Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease

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Abstract | Mononuclear phagocytic cells in the CNS used to be defined according to their anatomical location and surface marker expression. Recently, this concept has been challenged by the results of developmental and gene expression profiling studies that have used novel molecular biological tools to unravel the origin of microglia and to define their role as specialized tissue macrophages with long lifespans. Here, we describe how these results have redefined microglia and helped us to understand how different myeloid cell populations operate in the CNS based on their cell-specific gene expression signatures, distinct ontogeny and differential functions. Moreover, we describe the vulnerability of microglia to dysfunction and propose that myelomonocytic cells might be used in the treatment of neurological and psychiatric disorders that are characterized by primary or secondary 'microgliopathy'.

Macrophages
Tissue-resident cells of the mononuclear phagocyte system that are characterized by their ability to phagocytose foreign particulate material, debris and colloidal material.

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doi:10.1038/nrn3722
Published online 9 April 2014

Microglia are tissue-resident macrophages in the CNS. They belong to a group of mononuclear phagocytes that comprises peripheral tissue macrophages, CNS-associated macrophages, dendritic cells and monocyte-derived cells[1,2]. As such, they are critical effectors and regulators of changes in CNS homeostasis during development and in health and disease.

All mononuclear cells originate from haematopoietic stem cells (HSCs) and develop along distinct differentiation pathways in response to endogenous and environmental cues[3,4]. The body's mononuclear phagocyte system was for a long time believed to derive from a subgroup of white blood cells called leukocytes that are produced from HSCs. It was assumed that bone marrow-derived monocytes, a subgroup of leukocytes, circulate in the blood and enter the tissues (where they differentiate into tissue-resident macrophages) in non-pathological conditions and during inflammation. However, this view has changed in recent years as a result of the discovery of new subtypes of mononuclear phagocytes that have different origins and distinct roles in CNS disorders[5]. Furthermore, the findings of recent population-based gene expression studies have highlighted macrophage heterogeneity[6–8].

Emerging evidence from such studies suggests that microglia differ considerably from the macrophages that reside in other tissues[6]. Indeed, some genes that distinguish them from their peripheral relatives have been identified[9]. Moreover, recent fate-mapping studies of several macrophage populations in the body have provided elegant evidence that, under homeostatic conditions, microglia are not derived from the bone marrow but originate from HSCs in the yolk sac[10–12]. Thus, the existing model of tissue macrophage development needs to be extended to highlight the different origins of many macrophage populations, including microglia.

Several other important distinctions between different macrophage populations have been identified. Novel myeloid-specific gene targeting techniques targeting CX3C chemokine receptor 1 (*Cx3cr1*; also known as the fractalkine receptor) have enabled researchers to examine the kinetics of myeloid cell turnover during homeostasis and disease[13–15]. These new transgenic approaches have helped to establish the major kinetic features of microglia that distinguish them from other macrophages: namely, that they are long-lived, that they are not normally replaced by peripheral cells from the circulation and that they are able to self-renew in a context-dependent manner to ensure cell expansion. Previous studies used genes such as *Cd11b* (also known as *Itgam*), lysozyme M (*Lysm*; also known as *Lyz2*) and ionized calcium-binding adaptor molecule 1 (*Iba1*; also known as *Aif1*) to target myeloid cells in the brain[16–18]. However, these experiments either used transgenic mouse lines that showed low levels

Mononuclear phagocytes
A mononuclear cell type of the myeloid lineage (macrophages, monocytes or dendritic cells) that have the ability to phagocytose.

Dendritic cells
Also known as an interdigitating reticular cells because of their branched morphology. Dendritic cells are the most potent stimulators of T cell responses.

Monocyte
A type of mononuclear leukocyte that is derived from the bone marrow and circulates in the bloodstream. Monocytes typically migrate into tissues, where they can differentiate into various types of macrophages.

Haematopoietic stem cells
(HSCs). Rare multipotent cells that give rise to all blood cells, including myeloid and lymphoid lineages.

of recombination in microglia compared with circulating myeloid cells (such as in the *Lysm*-Cre line)[13] or used the antiviral drug ganciclovir, which has tremendous drug-induced effects on microglia proliferation *in vivo*[19].

Bone marrow-derived phagocytes often have disease-related functions that are distinct from those of yolk sac-derived microglia[1]. The differential roles of microglia and other brain macrophages have tremendous clinical implications for the treatment of severe brain diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS; also known as Lou Gehrig's disease), multiple sclerosis and several psychiatric disorders[1,20,21]. In theory, if practical hurdles can be overcome, specific myeloid populations — such as phagocytes derived from the yolk sac, bone marrow or blood — might be used to deliver therapeutic molecules to the CNS in order to ameliorate disease.

In this Review, we provide an overview of the progress in our understanding of the origin, fate and function of microglia and compare this with other brain macrophage populations. This information may help us to design new strategies to promote restoration of tissue homeostasis in the most complex organ of the body, the brain.

Diversity of CNS myeloid cell origins

The brain hosts several myeloid populations. In addition to the parenchymal microglia, these cells include perivascular cells, meningeal macrophages and choroid plexus macrophages (FIG. 1). Despite the fact that all of these macrophage populations share numerous myeloid- and macrophage-specific markers (such as IBA1, F4/80 (also known as EMR1) and CX3CR1 (REF. 1)) and exhibit similar immune regulatory functions (such as local immune surveillance and removal of debris), early results suggested that they have distinct ontogenesis[22,23].

The precise origin of microglia during brain development has been a matter of controversy for decades. As shown in FIG. 2, HSCs are the founders of the haematopoietic system, which is responsible for blood production. Starting at embryonic day 10.5 (E10.5), they emerge from ventral aortic haematogenic endothelial cells in the aorta–gonad–mesonephros (AGM) region of the embryo. At E10.5, HSCs are only found in the embryonic AGM region, and HSC expansion activity becomes detectable[24,25]. HSC-derived myeloid cells are then produced abundantly in the fetal liver by E12.5. HSCs from these two sources also develop into myeloid cells such as monocytes and potentially perivascular, choroid plexus and meningeal macrophages. After birth, these types of myeloid cell continue to be formed in the bone marrow from HSCs via myeloid precursors and macrophage and/or dendritic cell progenitors. However, the precise lineage relationship between parenchymal and non-parenchymal CNS macrophages during ontogeny and in adult animals needs to be further determined in future studies.

The discovery that the brain of the developing mouse embryo already contains microglia at E9.5 (REFS 26–29) suggested that microglial precursors may originate from the yolk sac (a structure that is present from an earlier stage of embryogenesis) rather than from HSCs in the fetal liver or bone marrow. Indeed, a population of maternally derived committed CD45-expressing macrophages can be found in the yolk sac of the embryo as early as E7.5 (REFS 11,24) . However, this population subsequently decreases in number; it becomes almost undetectable at E9.0 and is later absent in the embryo, suggesting that these cells have a temporal protective effect against intrauterine infections in the embryo[24]. A second population of extra-embryonic haematopoietic cells of zygotic origin in the yolk sac differentiates into anucleated red blood cells and macrophages[24].

