Cold Spring Harbor Perspectives in Biology
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# Origin of Microglia: Current Concepts and Past Controversies

**Florent Ginhoux[1] and Marco Prinz[2,3]**

[1]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore 138648

[2]Institute of Neuropathology, University of Freiburg, 79106 Freiburg, Germany

[3]BIOSS Centre for Biological Signalling Studies, University of Freiburg, 79106 Freiburg, Germany

*Correspondence:* florent_ginhoux@immunol.a-star.edu.sg; marco.prinz@uniklinik-freiburg.de

Microglia are the resident macrophages of the central nervous system (CNS), which sit in close proximity to neural structures and are intimately involved in brain homeostasis. The microglial population also plays fundamental roles during neuronal expansion and differentiation, as well as in the perinatal establishment of synaptic circuits. Any change in the normal brain environment results in microglial activation, which can be detrimental if not appropriately regulated. Aberrant microglial function has been linked to the development of several neurological and psychiatric diseases. However, microglia also possess potent immunoregulatory and regenerative capacities, making them attractive targets for therapeutic manipulation. Such rationale manipulations will, however, require in-depth knowledge of their origins and the molecular mechanisms underlying their homeostasis. Here, we discuss the latest advances in our understanding of the origin, differentiation, and homeostasis of microglial cells and their myelomonocytic relatives in the CNS.

Microglia are the resident macrophages of the central nervous system (CNS), which are uniformly distributed throughout the brain and spinal cord with increased densities in neuronal nuclei, including the *Substantia nigra* in the midbrain (Lawson et al. 1990; Perry 1998). They belong to the nonneuronal glial cell compartment and their function is crucial to maintenance of the CNS in both health and disease (Ransohoff and Perry 2009; Perry et al. 2010; Ransohoff and Cardona 2010; Prinz and Priller 2014).

Two key functional features define microglia: immune defense and maintenance of CNS homeostasis. As part of the innate immune system, microglia constantly sample their environment, scanning and surveying for signals of external danger (Davalos et al. 2005; Nimmerjahn et al. 2005; Lehnardt 2010), such as those from invading pathogens, or internal danger signals generated locally by damaged or dying cells (Bessis et al. 2007; Hanisch and Kettenmann 2007). Detection of such signals initiates a program of microglial responses that aim to resolve the injury, protect

Editors: Ben A. Barres, Marc R. Freeman, and Beth Stevens
Additional Perspectives on Glia available at www.cshperspectives.org

 doi: 10.1101/cshperspect.a020537
Cite this article as *Cold Spring Harb Perspect Biol* 2015;7:a020537

the CNS from the effects of the inflammation, and support tissue repair and remodeling (Minghetti and Levi 1998; Goldmann and Prinz 2013).

Microglia are also emerging as crucial contributors to brain homeostasis through control of neuronal proliferation and differentiation, as well as influencing formation of synaptic connections (Lawson et al. 1990; Perry 1998; Hughes 2012; Blank and Prinz 2013). Recent imaging studies revealed dynamic interactions between microglia and synaptic connections in the healthy brain, which contributed to the modification and elimination of synaptic structures (Perry et al. 2010; Tremblay et al. 2010; Bialas and Stevens 2013). In the prenatal brain, microglia regulate the wiring of forebrain circuits, controlling the growth of dopaminergic axons in the forebrain and the laminar positioning of subsets of neocortical interneurons (Squarzoni et al. 2014). In the postnatal brain, microglia-mediated synaptic pruning is similarly required for the remodeling of neural circuits (Paolicelli et al. 2011; Schafer et al. 2012). In summary, microglia occupy a central position in defense and maintenance of the CNS and, as a consequence, are a key target for the treatment of neurological and psychiatric disorders.

Although microglia have been studied for decades, a long history of experimental misinterpretation meant that their true origins remained debated until recently. Although we knew that microglial progenitors invaded the brain rudiment at very early stages of embryonic development (Alliot et al. 1999; Ransohoff and Perry 2009), it has now been established that microglia arise from yolk sac (YS)-primitive macrophages, which persist in the CNS into adulthood (Davalos et al. 2005; Nimmerjahn et al. 2005; Ginhoux et al. 2010, 2013; Kierdorf and Prinz 2013; Kierdorf et al. 2013a). Moreover, early embryonic brain colonization by microglia is conserved across vertebrate species, implying that it is essential for early brain development (Herbomel et al. 2001; Bessis et al. 2007; Hanisch and Kettenmann 2007; Verney et al. 2010; Schlegelmilch et al. 2011; Swinnen et al. 2013). In this review, we will present the latest findings in the field of microglial ontogeny, which provide new insights into their roles in health and disease.

