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# A story of birth and death: insights into the formation and dynamics of the microglial population

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**Abstract**

Microglia are the main resident immunocompetent cells of the brain with key roles in brain development, homeostasis and function. Here we briefly review our current knowledge of the homeostatic mechanisms regulating the composition and turnover of the microglial population under physiological conditions from development to ageing. A greater understanding of these mechanisms may inform understanding of how dysregulation of microglial dynamics could contribute to the pathogenesis and/or progression of neurological disorders.

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**Introduction**

Microglia are the main resident immunocompetent cells of the central nervous system (CNS); specialised myeloid cells which, along with other tissue-resident macrophage populations, are derived from erythro-myeloid progenitor cells originating in the yolk sac during early embryogenesis[1]. Although microglial cells display marked differences from other cells of the myeloid lineage, they express a range of 'classical' macrophage markers including the glycoproteins F4/80 and CD68, the fractalkine receptor CX3CR1, the integrin CD11b and the macrophage colony-stimulating factor-1 receptor (CSF1R) [2]. Microglia also express CD45, a pan-leukocyte marker, albeit in relatively lower levels compared to other CNS macrophage populations such as perivascular or meningeal macrophages, allowing the different cell populations to be distinguished phenotypically [3,4].

Microglia can adopt diverse morphologies depending on their functional states, the most commonly observed in the healthy brain being characterised by a small rod-shaped soma with elongated, ramified processes covered in fine protrusions that continuously survey the local microenvironment to detect perturbations in homeostasis [5]. Microglial function is critical to maintaining the CNS compartment in health and disease, with a range of functional states observed in the parenchyma that reflect the diverse roles of these dynamic cells. In the steady state, microglia phagocytose dying cells and debris to avoid generation of deleterious pro-inflammatory responses, prune excess neuronal connections in development, monitor synapses and control adult neurogenesis[6]. In the diseased or injured CNS they are the master regulators of the neuroimmune response and control the inflammatory status of the brain [6].

Whilst significant advancements have been made in the understanding of microglial functions, there are many aspects of their basic physiology that remain incompletely understood. Included amongst these are the mechanisms regulating the homeostatic composition and turnover of the microglial population from development to ageing. In this review we will recapitulate existing knowledge of the mechanisms regulating the dynamics of this cell

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population under physiological conditions, aiming to understand their roles during homeostasis and disease.

**Developmental origins of the microglial population**

Historically, the developmental origins of microglia have been the subject of intense debate, with hypotheses of mesodermal or monocytic origin dominating the field[7] until fate-mapping studies confirmed that these cells are derived from erythro-myeloid progenitors (EMPs) originating in the yolk sac (YS)[8]. The yolk sac origin of the microglial population was initially suggested by Alliot, et al. [9], who demonstrated the presence of F4/80$^+$/CD11b$^+$ 'microglial progenitors' in the brain at embryonic day (E) 8.0 after their appearance in the yolk sac. The authors observed an average of three microglial progenitors in the brain primordium at E8.5, which were postulated to undergo massive proliferation to produce 1200 cells by E10.5. Definitive haematopoiesis begins at E10.5 in the aorta-gonad-mesonephros (AGM) region of the embryo, producing haematopoietic stem cells (HSCs) that migrate to the foetal liver and bone marrow before differentiating into a multitude of myeloid cell lineages[2]. The presence of microglia in the embryonic brain at E9.5 precedes this stage of haematopoiesis, suggesting an alternative origin for microglial precursors.

Primitive haematopoiesis occurs from E7.0 to E9.5 in the yolk sac, giving rise to primitive macrophages and erythrocytes, including a subset of EMPs, which differentiate into microglial cells [8,10](Figure 1A). These immature uncommitted CSF1R$^+$ c-kit$^+$ CD45$^{low}$ AA4.1$^+$ progenitors initiate a core macrophage transcriptional program and develop into 'premacrophages' (pMacs), upregulating expression of CSF1R, CX3CR1, and Fc gamma receptors, amongst other markers [1,11](Figure 1A). pMacs colonise the whole embryo from E9.5 in a CX3CR1-dependent manner, giving rise to all resident tissue macrophage populations through induction of tissue-specific transcriptional programs[1]. From E10.5, pMacs in the brain differentiate into microglia, downregulating expression of *Timd4* and CD206 whilst upregulating expression of the microglial-specific transcription factors *Sall1* and *Sall3*[1]. Fate-mapping studies have convincingly shown that these embryonic cells, seeding the prenatal, brain persist into

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adulthood to form the resident microglial population in the adult brain[8,12]. Microglial cells develop in three temporal stages, in synchrony with the brain (early, pre-, and adult microglia), which are governed by distinct regulatory circuits[13]. For example, the transcription factor MAFB coordinates the adult microglia program, with its knockout leading to disruption of homeostasis[13]. Microglia reach its adult transcriptional identity by the second postnatal week, as demonstrated profiling TMEM119$^{+}$ microglia, a remarkably specific marker of these cells[14].

