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INSIGHTS

Microglia: Same same, but different

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Microglial identity in the central nervous system (CNS) is dependent on colony stimulating factor 1 receptor (CSF-1R) signaling and its ligands IL-34 and colony stimulating factor 1 (CSF-1). In this issue of *JEM*, Kana et al. (https://doi.org/10.1084/jem.20182037) make the important discovery that CSF-1, but not IL-34, orchestrates cerebellar microglial homeostasis in mice, and its deficiency resulted in severe cerebellar dysfunctions accompanied by defects in motor function and social behavior.

Microglia are tissue macrophages of the central nervous system (CNS) parenchyma that control tissue homeostasis. Microglia dysregulation is thought to be the cause of numerous neuropsychiatric, neurodegenerative, and neuroinflammatory diseases. Understanding the molecular pathways that regulate microglia identity is key for the future treatment of CNS disorders (Priller and Prinz, 2019). Colony stimulating factor 1 receptor (CSF-1R) is a receptor tyrosine kinase that is predominantly found throughout the entire mononuclear lineage including circulating monocytes, but also tissue macrophages such as osteoclasts in the bone, Langerhans cells in the epidermis, and microglia in the CNS (Dai et al., 2002). Microglia in the CNS have been described as highly dependent on CSF-1R signaling, especially for their differentiation and maintenance (Ginhoux et al., 2010; Prinz et al., 2017). Consequently, CSF-1R–deficient mice have been described as almost completely void of microglia in all brain regions analyzed (Ginhoux et al., 2010). In line with this phenotype, CSF-1R inhibitors can efficiently deplete adult microglia entirely in mouse brains within a few days (Elmore et al., 2014; Hagemeyer et al., 2017). The consequences of deficiencies in this signaling pathway in mouse models have also been identified in patients, where heterozygous mutations in the CSF-1R have been linked to a severe neurodegenerative condition referred to as hereditary leukodystrophy with axonal spheroids (Rademakers et al., 2012). A more recent study even describes two patients with homozygous mutations in the CSF-1R gene resulting in a complete loss of microglia in the brain resulting in severe structural brain defects (Oosterhof et al., 2019).

Interestingly, a complete ablation of microglia is not observed in animals deficient for the CSF-1R ligand CSF-1. In particular, *Csf1*$^{op/op}$ animals, a mutant mouse line carrying a spontaneous mutation in the *Csf1* gene, show only partial depletion of microglia (Wegiel et al., 1998). Identification of IL-34 as a second ligand for CSF-1R and analysis of *Il34*-deficient animals revealed a heterogeneity in microglia for one of the two ligands and also the heterogeneous expression of the ligands throughout the CNS. Although microglial cell numbers do not seem to be affected during development in *Il34*-deficient animals, adult mice show a strong reduction in forebrain microglia, whereas microglia in the cerebellum were not affected (Greter et al., 2012; Wang et al., 2012). Collectively, these results point toward a heterogeneous dependence on either CSF-1 or IL-34 for microglia in the CNS.

In their study in this issue of *JEM*, Kana et al. elegantly deciphered the causes and consequences of this constitutive heterogeneity of cerebellar microglia compared with their counterparts in the forebrain. In sum, the authors explored the role of CSF-1 in cerebellar microglia maintenance and their identity and function. In fact, the function of cerebellar microglia is poorly understood to date, as current literature focuses primarily on microglia in the forebrain, hippocampus, or cortex. Kana et al. (2019) first performed bulk RNA sequencing of human forebrain and cerebellar microglia and identified that

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cerebellar microglia have distinct transcriptional profiles compared with forebrain microglia. Similar transcriptional differences of microglia in different brain regions were also found in mice. This is in line with a recent single-cell RNA sequencing study that revealed considerable heterogeneity of adult murine microglia states across different brain regions (Masuda et al., 2019). The specific tissue factors involved in imprinting microglial identity are also not yet well defined, especially regarding how microglial cells in different brain regions develop their regional specificity. Kana et al. (2019) nicely demonstrated that the two CSF-1R ligands are differentially expressed across the rodent brain, with IL-34 dominating forebrain regions and CSF-1 being highly expressed in the cerebellum. They further demonstrated that the availability and heterogeneous expression pattern of these ligands found in mice is considerably conserved and also found in human brains. They next tested whether CSF-1 could be a potential factor for priming cerebellar microglia, whereas IL-34 is more involved in imprinting the forebrain