## HISTORICAL PERSPECTIVES ON MACROPHAGES AND MICROGLIA

In 1969, the original phagocyte classification systems based on the work of Metchnikoff and Ehrlich, and later Aschoff (Aschoff 1924; Minghetti and Levi 1998; Gordon and Taylor 2005; Kaufmann 2008), were superseded by the concept of the mononuclear phagocyte system (MPS) (van Furth and Cohn 1968; van Furth et al. 1972), a term that is still used today (Geissmann et al. 2010). The MPS today includes circulating monocytes in the bloodstream ("inflammatory" or Ly-6C$^{+}$ CCR2$^{+}$CX$_3$CR1$^{lo}$ monocytes and "patrolling" or Ly-6C$^{-}$CCR2$^{-}$ CX$_3$CR1$^{hi}$ monocytes in mouse), as well as dendritic cells and macrophages from both lymphoid and nonlymphoid organs. In this context, macrophages are the resident phagocytic cells in lymphoid tissues (spleen, lymph nodes) and nonlymphoid tissues, such as the brain (microglia), liver (Kupffer cells), lung (alveolar macrophages), bone (osteoclasts), kidney (kidney macrophages), and skin (Langerhans cells). Of note, epidermal Langerhans cells are a unique cell population in the sense that, although arising from macrophage progenitors, they acquire unique dendritic cell features on final differentiation compared with other macrophage populations (Ginhoux and Merad 2010; Hoeffel et al. 2012). At these sites, macrophages contribute to steady-state tissue homeostasis via the clearance of apoptotic cells and the production of growth factors, but, on infection, they become activated to phagocytose pathogens and produce inflammatory cytokines, as they are equipped with a broad range of pathogen-recognition receptors (Gordon 2002). Although the MPS has been a useful framework for considering phagocyte biology, it also led to the assumption that all tissue macrophages are identical in origin and function. However, this assumption has been challenged in recent years, especially in the case of microglia (Ginhoux et al. 2010; Hoeffel et al. 2012; Schulz et al. 2012).

It has taken more than 150 years of research for microglia to be formally recognized as a separate and specialized macrophage population in the CNS with distinct developmental origins.

del Río-Hortega was the first to clearly identify a small population of phagocytic, migratory cells within the CNS, which he proposed were of mesodermal origin (del Río-Hortega 1932). Some years later, he introduced the term "microglial cell," and refined his description of these cells as the nonneuronal, nonastrocytic element of the CNS, distinct from neurectodermal oligodendroglia and oligodendrocytes (del Río-Hortega 1939), which constitute the macroglia. Despite some controversies on microglia origin from either the mesoderm or the ectoderm in the history of microglial study (Rezaie and Male 2002; Chan et al. 2007; Ginhoux et al. 2013), several investigators followed del Río-Hortega's hypothesis and presented evidence supporting a mesodermal origin of microglia in light of their morphological and phenotypic similarities with macrophages, first by coupling light/electron microscopy and immunohistochemistry, which allowed identification of parallel morphological features of macrophages and microglia at various stages of development (Murabe and Sano 1982, 1983), and, second, by showing that microglial cells were recognized by antisera raised against monocyte/macrophage antigens (Hume et al. 1983; Perry et al. 1985).

## MARKERS OF MICROGLIA

Because of their mesodermal origin, microglia share many features with other myeloid cell types in the body. Observations of the phenotypic similarities between circulating monocytes, tissue macrophages, and microglia were first reported ~30 years ago in immunohistochemical studies, which showed microglial expression of macrophage markers, including F4/80, Fc receptor, and CD11b, in mouse (Perry et al. 1985) and, later, in human (Akiyama and McGeer 1990).

As the tissue-resident macrophage of the CNS, murine microglia have since been confirmed to express multiple macrophage markers, including the colony-stimulating factor (CSF)-1 receptor (CSF-1R, CD115), the integrin CD11b, the surface glycoproteins F4/80, the inhibitory immune receptor CD200R, the surface enzyme tyrosine-protein phosphatase non-receptor-type substrate or CD172α, the fractalkine receptor $CX_3CR1$, and the calcium-binding protein lba-1 (Prinz and Mildner 2011). However, few markers, which are specific to microglia, have been identified. CD39 (ectonucleoside triphosphate diphosphohydrolase) gene expression has recently been proposed to enable distinction of microglia from their peripheral relatives (Butovsky et al. 2012). Recent studies also reported that microglia have a unique transcriptomic signature, which distinguishes them from other CNS cells and peripheral macrophages or monocytes (Gautier et al. 2012; Chiu et al. 2013), and express a unique cluster of transcripts encoding proteins for sensing endogenous ligands and microbes, defined as the sensome (Hickman et al. 2013). Microglia also express lower levels of the panhematopoietic marker CD45 compared with tissue macrophages, which also permits their discrimination from monocytes in the bloodstream, whereas the hemoglobin scavenger receptor CD163 enables distinction from perivascular macrophages in the steady state (Dijkstra et al. 1985; Serrats et al. 2010).