From an initial limited number of infiltrating progenitors, the microglial population expands rapidly by proliferation, colonising all brain regions by birth. During the postnatal period, microglial numbers increase 2.2-fold, peaking at postnatal day 14 (P14)[15]. However, it remained unclear whether proliferation of the resident cell population alone accounts for this increase in cell numbers, or whether blood-derived monocytes infiltrate the parenchyma and differentiate into microglia[9]. To address this, our group utilised lentiviral vectors driving the expression of fluorescent proteins to label and track haematopoietic cells. *In utero* injection of vectors to the foetal liver at E14, when the liver is the main haematopoietic organ, allows specific labelling of haematopoietic cells produced at this developmental stage, which could then be analysed during the perinatal period. Infiltrating monocytes can be observed in the brain parenchyma from P0, reaching a peak at P3 (Figure 1B). From P6 to adulthood, these monocytes dramatically decline in number due to a prominent apoptotic response, with few cells surviving in the adult brain[16] (Figure 1B). These data indicate that monocyte infiltration and differentiation do not contribute to the postnatal expansion of the microglial population, providing further support for the YS-origin of adult microglial cells. However, it remains unknown if and how these monocytes contribute to postnatal development of the CNS. By six weeks of age, the microglial cell numbers have been refined to the density maintained throughout adulthood[15,16].

Microglia play an important role in embryonic and postnatal development of the brain. During brain development, microglia display an amoeboid morphology

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typically associated with phagocytic functions [17,18]. It has been shown that microglia in the prenatal brain actively phagocytose neural precursors cells (NPCs) during the late stages of cortical neurogenesis, regulating the thickness of the neocortex [19]. Selective depletion of microglia using clodronate liposomes or suppression of microglial activation results in a significant increase in the number of NPCs, presumably through a reduction in phagocytosis, which may affect the cytoarchitecture of the brain [19]. Absence of microglia during early embryonic stages also results in abnormal outgrowth of dopaminergic neurons to the striatum and perturbed localisation of a subset of neocortical interneurons [20]. These data demonstrate the importance of maintaining microglial dynamics during development, as the physiological changes associated with their loss of function could be implicated in the pathogenesis of neurodevelopmental disorders [21,22]. The phagocytic role of microglia in the healthy brain continues into adulthood, mostly restricted to the hippocampal neurogenic niche. The generation of an excess of hippocampal early neural precursor cells leads to an increased number of apoptotic cell, which are rapidly and efficiently removed by microglia in a non-inflammatory fashion[23]. Although microglia can accomplish this task even after significant increases in cell death, prolonged rates of apoptosis caused by an epileptogenic injury impair the phagocytic efficiency of microglia, further contributing to tissue damage[24].

Using *Csf1r*$^{-/-}$ mice, Erblich, et al. [25] demonstrated that microglial function in normal brain development is most important during the early postnatal period. At E16, no gross morphological changes are observed in the absence of microglia, however by the third postnatal week there is significant enlargement of the lateral ventricles and reduction in the size of the cortex, olfactory bulbs and hippocampus. An increase in neuronal density in the cortex was also observed, which could be attributed to increased presence of neural progenitor cells in the absence of microglia. However, the authors report no changes in the total number of neurons, suggesting that enlargement of the ventricles occurred as a result of hydrocephaly, which then caused pressure-induced compaction of the cortex [25]. These morphological changes coincide with the timeframe of the postnatal refinement of the microglial population [15]; developmental apoptosis during the first two postnatal weeks results in increased cellular debris that

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would usually be cleared by microglia, the population of which is undergoing significant expansion during this time. In the absence of microglia, due to CSF1R deficiency, the lack of phagocytosis could lead to a build-up of debris that perturbs normal cerebrospinal fluid drainage, resulting in hydrocephaly; however, this has not been investigated by subsequent studies. Overall, compelling evidence supports that microglia are critical for the correct development of the brain, evidenced by the fact that its depletion during neonatal development leads to persistent and several behavioural abnormalities[26,27].

