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Ontogeny and homeostasis of CNS myeloid cells

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Myeloid cells in the central nervous system (CNS) represent a heterogeneous class of innate immune cells that contribute to the maintenance of tissue homeostasis differentially during development and adulthood. The subsets of CNS myeloid cells identified so far, including parenchymal microglia and non-parenchymal meningeal, perivascular and choroid-plexus macrophages, as well as disease-associated monocytes, have classically been distinguished on the basis of their surface epitope expression, localization and morphology. However, studies using cell-specific targeting, *in vivo* imaging, single-cell expression analysis and other sophisticated tools have now increased the depth of knowledge of this immune-cell compartment and call for reevaluation of the traditional views on the origin, fate and function of distinct CNS myeloid subsets. The concepts of CNS macrophage biology that are emerging from these new insights have broad implications for the understanding and treatment of CNS diseases.

Microglia have historically dominated the field of neuroimmunology, perhaps because they are the only myeloid cell type in the central nervous system (CNS) parenchyma under steady-state conditions. However, there are many other types of CNS myeloid cells. These largely neglected non-parenchymal counterparts include leptomeningeal, perivascular and choroid-plexus macrophages and have important functions in health and disease[1,2]. Collectively, these CNS myeloid cells belong to the larger family of mononuclear phagocytes, which also includes peripheral-tissue-specific macrophages, various subsets of dendritic cells and circulating monocytes[3,4]. Macrophages are present in most organs and compartments of the body and, as innate immune cells, are fundamental effectors and regulators of immune responses. Moreover, macrophages have a crucial role in maintaining tissue homeostasis during development and in adulthood[1,2]. Despite the similarities of microglia with various other tissue-resident macrophages, two remarkable properties of microglia are their restricted prenatal origin and their longevity. In contrast to microglia, circulating monocytes and other tissue macrophages in organs such the heart[5], aorta[6], spleen, lungs and intestine[3] are continually replaced by circulating myeloid cells after birth. Intriguingly, studies now suggest that the family ties between microglia and other CNS macrophages are much closer than previously appreciated. Macrophages at the CNS interfaces, particularly perivascular macrophages, share the prenatal origin, the lack of replacement by circulating blood cells, and major parts of the transcriptional profile of microglia[7]. The longevity of microglia and these non-parenchymal CNS macrophages makes them particularly vulnerable to environmental insults. The maintenance of these populations also requires tight control of their self-renewal during tissue development and homeostasis. However, the precise mechanisms for the self-renewal of CNS macrophages are not yet understood.

Microglia were first decribed by Pío del Río-Hortega in 1919, but their origin, fate and functions remained unclear for many decades[8–10]. The existence of non-parenchymal CNS macrophages was first recognized in 1921, when the Austrian physiologist Walter Kolmer described epiplexus cells (later called 'Kolmer cells'), which sit on the ventricular surface of the choroid plexus[11]. The presence of additional macrophages in the choroid-plexus stroma and leptomeninges expressing F4/80$^+$ was shown later[12]. Perivascular macrophages were described first in 1988 as 'perivascular microglia'[13] but were defined soon after as 'perivascular macrophages'[14].

CNS macrophages have been characterized and classified mainly according to their localization, morphology and surface-marker expression and *in vitro* responses. All myeloid cells residing in the healthy CNS share several surface markers, such as Iba-1, F4/80, CD11b and the fractalkine receptor CX3CR1, so their morphology and distinct localization and cellular neighbors in different anatomical sites of the brain have been used to distinguish the various subsets. For example, perivascular macrophages are found next to endothelial cells; choroid-plexus macrophages are adjacent to the epithelial cells or fibroblasts in these structures; meningeal macrophages are close to meningeal fibroblasts; and microglia are surrounded by neurons, astrocytes and oligodendrocytes[1,15]. Thus, perivascular macrophages are sandwiched between the laminin-positive endothelial and glial basement membranes[16]. In contrast, subdural meningeal macrophages are in close proximity to ER-TR7$^+$ fibroblast-like cells that line the meninges and meningeal vasculature[17]. Choroid-plexus macrophages are found exclusively in the stroma and epithelial layer of the choroid plexus. In addition to the localization, morphology and surface-marker expression of CNS macrophages, *in vitro* responses have also been used to distinguish CNS macrophage subsets. However, these properties

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neither do justice to the complexity of these cells nor truly reflect their functional repertoire in the healthy and diseased brain.

Several reviews have comprehensively discussed the role of myeloid cells in CNS disorders[18,19]. Here, we summarize how studies have now drastically remodeled the view of CNS macrophages as immune cells with characteristic functions. We also highlight other developments in the field, with a focus on the unique ontogeny and kinetics of CNS macrophages and their divergent functions in CNS homeostasis and disease.

The 'yolky' beginnings of CNS macrophages

The origin of CNS macrophages was controversial for a long time; however, studies of novel transgenic mouse models have shed new light on the origin of parenchymal microglia and have shown that they are derived exclusively from prenatal hematopoietic progenitor cells found in the yolk sac and fetal liver (**Fig. 1**). Most of these studies have taken advantage of mouse lines with tamoxifen-inducible expression of Cre recombinase, in which a fusion protein of the mouse estrogen receptor and Cre expressed from an estrogen-receptor-encoding gene (CreER) is expressed under the control of either hematopoietic-cell-specific promoters (*Runx1* or *Tie2*) or myeloid-cell-specific promoters (*Csf1r* or *Cx3cr1*)[20–29]. Crossing those mice with specific gene-reporter mouse lines, such as mice expressing yellow fluorescent protein (YFP) from the ubiquitous *Rosa26* locus (*R26*-YFP), with a *lox*P-flanked 'stop' cassette that is eliminated in the progeny via tamoxifen-induced Cre expression, enables sophisticated fate-mapping studies at developmental stages of interest. However, all such studies have focused on parenchymal microglia and have completely neglected the origin of macrophages in the CNS interfaces: the subdural meninges, choroid plexus and perivascular space.

Whereas some historical studies initially investigated the kinetics and presumably the origin of perivascular and subdural meningeal macrophages[13,30–34], less attention was paid to choroid-plexus macrophages[35]. Notably, most of these studies were based on bone-marrow transplantation after whole-body irradiation of the recipients. Even under non-disease conditions, these experiments revealed that the donor-derived cells (identified, for example, by expression of green fluorescent protein) are localized in the perivascular space, leptomeninges and choroid plexus. However, it has been demonstrated that whole-body irradiation disrupts the blood–brain barrier (BBB) and leads to local priming, which results in an artificial attraction of injected bone-marrow cells into the CNS[36–39]. Thus, the results of such studies must be interpreted with caution. With the genetic fate-mapping tools now available, it has become possible to reconsider the origin of CNS macrophages.

