

Regulation of Microglia Development and Homeostasis

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ABSTRACT

Microglia represent the resident macrophages of the central nervous system (CNS) and account for 10% of the adult glial cell population in the normal brain. Although microglial cells are thought to contribute to most pathological conditions including CNS infections, neuroinflammatory lesions, brain tumors, and neurodegenerative diseases, their exact role in CNS development, homeostasis, and disease remains poorly understood. In contrast to most macrophage populations, microglia survive high-dose ionizing radiation and maintain themselves locally and independently of circulating precursors in the steady state. However, controversies remain on the origin of microglia in the brain and whether they could potentially be repopulated by circulating myeloid precursors after brain injury. Microglia-targeted therapies through the use of genetically modified circulating hematopoietic cells proved to be a promising therapeutic strategy for the treatment of brain diseases. It is thus of great importance to understand the contribution and developmental cues of circulating myeloid cells as potential microglia progenitors to the adult pool of microglia in the steady state and under inflammatory conditions. © 2012 Wiley Periodicals, Inc.

HISTORICAL PERSPECTIVE

Glial cells represent all non-neuronal cells in the central nervous system (CNS). They were termed glial cells from the Greek word “glia” meaning glue, to suggest the “supportive” role provided by non-neuronal cells to neuronal cells. Glial cells represent more than 90% of the human brain and consist of two main populations: the macroglia, which consist of astrocytes and oligodendrocytes, and the microglia.

Pío del Río-Hortega was the first to distinguish microglia from macroglia and to suggest the mesodermal origin of these cells in 1932 (del Río-Hortega, 1932). In this seminal study, del Río-Hortega also suggested that microglia arise early during embryonic development, which indicates that they might represent a distinct developmental lineage even though they share many properties with macrophages in other tissues. Interestingly, these early studies are still valid today and although it is now accepted that microglia are derived from myeloid precursors, the nature of their direct precursor, the time point at which myeloid precursors first invade the CNS,

and how they are maintained during adult life have long been a subject of debate. Here, we will discuss recent developments in our understanding of the ontogeny of microglia, which clarified some long-standing questions on the origin of this unique and distinct cell population.

MICROGLIA AND THE MONONUCLEAR PHAGOCYTE SYSTEM

First defined by van Furth et al., the mononuclear phagocyte system (MPS) refers to highly phagocytic cells with similar morphology, origin, and function (van Furth et al., 1972). The MPS theory suggests that most tissue macrophages derive from circulating monocytes and their precursors in the bone marrow (BM). Adoptive transfer studies have shown that circulating monocytes could potentially give rise to spleen and gut tissue macrophages (Bogunovic et al., 2009; Geissmann et al., 2003; Varol et al., 2007). However, increasing evidence in the literature suggests that tissue phagocytes are more heterogeneous than originally assumed.

The best example of the MPS heterogeneity is represented by the microglial cell. Microglia originate from primitive macrophages that arise during early development and are maintained locally independently of circulating monocytes (see below) (Ginhoux et al., 2010). In addition to microglia, epidermal Langerhans cells (LCs), which are the dendritic cells (DC) that populate the epidermis, derive predominantly from fetal liver monocytes and can also self-renew locally throughout life (Hoeffel et al., 2012; Merad et al., 2002).

Macrophages also display great diversity in terms of cytokines and transcription factors necessary for their development, which can often be ascribed to the tissues in which they reside. Although it has been suggested that most tissue macrophages are dependent on the cytokine macrophage-colony stimulating factor (M-CSF or Csf-1) for their development, there is now evidence that microglia and LCs can form in the absence of Csf-1

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TABLE 1. Macrophage Populations in Lymphoid and Nonlymphoid Tissues

	CNS	Spleen	Lymph node	Lung	Liver
Marker	Microglia: CD45 ^{lo} F4/80 ⁺ CD11b ⁺ Cx3Cr1 ⁺ CD68 ⁺ Iba-1 ⁺	Red pulp MF: CD45 ^{hi} F4/80 ^{hi} CD11b ^{int} Cx3Cr1 ⁻	Subcapsular sinus MF: CD45 ^{hi} F4/80 ^{lo} CD11b ⁺ CD169 ⁺ CD11c ^{lo} Medullary MF: CD45 ^{hi} F4/80 ⁺ CD11c ^{lo} CD169 ⁺	Alveolar MF: CD45 ^{hi} F4/80 ⁺ CD11b ^{lo} SiglecF ⁺ CD11c ^{hi} Cx3Cr1 ⁻ Interstitial MF: CD45 ^{hi} F4/80 ⁺ CD11c ⁻ CD68 ⁺ MHCII ⁺	Kupffer cells: CD45 ^{hi} F4/80 ⁺ CD11b ^{lo} CD169 ⁺ CD68 ⁺
References	(Ginhoux et al., 2010; Prinz et al., 2011)	(Hashimoto et al., 2011a; Kohyama et al., 2009)	(Asano et al., 2011; Hashimoto et al., 2011a; Phan et al., 2009)	(Kirby et al., 2009; Lagranderie et al., 2003)	(Crocker and Gordon, 1989; Flotte et al., 1983)

Table summarizes markers of macrophages in the spleen, lymph node, lung, and liver and of microglia in the CNS (Hashimoto et al., 2011b).

ligand (Ginhoux et al., 2006, 2010; Kondo et al., 2007; Wegiel et al., 1998). In addition, the cytokine GM-CSF thought to control mostly DC development also controls lung alveolar macrophage function (Dranoff et al., 1994).