Microglia are also able to actively engulf synaptic material and modulate synapse numbers in a CX3CR1-$CX_3CL_1$-dependent manner during postnatal development. *CX3CR1*$^{-/-}$ mice have a significant, but transient, decrease in microglial density in the postnatal brain, which correlates with increased spine density and altered electrophysiological activity [28], suggestive of reduced synaptic pruning and delayed development of the brain circuitry. Lack of microglial-mediated synaptic pruning has long-term effects on brain connectivity, evidenced by synaptic dysfunction and reduced functional connectivity between brain regions in *CX3CR1*$^{-/-}$ mice, resulting in behavioural deficits [29]. CX3CL1-CX3CR1 signalling by microglia is also important in providing trophic support for neurons during early postnatal development [30], highlighting the importance of this signalling pathway for neuron-microglia crosstalk throughout development. The classical complement cascade, which typically functions to target cellular debris for removal by the immune system, has also been implicated in microglial-mediated synaptic pruning in the retinogeniculate system during postnatal development. C3, a component of the complement system, localises to 'weak' synapses with lower neuronal activity, resulting in complement receptor 3 (CR3)/C3-dependent microglial phagocytosis [31]. Disruption of CR3/C3 signalling results in deficient synaptic remodelling during development, which leads to increased synaptic density within the dorsal lateral geniculate nucleus that persists into adulthood in mice. This signalling pathway has recently been implicated in early synapse loss in mouse models of AD-like pathology, suggesting that

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inappropriate activation of microglia by pathogenic proteins result in aberrant phagocytosis of functional synapses [32].

**Microglial dynamics in the adult brain**

The microglial population in the adult brain is highly heterogeneous, accounting for between 5% and 12% of the total population of glial cells in the healthy murine CNS[33]. Cell density remains stable throughout the lifetime of the mouse[16], with a higher density present in grey matter-enriched regions compared to white matter[33]. In comparison, the human CNS has a higher cell density in the white matter, with microglia making up 0.5% to 16.6% of the total cells in the brain[34]. In addition to heterogeneity in cell density, microglia have regionally distinct transcriptional identities, with a degree of variability comparable to that seen in the transcriptomes of different peripheral macrophage populations[35]. Microglia from the cortical and striatal regions are relatively similar, whereas cerebellar microglia have a distinct transcriptomic profile in the healthy adult brain. The greatest diversity in expression was found in genes associated with the 'immune response' or 'immune effector response', including multiple genes from the MHC-I (*H2-D1*, *H2-K1*) and MHC-II (*H2-Aa, H2-Eb1, Cd74*) antigen processing and presentation pathways, in addition to genes such as *Camp*, encoding the antimicrobial peptide mCRAMP and *Ngp*, neutrophilic granule protein. Enriched expression of these genes was found in the cerebellum compared to other regions, suggestive of greater 'immune-vigilance'[35]. Interestingly, expression of immunoregulatory molecules such as *Cd47* and *Cd300a*, which encode molecules limiting the strength of myeloid cell responses to external stimuli, was reduced in the cerebellum compared to the cortex. This indicates that there could be differential activity of certain immunoregulatory pathways across different brain regions. The gene networks underpinning this regional heterogeneity show differential sensitivity to healthy ageing of the brain. Cerebellar microglia become increasingly more distinct, displaying a striking age-related increase in expression of immune-related gene transcripts compared to other regions, indicative of increased immune sensitivity. Hippocampal microglia, which display an 'intermediate' profile between cortical/striatal and cerebellar microglia in the young brain, have a more similar

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transcriptome to cortical and striatal microglia in the aged brain[35]. An age-related shift in the transcriptional identity of human glial cells has recently been demonstrated using post-mortem tissue, with an upregulation of microglial-specific genes found across multiple brain regions in the aged brain [36]. It is thought that heterogeneity in the transcriptional identity of microglia may underlie the region-specific demands of brain tissue under homeostatic conditions. Heterogeneity in the gene expression of immunoregulatory markers corroborates previous evidence demonstrating region-specific differences in their cell-surface expression [37]. This immunological diversity of microglia across the CNS is interesting in the context of their potential functions in neurodegenerative conditions, which are characterised by a region-specific pattern of pathology. Despite differences in the transcriptomes of microglia across brain regions, Hickman, et al. [38] demonstrated that all cells express a 'core profile' of genes, including *CX3CR1, P2ry12*, encoding a purinergic receptor that regulates microglial activation [39], *Trem2*, which functions in microglial phagocytosis [40], *Hexb*, encoding a subunit of the lysosomal enzyme β-hexosaminidase [41] and *Tmem119*, a transmembrane protein of unknown function in microglia [42]. Tmem119 expression has recently been validated to be a useful experimental tool to distinguish resident microglia from other tissue resident macrophages, meningeal and perivascular macrophages in the CNS [14]. This core profile transcriptionally defines microglia and distinguishes them from other resident macrophage populations and CNS cells [14,38,43].