## THE YS ORIGIN OF MICROGLIA

The evident phenotypic similarities between microglia and other macrophage populations led to ready acceptance of the notion of their myeloid origin, although the true identity of microglial progenitors remained controversial until recently. Initial studies described the presence of microglial cells during early development, suggesting that microglia arise from embryonic progenitors. These progenitors were first proposed by del Río-Hortega to be meningeal macrophages infiltrating the brain during early embryonic development. However, the majority of the scientific community at that time, including del Río-Hortega himself, believed that microglia could also be derived from blood monocytes. Monocytes are indeed recruited to the neonatal and adult brain, in the latter case, most often under inflammatory conditions, where they can differentiate into microglia-like cells. These observations long supported the prevailing viewpoint that blood-

circulating monocytes represented microglial progenitors, replacing those seeding the brain during embryonic development. In fact, until recently, the most consensual hypothesis was that embryonic and perinatal hematopoietic waves of microglial recruitment and differentiation occurred in the CNS.

When del Río-Hortega first described microglia, he also noted their presence in early development and proposed that, near this time, they might initially arise from mesodermal cells of the pia mater, the innermost layer of the meninges (the membranes surrounding the CNS). He reported the "migration of embryonic corpuscles from the pia into the nerve centres," but simultaneously proposed that "microglia may eventually arise from other related elements, chiefly the blood mononuclears," based on the similarities in morphology and phagocytic activity between microglia and monocytes (del Río-Hortega 1939). These two statements were the founding of the "origin of microglia" controversy that was to last for the next 50 years. Later, the observation that the brain rudiment already contains microglia at E9.5 of the 20 d of murine embryonic development (Alliot et al. 1991, 1999) forced developmental neuroscientists to delve more deeply into the complex subject of embryonic hematopoiesis. Our current understanding of the multifaceted process of embryonic hematopoiesis was extensively reviewed (Cumano and Godin 2007; Orkin and Zon 2008). The appearance of microglia in the neuroepithelium at E9.5 days suggested that their precursors might originate from the YS (Fig. 1). The murine embryonic YS produces early primitive macrophages and erythrocytes as part of the process of "primitive hematopoiesis," occurring from E8.5, as opposed to the generation of definitive hematopoietic stem cells (HSCs), which occurs in the aorta–gonad–mesonephros (AGM) region of the embryo around E10.5. These AGM-derived HSCs then migrate to the fetal liver (FL) and bone marrow (BM) and differentiate therein into all lineages, including monocytes, macrophages, and lymphocytes, which are generated as part of "definitive hematopoiesis" (Bertrand et al. 2005; Cumano and Godin 2007). In addition, from E8.25, multilineage erythromyeloid progenitors (EMP) and lymphomyeloid progenitors also emerge in the YS as a "second wave," which is already considered as part of the definitive hematopoietic wave but called the transient definitive stage. The contribution of such progenitors to macrophage populations via a fetal monocyte intermediate remain to be investigated. HSC-derived myeloid cells, such as monocytes, are produced abundantly in the FL only from E12.5/E13.5 (Fig. 1), days after the initial colonization of the brain rudiment by YS macrophages at E9.5. Of interest, a population of maternally derived macrophages can be found in the YS of the embryo as early as E7.5. This population, however, subsequently decreases in number, becomes almost undetectable at E9.0, and is later absent in the embryo (Bertrand et al. 2005; Kierdorf et al. 2013a).

At E8.5–9.0, the first immature macrophages are found in the YS (Takahashi et al. 1996; Lichanska and Hume 2000), and they develop through a nonmonocytic pathway (Takahashi et al. 1996; Lichanska and Hume 2000). The first macrophage-like cells with an amoeboid shape appear in the rodent neuroepithelium at a similar time point (Ashwell 1990; Ashwell and Waite 1991; Chan et al. 2007) and were suggested to be the precursors of microglial cells (Alliot et al. 1999). A clear requirement for the circulatory system for brain colonization by YS macrophages was determined using E9.5–10 Ncx-$1^{-/-}$ embryos, which have no functional blood circulation (Koushik et al. 2001), and were found to lack microglial progenitors, as well as other fetal macrophages, despite normal YS hematopoiesis (Ginhoux et al. 2010). At E13.5, when the FL is already the primary hematopoietic organ and the main site of HSC expansion and differentiation (Lichanska and Hume 2000), microglial precursors can be detected in significant numbers within the lining of the fourth ventricle (Chan et al. 2007). As a result of the finding that tissues, such as the CNS, contain YS-derived macrophages, but not HSC or maternal macrophages, it became reasonable to believe that microglia originate from YS macrophages rather than from HSC in the FL or BM.

