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REVIEW

Microglia: unique and common features with other tissue macrophages

Marco Prinz · Tuan Leng Tay · Yochai Wolf · Steffen Jung

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Abstract Microglia are highly specialized tissue macrophages of the brain with dedicated functions in neuronal development, homeostasis and recovery from pathology Despite their unique localization in the central nervous system (CNS), microglia are ontogenetically and functionally related to their peripheral counterparts of the mononuclear phagocytic system in the body, namely tissue macrophages and circulating myeloid cells. Recent developments provided new insights into the myeloid system in the body with microglia emerging as intriguing unique archetypes. Similar to other tissue macrophages, microglia develop early during embryogenesis from immature yolk sac progenitors. But in contrast to most of their tissue relatives microglia persist throughout the entire life of the organism without any significant input from circulating blood cells due to their longevity and their capacity of self-renewal. Notably, microglia share some features with short-lived blood monocytes to limit CNS tissue damage in pathologies, but only bone marrow-derived cells display the ability to become permanently integrated in the parenchyma. This emphasizes the therapeutic potential of bone marrow-derived microglia-like cells. Further understanding of both fate and function of microglia during CNS pathologies and considering their uniqueness among other tissue macrophages will be pivotal for potential manipulation of immune cell function in the CNS, thereby reducing disease burden. Here, we discuss new aspects of myeloid cell biology in general with special emphasis on the brain-resident macrophages and microglia.

Keywords Microglia · Yolk sac · Bone marrow · Inflammation · Monocytes · Neurodegeneration · CX3CR1 · CX3CR1Cre

M. Prinz (✉) · T. L. Tay
Institute of Neuropathology, University of Freiburg, Breisacher Str. 64, 79106 Freiburg, Germany
e-mail: marco.prinz@uniklinik-freiburg.de

M. Prinz
BIOSS Centre for Biological Signalling Studies, University of Freiburg, Freiburg, Germany

Y. Wolf · S. Jung (✉)
Department of Immunology, The Weizmann Institute of Science, 234 Herzl St, 76100 Rehovot, Israel
e-mail: s.jung@weizmann.ac.il

The origin of microglia compared to other tissue macrophages

Tissue macrophages are part of the mononuclear phagocytes and were classically thought to derive from blood monocytes [30]. This assumption was based on the fact that monocytes are leukocytes with a very short circulation half-live and they were considered to act mainly as precursors of peripheral mononuclear phagocytes. Secondly, monocytes recruited to sites of inflammation, such as for instance a challenged peritoneum, were shown to give rise to macrophages [64]. Finally, also in vitro cultured monocytes were shown to differentiate into cells with macrophage features [125]. However, the recent past has seen a major revision of this established dogma. As a tissue macrophage archetype defined by its seclusion behind the blood–brain barrier (BBB), microglia played a major role in these discoveries. Early studies involving chick-quail chimeras had already suggested that microglia are established during embryonic development [63]. However, it took the advent of novel fate mapping methods to firmly establish the origins of adult microglia in mammals. Specifically, these novel findings are based on a combination of transgenic mice that harbor cell-type restricted constitutive

or conditional Cre recombinase activity with mice harboring reporter gene alleles that are activated upon excision of loxP-flanked ('floxed') STOP cassettes. As the Cre-mediated DNA rearrangements in the given cells are permanent, this approach reveals information about the ontogeny and history of the studied cells. Applying this approach to the study of microglia, Ginhoux and colleagues discovered that the microglia compartment is established well before birth from primitive macrophages that are generated during an early 'primitive' wave of hematopoiesis in the yolk sac [35]. Moreover, the authors showed that after birth, microglia remain independent from input from bone marrow-derived monocytes and adult hematopoiesis, but rather maintain itself by longevity and limited self-renewal. These findings were subsequently corroborated and extended to establish the critical role of Irf8 in the generation of yolk sac-derived microglia [58]. Moreover, additional studies by us and others showed that the embryonic origin is a common feature of most tissue macrophages [47, 116, 141]. Of note, recent work on microglia development in mouse was preceded by live time-lapse recordings that fate-mapped early hematopoietic precursors from the yolk sac to the mesenchyme of the head and the depths of the brain in transparent embryonic zebrafish [49].

As opposed to the microglia that seems almost exclusively yolk sac-derived, other tissue macrophage compartments display, however, various contributions from cells derived at a later time point from the fetal liver (Ginhoux and Jung, in press) (Fig. 1). Interestingly, Geissmann and colleagues showed that their unique yolk sac origin is reflected in the independence of microglia from the transcription factor Myb [116]. It is currently unclear to what extent the generation of fetal monocytes is dependent or independent of Myb and its link to primitive or definitive hematopoiesis. Of note, the number of tissue macrophages that have been studied in sufficient detail using the fate mapping approach is still limited. However, for epidermal Langerhans cells, as

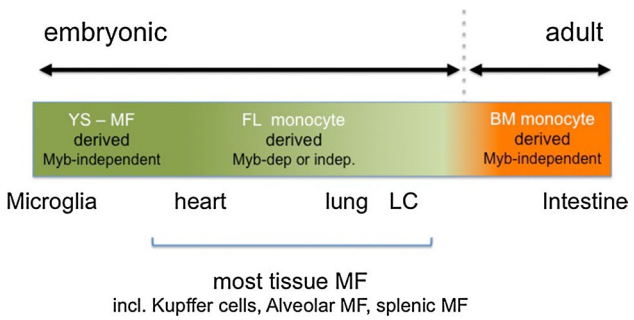

Fig. 1 Microglia origin compared to other tissue macrophages. Developmental scale of macrophage development from embryonic stages that contain yolk sac (*YS*)-derived macrophages (*MF*), fetal liver (*FL*) monocytes until postnatal (*adult*) bone marrow (*BM*)-derived macrophages. *Dotted line* indicates the time point of birth