The mechanisms by which microglial cell density is maintained in the healthy brain has been the subject of much controversy, with two main conflicting hypotheses; firstly, that the self-renewal of the resident cells is responsible for the maintenance of the population, or secondly that peripheral bone marrow derived cells (BMCs) infiltrate the parenchyma and differentiate into microglia.

The relative contributions of self-renewal and infiltration to the maintenance and/or expansion of the microglial population in health and under disease conditions have been investigated using bone marrow chimera studies [44,45]. However, increased blood-brain barrier (BBB) permeability and numbers of

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infiltrating BMCs are caused by the irradiation protocols used in these studies, thus confounding the interpretation of experimental results. The use of surgical parabionts, which share a joined circulatory system, allows for the contribution of circulating cells from one animal to the other to be analysed under near-physiological conditions without lethal irradiation or transplantation. In healthy adult parabiotic mice, there was no recruitment of circulating progenitors to the microglial pool[46]. These results were in stark contrast to mice subjected to myeloablation and bone marrow transplantation, suggesting that recruitment of circulating monocytes to the CNS only occurs following experimental preconditioning.

Targeted irradiation protocols, which specifically include or exclude the brain from irradiation before bone marrow transplantation, further support the enabling role of preconditioning prior to BMC infiltration. Protection of the brain from the irradiation protocol resulted in the absence of BMCs in the brain (identified as Ly-6C$^{hi}$ CCR2$^{+}$)[47]. More recent studies using sub-lethal irradiation and transplantation protocols have confirmed these findings[8]. Using a novel experimental approach combining irradiation and parabiosis without bone marrow transplantation, Ajami, et al. [48] demonstrated that monocytes infiltrate the parenchyma under specific disease conditions, in this case the experimental autoimmune encephalitis (EAE) model of multiple sclerosis (MS), however their contribution is transient and they do not form part of the resident microglial pool after remission. The authors found that only uncommitted HSCs are able to generate long-lived microglia under experimental conditions following lethal irradiation and bone marrow transplantation. These findings conclusively indicate that the contribution of peripheral bone marrow-derived cells to the long-term microglial population in the brain is minimal or absent. However, recent evidence has indicated that monocytes can colonize the CNS following depletion of microglia. The CD11b-HSVTK$^{mt30}$ system allows ~90% ablation of the microglial population through inhibition of DNA synthesis and induction of apoptosis in proliferating myeloid cells [49,50]. Following microglial depletion, expression of CCL2 in the brain increased, followed by waves of infiltration of circulating monocytes expressing high levels of CD45 and CCR2 [51]. The authors

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state that it is possible that the BBB was compromised in this model, leading to engraftment of peripheral cells. However as monocyte infiltration occurred only in regions where the microglial population had been depleted, it is possible that in the absence of a resident myeloid population there is a 'drive' to re-populate this element of the CNS.

Under normal physiological conditions, the evidence suggests that microglia, like other resident tissue macrophage populations [52], are maintained by self-renewal of resident cells in the steady state [53]. Microglial proliferation is a complex process regulated by many endogenous and exogenous factors. The CSF1R pathway is a key regulator of microglia proliferation in both the healthy and diseased CNS. CSF1R, encoded by the *c-fms* proto-oncogene, is expressed on both surveillant and activated microglia and is activated through binding of its ligands, colony-stimulating factor-1 (CSF1) and interleukin-34 (IL-34). *Csf1r*$^{-/-}$ mice have impaired development of YS-derived macrophage and microglial populations, with ~99% reduction in microglial numbers[8], whereas deficiency in CSF1 or IL-34 leads to a ~30%[8] or ~50%[54] reduction in microglia, respectively. This suggests that while CSF1R is crucial for cell survival, the two ligands may have complementary functions. Upregulation of cell cycle-associated proteins, including proliferating cell nuclear antigen (PCNA), cyclin A and cyclin D, in the presence of CSF1 provides evidence for the mitogenic capability of the CSF1R pathway [55], whilst its pro-survival signalling is mediated through activation of AKT kinase which directly phosphorylates and inhibits pro-apoptotic proteins as well as inhibiting activity of transcription factors that induce expression of pro-apoptotic genes [56]. Pharmacological blockade of CSF1R inhibits microglial proliferation in the healthy brain[16] and can induce apoptosis, resulting in depletion of ~99% of the population[57].