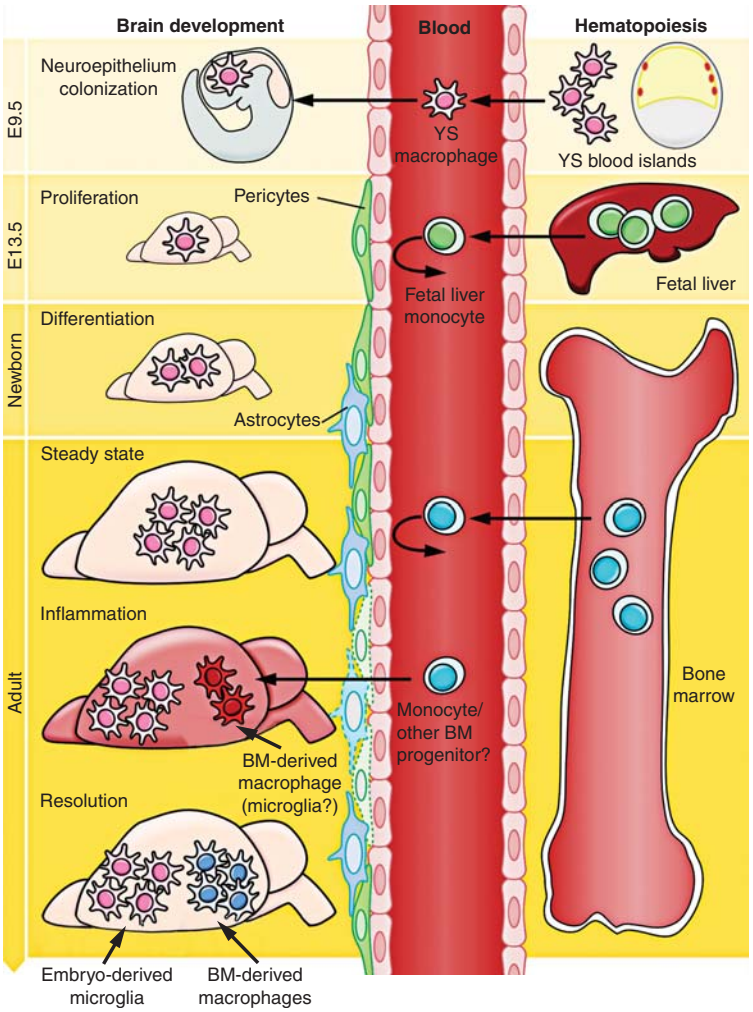


**Figure 1.** Brain development and microglial ontogeny. Primitive macrophages generated in the yolk sac (YS) blood islands around E8.0 spread into the embryos at the onset of blood circulation established around E8.5 and colonize the neuroepithelium from E9.0/E9.5, giving rise to embryonic microglia. In parallel, definitive hematopoiesis arises in the AGM and gives rise to progenitors that colonize the fetal liver (FL) from E10.5. The blood–brain barrier (BBB) starts to form from E13.5 and may isolate the developing brain from the contribution of FL and, later, of bone marrow (BM) hematopoiesis. Embryonic microglial cells expand, colonize the whole CNS, and will maintain themselves until adulthood via local proliferation during late gestation and postnatal development, as well as in the injured adult brain in reaction to inflammation. Nevertheless, under certain inflammatory conditions found, for example, after BM transplantation, the recruitment of BM-derived progenitors can supplement the microglial population to some extent.

Recent studies formally confirmed the YS origin of microglia using a range of different strategies (Ginhoux et al. 2010; Kierdorf et al. 2013a), including a fate-mapping mouse model, expressing a fluorescent protein exclusively in YS progenitors and their progeny, including YS macrophages (Ginhoux et al. 2010). Briefly, this mouse model was designed to express a tamoxifen-activated MER-Cre-MER recombinase gene under the control of one of the endogenous promoters of the runt-related transcription factor 1 (Runx1) locus (Samokhvalov

et al. 2007). When crossed with a Cre-reporter mouse strain, recombination can be induced in embryos by a single injection of 4-hydroxytamoxifen (4′OHT) into pregnant females. Active recombination in these knockin mice occurs in a short time frame, which does not exceed 12-h postinjection, and leads to irreversible expression of fluorescent protein in Runx1$^+$ cells and their progeny (Samokhvalov et al. 2007). Although both YS and FL hematopoietic progenitors express Runx1, YS progenitors are the only Runx1$^+$ cells present at E7.5, and, so, injection of 4′OHT at this time specifically and irreversibly tags YS progenitors and their progeny, but not FL-derived progeny. In contrast, injection of tamoxifen at E8.5 or later will favor the tagging of AGM-derived hematopoietic progenitors and not the YS progenitors (North et al. 1999; Samokhvalov et al. 2007). Thus, if microglia were predominantly derived from YS-tagged progenitors, they should express enhanced yellow fluorescent protein (eYFP) in the adult CNS when 4′OHT is injected at E7.25 and not at E8.5. Strikingly, the relative number of tagged microglia in mice injected at E7.25 was much greater than that of blood monocytes or other circulating leukocytes (Ginhoux et al. 2010). In contrast, the relative number of tagged microglia in mice injected from E8.0 onward decreased dramatically, reaching undetectable levels in mice injected as close as E8.5, whereas the relative number of eYFP$^+$ leukocytes, including monocytes, increased progressively in adult blood. Further confirmation of the YS origin of microglia later came from another study using myeloid-specific CSF-1R-Cre mice (Schulz et al. 2012). Importantly, this study also highlighted further differences between primitive and definitive hematopoiesis, showing that the latter relies on the transcription factor myeloblastosis (MYB), whereas YS-derived macrophages are MYB independent, but PU.1 dependent (Schulz et al. 2012), as described earlier (Sumner et al. 2000). This is in contrast to a previous study, which reported that mice with null mutations in PU.1 had normal numbers of Csf-1r$^+$ phagocytes at E11.5 (Lichanska et al. 1999). This further underlines the fact that YS-derived macrophages constitute an independent lineage, distinct from the progeny of definitive HSCs.

