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The force awakens: insights into the origin and formation of microglia

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Microglia are tissue resident macrophages of the central nervous system (CNS) that maintain homeostasis and respond to immune challenges. New genetic fate mapping tools have revealed a yolk sac origin of microglia. Once established in the CNS, microglia persist throughout the lifetime of the organism behind the blood–brain barrier and maintain themselves by self-renewal. Recent studies uncovered a broad spectrum of microglial functions that are influenced by the dynamism of brain formation and neuronal wiring. This review focuses on current findings concerning microglia origin and formation during development and discusses the factors important for microglia survival and function.

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Introduction

To understand the complex function of the central nervous system (CNS) and seek avenues to treat a plethora of brain disorders, it is imperative to understand the interaction between neurons and glia. Until recent years, the origin and physiological functions of the glial cell type microglia were under appreciated. The past decade has seen a boom in the literature on microglia, uncovering the critical roles of these brain macrophages for CNS development and maintenance of homeostasis. Gene expression analyses have suggested the uniqueness of microglia in comparison to other myeloid cells [1,2,3•,4,5]. On the basis of studies from the last five years, this review focuses on the development of microglia in perinatal brain, the intrinsic factors and environmental signals involved in this process, the roles of microglia in CNS ontogeny, and their persistence in the established brain network of adult CNS.

Microglial origins: from yolk sac to brain

Unlike all other brain cell types of neuroectodermal origin, microglia originate from yolk sac (YS) multi-lineage c-kit^{+} erythromyeloid progenitor (EMP) cells [6] that seed the primitive brain and remain throughout adulthood via constant self-renewal [7]. The colonization of the CNS by YS EMPs has been detailed in several seminal studies that applied cell type-specific genetic fate-mapping techniques [8••,9–11,12•]. YS precursor cells invade the brain anlage via the leptomeninges and lateral ventricles by 9.5 days post-conception (dpc) and spread throughout the organ from each direction at varying speeds with differential rates of proliferation [8••,13,14]. Using Ncx1 knockout mouse in which blood circulation was absent, it was shown that the recruitment of YS macrophages into the brain proper is dependent on a functioning circulatory system [8••]. Rapid occupation of the CNS by amoeboid YS-derived macrophages, which evolve into ramified microglia, is made possible by proliferation of 40–80% of the population between 10.5 dpc and postnatal (P) day 0 [6]. Microglial cell numbers increase until about P14 in mouse, following which a steady decline of half the population in the following month leads to stabilization of microglial density, reportedly due to concomitant increase in microglial apoptosis and decrease in their proliferation [15]. Overall it is believed that the recruitment of YS macrophages into the CNS is conserved across vertebrate species from fish to human [14,16,17].

The steady state contribution of definitive hematopoiesis to brain microglia was thus far excluded based on specific targeting of hematopoietic stem cells (HSCs) generated in the fetal liver or the aorta-gonad-mesonephros (AGM) in different myeloid-specific genetic reporter models for *Csf-1r* (colony-stimulating factor-1 receptor), *Flt3* (Fms-like tyrosine kinase 3), *Myb* (myeloblastosis), *Runx1* (runt-related transcription factor), and *Tie2* (angiopoietin receptor) [8••,9,11,12•]. The argument was further strengthened by findings from the $Kit^{MercreMer}$ fate mapping investigation, which demonstrated a HSC-origin of tissue resident macrophages with the exception of microglia, which appeared to be the only resident macrophages derived entirely from YS progenitors in this study [18••]. Ironically the conclusion put forth by the authors regarding the non-YS but HSC-origin of other adult tissue resident macrophages sparked off a lively exchange of opinions [19•,20•] as the statement is in stark contrast to the prevailing understanding of macrophage ontogeny which includes a YS source [11,12•].

Interestingly, a detailed study that specifically labeled microglia precursors in transparent zebrafish larvae for temporal-spatial fate-mapping at later time points suggests that a minor non-YS contribution of adult microglia [21] cannot be entirely dismissed. Furthermore it has been shown during homeostasis that hematopoietic mononuclear cells of the *Hoxb8* lineage exist in newborn mouse pups and contribute to a significant percentage of the total adult microglia population in regions where they are found [22]. Since *Hoxb8*$^+$ microglia were observed in a gradual distribution from the pial surface and ventricular lining during the first two postnatal weeks [22], it is conceivable for these cells to enter the parenchyma via the ventricular choroid plexus [23] or before closure of the blood–brain barrier (BBB) throughout the CNS [24,25], even in the absence of neuroinflammation. Fetal liver, AGM and/or bone marrow (BM)-derived populations of microglia with uncharacterized markers may have thus far been undetected in the approaches used in mouse models that lack temporal-spatial resolution. Notably, the strategies used by Ginhoux, Sheng and colleagues labeled approximately 30% [8••] and >50% [18••] of adult microglia, respectively. Until the development of techniques that demonstrate full penetrance in targeting all microglia in mammals, the non-YS source of these brain resident macrophages remains an open question. While current mouse models have vastly aided us in decrypting the primitive hematopoietic contribution to brain resident microglia, it is critical that we understand their limitations and carefully interpret our observations, as was very elegantly reviewed recently in [26••,27] (Figure 1).