The requirement for CSF1R signalling in the survival of the microglial population in the healthy adult brain has recently been demonstrated following pharmacological blockade of the receptor. PLX3397, a specific CSF1R inhibitor, reduced microglial numbers in the healthy brain by more than 90% after 7 days of treatment and almost completely depleted the population following longer

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treatment regimens [57]. The authors demonstrated that this reduction in cell numbers was not simply due to reduced proliferation but due to induction of apoptosis, evidenced by expression of active caspase-3 and positive propidium iodide staining. In this model, the microglial population began to reconstitute 3 days after stopping treatment, however repopulating cells display different morphology and expression of cell markers from control microglia, including transient expression of CD34 and c-Kit, markers of HSCs, and nestin, a marker of stem cell populations. By 14 days of recovery, microglial numbers, phenotype and morphology were indistinguishable from that in control brains [57]. Similar results were obtained after transgenic ablation of microglia: the microglial population is rapidly reconstituted by proliferation of resident cells. The use of a *CX3CR1*$^{CreEr}$-based system crossed with an inducible diphtheria toxin receptor (iDTR) system allowed specific ablation of microglial cells following tamoxifen and diphtheria toxin injections [58]. An 80% reduction in microglia was observed 3 days after induction, but after 14 days cell numbers returned to baseline levels. In agreement with the pharmacological depletion model, after the transgenic ablation of microglia pools of resident cells, which rapidly proliferate to colonize the parenchyma, rapidly reconstituted the population. These CNS-derived microglia also expressed nestin, however this expression is transient and is no longer seen once cell numbers have returned to normal at day 14. In the steady-state, nestin is expressed by neural stem cells [59], pericytes [60] and NG2$^+$ oligodendrocyte precursor cells [61] within the CNS, with expression rarely found in other cell types [62]. Genetic ablation of microglia was associated with a 'cytokine storm' in the brain, evidenced by increased expression of proinflammatory cytokines, chemokines and co-stimulatory molecules[58].

The aforementioned studies are repopulation paradigms, thus it is possible that expression of nestin on microglia is induced only following disruption of population homeostasis, as microglial expression of nestin under physiological conditions has not yet been reported [35,38,43], and in the steady state proliferating microglia do not express nestin[16]. Therefore, these data suggest that nestin expression in microglia reflects a transient repopulating phenotype, rather than a subpopulation of 'microglial progenitor cells' [63]. Furthermore, the cytokine

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storm observed by Bruttger *et al.* highlights how far from a physiological system these paradigms are, as the inflammatory milieu of the CNS is markedly affected following massive microglial cell death. However, they do support previous data indicating that proliferation of CNS resident cells contributes to the resident microglial population [53].

Maintenance of the microglial population by slow turnover of long-lived cells has been assumed for many years, since Lawson, et al. [53] reported a low turnover rate of 0.05% of microglia using $^{3}$H-thymidine labelling combined with F4/80 immunohistochemistry. However, as acknowledged by the authors at the time, limitations associated with this method are likely to have led to an underestimation of the proliferation rate. These studies have since been revisited using more sensitive techniques, such as incorporation of BrdU, which is considered to be the 'gold standard' in the field. We have shown that the turnover rate of mouse microglia is much higher than expected, with an average of 0.69% of the population proliferating at any one time, allowing for an estimated complete renewal of the population once every 96 days [16] (Figure 1C). In order to maintain a stable cell density throughout lifetime, there is a tight coupling between proliferation and apoptosis (Figure 1C), with cells more likely to proliferate in the vicinity of a dying cell immediately after it has undergone apoptosis, as evidenced by chronic two-photon microscopy[16]. This leads to a constant reorganisation of the microglial landscape, challenging the idea of individual cells being long-lived, which has implications for the concept of 'microglial memory'. The idea that cells can remember the particular signals or activity within its local microenvironment and use this to shape the activity of surrounding cells, for example in the monitoring and modulation of specific synapses[64] or the phenomenon of microglial priming[65], needs to be revisited. With cells turning over more rapidly than previously thought, it is likely that memory is stored within individual cells thanks to epigenetic modification or perhaps elsewhere in the neural/glial network.

**Microglial dynamics in the diseased brain**

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The role of the innate immune system in the pathogenesis of neurodegenerative diseases is complex and not fully understood. Evidence suggests that neuroinflammation is a key component driving disease progression in a number of neurodegenerative conditions, however its precise role, and the exact functions of microglial cells in disease aetiology, are still unclear [6]. In addition to the debate of self-renewal or infiltration maintaining the microglial population in the healthy brain, there is controversy over the roles of resident microglia and infiltrating monocytes in the diseased brain.