It took approximately two decades to confirm the yolk sac origin of microglia in sophisticated genetic fate-mapping experiments. By inducing Cre recombinase activity from the runt-related transcription factor (*Runx*) locus[10] or alternatively from the colony-stimulating factor 1 receptor (*Csf1r*) locus[12] via injections of tamoxifen into pregnant mice between E7.0 and E8.5 (when embryonic haematopoiesis is restricted to the yolk sac), the authors identified early yolk sac cells as the predominant source of microglia. Interestingly, myeloid progenitors from the blood did not significantly contribute to the pool of adult microglia after birth. Therefore, the vast majority of adult microglia seem to be derived from the yolk sac (specifically from cells born during a remarkably restricted time period in early embryogenesis). One limitation of the early work in this area was that only one-third of yolk sac macrophages could be genetically labelled, but this amount appropriately mirrored the percentage of labelled microglia during

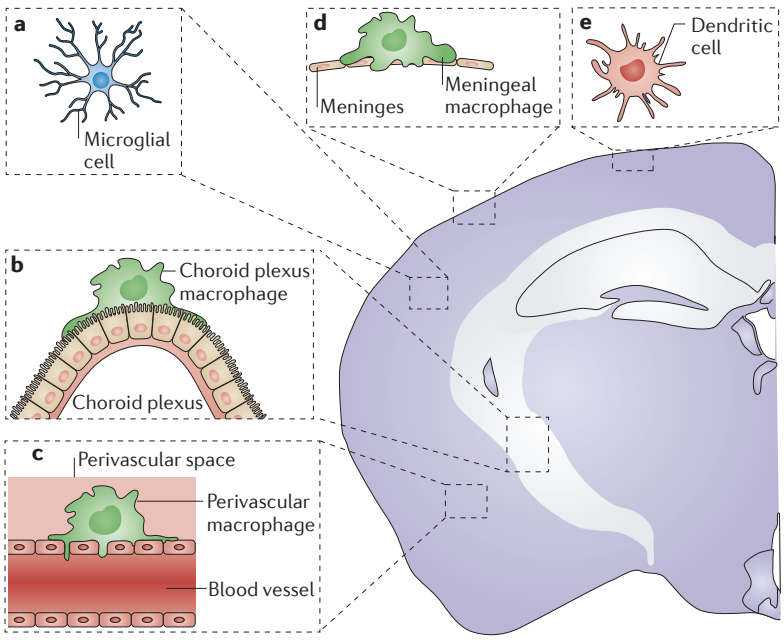

Figure 1 | **Myeloid cell types in the CNS.** Under homeostatic conditions, the brain hosts several heterogeneous populations of myeloid cells that are located at distinct sites, where they execute homeostatic and surveillance tasks. Within the brain parenchyma, microglia (part **a**) with small delineated processes actively screen the intraneuronal space for incoming threats, whereas macrophages can be found in the outer boundaries of the brain, such as the choroid plexus (part **b**), perivascular space (part **c**) and in the meninges (part **d**). Blood-derived dendritic cells (part **e**) are present at low numbers in the same locations as macrophages.

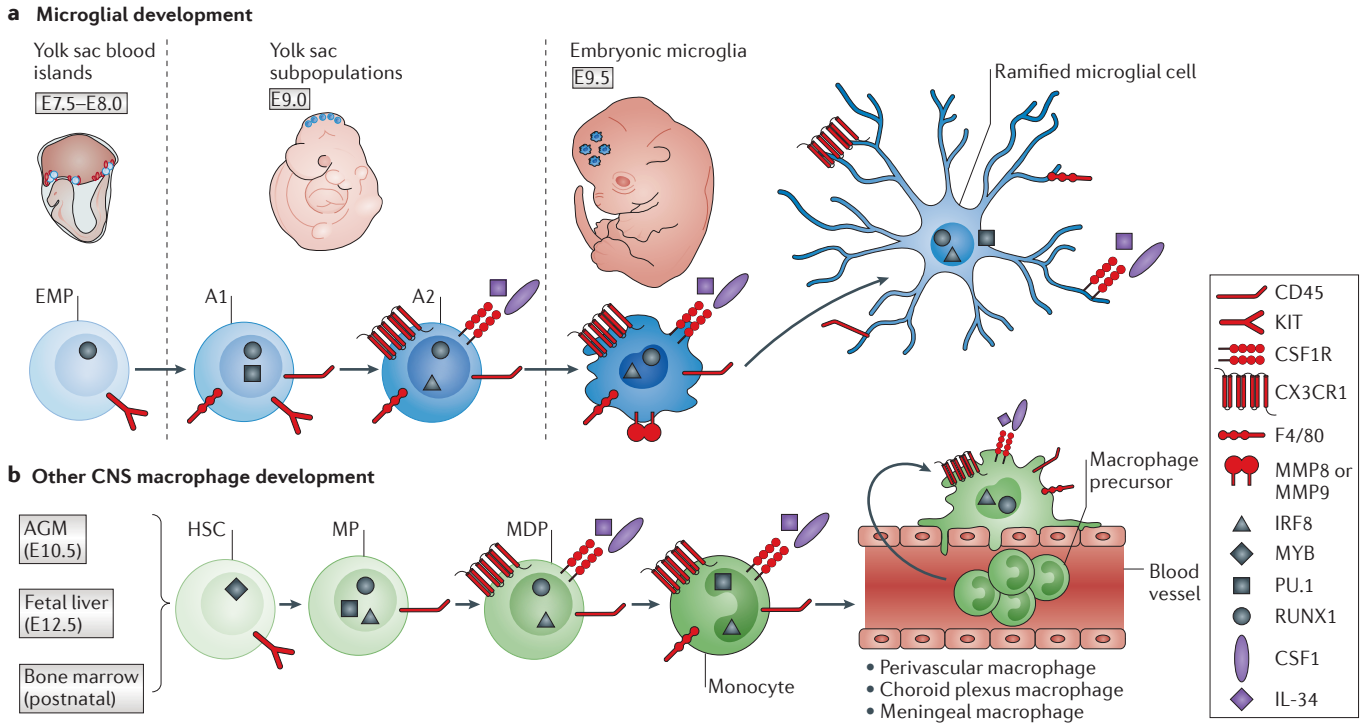

Figure 2 | **Embryonic and postnatal development of microglia in mice.** The figure illustrates the precursor cells, transcription factors (black symbols), cytokines (purple symbols) and cytokine receptors (red symbols) that are expressed by each cell population during the development of microglia and other CNS macrophages. **a** | Microglia derive from immature, uncommitted KIT$^+$ erythromyeloid progenitors (EMPs): that is, stem cells that are formed during primitive haematopoiesis, which begins at embryonic day 7.5 (E7.5)–E8.0 in the yolk sac in mice[11]. These cells start to upregulate the CD antigen CD45 but do not yet express myeloid markers (A1 stage). Later, myeloid cell markers, such as F4/80, CX3C chemokine receptor 1 (CX3CR1) and colony-stimulating factor 1 receptor (CSF1R) are expressed by migrating A2 cells (differentiated from A1 cells) that populate the brain mesenchyme. These early microglia reside in the brain throughout life and are thought to sustain the microglial population locally. **b** | By contrast, other CNS macrophages found in the meninges, choroid plexus and perivascular spaces are thought to be derived from the definitive haematopoiesis that starts at E10.5, first in the aorta–gonad–mesonephros (AGM) region or later, at E12.5, in the fetal liver. Postnatally, monocytes are formed in the bone marrow. Haematopoietic stem cells (HSCs) generate monocytes from myeloid precursors (MPs) and macrophage and/or dendritic cell progenitors (MDPs). IL-34, interleukin-34; IRF8, interferon regulatory factor 8; MMP, matrix metalloproteinase; MYB, transcriptional activator MYB; PU.1, transcription factor PU.1; RUNX1, runt-related transcription factor 1.

Leukocytes
White blood cells derived from multipotent haematopoietic stem cells in the bone marrow. Leukocytes are of myeloid or lymphoid lineage and are found in the blood and lymphatic system.

Yolk sac
A membranous sac attached to the embryo that provides early nourishment in the form of yolk. It functions as the developmental circulatory system of the embryo before internal circulation begins.

CD45
(Also known as leukocyte common antigen and PTPRC). A type I transmembrane protein present on all haematopoietic cells that assists in cell activation and the levels of which are reduced in mature parenchymal microglia.