Alternative microglial origins: repopulation in adult brain

In chronic neurodegeneration the increase in microglial cell number around disease sites such as experimental autoimmune encephalitis lesions or β-amyloid deposits in Alzheimer's disease mouse models occurs via proliferation of resident microglia and CNS preconditioning-dependent recruitment of BM-derived phagocytes [28–30]. With intact BBB the expansion of local microglia pool in neurodegeneration arises solely from division of adult microglia [31]. What happens when the established microglial network in the adult CNS is completely abolished? Inducing diphtheria toxin-mediated cell death in juvenile and adult mice with microglia-specific surface expression of the diphtheria toxin receptor led to about 99% reduction of microglia and thereafter changes in synaptic plasticity associated with motor learning within a week of microglia depletion [32]. Surprisingly the authors reported the absence of disturbed homeostasis such as a cytokine storm or astrogliosis [32], which starkly contradicted the findings from a later study where 80% reduction of microglia was achieved in three days using a similar approach [33]. This difference was attributed to a variation in experimental paradigm, namely the application of diphtheria toxin to ablate microglia cells earlier during CNS development in the former study [33]. In the latter study microglia briskly recovered to normal numbers in two weeks from clusters of transient Nestin-expressing proliferative microglia in an interleukin (IL)-1 signaling-dependent manner [33]. The near complete elimination of microglia for three weeks by oral application of a Csf-1R inhibitor resulted in no overt changes in cognition and behavior [34•]. Furthermore, an inflammatory response to the microglial ablation was generally absent. In this paradigm complete repopulation of microglia was surprisingly achieved one week after withdrawal of the Csf-1R inhibitor from the diet via highly proliferative Nestin-positive progenitor cells that subsequently differentiate into microglia [34•]. The plasticity of the mature microglial network was further demonstrated in a recent study where spatial memory and social behavior affected by hippocampus-specific or global microglial depletion were shown to be reversible upon microglia repopulation [35]. The BBB integrity was not compromised in both microglia elimination approaches [32,34•]. Together these studies point to a conceivable innate existence of microglial 'stem cells' in the adult CNS, independent of peripheral myeloid contribution, and yet unknown signals that regulate the steady state maintenance of microglia cell density (Figure 1). Of note, the repopulation of microglia took place under non-physiological conditions. However the outcomes of these studies suggest that a temporary depletion of microglia may not necessarily lead to harmful consequences for the CNS and may aid the design of strategies to hinder prolonged neuroinflammation.

Microglial factors: intrinsic properties and intercellular signaling

Contrary to myeloid cells derived from the fetal liver, AGM or BM, the specification and expansion of microglia cells are independent of the transcription factors Myb, Id2 (inhibitor of DNA binding 2), Batf3 (basic leucine zipper transcription factor, ATF-like 3), and Klf4 (Kruppel-like factor 4) [6,9]. However the transcription factor Pu.1, which is necessary across all subtypes of tissue macrophages [9,36••], has been shown to act in concert as heterodimer with the interferon regulatory factor 8 for the specification of the microglial lineage [6,37,38]. Genetic labeling of *Runx1*-expressing primitive hematopoiesis-derived precursors revealed that YS microglia cells are specified between 7.0 and 7.5 dpc [8••]. About a day later c-kit$^+$ EMPs mature to CD45$^+$ c-kit^{lo}Cx3cr1^{lo} F4/80$^-$ A1 progenitors, progress to CD45$^+$ c-kit$^-$ Cx3cr1^{hi} F4/80^{hi} A2 amoeboid macrophages in the YS and brain primordium at 9.0 dpc, before adopting the phenotype of mature macrophages in the neuroepithelium at 10.5 dpc, and finally taking on a ramified form of CD45^{lo} c-kit$^-$ Cx3cr1^{hi} F4/80^{lo} microglia by around 14.0 dpc [6,39].

Several factors and signaling pathways have been found to be critical for microglia survival and proliferation.