In many neurodegenerative conditions, including AD, prion disease and amyotrophic lateral sclerosis (ALS), significantly increased numbers of microglia are present within the parenchyma [66-68]. Bone marrow chimera studies have shown that BMCs can be actively recruited in some disease models, however the limitations associated with irradiation protocols confound interpretation of these data. Ajami, et al. [46] demonstrated that in both facial nerve axotomy (acute) and ALS (chronic) models of neurodegeneration, bone marrow-derived cells do not contribute to microgliosis in the absence of irradiation. This was corroborated by evidence demonstrating that the increased production of chemo-attractants, including CCL2, after injury or neurodegeneration in the CNS is not sufficient to promote monocyte infiltration across an intact BBB [47]. Whether or not the BBB is compromised in neurodegenerative conditions remains controversial [69,70], thus evidence indicating a parenchymal role for peripheral monocytes under pathological conditions must be interpreted with care. In *Ccr2*$^{-/-}$ mice, which have a significantly reduced population of circulating monocytes, the expansion of the microglial population in response to prion disease occurs to a similar level as that in wild-type mice [71]. Further studies in APP/PS1 mice, a model of AD-like pathology, indicate that bone marrow-derived cells do not enter the parenchyma in the absence of pre-conditioning by irradiation [72], providing evidence that circulating monocytes do not contribute to microgliosis under pathological conditions.

Evidence indicating that proliferation of resident cells is solely responsible for the increased cell density observed in neurodegenerative conditions comes from

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studies utilizing CSF1R inhibitors as a therapeutic intervention. In murine models of prion[66], AD[67] and ALS[73], the use of CSF1R inhibitors significantly reduce microglial proliferation, thus decreasing cell numbers in the parenchyma. In the ME7 murine model of prion disease, treatment with the selective CSF1R inhibitor GW2580 reduces microglial proliferation in addition to slowing the progression of neurodegeneration and alleviating behavioural deficits [66]. The use of this inhibitor in the $SOD1^{G93A}$ model of ALS also attenuated disease progression, reducing the degeneration of motor neurons and extending survival [73]. Treatment with the CSF1R inhibitors GW2580, PLX3397[74] or PLX5622[75] in the APP/PS1 [67], 5xfAD [76] and 3xTg-AD[75] models of AD, respectively, improved learning and memory deficits despite having no impact on amyloid-$\beta$ burden in the brain.

**Does a dysregulation of microglial dynamics impact the neuroinflammatory response?**

Although microglial cells have critical roles in the maintenance of brain homeostasis, the impact of altering the population in the absence of pathology is not well understood. Given the diverse array of microglial functions in the healthy and diseased CNS, changes to population dynamics may be implicated in the development of neurological and neurodegenerative conditions.

Few studies have reported the impact of increased microglial numbers in the non-diseased CNS. Overexpression of CSF1 promotes microglial proliferation and thus results in a 2-fold increase in cell numbers in the parenchyma [77]. Whilst there were no reported morphological aberrations, CSF1 overexpression was associated with a small decrease in brain weight, although the impact of increased microglial numbers on other CNS cell populations was not investigated. Cytokine expression in the steady-state was not affected, however microglia from CSF1-overexpressing mice showed an impaired response to systemic inflammation (LPS). Expression of both pro-inflammatory and anti-inflammatory cytokines was significantly reduced compared to wild-type mice treated with LPS [77]. Similarly, germ-free (GF) mice, which lack a complex gut microbiota, also have significantly increased numbers of microglia throughout

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the parenchyma [78]. Microglia from GF mice also have an impaired response to parenchymal or systemic inflammatory challenges[78]. Compared to wild-type mice, intracerebral infusion of LPS or lymphocytic choriomeningitis virus (LCMV) induced no changes in microglial morphology or cell number typically associated with activation. Expression of genes involved in the innate immune response was reduced in GF mice in response to both pathogenic challenges. Systemic LPS treatment was also associated in reduced expression of pro-inflammatory cytokines in GF mice, when compared to wild-type LPS-treated mice [78].

Whilst these models of increased microglial numbers suggest that there is an impairment of the brain's immune response, they are likely to also be influenced by the systemic immune response, as the described modifications also affect the systemic compartment, with the immune response likely to be missregulated at baseline and upon stimulation. De, et al. [77] suggest that it is the presence of increased CSF1, not increased cell numbers, that impacts the microglial response to LPS, as this has previously been shown *in vitro* [79,80]. Erny, et al. [78] suggests that the lack of complex gut microbiota affects the ability of microglia to initiate an immune response as opposed to impairment by increased cell numbers. Our recent studies have aimed at shedding light into the impact of increased microglial density in the brain, using Vav-Bcl2 mice[16]. These mice have a 40% increase in microglial density in the adult brain, with a significant increase in the $CD45^{hi}$ microglial population compared to WT, thanks to prevented apoptosis through overexpression of Bcl-2 in cells from the myeloid lineage[16]. The transcriptional signature of microglia derived from Vav-Bcl2 is characterised by a dysregulated metabolic and inflammatory response, when compared to wild-type microglia, although the neuronal and astroglial populations are unaffected[16]. Although these studies provide a useful framework for understanding the impact of increased microglial numbers in brain physiology, the effects of selectively increasing the number of microglia in the brain remains to be fully established.

