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Microglial signatures and their role in health and disease

Oleg Butovsky[*], **Howard L. Weiner**[*]

Ann Romney Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Abstract

Microglia are the primary innate immune cells in the CNS. In the healthy brain, they exhibit a unique molecular homeostatic 'signature', consisting of a specific transcriptional profile and surface protein expression pattern, which differs from that of tissue macrophages. In recent years, there have been a number of important advances in our understanding of the molecular signatures of homeostatic microglia and disease-associated microglia that have provided insight into how these cells are regulated in health and disease and how they contribute to the maintenance of the neural environment.

Our understanding of the origin and functions of microglia has grown to such an extent that it is as if a new CNS cell has been described[1–5]. These advances have major implications for understanding normal CNS function and have opened up new avenues to understand the role of microglia in disease. Most importantly, they have created the opportunity to consider ways in which microglia may be imaged and targeted for the treatment of disease.

The origin of microglia has now been clearly defined and shown to relate to the early colonization of the CNS by mesodermal progenitors[6] that arise from the yolk sac[7,8] (Box 1). It is now recognized that monocytes and tissue macrophages are not, as had been previously proposed[9], microglia progenitors in either health or disease[10,11], and that, in adulthood, microglia are an independent self-renewing population[12–14] (FIG. 1a). Human microglia turn over at a yearly median rate of 28% and live, on average, for 4.2 years. Thus, most of the microglial population is renewed several times over the course of a lifetime[15]. In support of the importance of microglial self-renewal, a recent study demonstrated that the repopulated microglia that rapidly replenish the adult brain's microglial population after microglial depletion are solely derived from the proliferation of residual microglia and not

[*] obutovsky@rics.bwh.harvard.edu; hweiner@rics.bwh.harvard.edu.

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O.B. and H.L.W. researched data for the article, made substantial contributions to the discussion of content, wrote the article and reviewed and/or edited the manuscript before submission.

Competing interests

O.B. and H.L.W. hold patent applications entitled 'Targeting Apolipoprotein E (APOE) in Neurologic Disease' (PCT/US2015/056492), 'Micrornas in Neurodegenerative Disorders' (PCT/US2012/059671) and 'Mir-155 Inhibitors for Treating Amyotrophic Lateral Sclerosis (ALS)' (Application #20180161357), which have exclusive licensing rights from the Brigham and Women's Hospital. O.B. is an adviser and collaborator for Sanofi and Nanostring.

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from newly generated progenitors[16]. However, it has also been demonstrated that, in some circumstances, peripherally derived macrophages can replace depleted microglia with cells that maintain their own unique identity (distinct from that of microglia)[17] and that these cells may play a distinct role in the progression or resolution of neurological diseases (FIG. 1b). In support of this idea, it was shown that abrogation of transforming growth factor-βl (TGFβ1) signalling in peripherally recruited myeloid cells, which enter the brain after microglial depletion, led to rapid onset of a progressive and fatal demyelinating motor disease[18].

Monocytes

Mononuclear cells that are derived from the bone marrow and circulate in the bloodstream. They pass into the body tissues, where they differentiate into various types of macrophages.

Tissue macrophages

Tissue-resident macrophages that are distributed throughout the body, including microglia, liver Kupffer cells, lung alveolar cells and splenic and peritoneal macrophages. They are established before birth and maintain themselves during adulthood independent of replenishment by blood monocytes. They become mobile when stimulated by inflammation and migrate to affected areas.

Microglia exist in resting or activated states depending on the inflammatory milieu, which differs in the healthy CNS and in various disease states[19–22]. In addition, microglia exhibit both phenotypic and functional plasticity in healthy and diseased brains. In peripheral macrophages (the innate immune cells that are a homologue of brain microglia), cell phenotypes have been determined by the varied expression of cell surface receptors by these cells[23]. Similarly, microglial phenotypes were, in the past, characterized by the presence of particular cell surface molecules and the expression of specific sets of cytokines and were classified as either M1-like (exhibiting pro-inflammatory signalling and neurotoxicity) or M2-like (participating in the resolution of inflammation)[24]. However, it is now clear that this simplistic view of microglial phenotypes does not adequately describe the complex physiology of microglial cells[5]. The characterization of microglial diversity in health and disease has therefore been redefined in both mice[25–29] and humans[30–32] with the help of newly developed technologies, including RNA-sequencing, quantitative proteomics and epigenetic studies.

Cytokines

Proteins secreted by a cell that act in a paracrine or autocrine fashion and affect the behaviour of other cells.

RNA-sequencing

An unbiased whole transcriptome deep-sequencing technology that uses next-generation sequencing to detect novel or known features and to quantify RNA activity.

Epigenetic studies

Investigations of inheritance by mechanisms other than those that involve changes to the underlying DNA sequence. Types of epigenetic mechanisms include DNA methylation, post-translational histone modifications and non-coding RNAs.