In an another study, we further characterized the early YS progenitor that gives rise to microglia in the brain; we observed c-kit$^+$ lineage$^-$ progenitor cells within the YS that have the ability to differentiate into $CX_3CR1^+$ microglia in vitro, as well as in vivo (Kierdorf et al. 2013a). These cells also generated Ter119$^+$ erythrocytes and, thus, represent a common erythromyeloid progenitor (EMP) in the YS. Subsequently, these uncommitted EMPs disappear, and immature F4/80$^+$CX$_3$CR1$^-$ and F4/80$^+$CX$_3$CR1$^+$ macrophages develop and seed the surface of the developing brain at E9.0 (Kierdorf et al. 2013a).

A similar pattern of events may occur in humans. In human fetuses, microglia-like cells with a range of morphologies can be detected as early as 13 wk of estimated gestational age (Hutchins et al. 1990). However, it appears that maturation of the microglial compartment is ongoing throughout the majority of gestation. Colonization of the spinal cord begins at around 9 wk, the major influx and distribution of microglia commences at about 16 wk, and ramified microglia take up to 22 wk to become widely distributed within the intermediate zone (Rezaie and Male 1999; Rezaie et al. 2005). In fact, it is only close to term, at 35 wk, that well-differentiated microglial populations can be detected within the developing human brain (Esiri et al. 1991; Rezaie and Male 2002; Rezaie et al. 2005; Verney et al. 2010).

Importantly, microglial origin is unique among the wide spectrum of tissue macrophage populations (Ginhoux and Jung 2014; Prinz and Priller 2014). Microglia arise predominantly from YS-derived macrophages (Fig. 1) (Ginhoux et al. 2010; Kierdorf et al. 2013a), whereas Langerhans cells originate mainly from FL-derived monocytes, but retain a detectable YS-derived macrophage (MF) component (Hoeffel et al. 2012). In contrast, alveolar macrophages appear to derive mostly from FL-derived monocytes with minimal lasting contribution from YS-derived macrophages (Guilliams et al. 2013; G Hoeffel, J Chen, Y Lavin et al., in prep.). The origin of other tissue-resident macrophage

populations in the adult remains to be investigated.

## FACTORS DETERMINING THE DEVELOPMENT AND HOMEOSTASIS OF MICROGLIA

The transcriptional program that controls microglial differentiation is only partially understood (Kierdorf and Prinz 2013). A dramatic reduction in numbers of tissue macrophages, including microglia, occurs in mice that lack the CSF-1R (Dai et al. 2002; Ginhoux et al. 2010; Erblich et al. 2011) and in Csf-$1^{op/op}$ mutant mice (Yoshida et al. 1990; Wegiel et al. 1998), which have a natural null mutation in the Csf-1 gene. These studies clearly establish the importance of CSF-1 and its receptor in macrophage homeostasis in vivo (Fig. 2) (Pixley and Stanley 2004), although the precise role of CSF-1 and its receptor during microglial lineage commitment remains controversial. One hypothesis is that CSF-1 drives the microglial differentiation of phagocytic YS macrophages entering the embryo (Metcalf 1985), whereas an alternative theory is that CSF-1 provides a survival signal for the differentiating macrophages and that these surviving cells then respond to an intrinsic developmental program to become mature microglia (Lagasse and Weissman 1997). In favor of the latter hypothesis, macrophages are detected in the YS and the brain rudiment in E10.5 Csf-$1r^{-/-}$ mice, but not in embryos at E12.5 (Ginhoux et al. 2010; Hoeffel et al. 2012).