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The impact of reduced numbers of microglia has been investigated in more detail, due to the availability of multiple pharmacological and transgenic tools to reduce or deplete the population, as recently reviewed by Waisman et al [63]. Over 80% depletion of microglial numbers has been attained using different models: Asai, et al. [81] and Elmore, et al. [57] utilised PLX3397, achieving approximately 86% and 99% reduction, respectively. Parkhurst, et al. [82] used *CX3CR1*$^{CreER}$-iDTR model of genetic ablation to specifically deplete microglia, achieving approximately 99% depletion, however the cells began to repopulate to approximately 85% of wild-type levels 7 days post-ablation. A similar result in this model was observed by Bruttger, et al. [58], however the authors reported that microglial numbers returned to normal levels after 14 days. Pharmacological depletion of microglial numbers in the absence of pathology leads to a reduction in basal expression of pro- and anti-inflammatory cytokines in the CNS [57,81], whereas genetic depletion results in generation of a substantial pro-inflammatory response[58]. However, these experimental paradigms result in death of almost all of the microglial population, likely altering the inflammatory tone and confounding the ability to draw interpretations of how the inflammatory status of the CNS would be affected by reduction of microglial numbers alone. The effect of microglial depletion on cognitive functions is debated, with studies reporting deficits in learning and memory after seven days of depletion [82], but no deficits in cortical and hippocampal learning and memory, motor function or anxiety-related behaviour seen after three weeks of depletion [57,83]. Interestingly, CSF1R haploinsufficiency leads to elevated microglial numbers and cognitive, sensorimotor deficits and depression and anxiety-like behaviour[84], suggestive of a link of microglial density with behaviour. Loss of the microglial population appears to alter baseline, as well as learning-dependent, neuronal spine density and remodelling, along with expression of synaptic proteins [82]. Finally, the innate immune response is impaired in the absence of microglia, as evidenced by a significant reduction in production of pro-inflammatory cytokines following LPS treatment [57,83].

In contrast to other depletion models, a significant upregulation of pro-inflammatory genes is found in *CX3CR1*$^{CreER}$-iDTR mice following microglial

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depletion. As all of these models induce substantial cell death in the CNS, it is possible that other CNS-resident cells, such as astrocytes, respond to this disruption of homeostasis. Elevated numbers of astrocytes were observed in the *CX3CR1*$^{CreER}$-iDTR model [58], however this population remained unaffected after PLX3397 treatment [57]; therefore it remains unclear how microglial depletion impacts other cell populations within the parenchyma. Whilst these studies are interesting in terms of investigating the impacts of having no microglia in the brain, it remains unknown how long-term gradual depletion of microglia would impact development, physiology, homeostatic functions and neuroinflammation in the CNS.

**Conclusions**

Our understanding of the mechanisms controlling microglial population dynamics under physiological conditions remains incomplete, however significant advancements to improve our knowledge have been made in recent years. It is now widely accepted that the adult microglial population is produced from yolk sac-derived EMPs that seed the developing brain and persist into adulthood. The contribution of BMCs in the healthy developing and adult brain is virtually non-existent, with resident microglia self-renewing throughout life to maintain a stable population density in the CNS, due to a regional and temporal coupling of proliferation and apoptosis. It remains unclear how long-term deregulation of microglial dynamics would impact brain development and function, however understanding this cross-talk may provide insight to the pathogenesis of neurodevelopmental disorders and age-related neuropathologies.

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**Figure 1. The dynamics of the microglial population throughout lifetime.**

(A) Microglia derive from CSF1R$^{+}$ c-kit$^{+}$ CD45$^{low}$ AA4.1$^{+}$ erythromyeloid progenitor cells (EMPs) that originate in the yolk sac at embryonic day (E) 8.5. From E9.5, EMPs develop into premacrophages (pMacs) and colonise the embryo in a CX3CR1-dependent manner. pMacs then take up residence in the developing brain and differentiate into microglia from E10.5. These embryonic microglial cells persist into adulthood to form the resident microglial population of the brain. (B) During the first two postnatal weeks, the microglial population undergoes significant expansion due to proliferation of resident cells. Microglial cell numbers peak at postnatal day (P) 14, but by six weeks of age cell numbers have been refined to the density maintained throughout adulthood. From P0, blood-derived monocytes can be found in the brain parenchyma with cell numbers peaking at P3. These monocytes subsequently undergo apoptosis with very few cells surviving in the brain by P6. (C) In the adult brain, the microglial population is maintained by self-renewal of resident cells with no contribution from peripheral bone marrow-derived cells. An average of 0.69% of the microglial population is proliferating at any one time, resulting in the estimation of a complete renewal of the population once every 96 days. There is a tight temporal and spatial coupling between proliferation and apoptosis in order to maintain a stable cell density throughout lifetime, leading to a constant reorganisation of the microglial landscape.