The classic view that non-parenchymal CNS macrophages are derived from the bone marrow has been now disproved by fate-mapping experiments with mice with tamoxifen-inducible activation of Cre under control of the *Cx3cr1* locus that also express *R26*-YFP (*Cx3cr1*CreER:*R26*-YFP mice). In addition, those findings were confirmed by parabiosis experiments, *in vivo* imaging and single-cell RNA sequencing. The *Cx3cr1*CreER:*R26*-YFP line can be used to efficiently target both CX3CR1$^+$ tissue macrophages and circulating myeloid cells[7,24,29,40]. It has been found that microglia arise from an uncommitted CD31$^+$ c-kit$^+$ erythromyeloid precursor (EMP) cell located in the yolk sac, which further develops via the macrophage ancestor population A1 (CD45$^+$CX3CR1^{lo}F4/80^{lo}) into the A2 (CD45$^+$CX3CR1^{hi}F4/80^{hi}) progenitor population[23,25,41–43]. As the A2 macrophage ancestor population expresses CX3CR1, the application of tamoxifen to pregnant *Cx3cr1*CreER:*R26*-YFP mice at embryonic day 9.0 (E9.0) results in the recombination of CX3CR1$^+$ cells and therefore targets the A2 population of yolk-sac progenitor cells[7,29]. Through the use of this fate-mapping tool it has been shown that subdural meningeal, perivascular and also, to some extent, choroid-plexus macrophages are derived from CX3CR1$^+$ A2 progenitor cells before birth[7]. Additionally, it has been demonstrated that the transcription factor PU.1, which affects multiple lineages at an early stage during hematopoiesis, including the development of macrophages, is essential for the development of all CNS macrophages, whereas PU.1's heterodimer partner IRF8 ('interferon-regulatory factor 8') has a less-severe but distinct effect on the development of microglia[23,29,44,45] and meningeal macrophages[7] (**Fig. 1**). Similar to the development of microglia, the development of CNS macrophages does not require MYB, a master transcription factor of definitive hematopoiesis[7,21]. A published study has added another piece to the macrophage puzzle by demonstrating that MYB$^+$ EMP cells originate in the yolk sac and give rise to fetal monocytes in the fetal liver that then develop into tissue macrophages[42]. Despite the fact that all current fate-mapping studies suggest that microglia and CNS macrophage originate from yolk-sac EMP cells, the contribution of fetal-liver progenitor cells to the pool of inhabitant CNS myeloid cells in mammals is likely but has been shown so far only in zebrafish[26].

The application of tamoxifen to adult *Cx3cr1*CreER:*R26*-YFP mice has further demonstrated that subdural meningeal and perivascular macrophages are long-lived cells without any detectable exchange with circulating cells, which is comparable to parenchymal microglia[7]. In contrast, only choroid-plexus macrophages exhibit partial turnover over several months. The longevity of perivascular macrophages for years has been suggested by studies of mouse models of stroke, on the basis of the uptake of low-density lipoproteins and pinocytosis of India ink[46,47]. A similarly long life for perivascular macrophages and microglia in the human brain has been reported in a patient who received transplantation of umbilical cord blood that was tracked into the recipient's brain parenchyma[48].

In the mouse, only choroid-plexus macrophages have been found to be dependent on blood-derived immigrating Ly6C^{hi} monocytes, because their numbers are much lower in CCR2-deficient mice than in CCR2-sufficient mice, whereas perivascular and subdural meningeal macrophage populations remain unchanged[7]; this is in agreement with earlier reports on perivascular macrophages in *Ccr2*$^{-/-}$ mice[37].

In sum, non-parenchymal meningeal and perivascular CNS macrophages and microglia arise entirely from embryonic precursor cells without any input from the blood or bone marrow during adulthood. This unique situation, relative to that of other macrophages resident in most organs of the body, such as the heart and aorta[5,6], is most probably due to early closing of the BBB during embryogenesis, starting from E14.5 (ref. 49), that prevents further influx of myeloid cells into the healthy CNS. The astonishing finding of such close ontogeny between microglia and the other macrophages in the CNS prompts the question of whether non-parenchymal CNS macrophages should not simply be considered as microglia present in diverse regions of the brain.

Immunosurveillance functions of CNS macrophages

Macrophages at CNS interfaces not only support CNS development, homeostasis and function but also have important pathophysiological roles[15,50,51]. At around day 9.5 of embryonic development, which is when CNS vascularization starts, CX3CR1$^+$F4/80$^+$ myeloid cells infiltrate the brain through the pial surface and migrate along the abluminal surface of penetrating vessels[20]. Microglia and perivascular macrophages are therefore uniquely positioned to influence the early sprouting, migration, anastomosis and refinement of the growing CNS vasculature. Indeed, studies of angiogenesis after macrophage

