nature. 2021; 598: 214–219.
102. Yamasaki R, Tanaka M, Fukunaga M, Tateishi T, Kikuchi H, et al. Restoration of microglial function by granulocyte-colony stimulating factor in Als model mice. J. Neuroimmunol. 2010; 229: 51–62.
103. Basso L, Lapointe TK, Iftinca M, Marsters C, Hollenberg MD, et al. Granulocyte-colony-stimulating factor (G-Csf) signaling in spinal microglia drives visceral sensitization following colitis. Proc. Natl. Acad. Sci. U. S. A. 2017; 114: 11235–11240.
104. Chitu V, Gokhan S, Gulinello M, Branch CA, Patil M, et al. Phenotypic characterization of a Csf1r haploinsufficient mouse model of adult-onset leukodystrophy with axonal spheroids and pigmented glia (Alsp). Neurobiol. Dis. 2015; 74: 219–228.
105. Biundo F, Chitu V, Gökhan Ş, Chen E, Oppong-Asare J, et al. Trem2 enhances demyelination in the Csf1r+/− mouse model of leukoencephalopathy. Biomedicines. 2023.
106. Kempthorne L, Yoon H, Madore C, Smith S, Wszolek ZK, et al. Loss of homeostatic microglial phenotype in Csf1R-related leukoencephalopathy. Acta Neuropathol.

Commun. 2020; 8: 72.
107. Arreola MA, Soni N, Crapser JD, Hohsfield LA, Elmore MRP, et al. Microglial dyshomeostasis drives perineuronal net and synaptic loss in a Csf1R(+/−) mouse model of Alsp, which can be rescued via Csf1R inhibitors. Sci. Adv. 2021; 7: eabg1601.
108. Biundo F, Chitu V, Shlager GGL, Park ES, Gulinello ME, et al. Microglial reduction of colony stimulating factor-1 receptor expression is sufficient to confer adult onset leukodystrophy. Glia. 2021; 69: 779–791.
109. Rademakers R, Baker M, Nicholson AM, Rutherford NJ, Finch N, et al. Mutations in the colony stimulating factor 1 receptor (Csf1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. Nat. Genet. 2011; 44: 200–205.
110. Konno T, Yoshida K, Mizuno T, Kawarai T, Tada M, et al. Clinical and genetic characterization of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia associated with Csf1R mutation. Eur. J. Neurol. 2017; 24: 37–45.
111. Konno T, Tada M, Tada M, Nishizawa M, Ikeuchi T. Hereditary diffuse leukoencephalopathy with spheroids (Hdls): a review of the literature on its clinical characteristics and mutations in the Colony-stimulating Factor-1 receptor gene. Brain Nerve. 2014; 66: 581–590.
112. Miura T, Mezaki N, Konno T, Iwasaki A, Hara N, et al. Identification and functional characterization of novel mutations including frameshift mutation in exon 4 of Csf1R in patients with adult-onset leukoencephalopathy with axonal spheroids and pigmented glia. J. Neurol. 2018; 265: 2415–2424.
113. Leng C, Lu L, Wang G, Zhang Y, Xu Y, et al. A novel dominant-negative mutation of the Csf1R gene causes adult-onset leukoencephalopathy with axonal spheroids and pigmented glia. Am. J. Transl. Res. 2019; 11: 6093–6101.
114. Chitu V, Gokhan S, Stanley ER. Modeling Csf-1 receptor deficiency diseases - how close are we? FEBS J. 2022; 289: 5049–5073.
115. Hiyoshi M, Hashimoto M, Yukihara M, Bhuyan F, Suzu S. M-Csf receptor mutations in hereditary diffuse

leukoencephalopathy with spheroids impair not only kinase activity but also surface expression. Biochem. Biophys. Res. Commun. 2013; 440: 589–593.

116. Nicholson AM, Baker MC, Finch NA, Rutherford NJ, Wider C, et al. Csf1R mutations link Pold and Hdls as a single disease entity. Neurology. 2013; 80: 1033–1040.

117. Kraya T, Quandt D, Pfirrmann T, Kindermann A, Lampe L, et al. Functional characterization of a novel Csf1R mutation causing hereditary diffuse leukoencephalopathy with spheroids. Mol. Genet. Genom. Med. 2019; 7: e00595.

118. Tian WT, Zhan FX, Liu Q, Luan XH, Zhang C, et al. Clinicopathologic characterization and abnormal autophagy of Csf1R-related leukoencephalopathy. Trans. Neurodegener. 2019; 8: 32.

119. Delaney C, Farrell M, Doherty CP, Brennan K, O'keeffe E, et al. Attenuated Csf-1R signalling drives cerebrovascular pathology. EMBO Mol. Med. 2020; e12889.