well as the majority of heart macrophages and lung macrophages, it has been established that they are mostly fetal liver-derived [27, 43, 52]. Microglia therefore clearly represent an exception in that they are homogeneously yolk sac-derived (Fig. 1). Of note, the spectrum of tissue macrophage origins also includes the other extreme, that is, a population that is entirely monocyte-derived during steady state. Macrophages that reside in the intestinal lamina propria, the connective tissue underlying the gut epithelium, were shown to have a rather short-half life and rely on constant renewal from Ly6C^{+} blood monocytes [142]. Monocyte-dependent macrophage subpopulations have also been noticed in other steady-state tissues, such as the skin and heart [27, 129], but it remains to be shown whether these cells differ in function. Moreover, monocytes are well known to be prominently recruited to sites of inflammation and during pathology. The monocyte-derived macrophages that arise from these inflammatory monocyte infiltrates are however probably mostly short lived. This has most convincingly been shown in an elegant study involving EAE and the use of parabionts [3]. Again, exceptions seem however to exist, as it was recently demonstrated that monocyte-derived macrophages that reside in atherosclerotic plaques can persist and undergo considerable proliferative expansion [109].

At least in the mouse, microglia are established before the formation of the BBB and are independent from hematopoiesis in adulthood. However, it has been noted that following irradiation and bone marrow transplantation, the brain can be seeded by hematopoietic stem cell (HSC)-derived cells that persist and constitute a progressively increasing and considerable fraction of the brain macrophage compartment (Volasky and Jung, unpublished results). These cells originate clearly from cells that are distinct from monocytes, but might be earlier myeloid precursors that entered the damaged brain following the irradiation. Importantly, this finding indicates that the adult bone marrow hosts cells that can give rise to long-lived radio-resistant microglia. Interestingly, it has been shown that this population can reconstitute microglia defects [16, 23] and thus has therapeutic potential. However, it remains to be established whether these brain macrophages have all activities of bonafide microglia, both in homeostasis and pathological settings.

Microglial functions within the non-inflamed CNS

Since the early studies of Ilya (Eli) Metchnikov macrophages are well appreciated to have dual functions, both in inflammatory and pathological settings, as well as in 'housekeeping' that is critical to maintain tissue homeostasis under non-inflammatory conditions. Though early studies have emphasized the contributions of microglia to pathology, more recent studies demonstrate that microglia

are hardly 'quiescent' in steady state, but in fact actively participate both in the shaping of the developing and young CNS, and its maintenance, regardless of immune challenges [131, 137]. Microglia function is therefore similar to reports describing macrophages involved in tissue remodeling [101] and wound healing [89].

Once considered 'immune-privileged' and excluded from ongoing immune surveillance, the brain is now recognized to host not only resident microglia as the representative macrophage population, but also other immune cells in steady state [105]. Since neuronal damage is largely irreversible once it has occurred, the CNS parenchyma requires tightly regulated and orchestrated inflammatory reactions that aim at restoration, in which microglia participate in substantially. Genome-wide expression profiling of multiple immune cell subsets by the Immunological Genome Consortium has established that microglia stand out with a unique transcriptome among other tissue macrophages, such as the ones in the splenic red pulp, peritoneum or lung alveolar space [32]. Similar analysis has emphasized the differences of gene expression profiles of microglia and Ly6C^{+} monocytes [11, 12] (Fig. 2). Moreover, these recent studies have highlighted the role of TGFβ in imprinting this unique microglia signature, which includes known macrophage genes such as *cx3cr1*, *itgam1* and *aif1* and also microglia-specific genes *p2ry12*, *fcrls* and *tmem119*. These data support the notion that to perform within their unique and sensitive microenvironment, microglia require a characteristic gene expression distinct from other Myb-dependent or independent, embryo-derived macrophages. This probably includes the ability to phagocytose and secrete soluble factors in a regulated manner, with minimum inflammatory output.

During development, microglia can interact with neurons either by direct contact resulting in phagocytosis or by secretion of soluble factors. The capability of microglia to phagocytose apoptotic neurons [73, 99, 122], synaptic material [97, 115] and cellular debris [23] is well established. Moreover, in the developing cerebellum, microglia were reported not only to phagocytose, but also to actively induce apoptosis of developmental Purkinje cells by means of respiratory burst [73]. In adolescent mice, in which many redundant neurons undergo elimination, microglia actively phagocytose apoptotic neurons and thus participate in early postnatal neurogenesis [122] and modulate synaptic pruning during development [97].

Microglial–neuronal crosstalk can involve several signaling pathways, such as the CD200-CD200R [53], the