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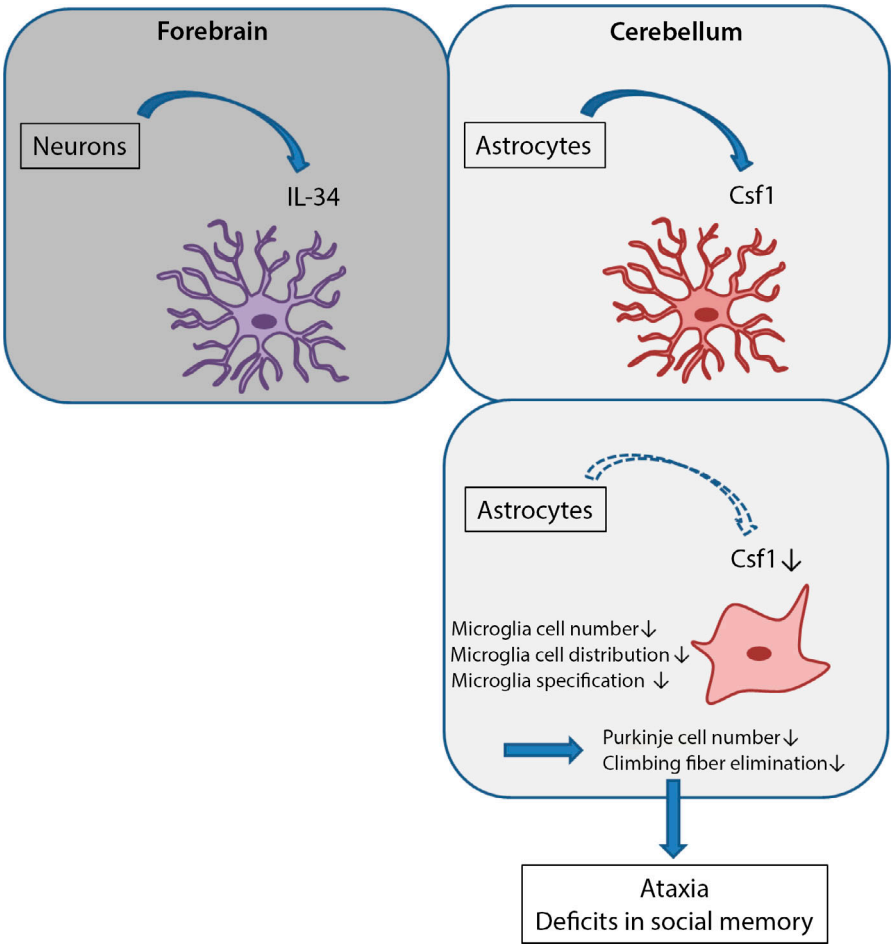

Different local requirements of the CSF-1R ligands IL-34 and CSF-1 for microglial homeostasis and function. The cytokine IL-34 produced by neurons is vital for microglial cells in the forebrain, whereas astrocyte-derived CSF-1 is essential for cerebellar microglia. Kana et al. (2019) elegantly show that CSF-1 depletion from neuroectodermal cells leads to dysfunctional microglia accompanied by altered Purkinje cell number and synaptic dysfunctions, ultimately leading to ataxia and deficits in social memory behavior.

microglial phenotype. To avoid the strong developmental defects developing in *Csf1*$^{op/op}$ mice, they used conditional *Nes*^{Cre} *Csf1*$^{fl/fl}$ animals, allowing deletion of CSF-1 in neural cells in the CNS. As predicted, these animals showed a severe reduction in microglial cell numbers in the cerebellum but not in forebrain regions, identifying CSF-1 as the major ligand for maintenance of cerebellar microglia.