Interestingly, the microglial population is more profoundly affected by the absence of the CSF-1R than in the absence of its ligand CSF-1 (Ginhoux et al. 2010), which suggested the possibility of a second ligand for CSF-1R that was later identified as interleukin-34 (IL-34) (Lin et al. 2008). In vitro IL-34 binds the CSF-1R at different regions than CSF-1 and with higher affinity (Chihara et al. 2010); IL-34 is also more highly conserved in mammalian and avian species than CSF-1, suggestive of an important role in macrophage homeostasis (Garceau et al. 2010). Recently, two groups generated IL-34 knockout (KO) mice (Greter et al. 2012; Wang et al. 2012) and reported that, in the brain, deficiency of IL-34 led to a significant decrease in microglial cell numbers. Interestingly, IL-34 possesses a spatiotemporal expression pattern that differs from that of CSF-1, permitting complementary activation of the CSF-1R in both embryonic and adult tissues (Wei et al. 2010). Of note, an alternate receptor for IL-34 was recently identified: the receptor-type protein tyrosine phosphatase (PTP)-ζ, which is a cell-surface chondroitin sulfate proteoglycan primarily expressed on neural progenitors and glial cells (Nandi et al. 2013), suggesting that IL-34 may have a wider repertoire of effects within the CNS than previously appreciated. A comparable reduction in microglial cell numbers was also reported in mice deficient for an adaptor protein for CSF-1R, DAP12 (Otero et al. 2009). DAP12 contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain and is highly expressed in natural killer (NK) and myeloid cells. In vitro, DAP12 controls proliferation and survival of macrophages stimulated with CSF-1, whereas in vivo in older mice deficient in DAP12 fewer microglia are present, in particular, regions of the CNS, which suggests a role for DAP12 in the long-term homeostasis of microglia. Humans with mutations in the DAP12 gene develop Nasu–Hakola disease, characterized by bone cysts and fractures and psychotic symptoms leading to severe neurodegeneration and encephalopathy (Paloneva et al. 2000).

The transcription factor PU.1, expressed exclusively in hematopoietic cells, is also involved in microglial development (Fig. 2). The *Pu.1* (*Sfpi-1*) gene is a member of the Ets family of transcription factors (Rosenbauer and Tenen 2007), and its disruption leads to multiple hematopoietic abnormalities, including a lack of mature B cells and macrophages (McKercher et al. 1996). In fact, PU.1-deficient mice are not only devoid of circulating monocytes and tissue macrophages (McKercher et al. 1996), but also parenchymal microglia in the brain (Beers et al. 2006). Similar data have been obtained in zebrafish PU.1 mutants that show a complete loss of brain macrophages (Herbomel et al. 2001). In addition, interferon regulatory factor (IRF)-8 has recently been found to regu-

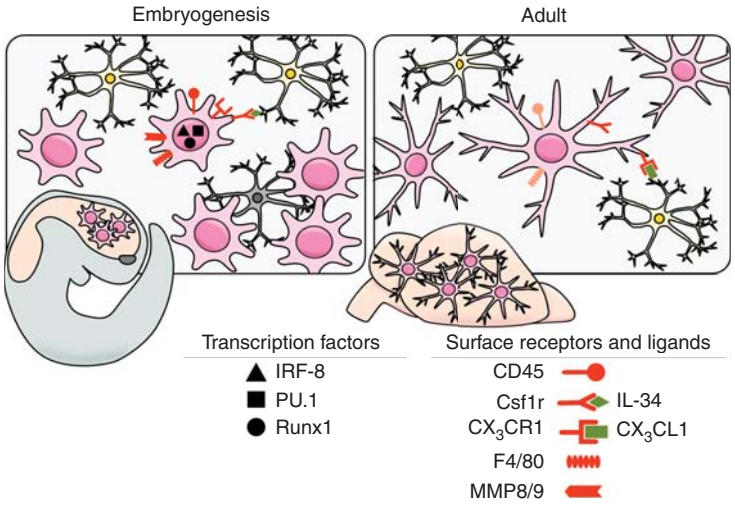


Figure 2. Morphology and markers of embryonic and adult microglia in the mouse. During embryogenesis (*left*), microglia show an activated and proliferating macrophage-like phenotype. Developing microglia with a round shape migrate throughout the maturing CNS phagocytizing neuronal debris. In adulthood (*right*), parenchymal microglia build a network of cells interacting with local neurons and display delineated small processes through which they actively survey the interneuronal space. The figure illustrates the precursors, transcription factors (black), and receptors (red) required for each developmental stage.

late the transcriptional programing of microglial development (Kierdorf et al. 2013a). IRF-8 is a heterodimeric partner of PU.1 with known roles in the development of B cells and myeloid cells in the BM (Holtschke et al. 1996). We recently found that YS-derived F4/80$^+$CX$_3$CR1$^+$ macrophages were dependent on the presence of IRF-8 for their early development, whereas other myeloid transcription factors, including MYB, ID2, BATF3, and KLF4, were redundant (Fig. 2) (Kierdorf et al. 2013a). Consequently, microglial density was significantly reduced in adult mice lacking IRF-8 (Kierdorf et al. 2013a). Moreover, it now seems that IRF-8 may have a role during the activation of adult microglia (Horiuchi et al. 2012; Masuda et al. 2012; Minten et al. 2012).