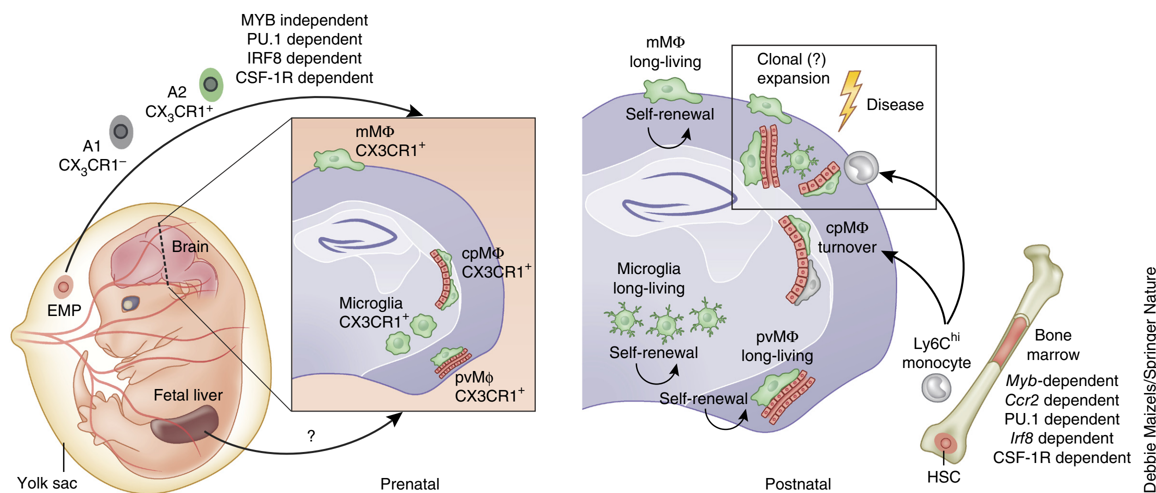

Figure 1 Origin and turnover of tissue macrophages in healthy CNS. CNS macrophages are derived from prenatal sources (left) and have no exchange with blood cells during in the healthy adult brain (right). A transient early wave of myeloid cell development called 'primitive hematopoiesis' (left) takes place at E7.0–E8.0. At this time, EMP cells develop in blood islands of the yolk sac. Their progeny (myeloid precursor cells) further proliferate, differentiate and populate several tissues, including the brain (left). CX3CR1+ A2 progenitor cells derive from c-Kit+CX3CR1− A1 cells and differentiate within the brain into microglia, perivascular macrophages (pvMΦ), meningeal macrophages (mMΦ) and choroid-plexus macrophages (cpMΦ). Factors important for proper CNS macrophage development are PU.1, IRF8 and CSF-1R, whereas all these cells develop independently of MYB. During further development, myelopoiesis is taken over by progenitor cells found from E12.5 in the fetal liver (which is part of the process of definitive hematopoiesis). Maturing myeloid cells continue to engraft in all tissues beyond E14.5–E15.5; however, due to the BBB, the microglia and largely non-parenchymal CNS macrophages are thought to be excluded from a fetal contribution. Starting around birth (right), myelopoiesis is thought to be restricted to bone marrow. Whereas choroid-plexus macrophages are the only cells with a substantial contribution from bone-marrow progenitors, meningeal macrophages and perivascular macrophages exhibit extreme longevity and self-renewal potential. Bone-marrow cells can enter CNS compartments only under disease conditions or following irradiation. HSC, hematopoietic stem cell.

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depletion, or in mice unable to produce microglia or perivascular macrophages, strongly support this concept[52]. Other studies of the branching of vascular sprouts in hindbrains of mice with homozygous deficiency in the gene encoding PU.1 have suggested that microglia and perivascular macrophages might bridge vascular sprouts during CNS development[53].

Perivascular macrophages promote vascular anastomoses not only during development[53] but also after the appearance of microlesions in blood vessels[54]. These cells might also have a surveillance role during homeostasis[19,46,55]. During adulthood, macrophages in the leptomeninges, choroid plexus and perivascular space are important effector and regulatory cells for the immune response of the CNS (**Fig. 2**). In the healthy CNS, only very few peripheral immune cells (T cells, B cells and monocytes) can reach the perivascular, leptomeningeal and ventricular space. The glia limitans strictly limits access of these cells to the CNS parenchyma under normal conditions[56]. However, neuroinflammatory conditions trigger defined molecular mechanisms that allow peripheral immune cells to breach the glia limitans and enter the CNS parenchyma[57,58].

Numerous immunological functions have been attributed to perivascular and subdural meningeal macrophages solely on the basis of their expression of immunological molecules. Early studies demonstrated, through the use of bone-marrow-chimeric rats, that these cells are the antigen-presenting cells of the CNS[13]. In addition to the immunological activation of perivascular macrophages in response to inflammation or neuronal injury[59], several reports have describe populations of perivascular and leptomenigeal macrophages that can perform phagocytosis or respond to cytokines and lipopolysaccharide in the peripheral blood[46,60]. Furthermore, perivascular and leptomenigeal macrophages are thought to be protective during bacterial meningitis[61]. Similarly, perivascular macrophages are thought to 'preferentially' take up viruses or pathogens into the CNS via the chemokine receptors CCR4 and CCR5 (ref. 62). In a mouse model of Alzheimer's disease, depletion of perivascular macrophages significantly increased the amyloid load on the cerebral vessels, indicative of an essential role for perivascular macrophages in neurodegeneration[63]. Likewise, perivascular macrophages have been found to be crucial for the cerebrovascular dysfunction of hypertension[64].

The choroid plexus is a multifunctional structure composed of a villous layer of cuboid epithelia surrounding an inner stroma with an extensive network of fenestrated capillaries that extend into the cerebrospinal fluid (CSF)-filled spaces of the third, fourth and lateral ventricles[65]. Choroid-plexus macrophages are located on the apical side of the choroid plexus epithelium facing the CSF and in the stroma. Like perivascular macrophages and microglia, these cells are also considered to be functional antigen-presenting cells[66,67] and are thought to have critical roles in immunosurveillance and regulation and in the phagocytosis of cellular debris and antigens in the CSF[68].

Although the full extent of their physiological role is still unknown, non-parenchymal CNS macrophages have been shown to act as antigen-presenting cells, perform phagocytosis and respond to CNS inflammation, neurodegeneration and peripheral inflammation.

Distinct transcriptional profiles of CNS macrophages

Because the brain is separated from the periphery by the BBB, CNS macrophages differ considerably from their peripheral-tissue macrophage relatives in gene-expression profile and chromatin state[69–72]. In addition to known genes that encode some common macrophage-related markers such as CD11b, CX3CR1, Iba-1, MERTK (which is important for the phagocytosis of apoptotic cells), and the master transcription factor PU.1, putative microglia-specific genes such as *P2ry12*, *Fcrls*, *Hexb*, *Tmem119*, *Tgfbr1 and Sall1* have been identified in large gene-expression studies[69,71,73–77] (**Fig. 2**). Notably, all those studies used bulk RNA for transcriptome analysis and did not include non-parenchymal