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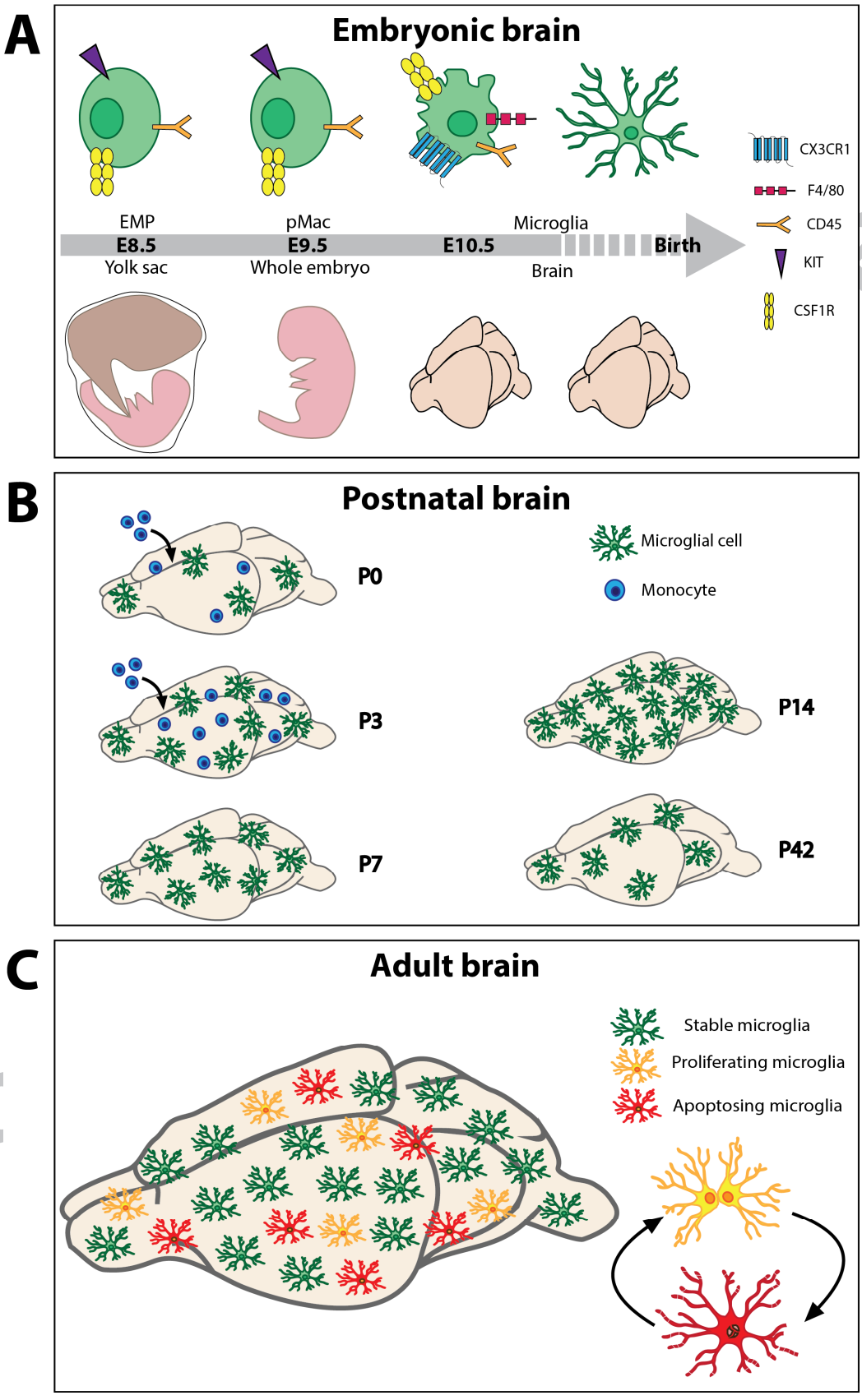

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**Highlights**

-The adult microglial population is formed exclusively from yolk sac derived progenitors

-The microglial population turns over several times during a lifetime, due to self-renewal of resident cells

-Microglial turnover is maintained by temporal and regional coupling of proliferation and apoptosis

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