Neuroepithelium
The ectodermal epithelium in the embryo from which the CNS and its main cellular constituents (neurons, astrocytes, oligodendrocytes and ependymal cells) are derived.

adulthood[10]. In a more recent study[12], mice lacking the transcriptional activator MYB, which do not develop HSCs or their progeny, were used. Yolk sac macrophages from these mice gave rise to a MYB-independent normal population of microglia in the adult. By contrast, MYB was required for stem cell development in the bone marrow. Further global transcriptional analysis showed a common signature of gene expression in embryonic microglia and F4/80$^+$ yolk sac macrophages[12].

A similar pattern of microglial cell development occurs in humans. In human foetuses, microglia-like cells with a range of morphologies can be detected as early as 3 weeks of estimated gestational age[30]. However, it seems that maturation of the microglial compartment is ongoing during most of the gestation period: colonization of the spinal cord begins at around 9 weeks, the major influx and distribution of microglia commences at about 16 weeks and ramified microglia take up to 22 weeks to become widely distributed within the intermediate zone[31,32]. In fact, it is only close to term, at 35 weeks, that well-differentiated microglial populations can be detected within the developing human brain[33] (for reviews, see REFS 31,32,34).

A recent study characterized the early yolk sac progenitors that give rise to microglia in the brain as KIT-positive, lineage marker-negative (meaning that no markers of mature haematopoietic cells were expressed) progenitor cells that have the ability to differentiate into CX3CR1-expressing microglia *in vitro* as well as *in vivo*[11,35]. These cells also generate Ter119-expressing erythrocytes, indicating that there is a common erythromyeloid progenitor (EMP) in the yolk sac for both lineages[11]. The uncommitted EMPs subsequently disappear and immature F4/80$^+$CX3CR1$^-$ (A1) and F4/80$^+$CX3CR1$^+$ (A2) macrophages develop and can be located on the surface of the developing brain at E9.0 in mice[11]. These macrophage-like cells, which have an amoeboid shape, were previously described in the rodent neuroepithelium[28,36,37]. At E13.5, when the fetal liver is already the primary haematopoietic organ and

Natural killer cells
A type of cytotoxic lymphocyte that are crucial for the innate immune system.

the main site of HSC expansion and differentiation[38], A2 macrophages can be detected in significant numbers within the lining of the fourth ventricle[37]. There is a 20-fold increase in the number of $CD11b^+F4/80^+$ microglial cells during the early postnatal period (between postnatal day 0 (P0) and P11) in rodents[27]. However, evidence suggests that this increase in microglial cell number is not induced by the recruitment of peripheral myeloid cells but instead results from the expansion of resident microglial cells[10,12].

Microglia and CNS macrophages thus represent two ontogenetically distinct myeloid populations. These differences imply that microglia and infiltrating macrophages have different functions, which are increasingly apparent in mouse models of disease (see below)[1].

Microglial development

The transcriptional programme that controls the differentiation of yolk sac progenitors into microglia is only partially understood. Indeed, many studies of the transcriptional control of macrophage differentiation have been carried out *in vitro* using progenitor-enriched cell populations. Thus, the precise role of the factors identified in these studies in driving the differentiation of yolk sac macrophages into typical microglia *in vivo* remains to be determined. This is particularly important given that functional macrophage specialization is likely to be regulated at the tissue level — that is, in the brain. Nevertheless, some molecules that are essential for the regulation of microglial cell development have already been described[35] (TABLE 1).

A dramatic reduction in the number of tissue macrophages, including microglia, has been observed in *Csf1r*-knockout mice[10,39,40] and in *Csf1*$^{op/op}$-mutant mice, which have a natural null mutation in *Csf1* (REFS 41,42). Thus, CSF1 and its receptor have a key role in microglial homeostasis in mice *in vivo*[43]. However, the mechanisms by which CSF1 and its receptor promote microglia commitment remain controversial. One hypothesis suggests that CSF1 drives the differentiation of phagocytic yolk sac macrophages that have entered the embryo into microglia[44], whereas a different theory proposes that CSF1 provides a survival signal for the differentiating macrophages and that surviving cells utilize an intrinsic developmental programme to become mature microglia[45,46]. Interestingly, microglia are more profoundly affected by the absence of CSF1R than by the absence of CSF1 (REF. 10). Of note, a second CSF1R ligand, interleukin-34 (IL-34), has recently been identified[47] and has been found to be highly expressed in the postnatal mouse brain[48]. In order to explore the role of this cytokine in microgliogenesis, mice deficient in IL-34 were generated[49,50]. Interestingly, the number of microglia was reduced in distinct regions of the brains of these mutants, suggesting that there is regional heterogeneity of CSF1R and/ or IL-34 expression[49,50]. Similar results were obtained in zebrafish, in which yolk sac-derived macrophages enter the developing brain and form early microglia that have high endocytic activity. This process was shown to depend on CSF1R expression: the *Panther* mutation of CSF1R led to a complete loss of brain macrophages[51]. Similarly, microglial cell numbers were reduced in mice deficient in an adaptor protein of the CSF1R, DNAX-activation protein 12 (DAP12; also known as TYROBP)[52]. DAP12, which has an important role in the human brain (see below), contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain and is highly expressed in natural killer cells and myeloid cells.

PU.1 is another transcription factor that is exclusively expressed in haematopoietic cells and is involved in microglial cell development. The gene encoding PU.1, *SFPI1* (also known as *SPI1*), is a member of the ETS family of transcription factors[53]. Its targeted disruption in mice leads to multiple haematopoietic abnormalities, including a lack of mature B cells and macrophages[54]. In fact, PU.1-deficient mice are devoid not only of circulating monocytes and tissue macrophages[54] but also of parenchymal microglia in the brain[55].

Additional transcription factors such as RUNX1 and interferon regulatory factor 8 (IRF8) are also indispensable regulators of the differentiation of microglia during embryonic development in mice[10,11]. A recent study carried out a detailed analysis of the function of RUNX1 in postnatal microglia[56] and showed that it not only regulates the differentiation but also the proliferation and homeostasis of postnatal microglia. The authors further suggested that RUNX1 might modulate the transition of activated amoeboid microglia into deactivated ramified microglia[56]. The transcription factors PU.1 and RUNX1 were shown to act in a negative feedback loop that governs the equilibrium between distinct myeloid fates by assuring an appropriate PU.1 dosage[57]. These results suggest that RUNX1 is a non-redundant transcription factor that is important for the activation of microglia.

The transcriptional programming of microglial cell development is also tightly regulated by transcription factors expressed in the myeloid lineage, such as IRF8. IRF8 is a heterodimeric partner of PU.1 and has known roles in the development of B cells and myeloid cells in the bone marrow[58]. A recent study investigated the role of IRF8 in microglial cell development in mice and found that yolk sac-derived A2 macrophages were particularly dependent on the presence of IRF8, whereas other myeloid transcription factors, such as MYB, DNA-binding protein

Table 1 | **Microglia phenotypes in animals lacking specific molecules**

Absent molecule	Microglia morphology	Microglia number	Refs
CSF1	↓	↓	41,169
CSF1 receptor	↓	↓	10,40
DAP12	ND	↓	52
Interleukin-34	ND	↓	49,50
IRF8	↓	→ or ↓	11,59–61
Transcription factor PU.1	NA	↓	51,55
RUNX1	↓	ND	56,57

↓ indicates a either dysmorphic or reduced number of microglia; → indicates no change. CSF1, colony-stimulating factor 1; DAP12, DNAX-activation protein 12; IRF8, interferon regulatory factor 8; NA, non-applicable; ND, not determined; RUNX1, runt-related transcription factor 1.