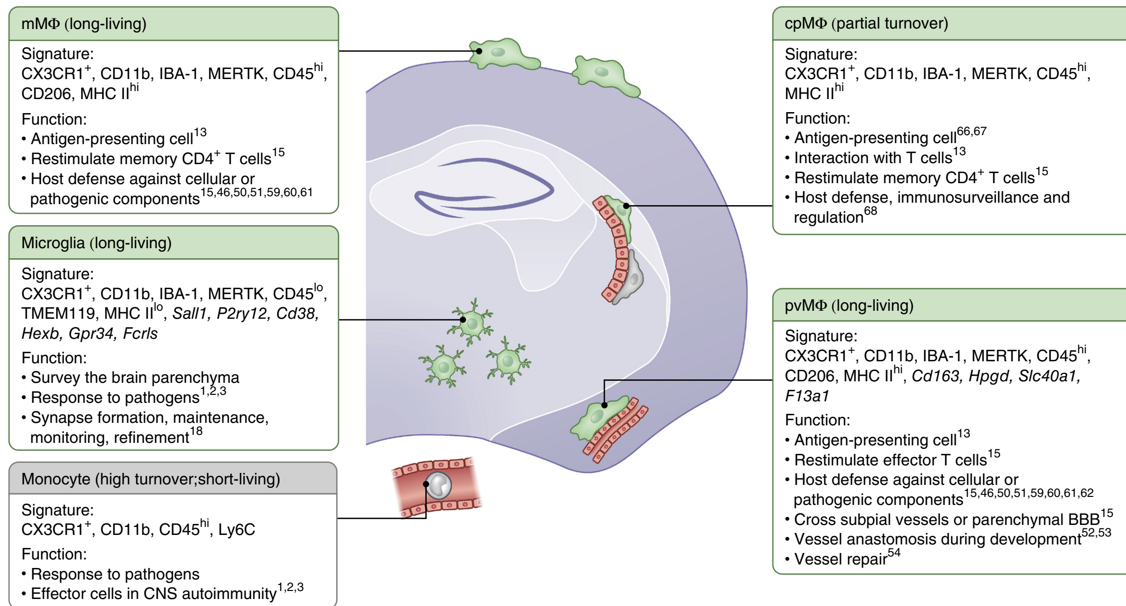

Figure 2 The localization and genetic signature of CNS macrophages. All CNS macrophages, as well as monocytes, share several surface markers, such as CD11b, Iba-1, MERTK and CX3CR1. Microglia are characterized by low expression of CD45 and their suggested unique expression (at least within the CNS) of P2RY12, Sall1 and Tmem119. In contrast, non-parenchymal macrophages have high expression of CD45 and major histocompatibility complex class II (MHC II). Moreover, perivascular macrophages and meningeal macrophages have high expression of CD206. CNS macrophages can be distinguished from monocytes by the expression of Ly6C, a marker present on monocytes but not on macrophages. All CNS macrophages have important roles in immunological defense.

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macrophages. Other single-cell RNA-sequencing studies have revealed highly cell-type-specific gene markers for microglia (*P2ry12*, *Gpr34*, *Cd83 and Hexb*) and perivascular macrophages (*Cd163*, *Hpgd*, *Mrc1*, *Slc40a1 and F13a1*)[7,28,75]. Selective expression of the receptor P2Y12 on microglia and not on perivascular or leptomeningeal macrophages has been confirmed in humans[78].

Non-parenchymal CNS macrophages have higher expression (relative to that in microglia) of the transmembrane tyrosine phosphatase CD45 and major histocompatibility complex class II; the latter suggests an antigen-presenting role for these cells[79]. Furthermore, perivascular and meningeal macrophages are characterized by their expression of the endocytic pattern-recognition receptor CD206 (also known as mannose receptor C type 1 or MRC1)[7]. Moreover, mainly perivascular macrophages and some meningeal and choroid-plexus macrophages have been shown to express the scavenger receptor CD163 (refs. 7,75,80,81). However, comparison of perivascular macrophages and microglia to bone-marrow-derived peritoneal macrophages or circulating monocytes has clearly highlighted a close relationship of CNS macrophages with each other that is independent of their peripheral counterparts[7,75]. Despite their close relationship, shared origin, and longevity, it has been shown that the microenvironment[70,71] and even physiological host microbiota-derived factors[43,82,83] shape macrophage properties. This fact makes it likely that the different compartments shape CNS macrophage signatures.

The purity of CNS macrophages preparations is of paramount importance for studies characterizing their gene expression and function. Contamination with other myeloid cells is a major problem, especially when widely expressed markers such as CD11b are used[84]. Likewise, microglial gene-targeting methods often inadvertently target other mononuclear phagocytes. The suboptimal specificity of such methods is therefore still a major obstacle in the investigation of microglia-specific biology *in vivo*. For example, use of the tamoxifen-inducible *Cx3cr1*CreER line also leads to the labeling of long-lived non-parenchymal CNS macrophages and therefore should not be considered 'microglia specific'. Combination of that line with the inducible diphtheria toxin receptor results in efficient depletion of microglia[85], but it remains unknown whether non-parenchymal macrophages additionally undergo depletion in this model. The results of studies using the *Cx3cr1*CreER line should therefore be interpreted with caution. Other microglial gene-targeting methods might provide a more specific alternative to the *Cx3cr1*CreER system. For example, *Sall1* encodes a zinc-finger transcription factor with high expression not only by microglia in the CNS[71,73] but also by cells of the liver, kidneys and heart[76]. The *Sall1*CreER line has been suggested to result in specific labeling of microglia in the CNS without targeting other CNS macrophages or, remarkably, infiltrating myeloid cells[76]. Tmem119 is a transmembrane protein of unknown function that has also been suggested to be a microglia-specific marker in mice and humans[86,87]. However, Tmem119 might not be expressed by all human microglia in different brain regions and during development[87]. Although it has been convincingly shown that Tmem119 is not expressed by infiltrating macrophages, the absence of Tmem119 expression in other CNS-resident macrophages still needs to be demonstrated[86].

In sum, there is an urgent need to elucidate in detail CNS myeloid-cell-specific features during health and disease. The discovery of specific expression signatures in CNS macrophage subsets will prove invaluable for the design of genetic methods to specifically targets these cell types.

Microgliopathies or macrophagopathies?

CNS-resident macrophages are involved in almost all brain diseases, ranging from neurodevelopmental disorders to neuroinflammatory, neurodegenerative, neurooncological and neuropsychiatric disorders[1]. In such diseases, CNS myeloid-cell responses include a plethora of characteristic macrophage functions such as phagocytosis,