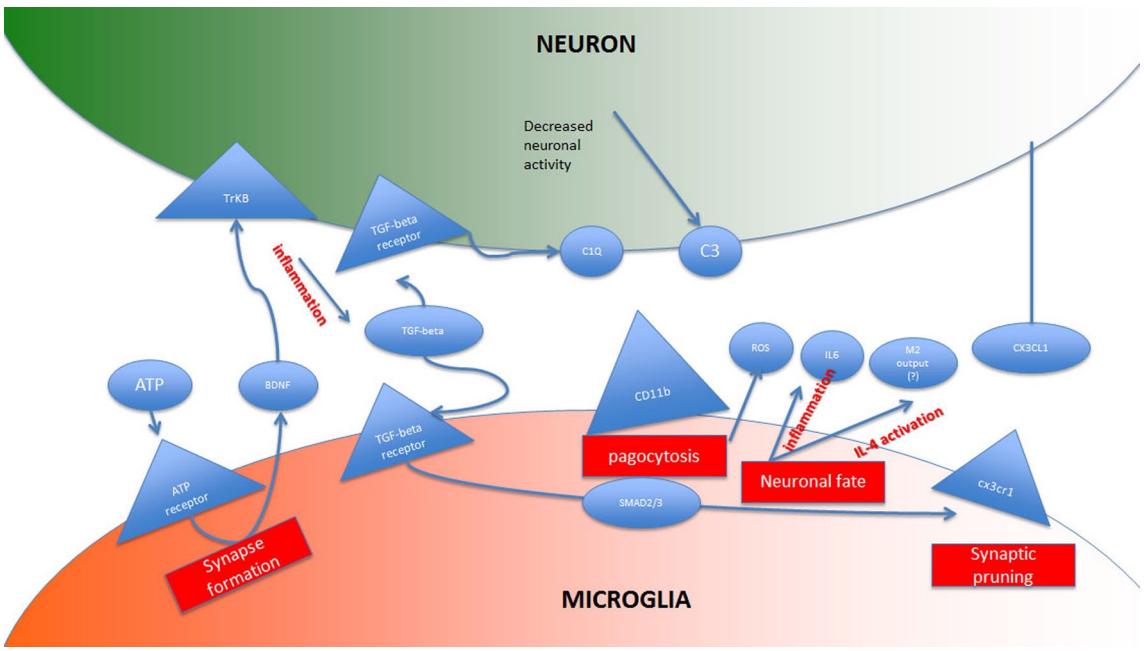

Fig. 2 Neuronal–microglial dialog during CNS homeostasis. Microglia participate in synaptic pruning during development via the CX_3CR1/CX_3CL1 axis, mediated either by transmembranal or soluble neuronal CX_3CL1, which may be influenced by TGFβ-mediated expression of CX_3CR1 via SMAD2/3 phosphorylation. Microglia can influence neuronal fate by inflammatory output, such as IL-6, which inhibits the differentiation of neuronal precursor cells (NPCs) into neurons during early postnatal days, or by IL-4-mediated activation, which may drive NPCs into newborn neurons. Microglia phagocytose weak synapses by the CR3 complement pathway (CD11b → C3/C1q), which is regulated by decreased neuronal activity or via TGFβ signaling. Neurons can also express TGFβ under inflammatory conditions, which may enhance microglial phagocytosis. Finally, ATP signaling can give rise to the secretion of microglial BDNF, which drive TrKB phosphorylation and thus contribute to the formation of glutamatergic synapses

CD47-CD172a [36] and the CX_3CR1-CX_3CL1 axis [60, 61, 107, 140] (Fig. 2). In the above cases, the ligand is expressed by neurons or other glial cells, with the receptor located on the microglia. It is generally thought that many of these signaling pathways maintain microglia in a quiescent state [10]. In support of this notion, disruption of such communication, such as for instance the CX_3CL1-CX_3CR1 axis, impairs the maturation of dendritic spines during development, probably by excessive synaptic pruning [97]. Importantly, the classical complement proteins, such as C1q, are present within the CNS [126], and microglia were shown to use the complement receptor 3 pathway to engulf presynaptic inputs during the pruning peak of retinal ganglion cells (RGCs) in an activity-dependent manner [115]. Several other reports have shown that microglia phagocytosis is dependent on neuronal activity, such as visual experience [130] or antagonist-mediated neuronal silencing, which decreases the contact frequency between microglia and synapses [136, 137]. It is therefore intriguing to postulate that neurons might be able to fine tune the inhibitory 'off' signal to microglia according to their activity levels or the strength of their synapses [10].

Direct microglial impact on neurons by secreted factors is less well established, although several such factors were proposed. The majority of the literature is focused on the interplay between adult neurogenesis and microglia-derived cytokines. Adult neurogenesis is confined to two locations in the brain, the subventricular zone (SVZ) in which the newborn olfactory neurons are generated and migrate into the olfactory bulb [85] and the subgranular zone of the hippocampus (SGZ) [122]. Microglia seem to be the key players for neurogenesis [34] as they phagocytose apoptotic neurons in the SGZ during early postnatal development without being activated by inflammation [122]. Importance of the robust microglia silence is highlighted by the fact that SGZ neurogenesis is inversely correlated with pro-inflammatory microglia activation [26]. Moreover, neuronal in vitro cultures supplemented with media from cultured LPS-activated microglia also display less DCX^+ immature neurons and this effect could be partially reversed using antibody-mediated IL-6 blockade [87]. Interestingly, IL-6 also blocks the differentiation of GFAP+ neuronal stem cells into mature neurons [6], and IL-6 overexpressing transgenic mice have reduced neurogenesis in the hippocampus [135]. A cytokine which is considered to promote neuronal differentiation is IL-4, which polarizes microglia to acquire M2-like, non-inflammatory features [117] and may contribute to the preservation of cognitive functions [31]. Accordingly, cultured neurons in the presence of conditioned medium of IL-4 activated microglia display more immature βIII tubulin+ neurons [17].

One cytokine, which recently gained particular focus with respect to microglia in the steady-state CNS, is the transforming growth factor (TGF) β (Fig. 2). TGFβ was reported to promote the development of bonafide microglia in vitro via the SMAD2/3 pathway and to regulate microglial CX_3CR1 expression [1]. TGFβ imprints a microglia gene expression signature and mice which lack TGFβ seem to have reduced numbers of microglia [12]. Neurons can, at least under inflammatory conditions, secrete TGFβ [72]. However, neurons also require TGFβ to fully express the complement protein C1q and thus initiate the CR3 pathway to induce microglial phagocytosis. As a result TGFβ-R2−/− mice exhibit an impaired segregation of contra- and ipsilateral retinal input in the RGC [9]. Thus, TGFβ is required both directly for microglia development and maturation, and also indirectly for their proper homeostatic functions.