PHENOTYPIC MARKERS

Similar to most tissue macrophages, microglia express the Csf-1 receptor (Csf-1R also called CD115), the fraktalkine receptor (Cx3cr1), the glycoproteins F4/80 and CD68, and the integrin CD11b. The calcium-binding protein Iba-1 is often used as a marker of microglia, although similar to the markers above, it is also expressed by most tissue macrophages and it cannot be used to differentiate between tissue resident microglia and other tissue macrophages. Microglia express much lower CD45 levels than perivascular macrophages in steady-state conditions, which represents the only phenotypical means available to distinguish the two populations (Table 1 and Fig. 1). The identification of microglia-specific markers would therefore prove extremely valuable for distinguishing the diverse subsets of myeloid cells during neuroinflammatory diseases and to assess their distinct contributions to brain homeostasis.

TURNOVER OF MICROGLIA IN THE STEADY STATE

Although it is now accepted that microglia are ontogenically related to macrophages, the exact contribution of BM-derived myeloid precursors including monocytes to the adult microglia pool in the steady state has been widely debated. In an early study, Ling showed that adoptive transfer of monocytes labeled with carbon particles in newborn rats gave rise to labeled microglia in the brain (Ling, 1979). The use of ³H-thymidine to study microglia turnover revealed the presence of a low number of F4/80⁺ and [³H]-thymidine-labeled cells in the CNS, which increased slowly after isotope injection, suggesting that adult microglia homeostasis results from both the recruitment of circulating monocytes and local microglia proliferation (Lawson et al., 1992).

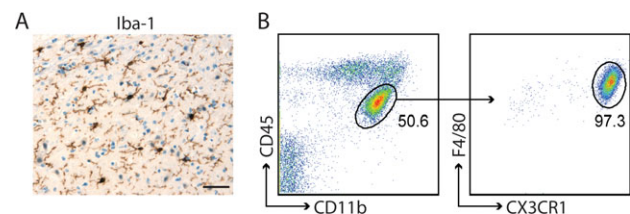


Fig. 1. (A) Image depicts Iba-1 staining (microglia) on brain sections from adult wild-type mice. Bar: 50 μm. (B) Plot shows the percentage of microglia (CD45^{lo}CD11b⁺F4/80⁺CX3CR1⁺) of adult wild-type mice analyzed by flow cytometry.

In contrast to most macrophage populations that are renewed by BM-derived precursors, microglial cells can survive high-dose γ -ray irradiation. These results were most clearly demonstrated in BM chimeric animals using congenic markers (CD45.1 and CD45.2) or GFP⁺ BM cells (Lassmann and Hickey, 1993; Priller et al., 2001). BM chimeric animals are generated upon lethal irradiation of recipient animals followed by their reconstitution with donor BM cells, which leads to the engraftment of most circulating as well as tissue leukocytes.

Several groups have suggested that BM-derived myeloid cells are constantly recruited to the brain in the steady state. Priller et al. have shown that upon BM reconstitution with GFP-transduced BM, GFP⁺ microglia could be found in the brain parenchyma in a site-specific manner (Priller et al., 2001). Eight weeks post-transplant, GFP⁺ microglia were detected in the olfactory bulb and the cerebellum (6 and 8% of total microglia, respectively). Fifteen weeks post-transplant, GFP⁺ microglia were detected in the hippocampus (5.5%) and cerebellum (12.5%) but rarely in the cortex (~2%). Simard and Rivest also detected GFP⁺ microglia in many regions of the CNS in wild-type mice transplanted with GFP⁺ BM, 7 weeks after transplant (Simard and Rivest, 2004). In another study, GFP⁺ cells were found only in the cerebellum and in injury sites (Vallieres and Sawchenko, 2003). In all of these reports, less than 10–20% of total microglial cells were donor derived even 1 year after transplant (Ginhoux et al., 2010).

Several reports have also argued against a replacement of adult microglia by circulating myeloid

precursors under physiological conditions (Ajami et al., 2007; Becher et al., 2001; Greter et al., 2005; Mildner et al., 2007). For example, it has been proposed that donor-derived microglia identified in BM chimeric animals are linked to radiation injuries because donor-derived microglial cells were undetectable in BM chimeric animals in which the brain was protected from irradiation (Mildner et al., 2007). Similar findings were made in parabiotic mice. Parabionts are surgically attached mice that share the same blood circulation for prolonged periods of time providing a model to assess the physiological turnover of circulating precursors in the absence of radiation-induced injuries. Microglia chimerism in parabionts never exceeded 5–10% (Ajami et al., 2007), even 1 year post parabiosis (Ginhoux et al., 2010).

We have found that in newborn mice reconstituted with donor congenic BM cells, less than 5% of microglia were of donor origin 3 months post-transplant, which is consistent with the results obtained in adult BM chimeras (Ginhoux et al., 2010). Interestingly, however, high levels of donor microglial chimerism were achieved upon transplantation of newborn mice deficient of microglia (PU.1^{-/-} mice, see below) with adult wild-type BM cells (Beers et al., 2006). This suggests that BM-derived precursors have the potential to repopulate the empty microglia niche in the CNS during the perinatal period. Whether blood-borne precursors could also repopulate the entire microglial pool in adult mice lacking microglia remains to be addressed. Taken together, these results suggest that microglia homeostasis is predominantly maintained through local self-renewal under physiological conditions.