In this Review, we discuss the features that define homeostatic microglia, including their transcriptional and surface protein signatures, and describe the factors that regulate these signatures. We relate the physiological functions of microglia, including their roles in synaptogenesis, trophic support, chemotaxis and neurogenesis, to their phenotypic diversity. Finally, we consider what microglial signatures can reveal about the role of these cells in ageing and disease as well as the potential impact of understanding these signatures in the development of therapeutic strategies to target microglia for the treatment of CNS disease.

Homeostatic microglia

Microglial transcriptional signature

Until recently, the molecular and functional characteristics of homeostatic microglia had not been identified, which had impeded the field as there was no basis to understand how microglia function in health and how they change and react to disease. A further complication was the lack of understanding of whether (and how) microglia fundamentally differ from peripheral myeloid cells. Taking advantage of new technological advances, including RNA-sequencing, quantitative proteomics, epigenetics and bioinformatics, several groups simultaneously identified a unique transcriptional signature for homeostatic microglia in adult mice[25,27–29]. Specifically, microglia — but not peripheral myeloid cells — were shown to express P2Y purinoceptor 12 (*P2ry12*), transmembrane protein 119 (*Tmem119*), sialic acid binding Ig-like lectin H (*Siglech*), probable G protein coupled receptor 34 (*Gpr34*), suppressor of cytokine signalling 3 (*Socs3*), β-hexosaminidase subunit β *(Hexb),* olfactomedin-like protein 3 (*Olfml3*) and Fc receptor-like S, scavenger receptor (*Fcrls*). Several of these microglial genes were also observed in human microglia, including *P2RY12* and *TMEM119* (REFs[25,33–36]). Identification of this homeostatic microglial transcriptional signature has enabled the development of robust tools, including microglia-specific antibodies and transgenic mice in which microglia are specifically targeted, the generation of microglia from induced pluripotent stem cells (iPSCs)[37–39] and the investigation of microglial biology in health and disease[40].

Peripheral myeloid cells

Cells originating from haematopoietic cells, as opposed to tissue-resident myeloid cells, such as microglia.

Induced pluripotent stem cells

(iPsCs). stem cells derived from skin or blood that have been reprogrammed into an embryonic-like pluripotent state.

Microglial surface protein signature

Microglia express macrophage markers, including adhesion G protein-coupled receptor E1 (ADGRE; also known as cell surface glycoprotein F4/80), crystallizable fragment (Fc) receptors, and αM integrin (ITGAM; also known as CD11b), in mice[41] and in humans[42]. Furthermore, murine microglia express a number of other macrophage markers, such as the macrophage colony-stimulating factor 1 receptor (CSF1R; also known as CD115), the inhibitory immune receptor cell surface glycoprotein CD200 receptor 1 (CD200R1), the surface enzyme tyrosine-protein phosphatase non-receptor type substrate 1 (SIRPA; also known as CD172a), the fractalkine receptor CX_3C-chemokine receptor 1 (CX_3CR1) and the calcium-binding protein allograft inflammatory factor 1 (AIF1; also known as IBA1)[43].

Although many of these proteins are expressed by all macrophages, the levels of expression of some protein markers can be used to distinguish microglia from related cell types. For example, microglia express lower levels of receptor-type tyrosine-protein phosphatase C (PTPRC; also known as CD45) than monocytes, allowing these two cell types to be discriminated, whereas the expression of scavenger receptor cysteine-rich type 1 protein M130 (CD163) by microglia enables their distinction from perivascular macrophages[35,44–47]. Finally, sialoadhesin (SIGLEC1; also known as CD169) is a marker for mature dendritic cells and macrophages in both mice and humans[48] and has been proposed as a marker for invading monocyte-derived macrophages in the CNS as it is not expressed in microglia in health or disease[49–52]. However, this method of distinguishing between cell types relies on relative surface marker expression as assessed by flow cytometry, which can have some limitations. For example, CD45 surface expression is known to be upregulated on microglia associated with amyloid-β (Aβ) plaques in the APP/PS1 mouse model of Alzheimer disease (AD)[52].

Microglia can also be distinguished from related cell types by their binding to specific antibodies. Vaccination of rats with adult microglia yielded two microglial-specific antibodies — an ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1; also known as CD39) antibody and 4D4, an antibody for which the protein specificity has not yet been identified — the binding of which could be used to distinguish between non-overlapping populations of monocytes and resident microglia in mice[49]. Furthermore, on the basis of identification of the unique transcriptomic signature of homeostatic microglia (see above)[25,27–29], several additional antibodies that label microglia have been developed and

characterized, including those recognizing receptors involved in microglial maintenance, such as FCRLS[25], P2RY12 (REFs[25,53]), TMEM119 (REFs[33,35]) and SIGLECH[54] (FIG. 1a).