Box 1 | **Microglia in the universe of mononuclear phagocytic cells**

Our knowledge of macrophage lineages was dramatically expanded by recent studies using both mutant mice and fate-mapping approaches. The first fate-mapping studies focused on microglia and Langerhans cells in the skin. Microglia are exclusively derived from KIT$^+$ erythromyeloid progenitors in the yolk sac as part of primitive haematopoiesis[10–12], whereas Langerhans cells predominantly originate in the fetal liver, with a smaller contribution from yolk sac macrophages[12,161]. Yolk sac-derived tissue macrophages were found to have a long lifespan, to be able to self-renew and to be present in several tissues such as brain (microglia), skin (Langerhans cells), liver (Kupffer cells), pancreas, lung (alveolar macrophages), spleen (red pulp macrophages) and kidney[12,14,161–165] (FIG. 3). In most tissues, except the brain and potentially also the liver, these yolk sac-derived tissue macrophages coexist with macrophages derived from definitive myelopoiesis in the fetal liver. It is not yet clear whether yolk sac-derived macrophages or fetal liver-derived populations are more dominant in normal adult tissues. Accordingly, it is also not known whether these ontogenetically different macrophages have distinct roles and functions. Microglia are an obvious exception in that they exclusively derive from one source (the yolk sac) before birth. More recently, mutations in GATA-binding protein 2 (*GATA2*)[166] and interferon regulatory factor 8 (*IRF8*)[167] have been associated with severe defects in bone marrow-derived myeloid cells but did not affect many tissue macrophages[167,168]. These data clearly indicate that different pathways regulate the development of myeloid cells by primitive and definitive haematopoiesis.

inhibitor ID2, basic leucine zipper transcriptional factor ATF-like 3 (BATF3) and Krüppel-like factor 4 (KLF4), were not essential for their development[11]. Consequently, the number of microglia is strongly reduced during adulthood in animals lacking IRF8 (REF. 11). Moreover, recent studies have indicated a role for IRF8 in the activation of adult microglia[59–61].

In summary, these data provide evidence that microglia develop from EMPs in the yolk sac in an IRF8- and PU.1-dependent manner but independently of MYB, ID2, BATF3 and KLF4. This unique genetic profile distinguishes microglia from other myeloid cells, such as circulating monocytes, CD11b$^+$ dendritic cells, CD8$^+$CD103$^+$CD11b$^-$ dendritic cells, endogenous brain macrophages and other tissue macrophages[62] (BOX 1; FIG. 3).

Microglial polarization

Like macrophages in the periphery, microglia act as the first line of defence in the nervous system. Every few hours, so-called 'resting' microglia screen the brain parenchyma with their highly motile processes[63]. Upon detection of signs of injury, such as extracellular calcium waves and release of adenosine triphosphate from neighbouring cells, microglial processes rapidly move towards the lesion site[64,65]. This is followed by the transformation of resting microglia into an activated state (FIG. 4). Depending on the particular signals detected and on the actions of modulators of microglial activation, a diversity of reactive microglial phenotypes can be generated[66].

Macrophages in non-neural tissues reprogramme their function in response to pathogens, tissue damage and lymphocyte interactions. This process, termed polarization, enables the adaptive responses of innate immunity to take place[67]. At least two distinct states of macrophage polarization have been recognized: M1 and M2 polarization. The T helper 1 (T_H1) cytokine, interferon-γ (IFNγ), and bacterial lipopolysaccharide (LPS) polarize macrophages towards the M1 phenotype. By contrast, the T_H2 cytokine, IL-4, drives M2 polarization. Notably, the chemokine CCL2 (also known as monocyte chemoattractant protein 1), which is strongly induced in neurodegenerative and neuroinflammatory conditions, also drives M2 macrophage polarization[68]. Macrophages activated in these ways are functionally distinct. M1-polarized macrophages produce pro-inflammatory cytokines such as IL-12, IL-23 and tumour necrosis factor-α (TNFα). They attract T_H1 cells by releasing the chemokines CXCL9 and CXCL10, and show enhanced antigen presentation capacity[67]. Moreover, they generate reactive oxygen and nitrogen species through activity of inducible nitric oxide synthase[67,69]. By contrast, M2-polarized macrophages produce anti-inflammatory cytokines such as IL-10 and promote T_H2 responses. They show increased phagocytic activity and expression of scavenger receptors such as CD163 (REF. 67). In addition, M2-polarized macrophages support tissue remodelling and promote fibrosis through increased arginase 1 activity[69,70]. However, as appealing as this dichotomous view of macrophage polarization may seem to be, it cannot account for the plasticity of myelomonocytic cells that has been observed[67,71]. In fact, various overlapping and novel phenotypes have been observed. Among M2-polarized macrophages, three subsets with different functional properties have been defined: M2a, M2b and M2c macrophages[67,71]. M2a and M2c macrophages dampen inflammation and promote tissue repair, whereas M2b macrophages (characterized by their downregulation of IL-12) have both pro- and anti-inflammatory functions[67,71]. In fact, there is probably a spectrum of plastic functional conditions of mononuclear phagocytes rather than a set of discrete activation states.

Despite their different ontogeny, microglia may also have the capacity to become polarized into M1-like and M2-like phenotypes. In an early *in vitro* study, T_H1- or T_H2-polarized T cells isolated from patients with multiple sclerosis differentially modulated human microglia and monocytes to become type 1 (M1-like) or type 2 (M2-like) antigen-presenting cells[72]. Interestingly, human microglia seem to be more restricted in their capacity to adopt the M2-like phenotype *in vitro* than monocyte-derived macrophages[73].

In animal models of stroke, traumatic brain injury or spinal cord injury, microglia and/or macrophages have been suggested to shift from a transient M2 phenotype to become M1-like phagocytes[74–76]. The NADPH oxidase-dependent redox state of the microenvironment plays a crucial part in the modulation of microglial phenotype *in vivo*. Inhibition or deletion of NADPH oxidase switches microglial activation from a classical (M1-like) to an alternative (M2-like) state in response to an inflammatory challenge[77]. Similarly, human gliomas instruct macrophages to shift towards an M2-like phenotype, as determined by immunostaining in glioma samples and *in vitro*[78]. Interestingly, CSF1R inhibitors were recently found to slow the growth of patient-derived glioma xenografts in mice by reducing the M2-like polarization of microglia and/or macrophages[79].

Myelopoiesis
The regulated formation of myeloid cells, including macrophages, monocytes, dendritic cells and granulocytes. Myelopoiesis takes place in the bone marrow or the yolk sac.