120. Hume DA, Caruso M, Ferrari-Cestari M, Summers KM, Pridans C, et al. Phenotypic impacts of Csf1R deficiencies in humans and model organisms. J. Leukoc. Biol. 2020; 107: 205–219.

121. Konno T, Tada M, Tada M, Koyama A, Nozaki H, et al. Haploinsufficiency of Csf-1R and clinicopathologic characterization in patients with Hdls. Neurology. 2014; 82: 139–148.

122. Konno T, Kasanuki K, Ikeuchi T, Dickson DW, Wszolek ZK. Csf1R-related leukoencephalopathy: a major player in primary microgliopathies. Neurology. 2018; 91: 1092–1104.

123. Chitu V, Biundo F, Oppong-Asare J, Gokhan S, Aguilan JT, et al. Prophylactic effect of chronic immunosuppression in a mouse model of Csf-1 receptor-related leukoencephalopathy. Glia. 2023; 71: 2664–2678.

124. Wang S, Lai X, Deng Y, Song Y. Correlation between mouse age and human age in anti-tumor research: significance and method establishment. Life Sci. 2020; 242: 117242.

125. Safaiyan S, Besson-Girard S, Kaya T, Cantuti-Castelvetri L, Liu L, et al. White matter aging drives microglial diversity. Neuron. 2020; 109: e10.

126. Oyanagi K, Kinoshita M, Suzuki-Kouyama E, Inoue T,

Nakahara A, et al. Adult onset leukoencephalopathy with axonal spheroids and pigmented glia (Alsp) and Nasu-Hakola disease: lesion staging and dynamic changes of axons and microglial subsets. Brain Pathol. 2017; 27: 748–769.

127. Ali ZS, Van Der Voorn JP, Powers JM. A comparative morphologic analysis of adult onset leukodystrophy with neuroaxonal spheroids and pigmented glia--a role for oxidative damage. J. Neuropathol. Exp. Neurol. 2007; 66: 660–672.

128. Kawakami I, Iseki E, Kasanuki K, Minegishi M, Sato K, et al. A family with hereditary diffuse leukoencephalopathy with spheroids caused by a novel c.2442+2T>C mutation in the Csf1R gene. J. Neurol. Sci. 2016; 367: 349–355.

129. Ivy GO, Killackey HP. The ontogeny of the distribution of callosal projection neurons in the rat parietal cortex. J. Comp. Neurol. 1981; 195: 367–389.

130. Wang Y, Berezovska O, Fedoroff S. Expression of colony stimulating factor-1 receptor (Csf-1R) by Cns neurons in mice. J. Neurosci. Res. 1999; 57: 616–632.

131. Luo J, Elwood F, Britschgi M, Villeda S, Zhang H, et al. Colony-stimulating factor 1 receptor (Csf1R) signaling in injured neurons facilitates protection and survival. J. Exp. Med. 2013; 210: 157–172.

132. Clare AJ, Day RC, Empson RM, Hughes SM. Transcriptome profiling of layer 5 Intratelencephalic projection neurons from the mature mouse motor cortex. Front. Mol. Neurosci. 2018; 11: 410.

133. Li X, Hu B, Guan X, Wang Z, Zhou Y, et al. Minocycline protects against microgliopathy in a Csf1r haplo-insufficient mouse model of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (Alsp). J. Neuroinflammation. 2023; 20: 134.

134. Mateen FJ, Keegan BM, Krecke K, Parisi JE, Trenerry MR, et al. Sporadic leucodystrophy with neuroaxonal spheroids: persistence of Dwi changes and neurocognitive profiles: a case study. J. Neurol. Neurosurg. Psychiatry. 2010; 81: 619–622.

135. Guerreiro R, Kara E, Le Ber I, Bras J, Rohrer JD, et al. Genetic analysis of inherited leukodystrophies: genotype-

phenotype correlations in the Csf1R gene. JAMA Neurol. 2013; 70: 875–882.

136. Karle KN, Biskup S, Schule R, Schweitzer KJ, Kruger R, et al. De novo mutations in hereditary diffuse leukoencephalopathy with axonal spheroids (Hdls). Neurology. 2013; 81: 2039–2044.

137. Riku Y, Ando T, Goto Y, Mano K, Iwasaki Y, et al. Early pathologic changes in hereditary diffuse leukoencephalopathy with spheroids. J. Neuropathol. Exp. Neurol. 2014; 73: 1183–1190.

138. Kim SI, Jeon B, Bae J, Won JK, Kim HJ, et al. An autopsy proven case of Csf1R-mutant adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (Alsp) with premature ovarian failure. Exp. Neurobiol. 2019; 28: 119–129.