Microglial heterogeneity and plasticity

Despite the existence of the molecular signatures described above, the presence of microglial heterogeneity has been recognized for many years. Initial descriptions noted microglia with different morphologies[55] and the differential responses of microglia to various stimuli[22] and brain irradiation[56]. Of note, in a number of different CNS areas, it has been observed that not all microglia express triggering receptor expressed on myeloid cells 2 (TREM2)[57], an immune receptor that is found in brain microglia and, when mutated, is a risk factor for non-familial AD[58]. A study in which a genome-wide analysis of gene expression in microglia from different brain regions across the adult lifespan of the mouse was performed found that there are region-specific transcriptional profiles and age-dependent regional variability in gene expression[59]. Interestingly, in the young-adult brain, microglia in different brain regions exhibited differences in the expression of genes in bioenergetic and immunoregulatory pathways[59]. The findings further suggested that cerebellar and hippocampal microglia exist in a more ‘immune-vigilant’ state than microglia in forebrain regions. Cerebellar and hippocampal microglia were found to undergo a region-specific response to age-related neurodegeneration[59] that was associated with reduced expression of genes associated with TGFβ signalling[52]. Indeed, another study confirmed that microglia in the white matter of the cerebellum undergo substantial changes during ageing, including alterations in morphology and gene expression[60]. However, a study that employed high-dimensional single-cell cytometry to characterize myeloid subsets in the brain in health, ageing and in mouse models of AD and multiple sclerosis (MS) found that all microglia are homogenously affected in neuroinflammatory diseases[61].

The diversity of microglial phenotype and function enables them to adapt and face challenges. For example, during inflammation, microglia change from a ‘bushy’ shape with extended processes to become amoeboid and produce inflammatory factors[62,63]. Microglial plasticity and its regulation are crucial features of their biology because these cells have several functions in the brain, including both normal brain maintenance and response to injury (see below)[64–67]. However, microglial plasticity in inflammatory conditions is a more complex phenomenon than was assumed by initial hypotheses that were based on macrophage morphoplasticity alone[23]. It is now known that the regulation of microglia involves several different classes of transcription factors that interact to select and activate various regulatory elements.

Mechanisms of homeostatic microglia regulation

In order to understand how microglial plasticity comes about, we must first understand the mechanisms that establish and maintain the phenotype of homeostatic microglia. For example, it has been shown that the cytokine CSF1, acting via its receptor CSF1R, is important for microglial development[8]. Consistent with this, it has been demonstrated that interleukin-34 (IL-34), which is an additional ligand for CSF1R, promotes the tissue-specific signalling pathway that is required for the differentiation of myeloid cells that populate the skin and microglia in the CNS[68]. In addition, it has been shown that tonic activity of

potassium channel subfamily K member 13 (KCNK13; also known as THIK1) promotes microglial ramification and surveillance of the brain parenchyma[69]. Given that THIK1 is also required for the release of IL-1β by microglia, this finding may have therapeutic implications: these may include stimulation of THIK1 signalling to induce microglial motility and eliminate Aβ plaque deposition in AD or inhibition of THIK1 signalling to reduce microglial-mediated damage to bystander neurons in other diseases.

Studies of factors that regulate microglia have revealed a number of molecular mechanisms that regulate microglial transcription programmes. The transcription factor PU.1 serves as a master regulator of myeloid cells[70]. PU.1 plays a crucial role in determining macrophage lineages and microglial genesis and is a major factor in selecting the set of enhancers expressed by microglia. Of note, PU.1-deficient mice have several haematopoietic abnormalities, including the loss of B cell and macrophage populations[71]. PU.1 deficiency is also associated with loss of microglia in mice and in zebrafish[72]. PU.1 binds to the CSF1R promoter to drive gene transcription[73] and to most enhancers in both mouse and human microglia. PU.1 has an important role as an essential lineage-determining transcription factor (LDTF)[74]. Motif analysis of PU.1 binding sites has shown enrichment for consensus sequences for mothers against decapentaplegic homologue 3 (SMAD3), myocyte-specific enhancer factor 2A (MEF2A) and transcription factor MafB (MAFB), suggesting that these transcription factors cooperate with PU.1 to establish microglial-specific enhancer profiles[75]. Consistent with these findings, PU.1 binds microglial-specific enhancer regions of genes encoding the MEF2 family of transcription factors, which are expressed in microglia but not in other peripheral immune cells[76]. These factors partner with PU.1 to establish the microglial-specific molecular signature that is described above.

Enhancers

Short regions of DNA, typically occupied by multiple transcription factors, that are sufficient to drive expression of a gene with temporal and/or cell-type specificity.

Lineage-determining transcription factor

(LDTF). Transcription factors that create regions of open chromatin, which enables the recruitment of secondary transcription factors required for cell-type-specific gene expression.