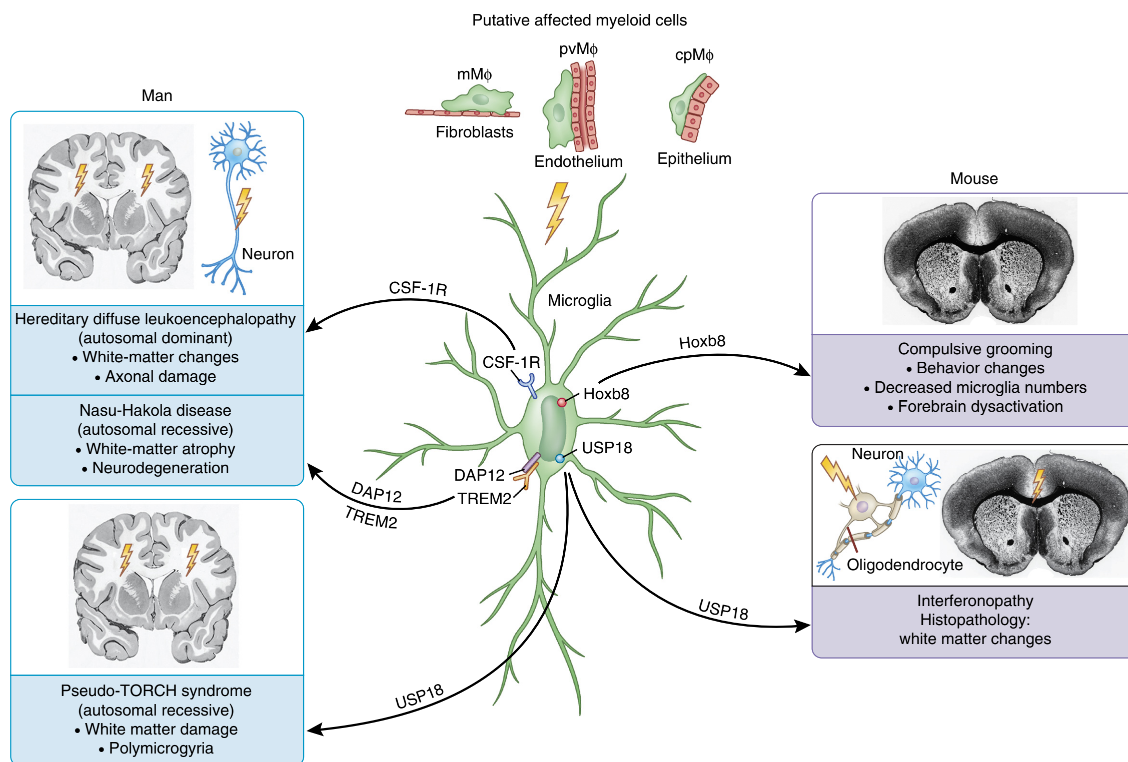

Figure 3 Myeloid-cell dysfunction in the adult CNS. Several mutations in microglia and CNS-resident macrophages, encompassing genes encoding CSF-1R plus TREM2 in humans or Hoxb8 plus USP18 in mice, are causative for a distinct group of monogenetic neurodegenerative brain diseases. Middle, localization of surface receptors (CSF-1R and TREM2) and cytoplasmic factors (USP18) or nuclear factors (Hoxb8) in microglia (center), leptomeningeal macrophages, perivascular macrophages and choroid-plexus macrophages. Loss of nutritive function of microglia and other CNS macrophages leads to pronounced neuropathological alterations, such as axonal damage accompanied by neuronal loss and white-matter changes initiated by oligodendrocytic changes. Notably, the role of non-parenchymal macrophages in these diseases is highly probable. TORCH, toxoplasmosis, other agents, rubella, cytomegalovirus and herpes simplex virus.

antigen presentation and the production of immunomodulatory factors. These properties make them attractive targets for the modulation of CNS diseases[88].

Their extreme longevity and the lack of replacement by peripheral myeloid cells (with the sole exception of choroid-plexus macrophages) render CNS-resident macrophages exquisitely vulnerable to developmental disturbances, aging and cell-intrinsic alterations. In fact, emerging evidence suggests that macrophage homeostasis can be strongly influenced by single-gene defects. The conditions in which usually single-gene mutations occur in the myeloid lineage are extremely rare, but given the importance of microglia and other CNS macrophages in physiological brain function, it is not surprising that an increasing number of macrophage-related genes associated with neuropsychiatric or neurological disorders have now been identified. In this expanding group of brain diseases, classically called 'microgliopathies', single-gene mutations in microglia were once thought to severely derail their function and drive subsequent CNS pathologies[1,89,90] (**Fig. 3**). However, with increasing understanding of the close family ties between CNS macrophages, the possible contribution of non-parenchymal macrophages to neurological diseases classically attributed to mutations in microglia needs to be carefully considered.

Hereditary diffuse leukoencephalopathy with spheroids was first described in 2012 as an autosomal dominant CNS disease induced by various mutations in the gene locus encoding the tyrosine-kinase domain of the cytokine receptor CSF-1R (http://www.omim.org/entry/221820)[90]. In the brain, CSF-1R is expressed predominantly by resident myeloid cells such as microglia, but it is also expressed on non-microglia macrophages. Although the brain lesions are located mainly in the white matter, the clinical symptoms are remarkably heterogeneous; patients suffer from personality and behavioral changes, dementia, depression, parkinsonism and seizures, among several other symptoms. Patients often die of dementia within 6 years of onset, and their brains display characteristic features such as demyelination, strongly activated astrocytes and the eponymous neurofilament-positive axonal spheroids as correlates of neuronal loss. These neuropathological features could be explained by dysfunctional microglia, but vascular changes that lead to stroke-like episodes[91] might arise from the impairment of perivascular macrophage function. However, it should also be noted that CSF-1R is expressed by a subset of neural progenitor cells and has been linked to neural differentiation[92]. In addition, a small number of neurons in the adult have also been suggested to express CSF-1R under physiological conditions and to upregulate CSF-1R expression after neural injury[93]. Whether putative neuronal mutations in the gene encoding CSF-1R contribute to disease is currently unclear.

Another disease ascribed to mutations in CNS myeloid cells is Nasu-Hakola disease; there are about 200 reported cases of this worldwide[94,95]. It is an autosomal recessive chronic neurodegenerative disease characterized by psychotic symptoms and bone cysts (http://www.omim.org/entry/221770)[94,95]. The pathogenesis of this disease is loss-of-function mutations in either *Tryobp* (which encodes the accessory protein DAP12) or *Trem2* (which encodes the cell-surface receptor TREM2)[96]. DAP12 is an adaptor that bears an immunoreceptor tyrosine-based activation motif that couples to CSF-1R and several

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other receptors. This again underlines the importance of CSF-1R signaling for microglial homeostasis. DAP12 can also form a receptor signaling complex with TREM2 and triggers activation of immune responses in macrophages. Because osteoclasts and potentially other non-parenchymal brain macrophages also display high expression of TREM2, they might also be key participants in the pathogenesis of Nasu-Hakola disease[97].

Mutation of *Hoxb8* (which encodes the transcription factor Hoxb8) in mice leads to an excessive grooming phenotype similar to the obsessive-compulsive disorder trichotillomania (http://www.omim.org/entry/142963)[98,99]. In fact, resident microglia are the only Hoxb8-expressing cells in the CNS parenchyma and are therefore thought to be critically involved in pathogenesis[100]. Notably, the compulsive grooming behavior of *Hoxb8*-deficient mice was restored through the transplantation of wild-type bone marrow. Given the contribution of bone marrow cells to perivascular and meningeal macrophage populations but not to microglial populations following irradiation[7,32], we are tempted to speculate that non-microglia macrophages contribute to the CNS pathology and the pathological grooming behavior caused by *Hoxb8* mutations.

The concept of human 'interferonopathies' as a broader set of Mendelian disorders, in which a constitutive upregulation of the activity of type I interferons directly relates to disease pathology, has now been established[101]. Several mutations have been discovered in this disease entity. The ubiquitin-specific peptidase USP18 has been discovered as a microglia-specific protein that leads to a fatal encephalopathy mediated by the type I interferon receptor IFNAR1 (ref. 102). Interestingly, in mice, *Usp18* has been found to be important only for white-matter microglia, suggestive of region-specific vulnerability. Autosomal recessive mutations in *USP18* have been identified in a few patients that display, in addition to a strong type I interferon signature in the brain parenchyma, extensive angiopathies and intracerebral bleeding, which again points to involvement of perivascular macrophages in human CNS pathologies[103].

In conclusion, evidence increasingly supports the possibility of a potential pathophysiological role for both microglia and non-parenchymal CNS macrophages in the pathogenesis of distinct monogenetic neurodegenerative disorders. Thus, both types of macrophages might be useful targets for cell-specific therapeutic approaches for these disorders.