## THE ADULT MICROGLIAL POPULATION: SELF-RENEWAL RATHER THAN REPLENISHMENT BY THE BLOOD

### During Homeostasis

Within the first week after birth, the microglial population expands so dramatically (Alliot et al. 1999; Tambuyzer et al. 2009) that it was presumed that the proliferation of embryonic microglial cells alone could not account for such a steep increase in numbers, and so there must be a fresh influx of cells from another compartment. As initially suggested by Del Rio-Ortega, blood monocytes were believed to invade the CNS in the perinatal period and give rise to microglia, replacing the embryonic microglial cells. Several studies supported this hypothesis, notably an early report (Ling 1976) in which round, amoeboid, phagocytic cells were seen in rat corpus callosum during the first few days of life and then disappeared coincident with the appearance of ramified microglia. However, this view has been radically revised in recent years; unequivocal evidence from fate-mapping mouse models revealed that microglia are not BM-derived under homeostatic conditions, but originate from the embryonic YS (Ginhoux et al. 2010). Furthermore, new myeloid-specific gene-targeting approaches that focused on the chemokine receptor CX$_3$CR1 have, for the first time, enabled the study of the kinetics of true homeostatic microglial turnover without the

need for irradiation or chemotherapy (Goldmann et al. 2013; Yona et al. 2013). Using this technique, microglia were found to be long lived with labeled cells traceable for several months, which further argues against replacement by blood cells. In contrast, short-lived circulating Ly-6C$^{hi}$ and Ly-6C$^{lo}$ monocytes were quickly replaced by their nonlabeled progeny (Goldmann et al. 2013; Yona et al. 2013).

Additional evidence for the lack of significant contribution of monocytes or other BM-derived progenitors to the adult microglial pool came from prolonged experiments performed in parabiotic mice, in which two adult congenic mice undergo surgery to physically link their circulatory systems. In reality, even after up to 12 months of parabiosis, although monocytes in the blood of the parabionts originate from both animals, microglia remained totally of host origin, clearly illustrating the absence of contribution of monocytes or BM-derived cells to the CNS microglial population (Ajami et al. 2007, 2011; Ginhoux et al. 2010; Hashimoto et al. 2013).

Taken together, these new genetic approaches helped to firmly establish the major features of microglial population, namely, that they are long lived in vivo and not replaced by peripheral cells from the circulation, but are able to perform context-dependent self-renewal to ensure population maintenance.

## During Disease

One of the most pressing questions in the field of microglial research during recent years has been whether "BM-derived microglia" exist in the adult brain and, if so, whether they are functional. The answer could have profound clinical implications because it determines whether it might be possible to use peripheral microglial precursors as carriers for neuroprotective or immune-modulatory genes into the diseased CNS to treat conditions, such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and Parkinson's disease (PD) (Prinz et al. 2011; Prinz and Priller 2014).

The first seminal cell transplantation experiments in rats showed that, following BM transplantation, it was only perivascular macrophages that were replaced and not those cells with ramified microglial characteristics in the parenchyma (Hickey and Kimura 1988). Similar results were seen in female patients who underwent sex-mismatched BM transplantation and were subsequently examined for the engraftment of Y-chromosome-positive microglial cells; donor-derived perivascular macrophages were present, but no parenchymal microglia could be detected (Unger et al. 1993). Although informative, all these studies relied on immunohistochemical approaches, which are substantially less sensitive than modern cell transfer experiments using genetically labeled cells. Priller et al. (2001) were among the first who used hematopoietic cells transduced to express green fluorescent protein (GFP) to ask about the long-term fate of myeloid cells in the murine CNS after whole body irradiation and BM transplantation (Fig. 3). They found GFP-expressing parenchymal microglia deep in the cerebellum, striatum, and hippocampus several weeks after transplantation (Eglitis and Mezey 1997; Priller et al. 2001). Despite the differences to the other studies mentioned above, the concept of BM-derived phagocytes in the CNS was born. In the following years, a plethora of publications appeared that examined the assumed function and fate of BM-derived mononuclear phagocytes in different neurological models using similar experimental paradigms. Remarkably, CNS infiltration of BM-derived phagocytes was also shown in animal models of disease with no obvious BBB damage, such as ALS (Solomon et al. 2006), AD (Malm et al. 2005; Simard et al. 2006; Mildner et al. 2011), scrapie (Priller et al. 2006), and many more (Priller et al. 2001; Djukic et al. 2006). However, later studies elegantly showed that the irradiation regimen used to prepare recipient animals for BM transplants is necessary for the recruitment and differentiation of BM cells into microglia-like cells (Fig. 3) (Ajami et al. 2007; Mildner et al. 2007, 2011). Importantly, Mildner showed that recipient mice, in which the CNS was shielded to protect from the irradiation and associated release of proinflammatory cytokines and chemokines, did not experience a significant invasion