TURNOVER OF MICROGLIA IN THE INFLAMED STATE

In contrast to the steady state, inflammatory conditions have been thought to promote the recruitment of circulating myeloid progenitors and their differentiation into microglia. Mildner et al. showed that Ly6C^{hi} monocytes differentiate into microglia in CNS pathologies associated with a blood–brain-barrier disruption (Mildner et al., 2007) (Fig. 2). However, it is difficult to phenotypically distinguish activated macrophages from microglial cells in the inflamed CNS, and therefore it remains possible that donor-derived myeloid cells were mostly perivascular macrophages or transient microglial-like cells. In addition, it is unclear whether newly engrafted microglia are functionally and morphologically equivalent to the resident microglia. A recent study, in which parabiosis and myeloablation were elegantly combined, revealed that although circulating monocytes infiltrate the CNS during inflammation, they only give rise to a transient population of macrophages that disappears once the inflammation resolves (Ajami et al., 2011). This study also compared the ability of multipotent progenitors contained in the lineage-negative, c-Kit-positive, Sca-1-positive (KLS) subset and committed myeloid progenitors expressing the fractalkine receptor

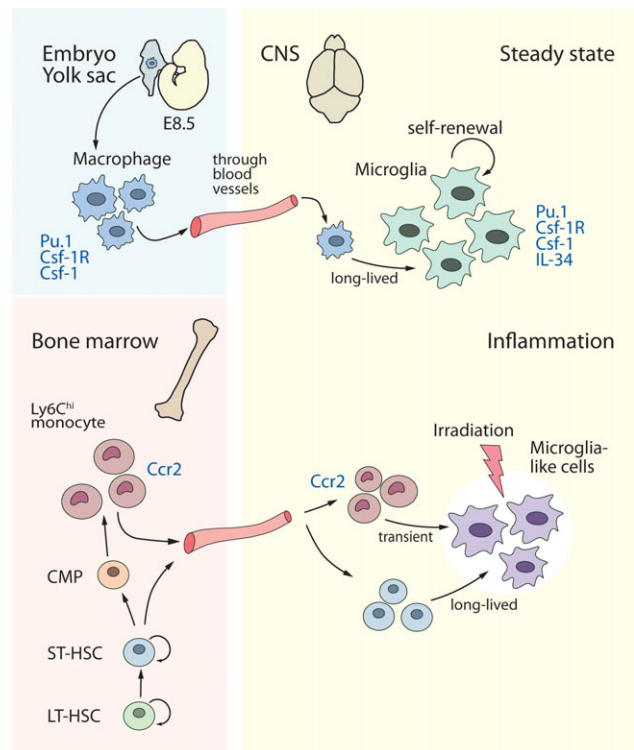


Fig. 2. Schematic for the development of microglia. In the steady state, microglia in the adult CNS are derived from yolk sac primitive macrophages that arise before E8 and migrate through blood vessels into the brain. Microglia development is highly dependent on Csf-1R signaling. During inflammation, circulating Ly6C^{hi} monocytes can give rise to microglia-like cells in a CCR2-dependent manner or ST-HSC can differentiate into long-lived microglia directly. (LT-HSC, long-term hematopoietic stem cells; ST-HSC, short-term hematopoietic stem cells; CMP, common myeloid progenitor).

CX3CR1 to give rise to long-lived microglia (Ajami et al., 2011) (Fig. 2). Adoptive transfer of KLS progenitors but not CX3CR1⁺ progenitors gave rise to detectable levels of long-lived microglia that persisted during EAE remission leading the authors to hypothesize that the microglial potential is retained in multipotent progenitors and lost during commitment to the myeloid lineage. Another possible explanation is that KLS cells are more resistant to inflammation-induced injuries compared to committed progenitors and have a higher proliferation potential, thereby giving rise to higher levels of committed progenitors found in the CX3CR1⁺ fraction.

Mice that carry null mutations in the methyl-CpG-binding protein (MECP2^{-/-}) provide a clinically relevant model for Rett Syndrome, an X-linked autism spectrum disorder, characterized in most cases by a mutation of the MECP2 gene. Interestingly, MECP2^{-/-} microglia display impaired phagocytic functions and reconstitution of MECP2^{-/-} animals with wild-type BM cells improved the clinical symptoms and reduced disease progression (Derecki et al., 2012). These results revealed a potential key role for microglia/microglia-like cells in Rett syndrome and support the use of BM transplants as a potential strategy for the treatment of clinical autism disorders.

Altogether, these studies highlight the importance of identifying microglial precursors and the mechanistic cues that control their recruitment to the brain parenchyma for the design of novel therapeutic strategies using myeloid precursors as therapeutic vehicles to deliver genes into the CNS.

ORIGIN OF MICROGLIA

As described above, congenic BM transplant studies in newborn mice fail to repopulate microglia in recipient mice, suggesting that a postnatal wave of circulating precursors does not contribute to microglial homeostasis under steady-state conditions establishing that microglia take residence in the brain before birth. In the mammalian embryo, the first site of hematopoiesis occurs in the extra-embryonic YS blood islands at around embryonic age 7.5 (E7.5), which is called primitive hematopoiesis (Orkin and Zon, 2008). The main function of the primitive hematopoiesis is to generate primitive red blood cells and macrophages (Lichanska and Hume, 2000; Lichanska et al., 1999). Primitive hematopoiesis declines over time and is progressively replaced by definitive hematopoiesis, which occurs in the aorta-gonad-mesonephros region at E10.5 and then later in the fetal liver starting at E11.5 until birth.