In addition to establishing microglial transcriptional signatures, these transcription factors have a number of additional roles in the maintenance of the homeostatic microglial phenotype. In one study, it was reported that the transcription factor MEF2C acts in homeostatic microglia to suppress the inflammatory response and that its expression declines during ageing in an interferon (IFN)-1-dependent manner[77]. This study showed that loss of microglial MEF2C was linked to downregulation of CX_3CR1, a molecule that is crucial for restraining immune responses in microglia[78,79]. On the other hand, MAFB is part of a family of transcription factors that binds to the transcription factor Maf (MAF) recognition element and forms heterodimers with the transcription factors MAF, AP-1 (JUN)

and proto-oncogene c-Fos (FOS). In macrophages, MAFB promotes an anti-inflammatory phenotype, and its loss exacerbates inflammatory conditions[80] and controls self-renewal[81]. Expression of MAFB increases during development, and its deletion in transgenic mice disrupts gene expression in adult microglia to a greater extent than it does in immature microglia[82]. MAFB also antagonizes granulocyte-macrophage colony-stimulating factor (GM-CSF), which represses microglial differentiation[83]. Furthermore, its expression is downregulated in the superoxide dismutase 1 (SOD1) mouse model of amyotrophic lateral sclerosis (ALS), in which inflammation is increased[52,84]. In primary microglia isolated from the newborn mouse brain, MAFB was shown to regulate cell division by dampening the response to CSF1 (REF.[83]). Thus, MAFB acts to maintain a healthy microglial phenotype by inhibiting both inflammatory and proliferative responses.

In addition to the factors described above, there are a number of secondary transcription factors involved in microglial homeostatic regulation. These include the SMAD proteins, which are TGFβ1-dependent and contribute to establishing transcription of specific microglial genes[75]. Furthermore, the development of microglia is dependent on CSF1R, which transmits signals upon activation by its ligands CSF1 and IL-34 and serves as a survival factor[68]. It has also been shown that the development of microglia relies on the secondary transcription factor IFN regulatory factor 8 (IRF8), which is essential for the development of the mature microglia precursors known as A2 cells[10] (Box. 1). Another secondary transcription factor, Sal-like protein 1 (SALL1), regulates cortical neurogenesis and is expressed in human and mouse microglia[25]. Mutations in *SALL1* result in neural or behavioural abnormalities in humans[85] and abnormal microglial morphology and downregulation of the microglial homeostatic signature in mice[86], suggesting that SALL1 inhibits reactive microglia and promotes a physiological surveillant phenotype. At this time, mechanisms connecting extrinsic signals to transcriptional responses remain poorly defined.

Finally, it has been shown that the absence of TGFβ1, a factor known to be required for the in vitro development of the molecular signature characteristic of adult microglia, in the CNS results in a paralytic neurologic disease in mice[25]. Importantly, TGFβ1 signalling has also been shown to be inhibited in microglia that are associated with neurodegeneration, in which the homeostatic signature is also suppressed[52], and an additional study has further highlighted the critical function of TGFβ1 in preventing microglia and/or macrophage-mediated CNS pathology[18]. Similarly, CNS-conditional *Tgfb1*$^{-/-}$ mice exhibit motor deficits, a loss of homeostatic microglia and the appearance of peripherally derived monocytes in the CNS[25]. Mice deficient in negative regulator of reactive oxygen species (NRROS), which is preferentially expressed in myeloid cells (including macrophages, neutrophils, microglia and perivascular macrophages) and regulates the production of reactive oxygen species, lack microglia and instead are populated by perivascular macrophage-like cells[87]. The neurological abnormalities observed in TGFβ1-deficient mice were also observed in NRROS-deficient mice and were associated with the loss of the homeostatic microglial signature and a defect in TGFβ and SALL1 signalling. The lack of microglia in mice lacking NRROS shows that this factor is important for the programme that generates the homeostatic microglial phenotype.

Microglial physiological functions

The features of homeostatic microglia discussed above make them well suited to their physiological functions in the CNS.

Synaptic plasticity and synaptogenesis

Microglia play a crucial role in synaptic and axonal pruning during development by phagocytizing inappropriate synaptic connections and axons, thereby shaping neural circuits in the developing brain[41,88–90]. Similarly, it has been shown that microglia shape postnatal neural circuits in an activity-dependent and complement-dependent manner that is dependent on neural activity[91]. In follow-up studies, the contribution of complement and microglia to synaptic loss in mouse models of AD has been investigated[92,93]. It is known that synapse loss correlates with cognitive decline in AD and that microglia may play an important role in neural inflammation. Inhibition of complement reduces the number of phagocytic microglia as well as the extent of early synapse loss. Furthermore, the complement component C1q is needed for the toxic effects of Aβ oligomers[92]. These findings suggest that, in AD, the ability of microglia to prune excess synapses in development is inappropriately activated and participates in synapse loss.