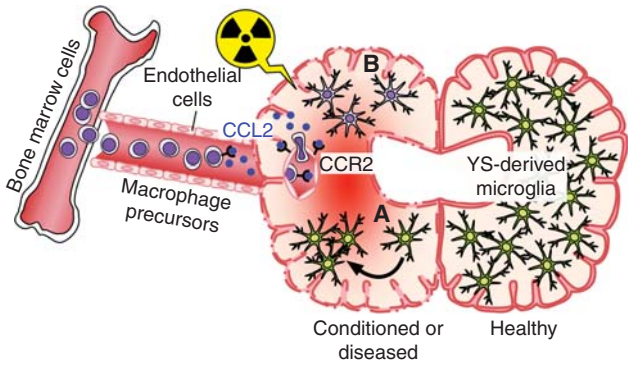


**Figure 3.** Formation of BM-derived microglia in the adult mouse brain. Postnatal BM-derived microglia form only under defined host conditions in the CNS. BM cells (*left*) are released into the bloodstream in a chemokine receptor (CCR)-2-dependent fashion and may enter the conditioned CNS. Local conditioning of the CNS can occur via irradiation and neurodegeneration, which lead to both disruption of the BBB and induction of chemokines, such as CCL2, thus, allowing engraftment of BM-derived macrophages. (*A*) YS-derived microglia (green) perform self-renewal by undergoing proliferation (indicated by an arrow), and (*B*) BM-derived phagocyte (purple).

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of BM-derived cells into the brain in contrast to the unshielded mice (Mildner et al. 2007). Beyond the irradiation issue, these data also suggest that microglial engraftment from the blood requires preconditioning of the CNS that likely disrupts the BBB. Additional clarity came from experiments in parabiotic mice, which enabled the study of the turnover of hematopoietic cells for prolonged periods without the need for irradiation (Ajami et al. 2007). Ajami used such mice to show that, in contrast to irradiated and transplanted mice, there was no microglial progenitor recruitment from the circulation in either denervation or CNS neurodegenerative disease. Intriguingly, if just one parabiont was irradiated, no further contribution from the other parabiont occurred, in apparent contradiction to the results of Mildner. However, Ajami further clarified that, although irradiation is required for donor cells to engraft, it is not sufficient; another important but often overlooked requirement is the artificial and concomitant introduction of a critical number of donor BM cells into the blood circulation (where they are not normally found). This, in conjunction with the inflammation of the BBB caused by irradiation, creates the unique nonphysiological situation that is required for the BM-to-microglia pathway to prevail (Diserbo et al. 2002; Li et al. 2004; Capotondo et al. 2012). Taking this work further, the same group recently used a similar approach, combining parabiosis and myeloablation, to show that recruited monocytes do not persist in the CNS and, therefore, even under these specific conditions, do not stably contribute to the resident microglial pool (Ajami et al. 2011). However, recruited short-lived monocytes are essential drivers of disease severity in multiple sclerosis (MS) and the experimental mouse model of autoimmune encephalomyelitis (EAE) (King et al. 2009; Mildner et al. 2009). In conclusion, BM-derived microglia can engraft into the diseased brain and become an integral part of the cellular network in the CNS only under specific nonphysiological conditions. These specific conditions include irradiation and chemotherapeutic regimes, for example, the application of myeloablating agents, which all (1) alter the integrity of the BBB, and (2) induce local production of myeloattracting chemokines, such as CCL2 (Boettcher et al. 2008; Lampron et al. 2012; Kierdorf et al. 2013b). Taken together, these data also clearly indicate that BM-derived nonmonocytic cells are able to permanently engraft to the diseased brain, whereas short-lived monocytes are only transiently recruited to the CNS.

## CONCLUSION

Altogether, these seminal studies established that microglia arise from embryonic hematopoietic precursors that seed the CNS before birth and, more importantly, before the onset of BM hematopoiesis. It is now accepted that microglia derive from unique embryonic precursors, the YS macrophages, which are not found in the BM as predicted earlier by the founder of the microglial field, Pío del Río-Hortega. This knowledge has far-reaching implications for the understanding of microglial functions in CNS development. First, the conservation of primitive macrophages and their YS derivation, both throughout evolution and across diverse species, suggests that microglia play an important physiological role in the development of the CNS. Furthermore, microglial cells are present during all stages of brain development, including the early prenatal stages of neuronal circuit building, as well as the postnatal stage of synapse elimination. This implies a functional niche for microglia in the development of neuronal circuits of the brain and proposes intriguing possibilities regarding the integrated development of the neural and immune systems.

## ACKNOWLEDGMENTS

We apologize to all colleagues whose work was was not cited owing to space constraints. We thank Dr. L. Robinson for critical review and editing of the manuscript. F.G. is supported by a Singapore Immunology Network core grant. M.P. is supported by the Federal Ministry of Education and Research (BMBF)-funded competence network of multiple sclerosis (KKNMS), the Gemeinnützige Hertie-Stiftung (GHST), the Fritz Thyssen Stiftung, the competence network of neurodegenerative disorders (KNDD), and the The Deutsche Forschungsgemeinschaft (SFB 992, FOR1336).

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