Another microglial-derived secreted factor that has been proposed to be critical in microglia–neuron crosstalk is brain-derived neurotrophic factor (BDNF), which acts on the receptor tyrosine kinase *TrkB* (also known as *NTRK2*) expressed on dendritic spines and is involved in neuronal plasticity and synapse remodeling [83]. ATP stimulation induces microglia secretion of BDNF, which is involved in pain sensation [18]. By exploiting mice that harbor an insertion of a tamoxifen-inducible Cre recombinase under the control of the CX_3CR1 promoter, and achieving microglia-specific BDNF gene ablation, it was recently suggested that microglia participate in the learning-dependent synapse formation and elimination in the motor cortex [98]. Moreover, the authors suggested that microglial BDNF might be critical for plasticity and neurotransmission, as protein levels of glutamate receptor subunits GluN2B and GluA1 were reduced in the absence of microglial BDNF. In the developing barrel cortex, CX_3CR1-deficient mice exhibit delayed developmental switch of the glutamate receptor subunits GluN2B to GluNA, one of the main features of barrel cortex development [54]. The mechanism suggested was the elimination by a soluble factor. However, given the data of Parkhurst et al. [98], it is possible that BDNF is involved in the mechanism. However, a molecular link between CX_3CR1 and BDNF production remains to be shown. The detachment of afferent axonal endings from the surface membrane of regenerating motoneurons and their subsequent displacement by microglia in a process called "synaptic stripping" has been described in rodent models and patients of neurodegenerative conditions [88].

Bone marrow-derived microglia-like cells and tissue macrophages

Do bone marrow-derived microglia-like cells (BMDM) exist in nature and if so, are they functionally similar compared to yolk sac-derived bonafide microglia? The answer to this question could have tremendous clinical implications for the treatment of many diseases of the human CNS, such

as brain tumors, amyotrophic lateral sclerosis (ALS), Alzheimer's (AD), and Parkinson's disease (PD). Specifically, microglia precursors in the bone marrow could be used as Trojan horses to deliver neuroprotective or immune-relevant genes to the diseased CNS to modulate pathology. Upon injury or infection, BM-derived inflammatory or patrolling monocytes are summoned from the bloodstream to the inflammatory lesion, followed by terminal differentiation into macrophages [70]. The availability of the CX_3CR1^{GFP} mouse [56] enabled the identification of a short-lived blood monocyte subset characterized as $CX_3CR1^{lo}CCR2^{+}Gr1^{+}$ cells that are actively attracted to inflamed tissue, while an alternate $CX_3CR1^{hi}CCR2^{+}Gr1^{+}$ class of monocytes was shown to reside in the vascular lumen and regulate endothelial integrity [5, 33]. Davies et al. recently showed that both resident macrophages and recruited inflammatory monocytes expand their populations with different kinetics at the onset and recovery of the tissue insult to regain homeostasis [21, 22]. Does this also apply to the brain parenchymal microglia of the immune-privileged CNS?

Several approaches were taken to resolve this enigma. Also using incorporation of [^{3}H]-thymidine, in combination with F4/80 immunohistochemistry to identify resident microglia in the adult murine brain, researchers came to the conclusion that these tissue macrophages are a self-renewing population, which nevertheless derive in addition from circulating monocytes that continuously engraft the brain via an intact BBB and subsequently differentiate into resident microglia [68]. However, other reports involving BM chimeras with alternatively marked donor cells, such as MHC class I in rat [76] or Y chromosome containing cells in female BM transplant hosts [134], were not able to demonstrate engraftment of donor BM-derived microglia in the brain parenchyma. Studies indicated that perivascular macrophages in mouse [7], rat [50] and human [134] brains were donor-derived from circulating myeloid cells. In a meningitis infection model, we showed that GFP-labeled circulating cells entered the brain of a reconstituted host and differentiated into microglia with multiple processes reminiscent of a typical microglia cell [24]. Later studies in non-human primates [124] and a comparative investigation involving radiation chimeric mice and rats [65] demonstrated differential recruitment of donor BM-derived macrophages to the periphery of the CNS (perivascular space and choroid plexus) and engraftment into the brain (neocortex and cerebellum).

All previous reports, however, did not directly address the question of whether BMDM are recruited to the immune-privileged brain parenchyma under physiological conditions. In fact, the use of irradiation followed by BM transplantation leads to non-physiological permanent alterations of the BBB, induces a chemokine storm among other changes in the brain [59, 79], and elevates HSC count in blood, all of which promote entry of circulating myeloid cells into the CNS [104, 106] (Fig. 3).

Two groups further investigated the engraftment of BMDM while avoiding the induction of non-physiological conditions via head protection of irradiated hosts [79], or using parabiosis, where the circulation of both host and recipient is surgically conjoined to allow mixing of the blood elements [2]. In the head protection model, engraftment by peripheral GFP-labeled BM cells was absent even upon cuprizone-induced demyelination or transection of the facial motor nerve [79]. Both studies came to the conclusion that in the presence of an intact BBB, native microglia undergo local proliferation during homeostasis. The recruitment of BM cells into the brain was only possible with conditioning of the host via irradiation [79] (Fig. 3). In another study involving whole BM and HSC transplantation and parabiosis models, it was striking that nearly no GFP^{+} donor cells were detected, even with injury to the hippocampus induced by intraperitoneal application of kainate acid, in the parabiosis model where the BBB was left intact [74]. Of note, peripheral chimerism was rather low at 0.5–10.7 % even in transplanted mice [74]. Similarly, a recent report comparing brain conditioning by lethal dose irradiation and application of the alkylating agent busulfan indicated that damage to the BBB is required for engraftment of BMDM in both steady and challenged conditions [59]. Treatment with busulfan did not disrupt the BBB as drastically as by irradiation, which led to several-fold higher myeloid cell entry into certain brain regions of radiation chimeras [59]. Others also showed using only busulfan-based chemotherapy regime that the introduction of BMDM into the brain parenchyma required pathological conditions, such as in hypoxic–ischemic stroke and APP/PS1 AD models [66], or stress [139]. In the latter case, $CD45^{hi}$ cells entered the brain parenchyma in a CCR2-independent, social stress-driven manner, and eventually adopted endogenous microglia morphology in the dentate gyrus [139].