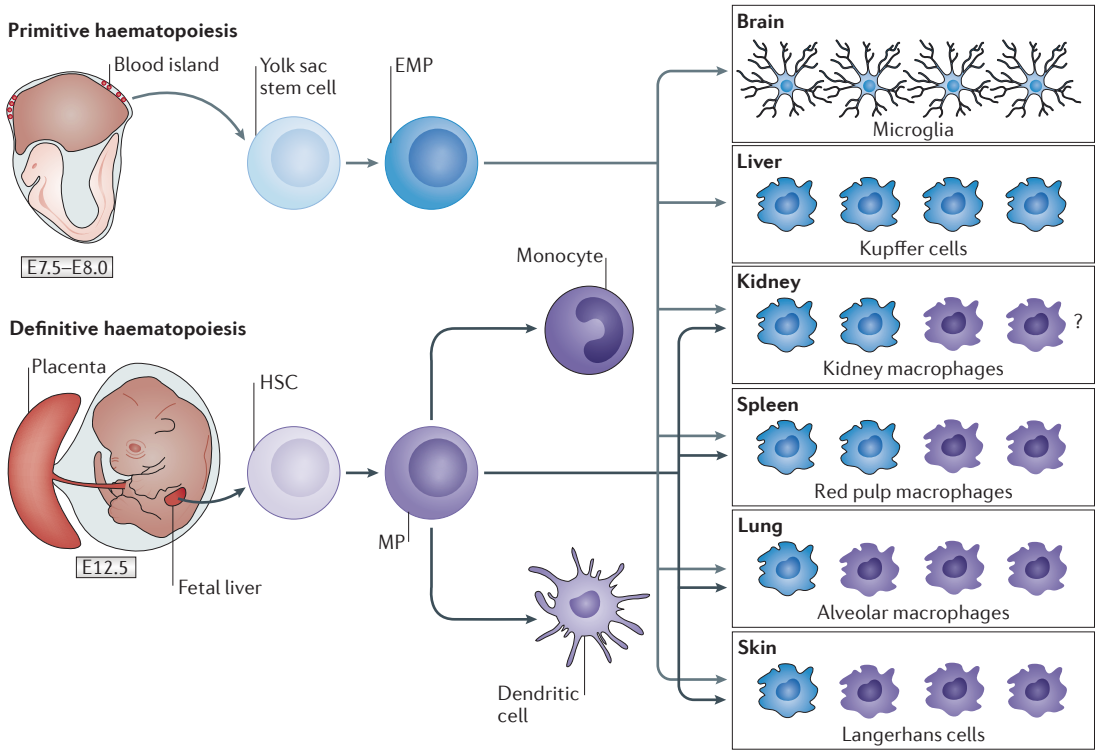

Figure 3 | **Development of resident macrophages in different tissues.** Several sources of myelopoiesis exist in the mouse. A transient early wave of myeloid cell development called primitive haematopoiesis takes place at embryonic day 7.5 (E7.5)–E8.0. At this time point, cells with stem cell properties develop in blood islands of the yolk sac. Their progeny (erythromyeloid progenitors (EMPs)) further differentiate and populate several tissues, including the brain, where they become tissue macrophages that potentially have longevity and a high capacity for self-renewal. Shortly thereafter, myelopoiesis is taken over by progenitors found in the aorta–gonad–mesonephros region (not depicted) and fetal liver (starting at E12.5), where it forms part of the process of definitive haematopoiesis. Maturating myeloid cells derived from definitive haematopoiesis are engrafted in all tissues except the brain, which is already disconnected from any cell recruitment owing to the establishment of the blood–brain barrier, and the liver. Between birth and senescence, myelopoiesis is thought to be restricted to the bone marrow (not depicted). HSC, haematopoietic stem cell; MP, myeloid precursor.

However, it has been much more difficult to find clear evidence of macrophage and/or microglia polarization in human inflammatory and neurodegenerative diseases: instead, overlapping phenotypes that co-express M1 and M2 markers predominate[80,81]. Interestingly, rare variants of the gene encoding triggering receptor expressed on myeloid cells 2 (TREM2), which promotes alternative (M2-like) activation of microglia and phagocytosis *in vivo*[82], have recently been associated with an increased risk of late-onset Alzheimer's disease[83]. Notably, deficiency of the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome, a multiprotein complex that is involved in host defence against invading pathogens, skews activated microglia towards an M2-like state in Alzheimer's disease transgenic mice, resulting in increased amyloid-β (Aβ) clearance and enhanced tissue remodelling[84]. Among the molecular signals that regulate myeloid cell polarization *in vivo*, microRNAs such as miR-155 have recently been identified[85].

M1-polarized microglia and/or macrophages exert cytotoxic effects on neurons and oligodendrocytes *in vitro*, whereas M2-polarized cells exhibit phagocytic capacity and promote neurite outgrowth[74,75,86]. Moreover, M2-polarized microglia and macrophages have been suggested to support CNS remyelination by driving oligodendrocyte differentiation[87]. In the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, relapse is characterized by an imbalance of monocyte activation profiles towards the M1 phenotype and suppression of immunomodulatory M2 macrophages and/or microglia at lesion sites[88]. Together, these findings have raised hopes that the promotion of myeloid cell M2 polarization may be a promising therapeutic avenue for neurological diseases by providing neuroprotective and regenerative effects (FIG. 4).

It is important to note that the simplistic dichotomous distinction of myeloid cell activation described above falls short of accommodating the complex environmental cues that microglia receive from a multitude of surrounding cell types. Moreover, analysis of candidate genes and marker proteins of macrophage polarization may not suffice to characterize the functional state of microglia. In fact, deep RNA sequencing of the microglial transcriptome in ALS transgenic mice recently failed to demonstrate M1- versus M2-type microglial polarization and instead revealed a neurodegeneration-specific signature[89].

Deep RNA sequencing
An approach enabled by next-generation sequencing technology that is particularly useful for identifying low-abundance RNAs or low-frequency mutations.

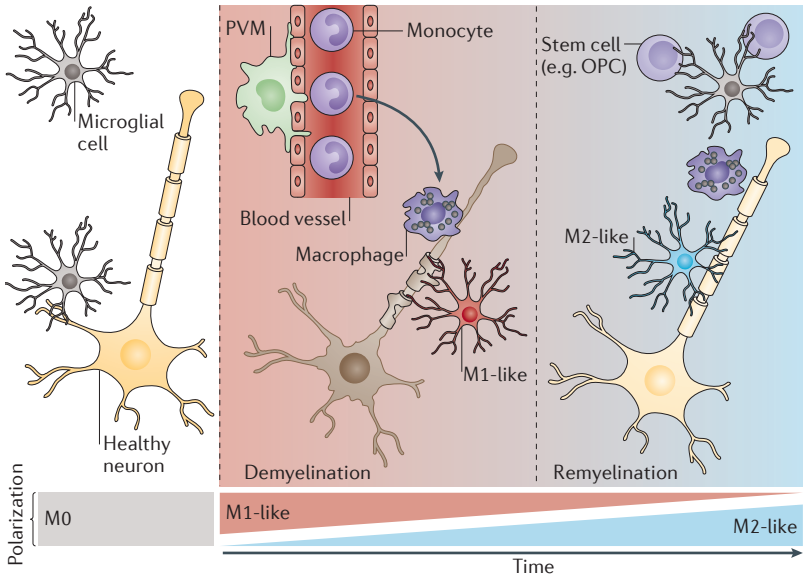

Figure 4 | **Functional reprogramming of microglia and macrophages in response to brain injury.** Under physiological conditions, microglia are continuously surveying their microenvironment. We have named this so-called 'resting' state of microglia M0. Neuronal dysfunction or damage can activate microglia to produce pro-inflammatory cytokines (M1-like polarization). Depending on the degree of homeostatic disturbances, leukocytes (not shown) may be recruited from the bloodstream. Peripherally derived macrophages (purple) and perivascular macrophages (PVMs) also participate in the inflammatory response. As a result of the passage of time, the type of brain injury or environmental factors, microglia and/or peripherally derived monocytes and macrophages may acquire an anti-inflammatory phenotype, which causes them to remove debris and promote regeneration (M2-like polarization). This may entail the recruitment and differentiation of local stem and progenitor cells, such as oligodendroglial progenitor cells (OPCs) for remyelination. However, it is important to note that the activation states of microglia and macrophages are not strictly dichotomous but are part of a spectrum of functional states.

Microglia in CNS disorders

Vulnerability of microglia and 'microgliopathies'. As described above, microglia are derived from primitive macrophages in the yolk sac, and postnatal haematopoietic progenitors do not significantly contribute to microglial homeostasis in the adult brain[10–12]. The longevity of murine microglia in non-pathological conditions is underscored by a recent study using mice in which tamoxifen-dependent Cre recombinase activity is induced from the *Cx3cr1* locus, which enables selective gene targeting in microglia[14]. After crossing these animals with ROSA26–yellow fluorescent protein reporter mice, a stable population of microglia that was not appreciably supplemented by blood cells over many weeks was observed, whereas other CX3CR1-expressing myeloid cells such as LY6C^{hi} or LY6C^{low} monocytes had lost the genomic recombination owing to higher levels of turnover[13]. These findings are in line with previous studies in parabiotic mice that were surgically joined together to generate a shared circulatory system, suggesting that the maintenance and expansion of microglia in healthy conditions, but also in neurodegenerative and neuroinflammatory conditions, depend on the self-renewal of CNS-resident cells[90,91].