Trophic support

Microglia secrete neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), fibroblast growth factor (FGF) and insulin-like growth factor 1 (IGF1), that control the activity of receptor tyrosine kinases and are associated with signalling through phosphoinositide 3-kinase (PI3K)[94]. It has been suggested that, at prenatal stages, the secretion of these factors mediates the effects of microglia on synaptogenesis[95], and it was shown that microglia promote learning-dependent synapse formation through the secretion of BDNF in mice[96]. In addition to secreting neurotrophins, microglia also respond to these factors with increased proliferation[97] or phagocytic activity[98].

Microglia also respond to injury by expressing specific growth factors. Following striatal injury, activated microglia and macrophages induce the sprouting of dopaminergic neurons in the injured striatum and express both BDNF and glia cell line-derived neurotrophic factor (GDNF)[99]. In another study, it was demonstrated that BDNF secreted by microglia caused a shift in the neuronal anion gradient underlying neuropathic pain[100]. The secretion of BDNF by microglia is suppressed by IL-1β[101]. Related to this, the suppression of synaptic plasticity by Aβ, which also disrupts the homeostatic microglial signature (see below), is also sensitive to IL-1β: excessive activation of IL-1β disrupts the formation of dendritic spines and thus interferes with memory consolidation[101]. Thus, the microglial molecular signature ultimately finds its expression in microglial function.

Chemotaxis and immune cell recruitment

Microglia attract monocytes to the brain in a CC-chemokine ligand 2 (CCL2)-dependent fashion. These monocytes play an important role in neuroinflammation but do not contribute to the resident microglial pool[102]. For example, in the SOD1 mouse model of ALS, splenic

monocytes expressing CC-chemokine receptor 2 (CCR2) and high levels of lymphocyte antigen 6C (LY6C) are recruited to the CNS by spinal cord microglia[49]. The recruitment of CCR2-expressing monocytes also plays an important role in mouse models of AD, in which genetic deletion of CCR2 in mice impairs monocyte accumulation and accelerates disease progression[103]. It has also been shown that infiltrating monocytes promote brain inflammation and exacerbate neuronal damage after status epilepticus[104]. Levels of CCL2 and other chemokines are expressed at the highest levels when microglia switch from homeostatic to disease-associated phenotypes. Thus, one of the functions of disease-associated microglia is to facilitate recruitment of monocytes to the CNS.

Neurogenesis

Microglia contribute to neurogenesis in two ways: through phagocytosis of excessive newborn cells and by promoting neurogenesis through the secretion of cytokines. Most newborn cells in the developing nervous system undergo apoptotic death as they transition from neural progenitors to neuroblasts and are rapidly cleared by microglia-mediated phagocytosis[105]. In addition, microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis[105]. Phagocytosis is linked to the homeostatic microglial phenotype and is specifically dependent on the expression of receptor tyrosine kinase MerTK (MERTK), which is responsible for routine non-inflammatory clearance of dead brain cells in the neurogenic niche[106].

In adult mice, hippocampal neurogenesis may be induced by exposure to an enriched environment, a process that is associated with the recruitment of T cells and the activation of microglia[107]. Similarly, activated microglia accumulate in the early postnatal subventricular zone, where they enhance neurogenesis via release of cytokines[108]. On the other hand, microglial activation is suppressed by the interaction of fractalkine, expressed by neurons, with microglial CX_3CR1. This may repress neurogenesis and is thus a target for the therapeutic modulation of microglia[109].

It is known that microglia are also involved in oligodendrocyte development. A unique molecular signature of the microglia involved in regulating the development and homeostasis of oligodendrocytes and their progenitors has been identified and shown to be essential for these functions, as confirmed by microglial depletion experiments in mice[110]. Interestingly, this signature included genes such as C-type lectin domain containing 7A (*Clec7a*), secreted phosphoprotein 1 (*Spp1*), *Igf1,* αX integrin *(Itgax)* and transmembrane glycoprotein NMB (*Gpnmb*), which have been described in ageing microglia and in disease models (see below)[52,111]. In addition, this ITGAX-expressing microglial subset has been shown to have a key role in myelinogenesis in the developing brain[112].

Inflammation also plays a role in regulating the influence of microglia on neurogenesis and oligodendrogenesis. Microglia activated by IL-4 or IFNγ induced oligodendrogenesis or neurogenesis, respectively, from adult stem cell progenitor cells both in vivo and in vitro[113]. High, but not low, levels of IFNγ induced a microglial phenotype that impeded oligodendrogenesis. IL-4 reduced this impediment and overcame the blockage of IGF1 production that was caused by IFNγ[113]. Consistent with these observations, an ITGAX-expressing microglial subset was identified as the source of IGF1 in the developing brain[112].

A better understanding of how microglia in different states of activation regulate adult neurogenesis under physiological and pathological conditions will provide the foundation for using modulation of microglial activation as a therapeutic strategy.