Interestingly, BM-derived myeloid cells and yolk sac-born microglia contribute differently to inflammatory response of the CNS [80] and neurodegenerative diseases as shown in mouse models of AD [82, 123] and multiple sclerosis (MS) [3, 81], as well as in a spinal cord injury model [120]. For example, $CCR2^{+}$ BMDM were suggested to be superior in plaque clearance and reduction of disease burden in AD models [82, 123]. Furthermore, recruitment of BM-derived $Ly6C^{hi}CCR2^{+}$ monocytes has been reported in the experimental autoimmune encephalomyelitis (EAE) animal model of autoimmune CNS disease mimicking MS and was suggested to exacerbate disease progression [81]. Notably, these infiltrating monocytes were short-lived and vanished several days after engraftment, thereby excluding their stable integration into the endogenous CNS microglial

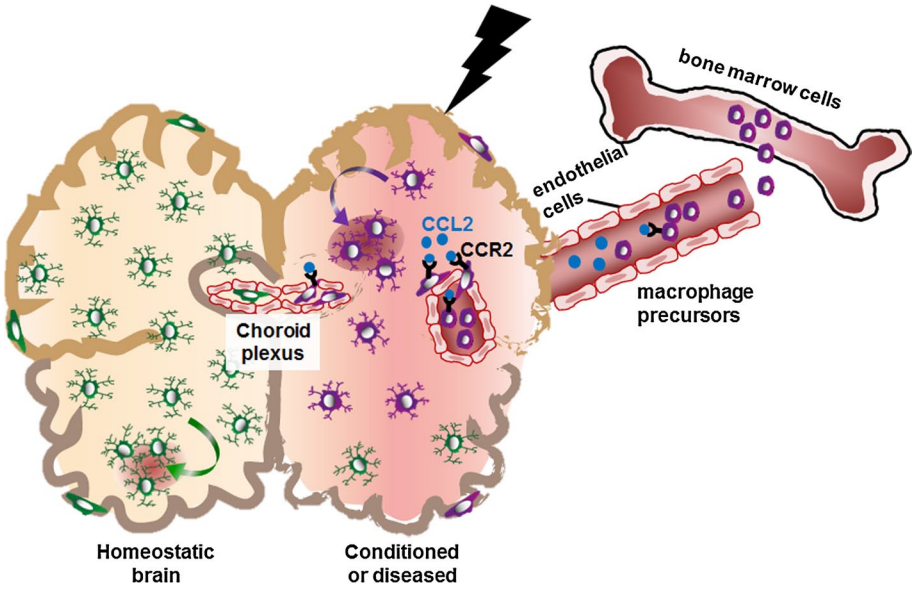

Fig. 3 Conditions for recruitment of bone marrow-derived microglia (BMDM). Postnatal BMDM form only under defined host conditions in the CNS. BM cells (*purple round cells*) are released into the bloodstream in a chemokine receptor (CCR) 2-dependent fashion and can enter the conditioned CNS. Local conditioning of the CNS can occur via irradiation or neurodegeneration, which lead to subsequent disruption of the blood–brain barrier and concomitant induction of chemokines such as CCL2, thus allowing engraftment of BM-derived macrophages (*purple elongated cells*). In conditioned CNS, BMDM (*purple ramified cells*) undergo hyperplasia in challenged or disease states (indicated by *purple arrow*). Yolk sac-derived microglia (*green*) are radio-resistant and perform self-renewal by undergoing homeostatic proliferation (indicated by *green arrow*)

network [3]. Using a CCR2-deficient mouse model, Serbina and Pamer demonstrated the necessity of this chemokine signaling for the extravasation of Ly6C^{hi} monocytes from the BM into the blood stream, but that it was not required for translocation to the tissue [118].

BMDM are characterized by the expression of Ly6C^{hi}CCR2^{+} surface markers, in contrast to Ly6C^{-}CCR2^{-} microglia [79, 81, 82, 86]. To date, however, it is still not completely clear whether BMDM are able to fulfill the whole range of functions of endogenous microglia. If the development of sophisticated microglial phenotypes is a consequence of long term co-evolution with other cell populations and networks in the CNS, could newly immigrated BMDM simply replace native microglia cells [92]? Interestingly, the route of entry of BMDM into the conditioned brain parenchyma was described to be via the choroid plexus [121]. Other studies hinted a preferential engraftment of BMDM in the olfactory bulb and cerebellum in contrast to the cortex, striatum and hippocampus [103].

Microglia as disease inducing cells

Despite the low number of microglia compared to other resident brain cell types, these small cells play significant roles in the developing early postnatal brain [97, 115, 133] as well as maintain homeostasis in the healthy adult brain [46, 131]. During challenges such as injury [20, 93], infection or disease [19, 24, 113], microglia nimbly navigate the altered brain physiological landscape via changes in their morphology, presentation of antigens and release of cytokines. Whether microglia contribute as beneficial or harmful mediators in autoimmune diseases [37] or neurodegenerative disorders [104, 127] is open to deeper investigation. Recent studies have linked microglia-related genetic mutations to neurological or behavioral abnormalities in humans and mouse models [14, 16, 23, 29, 62, 94, 96, 119]. On the contrary, some genetic depletions in microglia revealed neuroprotective outcomes in disease background [13, 48, 104, 106].