Cuadros et al. were the first to suggest the YS origin of microglial cells. These early studies used YS chimeras by grafting a chick embryo with a quail YS and the usage of histochemical stainings for acid phosphatase together with antibodies against quail hematopoietic cells. Their findings indicated that myeloid progenitors invade the CNS through the pial surface but independently of blood vessels (Cuadros et al., 1993). The hypothesis that YS-derived cells give rise to microglia was further supported by Pessac and coworkers (Alliot et al., 1999). They demonstrated that microglial precursors, characterized by high expression of F4/80⁺ and CD11b⁺ and the capability to adhere to an astroglial monolayer, were detectable first in the YS and subsequently in the brain rudiment as early as E8. Similar observations were made in zebrafish embryos. In this study, macrophage-like cells differentiated at an early stage (24 h postfertilization) in the YS of the zebrafish embryo, which resembled the YS primitive macrophages of avian and rodent embryos. These macrophage-like cells were found in the YS and subsequently migrated to the mesenchyme of the head (Herbomel et al. 1999).

Although these early studies suggested that YS macrophages can give rise to microglia, direct evidence of the YS contribution to adult microglia *in vivo* remained unknown. We recently used a fate mapping model to revisit the contribution of embryonic precursors to adult microglia. We used a mouse model developed by Samokhvalov and coworkers (Samokhvalov et al., 2007) in which mice expressing the tamoxifen-inducible Cre recombinase under the control of Runt-related transcription factor 1 (Runx1) were crossed to ROSA26-stop-EYFP or ROSA26-stop-LacZ mice (Ginhoux et al., 2010;

Samokhvalov et al., 2007). Runx1 expression is restricted to YS blood islands before E8, whereas after E8 Runx1 is also expressed in definitive hematopoietic precursors (Samokhvalov et al., 2007). Therefore, a single injection of tamoxifen before E7.5 leads to the long-term tagging of early YS cells allowing to trace YS-derived cells in adult mice. Using this model, we found that tamoxifen injection before E7.5 labels 30% of YS macrophages (likely because of incomplete Cre-mediated recombination). Strikingly, analysis of mice at different time points during development revealed that 30% of YS labeling was also associated with 30% labeling of microglia in E10 and E13 embryos and 30% labeling of microglial cells in the brain of 6-week-old animals, whereas no circulating leukocytes and monocytes were labeled in these mice. Importantly, injection of tamoxifen post-E8.5 failed to label YS macrophages and embryonic microglia as well as adult microglia, whereas it labeled adult monocytes and circulating leukocytes (Ginhoux et al., 2010). These results strongly suggest that microglia that populate the adult brain derive predominantly from YS primitive macrophages (Fig. 2). Using mice that are deficient for the sodium-calcium exchanger Ncx1 (Koushik et al., 2001), which lacks a heartbeat and thus a blood circulation, we further established that in contrast to earlier reports, the invasion of YS macrophages into the brain rudiment is highly dependent on blood vessels because microglia progenitors fail to enter the brain of *Ncx1*^{-/-} mice and rather accumulated in the YS (Ginhoux et al., 2010). A subsequent study recently established that in addition to microglia, a subset of most tissue macrophages also derives from the YS (Schulz et al., 2012).

TRANSCRIPTION FACTORS AND CYTOKINES THAT CONTROL MICROGLIA HOMEOSTASIS AND FUNCTION

Similar to most tissue macrophages, microglia are highly dependent on Csf-1R for their development (Hamilton, 2008). *Csf-1R*^{-/-} mice display greatly reduced macrophage populations in various tissues and completely lack microglia (Ginhoux et al., 2010) (Table 2). Interestingly, *Csf1*^{op/op} mice that carry a natural null mutation for Csf-1 (the ligand of Csf-1R) display only slightly reduced numbers of adult microglia, whereas macrophages in several tissues are drastically reduced (Dai et al., 2002; Wiktor-Jedrzejczak et al., 1990). The requirement for Csf-1R but not Csf-1 in microglia development suggested that microglia homeostasis is controlled by an alternative Csf-1R ligand. A new cytokine called interleukin-34 (IL-34) was recently identified in mice and humans and was shown to bind Csf-1R with high affinity (Lin et al., 2008). IL-34 was also found to support monocyte viability *in vitro* and to stimulate the differentiation and proliferation of macrophages reminiscent of Csf-1. Expression of IL-34 in a Csf-1-dependent manner rescued the phenotype of Csf-1-deficient mice (Wei et al., 2010), suggesting that Csf-1 and IL-34 com-

TABLE 2. Mice Models Lacking Cells of the MPS

	YS macrophages	Monocytes	Microglia	Macrophages	References
Csf-1R	↓↓↓	Ly6C ^{lo} ↓↓↓ Ly6C ^{hi} ↓	↓↓↓	↓↓↓	(Dai et al., 2002; Erlich et al., 2011; Ginhoux et al., 2010; Hashimoto et al., 2011a)
Csf-1	↓	↓	↓	↓↓↓	(Cecchini et al., 1994; Kondo et al., 2007; Wegiel et al., 1998; Wiktor-Jedrzejczak et al., 1990)
IL-34 Pu.1	n.d. ↓↓↓	- -	↓↓ ↓↓↓	- F4/80 ^{hi} CD11b ^{lo} ↓↓↓	(Wang et al., 2012) (Beers et al., 2006; Dakic et al., 2005; Lichanska et al., 1999; McKercher et al., 1996; Schulz et al., 2012)
Myb DAP12	- n.d.	n.d. -	- ↓	F4/80 ^{lo} CD11b ^{hi} ↓↓↓ n.d.	(Schulz et al., 2012) (Otero et al., 2009)