139. Kim EJ, Shin JH, Lee JH, Kim JH, Na DL, et al. Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia linked Csf1R mutation: report of four Korean cases. J. Neurol. Sci. 2015; 349: 232–238.

140. Meyer-Ohlendorf M, Braczynski A, Al-Qaisi O, Gessler F, Biskup S, et al. Comprehensive diagnostics in a case of hereditary diffuse leukodystrophy with spheroids. BMC Neurol. 2015; 15: 103.

141. Sundal C, Baker M, Karrenbauer V, Gustavsen M, Bedri S, et al. Hereditary diffuse leukoencephalopathy with spheroids with phenotype of primary progressive multiple sclerosis. Eur. J. Neurol. 2015; 22: 328–333.

142. Lynch DS, Jaunmuktane Z, Sheerin UM, Phadke R, Brandner S, et al. Hereditary leukoencephalopathy with axonal spheroids: a spectrum of phenotypes from Cns vasculitis to parkinsonism in an adult onset leukodystrophy series. J. Neurol. Neurosurg. Psychiatry. 2016; 87: 512–519.

143. Lynch DS, Rodrigues Brandão de Paiva A, Zhang WJ, Bugiardini E, Freua F, et al. Clinical and genetic characterization of leukoencephalopathies in adults. Brain. 2017; 140: 1204–1211.

144. Bonvegna S, Straccia G, Golfre Andreasi N, Elia AE, Marucci G, et al. Parkinsonism and nigrostriatal damage secondary to Csf1R-related primary Microgliopathy. Mov. Disord. 2020; 35: 2360–2362.

145. Porcher L, Bruckmeier S, Burbano SD, Finnell JE, Gorny N, et al. Aging triggers an upregulation of a multitude of cytokines in the male and especially the female rodent hippocampus but more discrete changes in other brain regions. J. Neuroinflammation. 2021; 18: 219.
146. Olah M, Patrick E, Villani AC, Xu J, White CC, et al. A transcriptomic atlas of aged human microglia. Nat. Commun. 2018; 9: 539.
147. Tipton PW, Stanley ER, Chitu V, Wszolek ZK. Is pre-symptomatic immunosuppression protective in Csf1R-related leukoencephalopathy? Mov. Disord. 2021; 36: 852–856.
148. Ali S, Tipton PW, Koga S, Middlebrooks EH, Josephs KA, et al. A novel Csf1R variant in a South Dakota family with Csf1R-related leukoencephalopathy. Parkinsonism Relat. Disord. 2022; 102: 51–53.
149. Dulski J, Heckman MG, Nowak J, Wszolek Z. Protective effect of glucocorticoids against symptomatic disease in Csf1R variant carriers. Move. Disord. 2023.
150. Konno T, Broderick DF, Mezaki N, Isami A, Kaneda D, et al. Diagnostic value of brain calcifications in adult-onset leukoencephalopathy with axonal spheroids and pigmented glia. AJNR Am. J. Neuroradiol. 2017; 38: 77–83.
151. Witt KA, Sandoval KE. Steroids and the blood-brain barrier: therapeutic implications. Adv. Pharmacol. 2014; 71: 361–390.
152. Schermer C, Humpel C. Granulocyte macrophage-colony stimulating factor activates microglia in rat cortex organotypic brain slices. Neurosci. Lett. 2002; 328: 180–184.
153. Xiao BG, Xu LY, Yang JS. Tgf-beta 1 synergizes with gm-Csf to promote the generation of glial cell-derived dendriform cells in vitro. Brain Behav. Immun. 2002; 16: 685–697.
154. Kostic M, Zivkovic N, Cvetanovic A, Stojanovic I. Granulocyte-macrophage colony-stimulating factor as a mediator of autoimmunity in multiple sclerosis. J. Neuroimmunol. 2018; 323: 1–9.
155. Mcginley AM, Sutton CE, Edwards SC, Leane CM, Decourcey J, et al. Interleukin-17A serves a priming role in

autoimmunity by recruiting Il-1beta-producing myeloid cells that promote pathogenic T cells. Immunity. 2020; 52: e6.

156. Rumble JM, Huber AK, Krishnamoorthy G, Srinivasan A, Giles DA, et al. Neutrophil-related factors as biomarkers in Eae and Ms. J. Exp. Med. 2015; 212: 23–35.

157. Werner K, Bitsch A, Bunkowski S, Hemmerlein B, Bruck W. The relative number of macrophages/microglia expressing macrophage colony-stimulating factor and its receptor decreases in multiple sclerosis lesions. Glia. 2002; 40: 121–129.