Thus, microglia are in the position of being particularly vulnerable to developmental disturbances and ageing. In fact, prenatal immune activation by exposure of mothers to the viral mimetic polyinosinic:polycytidylic acid has recently been suggested to increase the vulnerability of the pubescent offspring to stress, resulting in behavioural abnormalities and aggravated neuroimmune responses in mice that are characterized by increased numbers of activated microglia and increased levels of the pro-inflammatory cytokines IL-1β and TNFα in the hippocampus[92]. Although some concerns over the validity of the statistical analysis of this study have been raised[93], the findings are of interest for the 'multiple hit model' of psychiatric diseases, according to which two or more pathogenic factors act synergistically to cause disease.

Recently, microglia have been found to play a crucial part in synaptic pruning and remodelling during development and adulthood, a process that involves complement receptor 3, CX3CR1 and DAP12 (REFS 94–99). Microglia serve important physiological functions in learning and memory by producing brain-derived neurotrophic factor and other mediators that affect synaptic plasticity[15]. Notably, deep sequencing of RNA isolated from adult murine microglia suggested a prominent position for DAP12 in the microglial 'sensome', a unique cluster of proteins that enable microglia to carry out homeostatic functions, including sensing of chemokines, cytokines, purines, inorganic substances, changes in pH and amino acids[100]. Moreover, DAP12 is required for the long-term preservation of microglia in defined brain regions, and degenerative changes (such as cytorrhexis and nuclear condensation) and loss of microglia are observed in ageing DAP12-deficient mice[52]. Interestingly, loss-of-function mutations in *DAP12* or *TREM2* cause polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (Nasu-Hakola disease), which is characterized by psychotic symptoms and pre-senile dementia[101]. It should be noted that ageing of microglia can result in senescence, dystrophy, impaired movement, altered signalling, impaired phagocytosis and/or impaired proteostasis[102].

Given the importance of microglia in physiological brain function, it is not surprising that an increasing number of microglia-related genes have now been associated with neuropsychiatric or neurologic disorders. These include roles for *CD33* in Alzheimer's disease[103–105], *TREM2* in frontotemporal dementia[106], *CSF1R* in hereditary diffuse leukoencephalopathy with spheroids[107], and *TNFRSF1A* and *IRF8* in multiple sclerosis[108,109]. It is tempting to speculate that we shall soon discover new classes of 'microgliopathies' in which microglial dysfunction is the primary disease-causing mechanism.

Role in CNS disorders. Damage to the CNS commonly entails the recruitment of circulating immune cells, resulting in an innate immune response that consists of resident microglia and peripherally derived monocytes, macrophages and dendritic cells. The differential roles of these myeloid cell populations in CNS disorders have only recently been acknowledged. In healthy conditions, monocytes circulate in the blood,

Parabiotic mice
Mice in which shared blood circulation is created via surgical intervention. This procedure enables the fate of labelled donor cells to be followed in the parabiotic partner.

bone marrow and spleen without proliferating; they enter tissues under inflammatory conditions, giving rise to macrophages and inflammatory dendritic cells[3]. At least two functional subsets of murine blood monocytes exist: short-lived 'inflammatory' monocytes ($CCR2^+GR1^+CX3CR1^{low}LY6C^+$) and small 'patrolling' monocytes ($CCR2^-GR1^-CX3CR1^{hi}LY6C^-$)[110]. The patrolling population of monocytes originates from $LY6C^+$ blood monocytes[14]. Patrolling $LY6C^-$ monocytes act as intravascular housekeepers that orchestrate the neutrophil-mediated necrosis of endothelial cells and the phagocytosis of cellular debris[111].

Recently, experiments in Alzheimer's disease transgenic mice revealed an important role for patrolling $LY6C^-$ monocytes in vascular Aβ clearance[112]. Specifically, patrolling monocytes were found to crawl on $Aβ^+$ veins, internalize Aβ and then circulate back to the bloodstream. Consequently, depletion of $LY6C^-$ monocytes resulted in increased amyloid load in the brain[112]. It has also been suggested that perivascular macrophages expressing the chemokine receptor CCR2 clear Aβ in mouse models of Alzheimer's disease[113]. In line with these observations, depletion of perivascular macrophages increases the deposition of Aβ in leptomeningeal and cortical blood vessels of mice with cerebral amyloid angiopathy[114]. Conversely, stimulation of perivascular macrophage turnover by chitin administration results in the clearance of $Aβ_{42}$-immunoreactive vascular amyloid deposits via CD163-expressing macrophages[114]. Interestingly, normal human monocytes and/or macrophages also have a remarkable ability to phagocytose and clear Aβ, but they seem to lose this capacity in patients with Alzheimer's disease[115]. Results suggest that treatment with bisdemethoxycurcumin can correct this phenotype, perhaps by inducing the transcription of *MGAT3* and Toll-like receptor genes[116].

The role of resident microglia in Aβ clearance and Alzheimer's disease pathogenesis is more controversial. *CD33* has recently been identified as a risk factor for Alzheimer's disease[103–105]. In fact, CD33 directly inhibits the uptake of $Aβ_{42}$ in mouse primary microglial cells through its interaction with sialic acids[105]. Similarly, apolipoprotein E (*APOE*) and *TREM2* have been associated with late-onset Alzheimer's disease[83,117], and both gene products are directly or indirectly involved in Aβ clearance by microglia[118,119]. Murine microglia are functionally impaired by amyloid plaque deposition and show reduced directed process motility and phagocytic activity *in vivo*[120]. Moreover, microglia may contribute to Aβ-induced neurotoxicity *in vitro* by generating reactive oxygen species and peroxynitrite[121,122]. Conversely, transient ablation of microglia in Alzheimer's disease transgenic mice neither affected amyloid plaque formation and maintenance nor amyloid-associated neuritic dystrophy[123]. It has been suggested that microglia can only clear amyloid after antibody opsonization, but recent Phase 3 trials of Aβ-specific antibodies, bapineuzumab and solanezumab, failed to improve cognition or functional ability in patients with Alzheimer's disease[124,125]. Moreover, the clearance of amyloid plaques by the antibody AN1792 in patients with Alzheimer's disease did not prevent progressive neurodegeneration[126]. It therefore seems likely that resident microglia and subsets of myeloid cells may have distinct and non-redundant roles in Alzheimer's disease (FIG. 5).

Similarly, functional recovery from spinal cord injury in mice may depend more on infiltrating blood-derived macrophages than on microglia[127]. Activated $GR1^+LY6C^+CD11c^+$ macrophages accumulate at the margins of the lesion site after spinal cord injury and express IL-10. Depletion of these infiltrating monocyte-derived macrophages results in diminished recovery of hindlimb motor function and greater spread of damage. Conversely, augmentation of monocyte infiltration by vaccination with myelin peptide promotes functional recovery from spinal cord injury[127]. However, these results are at odds with the observation that intravenous clodronate treatment in rats reduces the infiltration of monocytes and/or macrophages into the injured spinal cord and promotes functional recovery[128]. Interestingly, the port of CNS entry seems to determine the fate of infiltrating monocytes[129]. Thus, early invasion of M1-type $LY6C^{hi}$ monocytes and/or macrophages occurs through the leptomeninges, whereas M2-polarized $LY6C^{low}CX3CR1^{hi}$ monocytes and/or macrophages are later recruited to the injured spinal cord via the choroid plexus. Notably, factors in the cerebrospinal fluid, such as IL-13, IL-10 and transforming growth factor-β, may instruct monocytes to acquire an anti-inflammatory, resolving phenotype.