Interestingly, transcriptional profiling of the few aberrant microglia remaining in the postnatal cerebellum showed a reduction of typical microglial homeostasis genes, as well as the genes involved in development, growth and metabolism. These changes in the transcriptional program were in contrast to changes found in forebrain microglia of *Il34*-deficient mice, which showed greater resemblance to the newly defined subtype of disease-associated microglia (Keren-Shaul et al., 2017; Krasemann et al., 2017). Microglia from white matter, however, have been reported to be more metabolically active, and their gene expression showed a high induction of metabolism-related genes especially during early postnatal development, a program which could potentially be driven by CSF-1. Kana et al. (2019) further provided evidence for this intriguing hypothesis by treating neonatal microglia in vitro with either CSF-1 or IL-34, respectively. Interestingly, CSF-1–treated microglia acquired a genetic signature similar to cerebellar microglia, whereas IL-34 imprinted a signature similar to forebrain microglia observed in vivo. These experiments clearly established that both IL-34 and CSF-1 are two key factors in forming microglial identities and specific local states in the adult brain. However, when they further analyzed embryonic development, they found that this regional dependence and distribution of the ligands is not yet established during embryogenesis. During embryonic development, forebrain microglia are also CSF-1 dependent, as shown by a strong reduction of forebrain microglia at E17.5 in *Nes*^{Cre} *Csf1*$^{fl/fl}$ mice. Notably, a switch from CSF-1 to IL-34 dependence for microglia in the forebrain is observed postnatally by as yet unknown mechanisms.

Until now, it was not clear what physiological function microglia might have during cerebellar development and homeostasis. In line with results from *Csf1r*-deficient animals or *Csf1*$^{op/op}$ mice, magnetic resonance imaging of *Nes*^{Cre} *Csf1*$^{fl/fl}$ mice showed an increase in brain mass as well as cerebellar volume, but a reduction in brain size and ventricle size. Detailed analysis of the cerebellum by Kana et al. (2019) further revealed a reduction in Purkinje cells, as well as an increase in aberrant dendrites emerging from these cells. Despite previous reports of cerebellar Purkinje cells expressing CSF-1R and their potential to be affected by the loss of CSF-1, the authors clearly excluded an expression of CSF-1R on calbindin-positive Purkinje cells that is in line with previous studies on the microglia-restricted expression of CSF-1R (Hagemeyer et al., 2017). Kana et al. (2019) further demonstrated that the loss of cerebellar microglia led to a developmental defect of Purkinje cells most likely due to decreased microglia-mediated elimination of climbing fibers during early postnatal development. This results in an increase in spontaneous miniature excitatory postsynaptic currents in the remaining Purkinje cells. At the age of 5–7 wk, these changes in the cerebellar signaling and structures further manifested into mild ataxia in *Nes*^{Cre} *Csf1*$^{fl/fl}$ mice, but surprisingly, these animals showed additional defects in a behavior test for social memory. Furthermore, *Nes*^{Cre} *Csf1*$^{fl/fl}$ mice exhibited a reduction in their preference to interact with a newly introduced mouse compared with a familiar interaction partner. Notably, such behavioral defects are often observed in mouse models for autism spectrum disorders and other neuropsychiatric diseases. In future studies, it would be interesting to use inducible mutants of CSF-1 deficiency to clearly delineate the precise time points for the induction of the observed pathologies.

Taken together, this landmark study elucidates a new key function for CSF-1 for

priming cerebellar microglial identity, and it further highlights the important role of microglia in cerebellar development, particularly in Purkinje cell function. The findings in this study further point to as yet unknown roles of both cerebellar microglia and CSF-1 for the development of autism spectrum disorders. These results also raise the unique hypothesis that the availability of CSF-1 during developmental stages of the brain and aberrations thereof could be a potential cause for neuropsychiatric diseases. These novel and unexpected findings on microglial biology will certainly spark new interest in these innate immune cells, allowing us to better understand and combat brain disorders in the near future.

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