Microglia in disease and ageing

It is now known that, during the course of many diseases, microglia lose their homeostatic molecular signature and functions[26,84,114], such as their roles in synaptic plasticity[115], and become chronically inflammatory[116]. At the present time, a common neurodegenerative microglial signature has been identified for diseases such as ALS, AD and MS. Investigations are beginning to probe the unique microglial signatures related to specific diseases and to specific stages of disease; however, these have yet to be defined.

Amyotrophic lateral sclerosis

One of the first descriptions of a disease-associated microglial signature was provided by studies that examined the SOD1 mouse model of ALS[28]. Tau, presenilin 2 and apolipoprotein E (ApoE) pathways were found to be activated in microglia in the SOD1 mouse, with *Apoe* being one of the transcripts that was the most significantly induced during disease progression. This upregulation was not seen in response to lipopolysaccharide (LPS) activation or in M1 or M2 macrophages. In concordance with these results, a later study also reported increased ApoE signalling in microglia in both SOD1 mice and human ALS[84]. Moreover, this study also revealed loss of the homeostatic microglial signature described above as early as 2 months before measurable disease onset in the SOD1 mouse.

Studies have also provided information on the mechanisms driving the changes in microglial signature in ALS. ALS is associated with neuronal death and the activation of TREM2-ApoE signalling in microglia by apoptotic neurons[52]. This signalling leads to the induction of the expression of miR-155, which has been shown to be a pro-inflammatory signal[117]. Indeed, genetic deletion of *Apoe* abrogated induction of miR-155 in microglia[52]. Importantly, genetic ablation of miR-155 itself restored the homeostatic microglial signature and extended survival in SOD1 mice[84]. Many of the microglial changes observed in ALS were also observed in an AD mouse model (see below), suggesting a common microglial response to different disease processes.

Ageing

Aged microglia display a dystrophic phenotype[118–120]. It is also known that ageing, similar to neurodegeneration, induces a hypersensitive, pro-inflammatory microglial phenotype that some have referred to as ‘primed microglia’[26]. In ageing mice, most of the ‘sensome’ genes (which encode proteins involved in sensing endogenous ligands and microorganisms) that are expressed by homeostatic microglia are downregulated[27]. These cells also express pro-inflammatory cell surface markers such as galectin 3 (LGALS3), tyrosine-protein kinase receptor UFO (AXL), CLEC7A, major histocompatibility complex (MHC) class II molecules and CXC-chemokine receptor 4 (CXCR4)[26] and appear immunologically activated but not polarized to a pro- or anti-inflammatory phenotype[25]. Microglia from transgenic mice that lack multifunctional protein 2 (MFP2) and thus model the peroxisomal

β-oxidation deficiency common to ageing express inflammatory surface markers and downregulate the homeostatic purinergic receptor P2RY12 (REF.[121]) in the same manner as aged microglia[27]. In MFP2-deficient mice TGFβ receptor 1 (*Tgfbr1*) is downregulated, suggesting that the loss of homeostatic properties in ageing microglia is also related to the downregulation of this receptor[121].

Alzheimer disease

Reactive gliosis and neural inflammation are hallmarks of AD[4,122–125]. In the past, microglial changes had been considered to be secondary events; however, it is now clear that, although not being the primary initiator of AD, microglia play an important cell-autonomous role in the disease. Immunohistochemical analysis has shown that microglia surround amyloid plaques in both human AD and in animal models of the disease and may be either beneficial or detrimental in halting disease progress[4]. Genetic studies show that a number of genes that have been linked to early-onset AD risk are expressed primarily in microglia. These include those encoding TREM2 (REFs[126,127]), membrane-spanning 4-domains subfamily A (MS4A) members[128], ATP-binding cassette subfamily A member 7 (ABCA7)[128], the ε4 allele of ApoE (ApoE4)[129–131], myeloid cell surface antigen CD33 (REFs[132–134]), PU.1 (REF.[135]) and Myc box-dependent-interacting protein 1 (BIN1)[116,136]. In addition, microglial expression of the complement receptor CR1 has been linked to late-onset AD[137,138] and may reflect the microglial phagocytosis of synapses that has been proposed to contribute to AD pathogenesis.