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Conclusions

Here we have summarized the evidence of close family ties between microglia and non-parenchymal CNS macrophages. Until now, it was thought that non-parenchymal CNS macrophages are derived from bone marrow and undergo modest but constant replacement by short-living blood monocytes. It is now known that microglia share their prenatal origin with leptomeningeal, perivascular-space and choroid-plexus macrophages and that the majority of these populations receive no contribution from the bone marrow but are instead long-lived cells with considerable self-renewal capacity.

The heterogeneous population of cells localized to CNS interfaces includes macrophages, dendritic cells responsive to the cytokine receptor FLT3 (ref. 104), and leptomeningeal mesothelial cells, which, in combination with pericytes and astrocytic foot processes, contribute to the formation of the immunological BBB[58]. Several immunological functions have been ascribed to macrophages at CNS interfaces. However, assessing the precise immunological and non-immunological functions of these cells *in vivo* is still hampered by their scarcity and the dearth of specific methods of isolation and targeting. Unbiased high-throughput gene-expression profiling and other molecular–biological tools are bringing about a renaissance in myeloid-cell research. Single-cell RNA-sequencing studies have revealed unique expression profiles of CNS macrophage subsets that will undoubtedly spur the development of new tools with which to target and analyze these cells in detail. With these developments, researchers are at the cusp of a deeper appreciation of the diverse properties and functions of microglia and their non-parenchymal siblings during development, homeostasis and disease.

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COMPETING FINANCIAL INTERESTS
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1. Prinz, M. & Priller, J. Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nat. Rev. Neurosci.* **15**, 300–312 (2014).
2. Prinz, M., Priller, J., Sisodia, S.S. & Ransohoff, R.M. Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat. Neurosci.* **14**, 1227–1235 (2011).
3. Ginhoux, F. & Jung, S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat. Rev. Immunol.* **14**, 392–404 (2014).
4. Gomez Perdiguero, E., Schulz, C. & Geissmann, F. Development and homeostasis of 'resident' myeloid cells: the case of the microglia. *Glia* **61**, 112–120 (2013).
5. Molawi, K. *et al.* Progressive replacement of embryo-derived cardiac macrophages with age. *J. Exp. Med.* **211**, 2151–2158 (2014).
6. Ensan, S. *et al.* Self-renewing resident arterial macrophages arise from embryonic CX3CR1+ precursors and circulating monocytes immediately after birth. *Nat. Immunol.* **17**, 159–168 (2016).
7. Goldmann, T. *et al.* Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nat. Immunol.* **17**, 797–805 (2016).
8. del Rio-Hortega, P. El 'tercer elemento' de los centros nerviosus. I. La microglia en estado normal. II. Intervencion de la microglia en los procesos patologicos (Celulas en bastoncito y cuerpos granuloadi-posos). III. Naturaleza probable de la microglia. *Bol. R. Soc. Esp. Hist. Nat. Secc. Biol.* **9**, 68–120 (1919).
9. del Rio-Hortega, P. Microglia in *Cytology and Cellular Pathology of the Nervous System* (ed. Peneld, W.) 483–534 (P.B. Hoeber, New York, 1932).
10. Tremblay, M.E., Lecours, C., Samson, L., Sanchez-Zafra, V. & Sierra, A. From the Cajal alumni Achucarro and Rio-Hortega to the rediscovery of never-resting microglia. *Front. Neuroanat.* **9**, 45 (2015).
11. Kolmer, W. Über eine eigenartige Beziehung von Wanderzellen zu den Chorioidealplexus des Gehirns der Wirbeltiere. *Anat. Anz.* **54**, 15–19 (1921).
12. Perry, V.H., Hume, D.A. & Gordon, S. Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. *Neuroscience* **15**, 313–326 (1985).
13. Hickey, W.F. & Kimura, H. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science* **239**, 290–292 (1988).
14. Graeber, M.B., Streit, W.J. & Kreutzberg, G.W. Identity of ED2-positive perivascular cells in rat brain. *J. Neurosci. Res.* **22**, 103–106 (1989).
15. Ransohoff, R.M. & Cardona, A.E. The myeloid cells of the central nervous system parenchyma. *Nature* **468**, 253–262 (2010).
16. Galea, I. *et al.* Mannose receptor expression specifically reveals perivascular macrophages in normal, injured, and diseased mouse brain. *Glia* **49**, 375–384 (2005).
17. Kim, J.V., Kang, S.S., Dustin, M.L. & McGavern, D.B. Myelomonocytic cell recruitment causes fatal CNS vascular injury during acute viral meningitis. *Nature* **457**, 191–195 (2009).
18. Ransohoff, R.M. How neuroinflammation contributes to neurodegeneration. *Science* **353**, 777–783 (2016).
19. Ransohoff, R.M. & Perry, V.H. Microglial physiology: unique stimuli, specialized responses. *Annu. Rev. Immunol.* **27**, 119–145 (2009).
20. Ginhoux, F. *et al.* Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**, 841–845 (2010).
21. Schulz, C. *et al.* A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* **336**, 86–90 (2012).
22. Hashimoto, D. *et al.* Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* **38**, 792–804 (2013).