Table summarizes phenotypes of mice lacking transcription factors or cytokines. n.d., not determined; -, no difference compared to wild-type mice; ↓, reduction compared to wild-type mice.

parably regulate Csf-1R signaling. However, Csf-1 and IL-34 display little homology, indicating that they bind different regions of the Csf-1R (Garceau et al., 2010). IL-34 is conserved in avian and mammalian species, which further emphasizes the potential key role of IL-34 in macrophage biology (Garceau et al., 2010). Interestingly, IL-34 enhances neuroprotective effects of microglia *in vitro* (Mizuno et al., 2011) and is expressed at high levels in the brain and skin of adult mice, suggesting a role for IL-34-mediated Csf-1R signaling in these tissues (Wei et al., 2010). Accordingly, IL-34-deficient mice have recently been found to have significantly fewer microglia than wild-type mice (Wang et al., 2012). However, the fact that IL-34 deficiency does not completely phenocopy Csf-1R deficiency suggests that Csf-1 might compensate for the absence of IL-34 in the CNS.

Csf-1R is not only highly expressed by microglia in adult mice but also by YS macrophages, and the absence of Csf-1R or Csf-1R blockade compromises microglia differentiation in embryos (Ginhoux et al., 2010). It is thus unclear whether continued Csf-1R expression is required to promote adult microglia homeostasis *in vivo*. In addition, which of the Csf-1R ligands, IL-34 or Csf-1, controls microglia development in embryos and homeostasis in the adults or whether they have complimentary or redundant roles remains to be established.

Another key molecule for the development of macrophages and microglia is PU.1. PU.1 is an essential transcription factor for adult myelopoiesis and has been shown to regulate Csf-1R among other genes. PU.1-deficient mice lack macrophages, monocytes, and microglia (Beers et al., 2006) (Table 2).

The adaptor protein DAP12 or the triggering receptor expressed on myeloid cells-2 (TREM2) is involved in myeloid cell differentiation, and mice deficient in DAP12 or TREM2 develop osteopetrosis because of the lack of osteoclasts (Kaifu et al., 2003; Neumann and Takahashi, 2007). Interestingly, humans with deletions in either of these genes develop the fatal Nasu-Hakola disease, which is characterized by bone cysts, bone fractures, and psychotic symptoms leading to severe neurodegeneration and encephalopathy (Paloneva et al., 2000). A recent study has shown that Csf-1 activates β -catenin via the adaptor protein DAP12, which results in proliferation and survival of macrophages *in vitro* (Otero et al., 2009). Interestingly, older mice deficient in DAP12 exhibit fewer

microglia in defined regions of the CNS, for example, in the basal ganglia and the spinal cord. These findings established a critical role for DAP12 in the long-term homeostasis of microglia, which might be a direct link to the pathology of patients with Nasu-Hakola disease.

ANTIGEN PROCESSING FUNCTION AND PATHOGEN RECOGNITION

In comparison to professional antigen-presenting cells like DCs, the capacity of microglia to prime naïve T cells is restricted. Similar to macrophages, in the steady state, microglia express undetectable levels of MHCII and T-cell costimulatory molecules such as CD40, CD80, and CD86 and are therefore not properly equipped to present antigen under physiological conditions (Ransohoff and Perry, 2009). *In vitro* microglia can rapidly upregulate these surface molecules in response to inflammatory stimuli, for example, IFN- γ or LPS, and can activate naïve T cells. Their efficiency at inducing T cell responses, however, is rather limited and poor in comparison to DCs. Under neuropathological conditions, microglia instantly convert to an activated state, upregulate MHCII and costimulatory molecules, and secrete proinflammatory cytokines such as IL-12, IL-23, TNF- α , and IL-1 β , which are considered essential for the initiation of CNS immunity. Pathogen-associated molecular patterns are recognized by cells of the innate and the adaptive immune system through the engagement of pattern recognition receptors such as Toll-like receptors or NOD-like receptors. Microglia express all of the nine TLRs reported to date in mice, which enables them to sense and respond to a wide variety of pathogens within the CNS (Lehnardt, 2010). The TLR-induced microglia activation causes a rapid production of chemokines and proinflammatory cytokine and the upregulation of MHCII (Olson and Miller, 2004). However, the excessive activation of microglia and their release of reactive-oxygen and -nitrogen species and iNOS lead to detrimental neurotoxic effects, which have to be tightly controlled to circumvent collateral damage in the CNS. Microglia express high levels of CX3CR1 (fractalkine receptor), and it was shown that if microglia are unable to respond to the ligand CX3CL1 expressed by neurons, neurodegeneration was exacerbated suggesting that the fractal-

kine receptor modulates microglia neurotoxicity (Cardona et al., 2006). Another example for a neuron-microglia-inhibitory signal is CD200-CD200R. CD200 is expressed by neurons, whereas CD200R is expressed by microglia. In CD200-deficient mice, microglia exhibit an activated phenotype under physiological conditions and an accelerated response to facial nerve transection and an earlier disease onset in EAE (Hoek et al., 2000).