A switch from an M1- to an M2-type response also occurs in microglia and peripherally derived macrophages after CNS demyelination, and this switch is associated with regeneration[87]. By contrast, $LY6C^{hi}$ monocytes have a strong disease-promoting effect in EAE[130,131]. Inflammatory monocytes are also activated in a mouse model of ALS, in which their progressive recruitment to the spinal cord mediates neuronal loss[9]. Interestingly, resident microglia seem to attract the M1-polarized $LY6C^{hi}$ monocytes by producing CCL2. At the same time, microglia succumb to apoptosis during disease progression.

In conclusion, the data suggest a complex interplay between microglia and peripherally derived monocytes, macrophages and dendritic cells in CNS disorders. However, it should be noted that the findings in animal models do not translate easily to the human condition. This is exemplified by the discrepancies between the dramatic therapeutic effects of Aβ vaccination in Alzheimer's disease transgenic mice and the lack of beneficial clinical effects observed in human Aβ immunotherapy trials so far[132].

Targeting myeloid cells to the CNS

Given that resident yolk sac-derived microglia are vulnerable and may be overwhelmed by CNS diseases and ageing, an interest in strategies to support microglia has developed. Using green fluorescent protein as a sensitive marker to track haematopoietic cells, it has been shown that genetically modified myeloid cells can be specifically targeted to sites of damage in the CNS[133]. Engraftment of ramified bone marrow-derived phagocytes (BMDPs) has been observed in the adult brain parenchyma of mice[133–139],

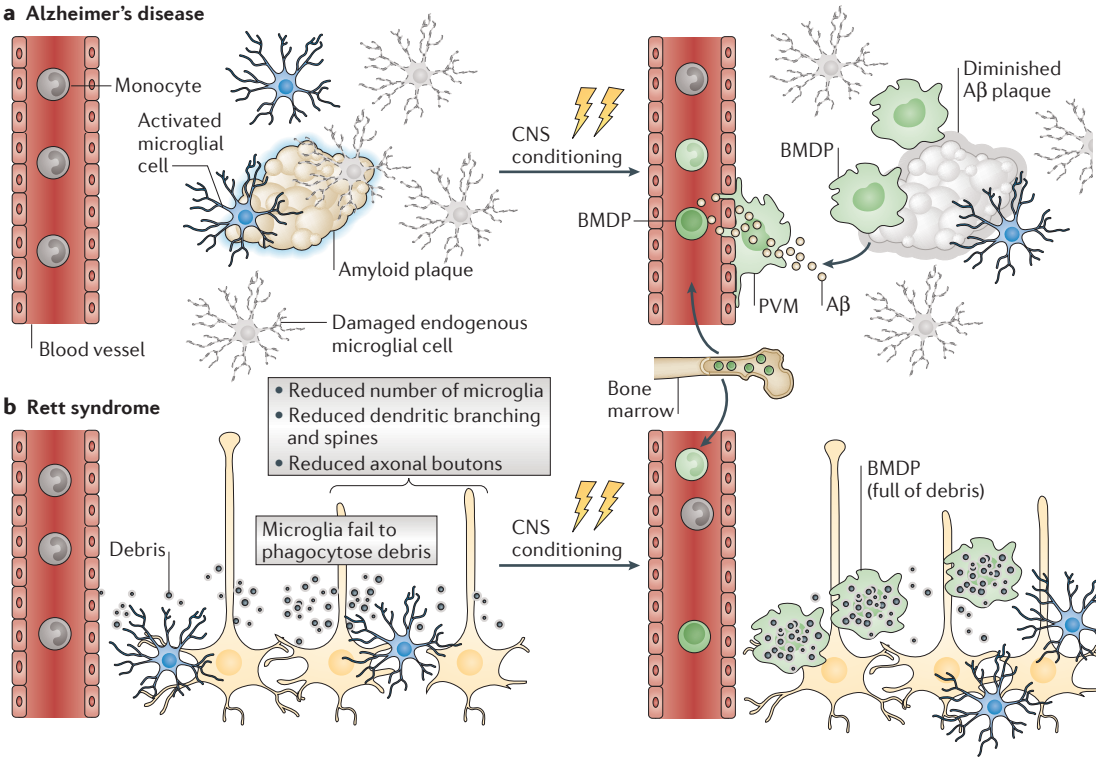

Figure 5 | **Disease-modulating roles of bone marrow-derived myeloid cells in the CNS.** Neuropsychiatric disorders are often associated with microglial dysfunction or demise. **a** | In Alzheimer's disease, activated microglia have been suggested to contribute to amyloid-β (Aβ)-induced neurotoxicity. At the same time, microglia are damaged by amyloid species. Transplantation of wild-type bone marrow cells (green) in transgenic mouse models of Alzheimer's disease results in the recruitment of bone marrow-derived phagocyte (BMDP) populations to the neurovascular unit and brain parenchyma after previous CNS conditioning (for example, as a result of irradiation)[113,156]. As a result, amyloid load in the brain may be reduced through enhanced clearance of Aβ from the CNS by perivascular monocytes and/or perivascular macrophages (PVMs). **b** | In Rett syndrome, microglia are reduced in numbers and fail to phagocytose debris[160]. This may contribute to neuronal dysfunction and structural abnormalities of neurons (reduced dendritic branching, spine density and axonal boutons). Transplantation of wild-type bone marrow cells in the methyl-CpG-binding protein 2 (*Mecp2*)-null mouse models of Rett syndrome results in the recruitment of BMDP populations to the brain and rescues the phenotype[160].

rats[140] and humans[141] after bone marrow transplantation (BMT). However, the vast majority of bone marrow-derived cells in these studies populated the perivascular spaces rather than the brain parenchyma. This is in line with earlier observations suggesting a high turnover of perivascular cells in rodents and humans[22,142–145]. It soon became apparent that the engraftment of BMDPs in the adult brain parenchyma is facilitated by the conditioning regimens used to prepare the recipients for BMT, such as total body irradiation. In fact, protecting the head from irradiation and the resulting CNS inflammation during BMT abrogated the engraftment of ramified BMDPs in the brain parenchyma[146].

However, irradiation is not sufficient to recruit BMDPs into the CNS, as demonstrated in parabiotic mice that were surgically joined together to generate blood chimerism without irradiation or BMT. Notably, no engraftment of ramified BMDPs was observed in the brain parenchyma of parabiotic mice more than 1 year after the surgery[10,90]. Moreover, neither irradiation nor neurodegeneration promoted the CNS engraftment of ramified BMDPs in parabiotic mice[90]. Together, these findings suggest that the BMDPs that are recruited to the brain parenchyma — in contrast to perivascular macrophages — do not derive from circulating precursors such as monocytes. Two recent studies lend support to this conclusion. First, EAE experiments in parabiotic mice revealed that inflammatory monocytes and/or macrophages are recruited from the bloodstream to the spinal cord in a CCR2-dependent manner and trigger disease progression[91]. Notably, the presence of these myelomonocytic cells in the CNS was transient, and only uncommitted lineage-negative KIT^+SCA1^+ stem or progenitor cells were capable of generating long-lived BMDPs in irradiated recipients. Second, HSC transplantation in mice revealed a short-term wave of brain infiltration by a fraction of the donor cells independently of whether irradiation or chemotherapy (using the agents busulphan or treosulphan) was used as a conditioning regimen[147]. However, only lethal irradiation and myeloablation with busulphan, which are capable of ablating brain-resident myeloid precursors (treosulphan does not cross the blood–brain barrier), enabled turnover of microglia with the donor. This was mediated by local proliferation of early immigrants rather than entrance of mature cells from the circulation. In line with these results, it was

recently shown that myeloid cells can be targeted to sites of brain damage even in the presence of very low levels of peripheral blood chimerism[148]. To date, the cellular origins of the precursors of BMDPs in the adult brain parenchyma remain unclear. Nevertheless, myelosuppressive conditioning using busulphan is sufficient to trigger the recruitment of BMDPs to the CNS[149,150]. This is an important observation, as myeloablation with busulphan has been successfully used in clinical trials[151].