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23. Kierdorf, K. *et al.* Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat. Neurosci.* **16**, 273–280 (2013).
24. Yona, S. *et al.* Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* **38**, 79–91 (2013).
25. Gomez Perdiguero, E. *et al.* Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* **518**, 547–551 (2015).
26. Xu, J. *et al.* Temporal-spatial resolution fate mapping reveals distinct origins for embryonic and adult microglia in zebrafish. *Dev. Cell* **34**, 632–641 (2015).
27. Sheng, J., Ruedl, C. & Karjalainen, K. Most tissue-resident macrophages except microglia are derived from fetal hematopoietic stem cells. *Immunity* **43**, 382–393 (2015).
28. Mass, E. *et al.* Specification of tissue-resident macrophages during organogenesis. *Science* http://dx.doi.org/10.1126/science.aaf4238 (4 August 2016).
29. Hagemeyer, N. *et al.* Transcriptome-based profiling of yolk sac-derived macrophages reveals a role for Irf8 in macrophage maturation. *EMBO J.* **35**, 1730–1744 (2016).
30. Hickey, W.F., Vass, K. & Lassmann, H. Bone marrow-derived elements in the central nervous system: an immunohistochemical and ultrastructural survey of rat chimeras. *J. Neuropathol. Exp. Neurol.* **51**, 246–256 (1992).
31. Kennedy, D.W. & Abkowitz, J.L. Kinetics of central nervous system microglial and macrophage engraftment: analysis using a transgenic bone marrow transplantation model. *Blood* **90**, 986–993 (1997).
32. Priller, J. *et al.* Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. *Nat. Med.* **7**, 1356–1361 (2001).
33. Flügel, A., Bradl, M., Kreutzberg, G.W. & Graeber, M.B. Transformation of donor-derived bone marrow precursors into host microglia during autoimmune CNS inflammation and during the retrograde response to axotomy. *J. Neurosci. Res.* **66**, 74–82 (2001).
34. Djukic, M. *et al.* Circulating monocytes engraft in the brain, differentiate into microglia and contribute to the pathology following meningitis in mice. *Brain* **129**, 2394–2403 (2006).
35. Vallieres, L. & Sawchenko, P.E. Bone marrow-derived cells that populate the adult mouse brain preserve their hematopoietic identity. *J. Neurosci.* **23**, 5197–5207 (2003).
36. Mildner, A. *et al.* Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat. Neurosci.* **10**, 1544–1553 (2007).
37. Mildner, A. *et al.* Distinct and non-redundant roles of microglia and myeloid subsets in mouse models of Alzheimer's disease. *J. Neurosci.* **31**, 11159–11171 (2011).
38. Ajami, B., Bennett, J.L., Krieger, C., Tetzlaff, W. & Rossi, F.M. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat. Neurosci.* **10**, 1538–1543 (2007).
39. Kierdorf, K., Katzmarski, N., Haas, C.A. & Prinz, M. Bone marrow cell recruitment to the brain in the absence of irradiation or parabiosis bias. *PLoS One* **8**, e58544 (2013).
40. Goldmann, T. *et al.* A new type of microglia gene targeting shows TAK1 to be pivotal in CNS autoimmune inflammation. *Nat. Neurosci.* **16**, 1618–1626 (2013).
41. Bertrand, J.Y. *et al.* Three pathways to mature macrophages in the early mouse yolk sac. *Blood* **106**, 3004–3011 (2005).
42. Hoeffel, G. *et al.* C-Myb^{+} erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity* **42**, 665–678 (2015).
43. Matcovitch-Natan, O. *et al.* Microglia development follows a stepwise program to regulate brain homeostasis. *Science* **353**, aad8670 (2016).
44. Masuda, T. *et al.* IRF8 is a critical transcription factor for transforming microglia into a reactive phenotype. *Cell Rep.* **1**, 334–340 (2012).
45. Minten, C., Terry, R., Deffrasnes, C., King, N.J. & Campbell, I.L. IFN regulatory factor 8 is a key constitutive determinant of the morphological and molecular properties of microglia in the CNS. *PLoS One* **7**, e49851 (2012).
46. Kida, S., Steart, P.V., Zhang, E.T. & Weller, R.O. Perivascular cells act as scavengers in the cerebral perivascular spaces and remain distinct from pericytes, microglia and macrophages. *Acta Neuropathol.* **85**, 646–652 (1993).
47. Kosel, S. *et al.* Long-lasting perivascular accumulation of major histocompatibility complex class II-positive lipophages in the spinal cord of stroke patients: possible relevance for the immune privilege of the brain. *Acta Neuropathol.* **94**, 532–538 (1997).
48. Takahashi, K. *et al.* Differentiation of donor-derived cells into microglia after umbilical cord blood stem cell transplantation. *J. Neuropathol. Exp. Neurol.* **74**, 862–866 (2015).
49. Daneman, R., Zhou, L., Kebede, A.A. & Barres, B.A. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* **468**, 562–566 (2010).
50. Brendecke, S.M. & Prinz, M. Do not judge a cell by its cover–diversity of CNS resident, adjoining and infiltrating myeloid cells in inflammation. *Semin. Immunopathol.* **37**, 591–605 (2015).
51. Williams, K., Alvarez, X. & Lackner, A.A. Central nervous system perivascular cells are immunoregulatory cells that connect the CNS with the peripheral immune system. *Glia* **36**, 156–164 (2001).
52. Checchin, D., Sennlaub, F., Levavasseur, E., Leduc, M. & Chemtob, S. Potential role of microglia in retinal blood vessel formation. *Invest. Ophthalmol. Vis. Sci.* **47**, 3595–3602 (2006).
53. Fantin, A. *et al.* Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. *Blood* **116**, 829–840 (2010).
54. Liu, C. *et al.* Macrophages mediate the repair of brain vascular rupture through direct physical adhesion and mechanical traction. *Immunity* **44**, 1162–1176 (2016).
55. Mato, M. *et al.* Involvement of specific macrophage-lineage cells surrounding arterioles in barrier and scavenger function in brain cortex. *Proc. Natl. Acad. Sci. USA* **93**, 3269–3274 (1996).
56. Prinz, M. & Priller, J. The role of peripheral immune cells in the CNS in steady state and disease. *Nat. Neurosci.* **20**, 136–144 (2017).
57. Engelhardt, B. & Ransohoff, R.M. Capture, crawl, cross: the T cell code to breach the blood-brain barriers. *Trends Immunol.* **33**, 579–589 (2012).
58. Engelhardt, B., Vajkoczy, P. & Weller, R.O. The movers and shapers in immune privilege of the CNS. *Nat. Immunol.* **18**, 123–131 (2017).
59. Streit, W.J., Graeber, M.B. & Kreutzberg, G.W. Expression of Ia antigen on perivascular and microglial cells after sublethal and lethal motor neuron injury. *Exp. Neurol.* **105**, 115–126 (1989).
60. Elmquist, J.K. *et al.* Intravenous lipopolysaccharide induces cyclooxygenase 2-like immunoreactivity in rat brain perivascular microglia and meningeal macrophages. *J. Comp. Neurol.* **381**, 119–129 (1997).
61. Polfliet, M.M. *et al.* Meningeal and perivascular macrophages of the central nervous system play a protective role during bacterial meningitis. *J. Immunol.* **167**, 4644–4650 (2001).
62. He, J. *et al.* CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* **385**, 645–649 (1997).
63. Hawkes, C.A. & McLaurin, J. Selective targeting of perivascular macrophages for clearance of beta-amyloid in cerebral amyloid angiopathy. *Proc. Natl. Acad. Sci. USA* **106**, 1261–1266 (2009).
64. Faraco, G. *et al.* Perivascular macrophages mediate the neurovascular and cognitive dysfunction associated with hypertension. *J. Clin. Invest.* **126**, 4674–4689 (2016).
65. Engelhardt, B., Wolburg-Buchholz, K. & Wolburg, H. Involvement of the choroid plexus in central nervous system inflammation. *Microsc. Res. Tech.* **52**, 112–129 (2001).
66. Schwartz, M. & Baruch, K. The resolution of neuroinflammation in neurodegeneration: leukocyte recruitment via the choroid plexus. *EMBO J.* **33**, 7–22 (2014).
67. Kunis, G., Baruch, K., Miller, O. & Schwartz, M. Immunization with a myelin-derived antigen activates the brain's choroid plexus for recruitment of immunoregulatory cells to the CNS and attenuates disease progression in a mouse model of ALS. *J. Neurosci.* **35**, 6381–6393 (2015).
68. Baruch, K., Kertser, A., Porat, Z. & Schwartz, M. Cerebral nitric oxide represses choroid plexus NFkappaB-dependent gateway activity for leukocyte trafficking. *EMBO J.* **34**, 1816–1828 (2015).
69. Gautier, E.L. *et al.* Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat. Immunol.* **13**, 1118–1128 (2012).
70. Gosselin, D. *et al.* Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* **159**, 1327–1340 (2014).
71. Lavin, Y. *et al.* Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* **159**, 1312–1326 (2014).
72. Becher, B. *et al.* High-dimensional analysis of the murine myeloid cell system. *Nat. Immunol.* **15**, 1181–1189 (2014).
73. Butovsky, O. *et al.* Identification of a unique TGF-β-dependent molecular and functional signature in microglia. *Nat. Neurosci.* **17**, 131–143 (2014).
74. Hickman, S.E. *et al.* The microglial sensome revealed by direct RNA sequencing. *Nat. Neurosci.* **16**, 1896–1905 (2013).
75. Zeisel, A. *et al.* Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science* **347**, 1138–1142 (2015).
76. Buttgereit, A. *et al.* Sall1 is a transcriptional regulator defining microglia identity and function. *Nat. Immunol.* **17**, 1397–1406 (2016).
77. Chiu, I.M. *et al.* A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. *Cell Rep.* **4**, 385–401 (2013).
78. Mildner, A., Huang, H., Radke, J., Stenzel, W. & Priller, J. P2Y12 receptor is expressed on human microglia under physiological conditions throughout development and is sensitive to neuroinflammatory diseases. *Glia* **65**, 375–387 (2017).
79. Lassmann, H., Zimprich, F., Vass, K. & Hickey, W.F. Microglial cells are a component of the perivascular glia limitans. *J. Neurosci. Res.* **28**, 236–243 (1991).
80. Linehan, S.A., Martinez-Pomares, L., Stahl, P.D. & Gordon, S. Mannose receptor and its putative ligands in normal murine lymphoid and nonlymphoid organs: In situ expression of mannose receptor by selected macrophages, endothelial cells, perivascular microglia, and mesangial cells, but not dendritic cells. *J. Exp. Med.* **189**, 1961–1972 (1999).
81. Polfliet, M.M., Fabriek, B.O., Daniels, W.P., Dijkstra, C.D. & van den Berg, T.K. The rat macrophage scavenger receptor CD163: expression, regulation and role in inflammatory mediator production. *Immunobiology* **211**, 419–425 (2006).
82. Erny, D. *et al.* Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* **18**, 965–977 (2015).