MICROGLIA AND EPIDERMAL LANGERHANS CELLS

LCs are the resident DCs of the epidermis and represent the first immune line of defense against skin-invading pathogens. Interestingly, LCs resemble microglia in several aspects. Similar to microglia, LCs express high levels of Csf-1R, and mice lacking Csf-1R are completely devoid of LCs (Ginhoux et al., 2006). In contrast, *Csf1^{op/op}* mice display normal numbers of LCs (Ginhoux et al., 2006; Wiktor-Jedrzejczak et al., 1990). This was explained by a recent study demonstrating that IL-34-mediated Csf-1R signaling is critical for the development of LCs (Wang et al., 2012). Like microglia, LCs are radio-resistant, maintain themselves *in situ*, and are not repopulated by blood-borne precursors during the steady state (Merad et al., 2002). So far, LCs and microglia are the only known hematopoietic cell types capable of self-renewal during homeostatic conditions. Upon severe skin inflammation, however, the resident LCs disappear and are reconstituted by Ly6C^{hi} monocytes in a Csf-1R-dependent manner (Ginhoux et al., 2006). Recent studies established that LC predominantly derive from fetal liver monocytes with some minor contribution from YS macrophages (Ginhoux and Merad, 2010; Hoeffel et al., 2012).

FUNCTIONAL RELEVANCE OF THE ONTOGENY DATA

Ample evidence now suggests that microglia form a separate developmental lineage of the MPS. Certain aspects of microglia ontogeny and their developmental requirements, for example, Csf-1R dependency, have been resolved. However, the precise underlying mechanisms for their homeostasis in healthy adult life as well as during pathologies remain unclear. Microglia play multifaceted roles not only during physiological conditions in embryonic as well as adult neurogenesis but also during CNS pathologies such as in neuroinflammation, neurodegeneration, and neural repair. Many neuroinflammatory diseases are characterized by microgliosis concomitant with the infiltration of blood-derived myeloid cells. Discriminating invading myeloid cells from resident microglia will be helpful for designing new therapeutic strategies for the treatment of CNS pathologies. The identification of YS-derived microglia-specific genes that are absent from newly engrafted microglia-like cells and CNS-associated macrophages should allow the specific targeting of tissue resident microglia and gain a better understanding of the homeostatic

mechanisms and the functional specialization of this critical brain support system.

REFERENCES

- Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. 2011. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat Neurosci* 14:1142–1149.
- Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. 2007. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci* 10:1538–1543.
- Alliot F, Godin I, Pessac B. 1999. Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res Dev Brain Res* 117:145–152.
- Asano K, Nabeyama A, Miyake Y, Qiu CH, Kurita A, Tomura M, Kanagawa O, Fujii S, Tanaka M. 2011. CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens. *Immunity* 34:85–95.
- Becher B, Durell BG, Miga AV, Hickey WF, Noelle RJ. 2001. The clinical course of experimental autoimmune encephalomyelitis and inflammation is controlled by the expression of CD40 within the central nervous system. *J Exp Med* 193:967–974.
- Beers DR, Henkel JS, Xiao Q, Zhao W, Wang J, Yen AA, Siklos L, McKercher SR, Appel SH. 2006. Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA* 103:16021–16026.
- Bogunovic M, Ginhoux F, Helft J, Shang L, Hashimoto D, Greter M, Liu K, Jakubzick C, Ingersoll MA, Leboeuf M, Stanley ER, Nussenzweig M, Lira SA, Randolph GJ, Merad M. 2009. Origin of the lamina propria dendritic cell network. *Immunity* 31:513–525.
- Cardona AE, Piro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee J, Cook DN, Jung S, Lira SA, Littman DR, Ransohoff RM. 2006. Control of microglial neurotoxicity by the fractalkine receptor. *Nat Neurosci* 9:917–924.
- Cecchini MG, Dominguez MG, Mocchi S, Wetterwald A, Felix R, Fleisch H, Chisholm O, Hofstetter W, Pollard JW, Stanley ER. 1994. Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. *Development* 120:1357–1372.
- Crocker PR, Gordon S. 1989. Mouse macrophage hemagglutinin (sheep erythrocyte receptor) with specificity for sialylated glycoconjugates characterized by a monoclonal antibody. *J Exp Med* 169:1333–1346.
- Cuadros MA, Martin C, Coltey P, Almdros A, Navascues J. 1993. First appearance, distribution, and origin of macrophages in the early development of the avian central nervous system. *J Comp Neurol* 330:113–129.
- Dai XM, Ryan GR, Hapel AJ, Dominguez MG, Russell RG, Kapp S, Sylvestre V, Stanley ER. 2002. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. *Blood* 99:111–120.
- Dakic A, Metcalf D, Di Rago L, Mifsud S, Wu L, Nutt SL. 2005. PU.1 regulates the commitment of adult hematopoietic progenitors and restricts granulopoiesis. *J Exp Med* 201:1487–1502.
- del Rio-Hortega P. 1932. Microglia. In: Penfield W, editor. *Cytology and cellular pathology of the nervous system*, Vol. 2. New York: Hoeber. pp 483–534.
- Derecki NC, Cronk JC, Lu Z, Xu E, Abbott SB, Guyenet PG, Kipnis J. 2012. Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* 484:105–109.
- Dranoff G, Crawford AD, Sadelain M, Ream B, Rashid A, Bronson RT, Dickersin GR, Bachurski CJ, Mark EL, Whitsett JA, Mulligan RC. 1994. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. *Science* 264:713–716.
- Erblich B, Zhu L, Etgen AM, Dobrenis K, Pollard JW. 2011. Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. *PLoS One* 6:e26317.
- Flotte TJ, Springer TA, Thorbecke GJ. 1983. Dendritic cell and macrophage staining by monoclonal antibodies in tissue sections and epidermal sheets. *Am J Pathol* 111:112–124.
- Garceau V, Smith J, Paton IR, Davey M, Fares MA, Sester DP, Burt DW, Hume DA. 2010. Pivotal Advance: Avian colony-stimulating factor 1 (CSF-1), interleukin-34 (IL-34), and CSF-1 receptor genes and gene products. *J Leukoc Biol* 87:753–764.
- Geissmann F, Jung S, Littman DR. 2003. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 19:71–82.
- Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M.