There is growing interest in long-term engraftment of myeloid cells in the mature CNS because these cells harbour great therapeutic potential for neurological and psychiatric disorders. This is particularly true for disorders in which microglia fail to support normal brain function or even 'turn against' their environment. Allogeneic HSC transplantation and autologous stem cell-based gene therapies have been tested in models of inborn errors of metabolism, such as lysosomal storage diseases[152,153]. The aim is to repopulate recipient haematopoietic and lymphoid compartments with cells expressing functional enzymes. However, HSC transplantation seems to benefit only a subset of patients who have not yet developed overt neurological symptoms, suggesting that BMDP engraftment in the CNS occurs at a slower pace than disease progression. In addition, graft failures and incomplete chimerism may limit the success of allogeneic HSC transplantation. Autologous HSC-based gene therapies offer immunological advantages in relation to graft rejection and graft-versus-host disease, and may benefit even those patients with early-onset and rapid disease progression. In the case of X-linked adrenoleukodystrophy, zones devoid of microglia and the occurrence of microglial apoptosis in perilesional white matter suggest that microgliopathy is an early pathogenic event[154]. HSC transplantation can arrest the neuroinflammatory demyelinating process with a characteristic delay of 12–18 months, which has been attributed to the slow replacement of microglia with BMDPs[153].

Non-cell-autonomous neurodegeneration has also been described for 'classical' neurodegenerative diseases. In ALS, upper and lower motor neurons are lost, which results in progressive tetraparalysis and death. The most commonly inherited form of ALS is caused by mutations in superoxide dismutase 1 (*SOD1*). Microglia derived from transgenic mice overexpressing mutant SOD1 produce more free radicals and induce more neuronal cell death *in vitro* than wild-type microglia[55]. Notably, expression of mutant SOD1 in motor neurons is not sufficient to trigger their degeneration, and SOD1-mutant mice survive longer when surrounded by wild-type microglia or BMDPs[55,155].

As described above, dysfunction of microglia has also been described in animal models of Alzheimer's disease[120]. Although resident microglia are recruited to sites of amyloid deposition in the brain, they may ultimately fail to restrict amyloid plaque formation (FIG. 5). However, peripheral myeloid cells may still possess the capacity to remove Aβ from the CNS. Following irradiation and BMT, peripherally derived monocytes and/or macrophages populate the perivascular spaces, and BMDPs are engrafted into the brain parenchyma, where they accumulate around amyloid plaques (FIG. 5). It has been suggested that BMDPs are specifically attracted to Aβ *in vivo* and are more efficient than resident microglia in eliminating this protein by phagocytosis[156]. Moreover, CCR2-expressing perivascular monocytes and/or macrophages clear Aβ from the brain[113], which is line with previous observations that CCR2 deficiency accelerates disease progression and promotes cerebral amyloid angiopathy in Alzheimer's disease transgenic mice[157].

Even behavioural disorders with less overt neuropathological changes may benefit from the engraftment of BMDPs in the brain[20,21]. Thus, the compulsive grooming and hair removal in *Hoxb8*-mutant mice has been linked to a defect in resident microglia, and the behavioural deficit is corrected by the transplantation of wild-type bone marrow cells[158]. Rett syndrome is an X-linked neurodevelopmental disorder with phenotypic overlap with autism spectrum disorders[159]. The patients are generally female and develop normally during the first year of their life, after which they start to suffer from deficits in language and communication, impaired social interactions, stereotypic behaviours and autonomic dysfunction. Rett syndrome is mainly caused by mutations in the gene encoding methyl-CpG-binding protein 2 (MECP2). The symptoms are believed to result from deficits at the microcircuit level in the brain, involving synaptic transmission and plasticity (FIG. 5). Recent experiments in *Mecp2*$^{-/y}$ male mice (in which the single X chromosome copy of *Mecp2* is mutated) and *Mecp2*$^{-/+}$ female mice (which carry only one functional copy of *Mecp2*) suggested that resident microglia may also be responsible for the disorder[160]. In fact, microglia are reduced in number in *Mecp2*-null mice and fail to phagocytose debris as effectively as those in wild-type mice (FIG. 5). Importantly, engraftment of wild-type BMDPs after BMT increased the lifespan of *Mecp2*-null mice and ameliorated the behavioural and autonomic phenotypes[160].

Conclusions and future directions

The advent of new transgenic animal models, intravital imaging and transcriptomic tools has enabled researchers to unequivocally determine the origin of microglia as well as to study their development and fate in the CNS. Microglia belong to the mononuclear phagocyte system of the body. They are more closely related to macrophages that reside in peripheral tissues than they are to the neuroectodermal-derived neurons, astroglia and oligodendroglia in the brain.

We are now able to study microglia as they develop in the embryo, acquire specialized functions, participate in neuronal network formation and activity, respond to damage and succumb to disease. Although many of the insights have come from studying laboratory animals and remain to be translated to the human condition, there is an increasing awareness of the diversity and plasticity of innate immune cells in the brain. Novel transcriptomic and epigenomic techniques will improve our knowledge even further.

Microglia are not a homogeneous population, and they do not respond uniformly to microenvironmental changes. Instead, they meticulously survey and weigh

Graft-versus-host disease
A complication following an allogeneic tissue transplant in which immune cells (white blood cells) in the tissue (the graft) recognize the recipient (the host) as 'foreign'.

X-linked adrenoleukodystrophy
A rare X chromosome-linked disorder resulting from mutations in *ABCD1* (ATP-binding cassette subfamily D member 1) that cause defects in peroxisomal β-oxidation and lead to the accumulation of very-long-chain fatty acids, particularly in the CNS and adrenal cortex.

the signals that instruct them to acquire specific functions. The same is true for brain macrophages and peripherally derived monocytes, macrophages and dendritic cells. All of these innate immune cells rarely act in isolation, and it may turn out to be a dangerous oversimplification to distinguish 'detrimental' M1- and 'healing' M2-type responses when there is actually a spectrum of spatiotemporally defined graded activation patterns of microglia and brain macrophages.

One of the most exciting findings of the latest research on myeloid cells in the brain is the changing concept of microglia from culprit to victim. We are beginning to recognize that disturbances or loss of microglial function may be a driving force in CNS disorders. It is tempting to speculate that we shall see an increasing number of primary or secondary 'microgliopathies', which will open up new avenues for the treatment of neurological and psychiatric disorders.

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Acknowledgements
The authors apologize to all of those colleagues whose work was discussed without proper quotation owing space constraints. The authors thank S. M. Brendecke for critically reading the manuscript and M. Knust for help with the artwork. M.P. and J.P. are joint coordinators of the DFG-funded research unit (FOR) 1336. In addition, M.P. is supported by the BMBF-funded competence network of multiple sclerosis (KKNMS), the Gemeinnützige Hertie-Stiftung (GHST), the Fritz Thyssen Stiftung, the competence network of neurodegenerative disorders (KNDD) and the DFG (SFB 992). J.P. receives additional funding from the DFG (SFB/TRR43 and the Cluster of Excellence NeuroCure), the BMBF (Forschungsnetz zu psychischen Erkrankungen) and the Berlin Institute of Health.

Competing interests statement
The authors declare no competing interests.