83. Erny, D., Hrabe de Angelis, A.L. & Prinz, M. Communicating systems in the body: how microbiota and microglia cooperate. *Immunology* **150**, 7–15 (2017).
84. Sharma, K. *et al.* Cell type- and brain region-resolved mouse brain proteome. *Nat. Neurosci.* **18**, 1819–1831 (2015).
85. Bruttger, J. *et al.* Genetic cell ablation reveals clusters of local self-renewing microglia in the mammalian central nervous system. *Immunity* **43**, 92–106 (2015).
86. Bennett, M.L. *et al.* New tools for studying microglia in the mouse and human CNS. *Proc. Natl. Acad. Sci. USA* **113**, E1738–E1746 (2016).
87. Satoh, J. *et al.* TMEM119 marks a subset of microglia in the human brain. *Neuropathology* **36**, 39–49 (2016).
88. Biber, K., Moller, T., Boddeke, E. & Prinz, M. Central nervous system myeloid cells as drug targets: current status and translational challenges. *Nat. Rev. Drug Discov.* **15**, 110–124 (2016).
89. Bianchin, M.M., Martin, K.C., de Souza, A.C., de Oliveira, M.A. & Rieder, C.R. Nasu-Hakola disease and primary microglial dysfunction. *Nat Rev Neurol* **6**, 2 p following 523 (2010).
90. Rademakers, R. *et al.* Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. *Nat. Genet.* **44**, 200–205 (2011).
91. Battisti, C. *et al.* Hereditary diffuse leukoencephalopathy with axonal spheroids: three patients with stroke-like presentation carrying new mutations in the CSF1R gene. *J. Neurol.* **261**, 768–772 (2014).
92. Nandi, S. *et al.* The CSF-1 receptor ligands IL-34 and CSF-1 exhibit distinct developmental brain expression patterns and regulate neural progenitor cell maintenance and maturation. *Dev. Biol.* **367**, 100–113 (2012).
93. Luo, J. *et al.* Colony-stimulating factor 1 receptor (CSF1R) signaling in injured neurons facilitates protection and survival. *J. Exp. Med.* **210**, 157–172 (2013).
94. Paloneva, J. *et al.* Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. *Nat. Genet.* **25**, 357–361 (2000).
95. Paloneva, J. *et al.* Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. *Am. J. Hum. Genet.* **71**, 656–662 (2002).
96. Neumann, H. & Takahashi, K. Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis. *J. Neuroimmunol.* **184**, 92–99 (2007).
97. Colonna, M. TREMs in the immune system and beyond. *Nat. Rev. Immunol.* **3**, 445–453 (2003).
98. Greer, P.L. & Greenberg, M.E. From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. *Neuron* **59**, 846–860 (2008).
99. Greer, J.M. & Capecchi, M.R. Hoxb8 is required for normal grooming behavior in mice. *Neuron* **33**, 23–34 (2002).
100. Chen, S.K. *et al.* Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell* **141**, 775–785 (2010).
101. Crow, Y.J. & Manel, N. Aicardi-Goutieres syndrome and the type I interferonopathies. *Nat. Rev. Immunol.* **15**, 429–440 (2015).
102. Goldmann, T. *et al.* USP18 lack in microglia causes destructive interferonopathy of the mouse brain. *EMBO J.* **34**, 1612–1629 (2015).
103. Meuwissen, M.E. *et al.* Human USP18 deficiency underlies type 1 interferonopathy leading to severe pseudo-TORCH syndrome. *J. Exp. Med.* **213**, 1163–1174 (2016).
104. Anandasabapathy, N. *et al.* Flt3L controls the development of radiosensitive dendritic cells in the meninges and choroid plexus of the steady-state mouse brain. *J. Exp. Med.* **208**, 1695–1705 (2011).

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Erratum: Ontogeny and homeostasis of CNS myeloid cells

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In the version of this article initially published, some of the arrows along the right side of Figure 1 were incorrect. The arrow from 'Ly6C^{hi} monocyte' to 'pvMΦ long-living' should be deleted, and an arrow should be added from 'Ly6C^{hi} monocyte' into the boxed area above (top right). The error has been corrected in the HTML and PDF versions of the article.

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