2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330:841–845.
- Ginhoux F, Merad M. 2010. Ontogeny and homeostasis of Langerhans cells. *Immunol Cell Biol* 88:387–392.
- Ginhoux F, Tacke F, Angeli V, Bogunovic M, Loubeau M, Dai XM, Stanley ER, Randolph GJ, Merad M. 2006. Langerhans cells arise from monocytes in vivo. *Nat Immunol* 7:265–273.
- Greter M, Heppner FL, Lemos MP, Odermatt BM, Goebels N, Laufer T, Noelle RJ, Becher B. 2005. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. *Nat Med* 11:328–334.
- Hamilton JA. 2008. Colony-stimulating factors in inflammation and autoimmunity. *Nat Rev Immunol* 8:533–544.
- Hashimoto D, Chow A, Greter M, Saenger Y, Kwan WH, Leboeuf M, Ginhoux F, Ochando JC, Kunisaki Y, van Rooijen N, Liu C, Teshima T, Heeger PS, Stanley ER, Frenette PS, Merad M. 2011a. Pretransplant CSF-1 therapy expands recipient macrophages and ameliorates GVHD after allogeneic hematopoietic cell transplantation. *J Exp Med* 208:1069–1082.
- Hashimoto D, Miller J, Merad M. 2011b. Dendritic cell and macrophage heterogeneity in vivo. *Immunity* 35:323–335.
- Herbomel P, Thisse B, Thisse C. 1999. Ontogeny and behaviour of early macrophages in the zebrafish embryo. *Development* 126:3735–3745.
- Hoeffel G, Wang Y, Greter M, See P, Teo P, Malleret B, Leboeuf M, Low D, Oller G, Almeida F, Choy SH, Grisotto M, Renia L, Conway SJ, Stanley ER, Chan JK, Ng LG, Samokhvalov IM, Merad M, Ginhoux F. 2012. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J Exp Med* 209:1167–1181.
- Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, Blom B, Homola ME, Streit WJ, Brown MH, Barclay AN, Sedgwick JD. 2000. Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science* 290:1768–1771.
- Kaifu T, Nakahara J, Inui M, Mishima K, Momiyama T, Kaji M, Sugahara A, Koito H, Ujike-Asai A, Nakamura A, Kanazawa K, Tan-Takeuchi K, Iwasaki K, Yokoyama WM, Kudo A, Fujiwara M, Asou H, Takai T. 2003. Osteopetrosis and thalamic hypomyelination with synaptic degeneration in DAP12-deficient mice. *J Clin Invest* 111:323–332.
- Kirby AC, Coles MC, Kaye PM. 2009. Alveolar macrophages transport pathogens to lung draining lymph nodes. *J Immunol* 183:1983–1989.
- Kohyama M, Ise W, Edelson BT, Wilker PR, Hildner K, Mejia C, Frazier WA, Murphy TL, Murphy KM. 2009. Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis. *Nature* 457:318–321.
- Kondo Y, Lemere CA, Seabrook TJ. 2007. Osteopetrotic (op/op) mice have reduced microglia, no Aβ deposition, and no changes in dopaminergic neurons. *J Neuroinflamm* 4:31.
- Koushik SV, Wang J, Rogers R, Moskophidis D, Lambert NA, Creazzo TL, Conway SJ. 2001. Targeted inactivation of the sodium-calcium exchanger (Ncx1) results in the lack of a heartbeat and abnormal myofibrillar organization. *FASEB J* 15:1209–1211.
- Lagranderie M, Nahori MA, Balazuc AM, Kiefer-Biasizzo H, Lapa e Silva JR, Milon G, Marchal G, Vargaftig BB. 2003. Dendritic cells recruited to the lung shortly after intranasal delivery of *Mycobacterium bovis* BCG drive the primary immune response towards a type 1 cytokine production. *Immunology* 108:352–364.
- Lassmann H, Hickey WF. 1993. Radiation bone marrow chimeras as a tool to study microglia turnover in normal brain and inflammation. *Clin Neuropathol* 12:284–285.
- Lawson LJ, Perry VH, Gordon S. 1992. Turnover of resident microglia in the normal adult mouse brain. *Neuroscience* 48:405–415.
- Lehnardt S. 2010. Innate immunity and neuroinflammation in the CNS: The role of microglia in Toll-like receptor-mediated neuronal injury. *Glia* 58:253–263.
- Lichanska AM, Browne CM, Henkel GW, Murphy KM, Ostrowski MC, McKercher SR, Maki RA, Hume DA. 1999. Differentiation of the mononuclear phagocyte system during mouse embryogenesis: The role of transcription factor PU.1. *Blood* 94:127–138.
- Lichanska AM, Hume DA. 2000. Origins and functions of phagocytes in the embryo. *Exp Hematol* 28:601–611.
- Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, Halenbeck R, Wu G, Zhou A, Behrens D, Hollenbaugh D, Linnemann T, Qin M, Wong J, Chu K, Doberstein SK, Williams LT. 2008. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. *Science* 320:807–811.
- Ling EA. 1979. Transformation of monocytes into amoeboid microglia in the corpus callosum of postnatal rats, as shown by labelling monocytes by carbon particles. *J Anat* 128:847–858.