Several molecular modulators of microglia homeostasis that may subsequently mediate or induce CNS diseases [60] include transcription factors and other nuclear proteins (Fig. 4). The development of yolk sac-derived microglia hinges on two important transcription factors PU.1 (also known as SFPI1) and interferon regulatory factor (IRF) 8 [8, 58]. PU.1 is exclusively expressed in the hematopoietic cell lineage and required for normal myeloid cell development among members of the ets family of transcription factors [110]. Mice deficient in PU.1 lack circulating monocytes and tissue macrophages including microglia [8, 77]. The regulation of the receptor of the macrophage colony-stimulating factor CSF-1, which is required for macrophage survival and proliferation, was shown in vitro

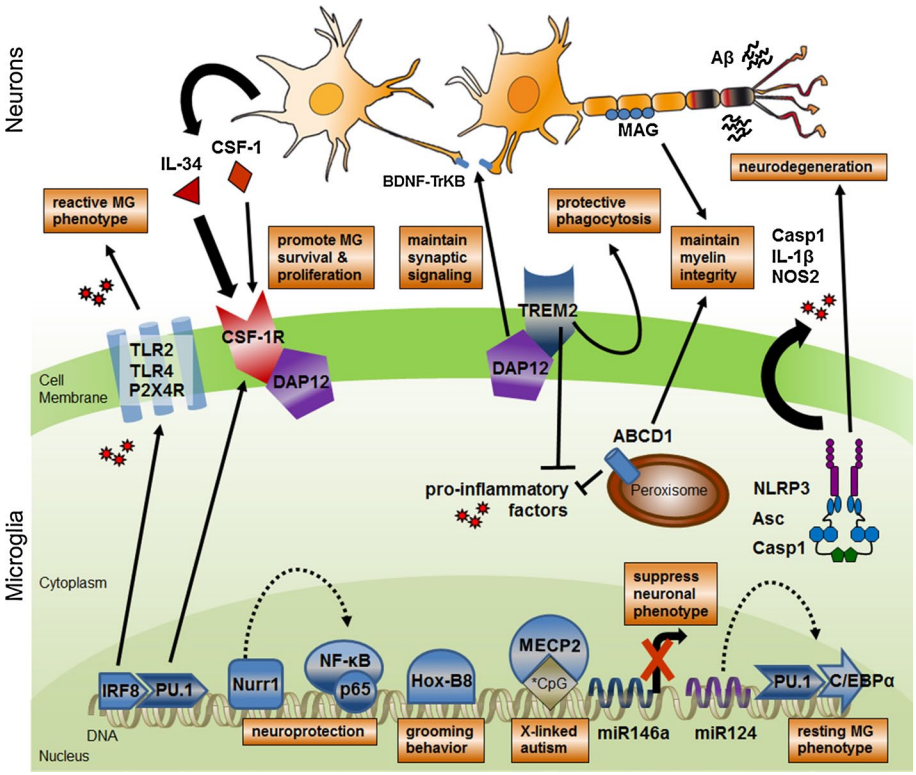

Fig. 4 Factors for microglia homeostasis and disease induction. Up-regulation of IRF8 expression promotes a reactive microglia (*MG*) phenotype via *TLR2*, *TLR4*, *P2X4R*, and inflammatory factors. IRF8 DNA-binding activity is dependent on other transcription factors, such as PU.1. PU.1 regulates the receptor for macrophage colony-stimulating factor CSF-1 (*CSF-1R*), which is required for microglia survival and proliferation. In the CNS, the ligand interleukin (*IL*)-34 is more prominent than CSF-1 in microglia–neuron interaction. Microglia-specific CSF-1R signaling requires the adaptor DNAX-activating protein of 12 kDa (*DAP12*). DAP12 maintains synaptic signaling via the brain-derived neurotrophic factor (*BDNF*)—tyrosine kinase receptor B (*TrKB*). Functional loss of DAP12 and triggering receptor expressed on myeloid cells 2 (*TREM2*) has been implicated in the pathogenesis of neurodegenerative diseases such as Alzheimer's, Parkinson's, Nasu–Hakola disease, and multiple sclerosis. TREM-2 suppresses the expression of pro-inflammatory factors and promotes phagocytosis of cellular debris. The ABCD1 protein, an adenosine triphosphate-binding cassette transporter located in the peroxisomal membrane, together with the myelin-associated glycoprotein (*MAG*) expressed in Schwann cells or oligodendrocytes is important for maintaining myelin integrity and modulating microglia activation state. In AD, the microglial inflammasome, comprising caspase 1 (*Casp 1*), apoptosis-associated speck-like protein containing a caspase recruitment domain (*Asc*), and nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3 (*NLRP3*), contributes to increased amyloid-β (*Aβ*) load, shift of microglia towards pro-inflammatory-activated phenotype, neurodegeneration, and cognitive defects. The orphan nuclear receptor Nurr1 may be neuroprotective via NF-κB-p65 signaling. Loss of Hox-B8 in microglia leads to excessive grooming. Deficiency in the methyl-CpG-binding protein (*MECP*) 2 leads to the X-linked autism spectrum disorder Rett syndrome. MicroRNA miR-146a could maintain microglia phenotype via suppression of neuron-specific genes. The resting state of microglia may be maintained by miR-124 via the transcription factors PU.1 and C/EBPα. Cell membrane, cytoplasm and nucleus of a microglia cell are depicted in *different shades of green*