Transcriptomic analysis of microglia in an AD mouse model and in human AD has revealed a proinflammatory signature present in Aβ-plaque-associated microglia[52,111,139,140] (FIG. 2a). This phenotype was not observed in microglia in non-plaque areas, suggesting that the neuroinflammation associated with AD predominantly occurs in plaque-associated microglia. The upregulated genes in Aβ-plaque-associated microglia, also known as disease-associated microglia (‘DAM microglia’), included *Axl*, *Apoe*, *Clec7a*, *Itgax*, *Lgals3* and *Cst7*, which are now known to be part of a common neurodegenerative[52] and disease-associated signature that has been observed by several groups[26,111,141,142] (see below; FIG. 2a). These DAM microglia were shown to be generated through a two-step activation process in which homeostatic microglia first transition to an intermediate stage (known as stage 1 DAM) in a TREM2-independent manner, followed by a second TREM2-dependent transition to stage 2 DAM[111]. These studies emphasize that cellular reprogramming of microglia occurs in response to neurodegeneration and that this process is related to unique underlying transcriptional programmes. DAM microglia have been observed in other conditions including ageing, ALS and frontotemporal dementia (FTD) and in an inducible CK-p25 mouse model of severe neurodegeneration, and it appears that the DAM expression phenotype is nonspecifically triggered by altered brain homeostasis[111]. In a mouse AD model, microglia typified by the expression of modules of co-regulated type 1 and type 2 IFN-responsive genes and their proliferation were shown to be present as an early response to neuronal injury[142]. Moreover, two distinct reactive microglial phenotypes were identified at later stages of neurodegeneration[142]. Independent of the specific disease, it appears that, over time, microglia may transform into an activated state and that this change is related to signals from the periphery that occur with ageing or disease[143].

Although it is clear that the DAM microglia identified in these studies are associated with Aβ plaques, it is not clear whether they have a protective or disease-inducing function (FIG. 2b). It is known that innate immune cells can protect against AD by reducing the accumulation of Aβ, which occurs during early stages of disease. For example, in mice, peripheral monocytes can enter the brain, clear Aβ and affect disease pathology[144], an effect that is lost in *Ccr2*$^{-/-}$ animals[103]. It may be that as microglia age they lose their ability to phagocytose Aβ in the brain[27,145]. Furthermore, with the deposition of Aβ, the phenotype of microglial cells becomes more neurotoxic[118,146–148]: they express several inflammatory cytokines, including IL-1β, IL-6, tumour necrosis factor (TNF) and CCL3 (also known as MIP1α)[146]. The neurotoxicity of microglial cells may also be related to their expression of tau, which is triggered by the deposition of Aβ in the brain and is known to be neurotoxic[149].

In summary, microglia are thought to have multiple functions in AD. The identification of the factors associated with both their beneficial and detrimental functions and the development of strategies to restore the homeostatic microglial signature or to induce the DAM microglial signature could improve our ability to target microglia and treat AD. It is important to note that there are likely to be multiple microglial phenotypes associated with disease and that DAM microglia as defined may have either beneficial or detrimental function. These functions will need to be defined in more detail in order to target microglia therapeutically.

Multiple sclerosis

MS is an inflammatory disease of the CNS in which microglia play an important role. In the experimental autoimmune encephalomyelitis (EAE) mouse model of MS, microglia have a transcriptional phenotype that is similar to that observed in ALS and AD models (see below)[52]. Aspects of this transcriptional profile may contribute to disease. For example, microglia have been shown to express chemokines (such as CCL2) that facilitate recruitment of inflammatory CCR2^{+} monocytes from the periphery. Microglia are the only cells in the brain to express CX_3CR1, which serves an immunomodulatory role in the homeostatic state and the genetic ablation of which results in neurotoxicity[78]. Moreover, suppression of microglial *Cx3cr1* expression is associated with disease progression in EAE[52]. The disease-associated microglial transcriptional phenotype in EAE also contributes to the inflammatory cascade by activating astrocytes[150]. In human MS, microglia lose the expression of the P2RY12 homeostatic microglial marker and adopt a pro-inflammatory phenotype that includes the expression of phagocytic-related markers (macrosialin (also known as CD68)), antigen presentation markers (MHC class I and II molecules and T lymphocyte activation antigen CD86) and proteins involved in the production of reactive oxygen species (cytochrome *b*-245 light chain (CYBA))[36]. During the progressive stages of MS, microglia have been shown to accumulate iron[151,152]. Minocycline has been shown to specifically affect microglia[153–155], and a trial in MS showed positive trends[156], although minocycline worsened patients with ALS[157]. These differences may be related to how minocycline differentially affects the microglial signature.

Parkinson disease

Parkinson disease (PD) is characterized by the loss of dopamine neurons in the substantia nigra and the presence of intracellular proteinaceous inclusions (Lewy bodies) in neurons composed of α-synuclein that activate microglia[158,159]. Although molecular profiling of microglia in animal models of PD has yet to be attempted, activated microglia are known to be prominent in mouse models and in human PD[160]. This activation includes the expression of AXL in disease associated microglia. Microglial activation appears to act via TAM (TYRO3, AXL and MERTK)-dependent mechanisms to have a detrimental role in a mouse model of PD[106]. Microglial activation in human PD has been demonstrated by positron emission tomography[161], and it has been reported that α-synuclein-dependent activation of microglia may be linked to changes in the microbiome in both animal models and patients[162].