- McKercher SR, Torbett BE, Anderson KL, Henkel GW, Vestal DJ, Barbault H, Klemsz M, Feeney AJ, Wu GE, Paige CJ, Maki R. 1996. Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. *EMBO J* 15:5647–5658.
- Merad M, Manz MG, Karsunky H, Wagers A, Peters W, Charo I, Weissman IL, Cyster JG, Engleman EG. 2002. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nat Immunol* 3:1135–1141.
- Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, Heikenwalder M, Bruck W, Priller J, Prinz M. 2007. Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci* 10:1544–1553.
- Mizuno T, Doi Y, Mizoguchi H, Jin S, Noda M, Sonobe Y, Takeuchi H, Suzumura A. 2011. Interleukin-34 selectively enhances the neuroprotective effects of microglia to attenuate oligomeric amyloid-beta neurotoxicity. *Am J Pathol* 179:2016–2027.
- Neumann H, Takahashi K. 2007. Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis. *J Neuroimmunol* 184:92–99.
- Olson JK, Miller SD. 2004. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. *J Immunol* 173:3916–3924.
- Orkin SH, Zon LI. 2008. Hematopoiesis: An evolving paradigm for stem cell biology. *Cell* 132:631–644.
- Otero K, Turnbull IR, Poliani PL, Vermi W, Cerutti E, Aoshi T, Tassi I, Takai T, Stanley SL, Miller M, Shaw AS, Colonna M. 2009. Macrophage colony-stimulating factor induces the proliferation and survival of macrophages via a pathway involving DAP12 and beta-catenin. *Nat Immunol* 10:734–743.
- Paloneva J, Kestila M, Wu J, Salminen A, Bohling T, Ruotsalainen V, Hakola P, Bakker AB, Phillips JH, Pekkarinen P, Lanier LL, Timonen T, Peltonen L. 2000. Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. *Nat Genet* 25:357–361.
- Phan TG, Green JA, Gray EE, Xu Y, Cyster JG. 2009. Immune complex relay by subcapsular sinus macrophages and noncognate B cells drives antibody affinity maturation. *Nat Immunol* 10:786–793.
- Priller J, Flugel A, Wehner T, Boentert M, Haas CA, Prinz M, Fernandez-Klett F, Prass K, Bechmann I, de Boer BA, Frotscher M, Kreutzberg GW, Persons DA, Dirnagl U. 2001. Targeting gene-modified hematopoietic cells to the central nervous system: Use of green fluorescent protein uncovers microglial engraftment. *Nat Med* 7:1356–1361.
- Prinz M, Priller J, Sisodia SS, Ransohoff RM. 2011. Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat Neurosci* 14:1227–1235.
- Ransohoff RM, Perry VH. 2009. Microglial physiology: Unique stimuli, specialized responses. *Annu Rev Immunol* 27:119–145.
- Samokhvalov IM, Samokhvalova NI, Nishikawa S. 2007. Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. *Nature* 446:1056–1061.
- Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SE, Pollard JW, Frampton J, Liu KJ, Geissmann F. 2012. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 336:86–90.
- Simard AR, Rivest S. 2004. Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. *FASEB J* 18:998–1000.
- Vallieres L, Sawchenko PE. 2003. Bone marrow-derived cells that populate the adult mouse brain preserve their hematopoietic identity. *J Neurosci* 23:5197–5207.
- van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. 1972. The mononuclear phagocyte system: A new classification of macrophages, monocytes, and their precursor cells. *Bull World Health Organ* 46:845–852.
- Varol C, Landsman L, Fogg DK, Greenshtein L, Gildor B, Margalit R, Kalchenko V, Geissmann F, Jung S. 2007. Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med* 204:171–180.
- Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, Barrow AD, Diamond MS, Colonna M. 2012. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nat Immunol* 13:753–760.
- Wegiel J, Wisniewski HM, Dziejatkowski J, Tarnawski M, Kozielski R, Trenkner E, Wiktor-Jedrzejczak W. 1998. Reduced number and altered morphology of microglial cells in colony stimulating factor-1-deficient osteopetrotic op/op mice. *Brain Res* 804:135–139.
- Wei S, Nandi S, Chitu V, Yeung YG, Yu W, Huang M, Williams LT, Lin H, Stanley ER. 2010. Functional overlap but differential expression of CSF-1 and IL-34 in their CSF-1 receptor-mediated regulation of myeloid cells. *J Leukoc Biol* 88:495–505.
- Wiktor-Jedrzejczak W, Bartocci A, Ferrante AW Jr, Ahmed-Ansari A, Sell KW, Pollard JW, Stanley ER. 1990. Total absence of colony-stimulating factor 1 in the macrophage-deficient osteopetrotic (op/op) mouse. *Proc Natl Acad Sci USA* 87:4828–4832.