to be dependent on PU.1 [15]. In a mouse model of ALS, microglia from wild-type BM-derived cells transferred to PU.1−/− neonates with a point mutation for ALS-causing gene were able to partially but significantly rescue the defects seen in knockouts [8]. IRF8 is a weak DNA-binding protein on its own and requires interaction with other transcription factors, such as PU.1 [84]. Interestingly, IRF8-deficient mice developed altered distribution of microglia with reduced cell surface area and different expression of microglia markers including Iba-1, CD45, CD11b, F4/80 and CX_3CR1 [84]. Thus, IRF8 was proposed as a key factor of microglia development and activation [58, 84]. In a peripheral nerve injury model, Masuda et al. showed that IRF8 is up-regulated and claimed that its expression shifts microglia towards a reactive phenotype via promotion of gene expression of innate responses such as TLR2 and TLR4, chemotaxis, CX_3CR1 and inflammatory factors IL-1β and P2X4R [75]. However, it is still open whether IRF8 is critical in regulating homeostasis of mature microglia. Point mutations in IRF8 were reported in three patients of immunodeficiency syndromes, characterized by the absence or abnormality of peripheral myeloid cells such as monocytes and dendritic cells, but skin samples revealed normal LCs, while brain biopsies were unavailable [44].

The immune-related role of another microglia-related transcription factor, Nurr1, was investigated in the context of inflammation-mediated neuronal cell death [112]. Nurr1 is an orphan nuclear receptor known to be required for the maintenance of post-mitotic midbrain dopaminergic neurons and their neuronal phenotype [4]. Human point mutations in the Nurr1-coding gene *NR4A2* were identified in patients of late onset familial, but not sporadic, PD [69]. In these affected patients, the levels of *NR4A2* mRNA were decreased in lymphocytes, together with altered expression of tyrosine hydroxylase, which is the rate-limiting enzyme in the biosynthesis of dopamine [69]. By analyzing the responses to LPS-induced inflammation in primary mouse and human microglia in which *Nurr1* was knocked down by silencing RNA or lentivirus small-hairpin RNA, it was proposed that Nurr1 played a neuroprotective role via the NF-κB-p65 signaling pathway in glia [112]. However, this putative function of microglial Nurr1 in vivo has not been directly addressed so far.

The loss of DNA-binding nuclear protein Hox-B8 in the hematopoietic lineage revealed a striking excessive pathologic grooming behavior in the mutant mice [16]. This behavior was reportedly similar to that of sufferers of the obsessive–compulsive spectrum disorder trichotillomania. Interestingly, the authors attributed this strong phenotype to microglia, as they were unable to detect reporter expression in other CNS cell types based on their *Hoxb8-IRES-Cre*-mediated reporting of Hox-B8 localization [16]. Furthermore, they demonstrated that wild-type BMDM could rescue the behavioral phenotype in their mutant model [16].

In a similar vein, Derecki et al. were able to ameliorate the pathology in a methyl-CpG-binding protein (MECP) 2-null mouse model of Rett syndrome, an X-linked autism spectrum disorder, by transplantation of wild-type BM [23]. Rett syndrome is severely debilitating in males, which have only one X chromosome. However, CNS engraftment of BMDM, which displayed properties of resting microglia, largely alleviated the symptoms in MECP2-null males and extended their life spans [23].

Microglial phenotype is also controlled by several microRNAs (miRNA) [42]. In a microarray analysis of miRNA expression in neurons, astrocytes, oligodendrocytes and microglia, cell type-specific enrichment of miRNAs such as miR-146a in microglia was described [55]. Binding of miR-146a to neuron-specific genes shown in luciferase reporter assays led the authors to speculate that this miRNA could be important for maintaining a non-neuronal phenotype in microglia [55]. In another study, miR-124 was proposed to maintain the quiescent state of microglia, where elevated levels of miR-124 kept activation markers MHC Class II and CD45 down-regulated through the C/EBPα-PU.1 pathway [102]. Therefore, it is reasonable to speculate that mutations in miRNA-coding regions could contribute considerably to neurological dysfunction.

An important factor for the survival, maintenance and proliferation of macrophages is CSF-1 that signals through its receptor CSF-1R (also known as CD115) [41, 67]. Impaired auditory and visual processing of *Csf1*$^{op/op}$ osteopetrotic mice, in which CSF-1 is deficient, has been described [78]. A comparison of CSF-1R null mutant and *Csf1*$^{op/op}$ survival rates 1-month after birth revealed complete mortality of CSF-1R knockouts and a 40 % survival of *Csf1*$^{op/op}$ [90]. CSF-1R mutants reportedly have smaller brains and atrophic olfactory bulbs with obvious strong defects in olfactory function [28, 90]. While embryonic brains appeared to develop normally in CSF-1R knockouts, microglia are completely absent in these mutants [28]. It was reported that in postnatal mouse brain, CSF-1R is only expressed on microglia but not in other neural cell types [28]. These findings suggested that development of microglia in the CNS is dependent on CSF-1R signaling albeit via a different ligand compared to other tissue macrophages. Indeed, the expression of CSF-1 was described to be low throughout the brain, with slightly higher expression in the cerebellum throughout development and in adult animals [78]. Subsequently, it was found that microglial phenotype in mutants of the cytokine IL-34, a recently discovered alternative ligand of CSF-1R [71], is closer to that of the CSF-1R null, as a severe reduction of microglia number as well as skin LCs was observed here [138]. Furthermore, a lack of alteration in blood monocytes, other tissue macrophages and DCs, apart from a subset of DCs in the lung, points to the tissue-restricted requirement of IL-34 for LC development and microglia maintenance [138]. Comparative analysis of both CSF-1R ligands revealed a broader regional expression of IL-34 than CSF-1 in the brain, with complementary expression of each gene in the neocortex [90]. Using a mouse model of prion disease and from analyses of clinical samples of variant Creutzfeldt–Jacob disease and AD, PU.1 and CSF-1R were postulated to be molecular factors regulating microglial proliferation in these pathologies, suggesting that a delay in neuronal damage and disease progression could be achieved by targeting CSF-1R signaling between microglia and neurons [40]. CSF-1R signaling-dependent survival and proliferation of macrophages were shown to be inhibited in mutant mice for the transmembrane tyrosine kinase-binding DNAX-activating protein of 12 kDa (DAP12); in particular, fewer microglia cells were found in certain brain regions [95].

Functional loss of the adaptor protein DAP12 (alternatively named KARAP or TYROBP) in conjunction with mutations in the surface receptor, triggering receptor expressed on myeloid cells 2 (TREM2), has been implicated in the pathogenesis of several neurodegenerative diseases, including AD, Nasu-Hakola disease (NHD), PD, and

MS [29, 62, 91, 94, 96, 100, 108, 119, 128]. Expression of DAP12 in the brain is detected in microglia only and its mutation was linked to altered synaptic plasticity due to a large decrease in signaling via the brain-derived neurotrophic factor—tyrosine kinase receptor B [111]. TREM-2 is a pattern recognition receptor located on the cell surface of dendritic cells, bone osteoclasts and brain parenchymal microglia [119]. It plays a role in suppressing the synthesis of pro-inflammatory factors [45, 132] and promotes protective phagocytosis of cellular debris, such as degenerated myelin, as demonstrated in an animal model of MS [128]. In support of these findings, another study detected enhanced expression of TREM-2 on microglia during EAE and that blockade of the receptor exacerbated disease progression [100]. Just recently, the mutant allele *p.R47H* of TREM-2 was identified in a genome-wide association study on thousands of patients to be a risk factor for frontotemporal dementia and PD [108], in addition to the known risk for AD [91].

Human mutations in TREM-2/DAP12 are known in the rare autosomal recessive NHD [29, 62, 94, 96], which is characterized by pathological lesions in the cortex, thalamus and basal ganglia, progressive early-onset dementia and formation of bone cysts, consistent with the known tissue distribution of this receptor–adaptor complex [119]. Notably, peripheral myeloid cells appear to be unaffected in loss of TREM-2/DAP12 functions [119]. An investigation on NHD-specific biomarkers expressed by microglia confirmed the absence of DAP12 expression in NHD brains while detecting DAP12 protein on quiescent microglia in control samples [114]. Interestingly, this study included a claim that human microglia do not express TREM-2, leading to the antithesis proposed by the authors that loss of microglial TREM-2/DAP12 function in humans may not be the primary cause of NHD phenotype [114].

Intracellular signaling pathways also influence microglia-mediated innate immune responses. Analysis of brain lysates from AD patients and controls clearly demonstrated elevated levels of cleaved caspase-1 (Casp1) in the pathology, which was reflected in the APP/PS1 transgenic mouse model for familial AD [48]. Casp 1 is a component of the microglial nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, together with NLRP3 and the adaptor molecule apoptosis-associated speck-like protein containing a caspase recruitment domain [38]. NLRP3 inflammasomes have been implicated in several chronic inflammatory diseases, because they act as a sensor for inflammatory compounds such as amyloid-β (Aβ) [38, 48]. To examine the contribution of NLRP3 inflammasome to AD, the authors bred mutant mice carrying a defective component of the inflammasome with APP/PS1 mice [48]. The absence of either NLRP3 or Casp1 in the AD model significantly improved clearance of Aβ and led to overall neuroprotection, apparently via a shift of microglia activation towards anti-inflammatory state (i.e., lower IL-1β and nitric oxide synthase 2 and higher arginase-1, IL-4 and found in inflammatory zone 1, compared to wild-type microglia in AD) [48].

Mutations in the *ABCD1* gene encoding the ALD protein, an adenosine triphosphate-binding cassette transporter, cause the clinically heterogeneous disorder X-linked adrenoleukodystrophy (ALD) [14]. In boys, ALD is a severe brain inflammation and demyelination disease. Transfusion of autologous HSCs that were ex vivo infected with *ABCD1*-encoding lentivirus in two patients showed a halt in disease progression in less than 2 years from onset of gene therapy, even while reconstitution of myeloid and lymphoid cell types was only 9–14 % [14]. This report indicates that *ABCD1*-deficient microglia in the CNS could be replaced by BMDM carrying the wild-type ABCD1 allele. It was also described in ABCD1-null and myelin-associated glycoprotein-deficient mice models of ALD that a combined lack of both functional genes in metabolic control and myelin integrity led to an additive effect on microglia activation and axonal degeneration [25].

Outlook

Recent developments in investigative tools for immunology, imaging and genetics have progressed our understanding of the unique nature of microglia in development, homeostasis and disease. However, we are just beginning to decipher the enigmatic nature of microglia. For future experiments in mouse model systems, it would be vital to take advantage of recently developed microglia-specific genetic systems [39, 98, 141]. In particular, the use of these inducible systems for gene depletion in microglia would reduce the observation of artifacts arising from peripheral myeloid cells or other brain cell types in generic knockout animals [19]. These investigations may potentially reveal new intercellular interactions modulated by microglia and may recapitulate aberrant phenotypes observed in humans. As multifaceted guardian of the CNS, microglia possess a specialized transcriptomic signature that encodes proteins for detecting endogenous ligands and infectious agents [51], and myriad membrane channels and receptors [57]. It is thus vital to continue exploring the possibilities where these cells could be harnessed for gene delivery to the CNS, or act as direct targets of pharmacological interventions.

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