Autism spectrum disorder

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that includes abnormalities in social interaction, difficulties with language and repetitive behaviour. Although considered primarily a neuronal disease, the role of microglia in sculpting and regulating neuronal synapses suggests that microglial dysfunction contributes to ASD. In humans, post-mortem genome-wide transcriptome analysis has revealed age-related changes in the trajectory of microglial and synaptic dysfunction that may reflect an early developmental alteration in microglial function[163]. Consistent with this, mice lacking CX_3CR1 exhibit a transient reduction in the numbers of microglia present in the brain during the early postnatal period and a subsequent deficit in synaptic pruning associated with impaired social behaviour[164].

Regulation of disease-associated microglial signatures

As described above, microglia change during ageing and in disease to acquire different phenotypes and activate specific transcriptional programmes as a result of processes that are complex and result in both protective and disease-associated microglia. To determine whether there are common patterns in the changes observed in different diseases, microglial transcriptomes in ageing and in mouse models of ALS, AD and MS have been analysed[52]. Across disease models, the induction of genes related to ApoE signalling and the suppression of TGFβ signalling in microglia were associated with neurodegeneration, resulting in a phenotype that was termed ‘MGnD’ (microglia neurodegenerative phenotype). The MGnD microglial phenotype overlaps to some extent with that of the DAM microglia found in models of AD (see above). In disease, several core homeostatic disease genes were suppressed, including those encoding the transcriptional regulators *Mef2a, Mafb, Sall1* and early growth response protein 1 (*Egr1*). At the same time, there was an increase in the expression of genes encoding transcription factors such as class E basic helix-loop-helix protein 40 (*Bhlhe40*), transcription factor EC (*Tfec*), protein C-ets-2 (*Ets2*) and cAMP-dependent transcription factor ATF3 (*Atf3*). Another study analysed 18 data sets from transcriptional profiles of CNS myeloid cells from diverse mouse models of neurodegeneration and identified a set of co-regulated genes associated with neurodegenerative disease in mice that were also observed in human AD brains[165]. Many of

these genes overlap with the MGnD microglial phenotype. Interestingly, some of the inflammatory genes that were observed in AD brains, were not observed in mouse models of AD.

A major question is how microglial signatures are regulated in disease. Phagocytosis of apoptotic neurons that were injected into the brain of naive mice suppressed the microglial homeostatic signature and resulted in a transcriptional profile that mimicked the molecular signature of MGnD microglia. This microglial phenotype switch was shown to be regulated by activation of TREM2-ApoE signalling via phosphatidylserine exposed on the apoptotic neurons[52]. Indeed, TREM2 is an important microglial molecule[25,27,28] thought to be involved in the regulation of microglial signatures in disease[1,58]. TREM2 is a receptor that detects damage-associated lipids, allowing microglia to sense Aβ accumulation, and its loss has been shown to augment Aβ accumulation in a mouse model of AD[166]. It has been reported that TREM2 promotes microglial survival by activating the WNT-β-catenin signalling pathway[167]. Consistent with this, TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury in mice[168]. A rare mutation of TREM2 (R47H) is associated with a substantial increase in the risk of developing AD[126].

Aβ-induced microglial activation is impaired in humanized transgenic mice expressing human R47H TREM2 (REF.[169]), and the R47H mutation impairs TREM2 binding to anionic lipids[166]. Furthermore, an elevation in the levels of soluble TREM2 in the cerebrospinal fluid in AD is coincident with markers of neural damage and the onset of clinical dementia[170]. It has been proposed that TREM2 signalling is anti-inflammatory for microglia and that agonizing TREM2 signalling would suppress neuroinflammation[58,171]. Indeed, in EAE it has been shown that TREM2 is upregulated in spinal cord microglia and that an anti-TREM2 antibody exacerbates disease[172]. Thus, stimulating TREM2 represents a potential therapeutic strategy in neurodegenerative disease. However, others have reported that *Trem2* deficiency eliminates $TREM2^+$ inflammatory macrophages and ameliorates pathology in AD mouse models[173], which is seemingly inconsistent with the link between TREM2 loss-of-function mutations and an increased risk of AD. One possible explanation for this discrepancy is that TREM2 is highly expressed on inflammatory monocytes, which are recruited to the plaques, and that genetic ablation of *Trem2* eliminates their recruitment. However, in parabiosis experiments it was found that amyloid-associated myeloid cells are brain-resident microglia rather than recruited peripheral monocytes[174]. In the absence of TREM2, it has been reported that microglia do not fully surround Aβ plaques and that Aβ plaques are more diffuse and associated with greater neuritic damage[174]. These findings are consistent with the interpretation that TREM2 protects from AD by enabling microglia to surround and alter Aβ plaque structure, thereby limiting neuritic damage.

To further explore these differences, the effects of *Trem2* deficiency at early and late stages of disease in AD mouse models have been explored. *Trem2* deficiency ameliorated amyloid pathology at early stages but exacerbated it at late stages, suggesting that TREM2 plays distinct roles at different stages of disease[175]. Given that TREM2 mutations are associated with increased risk of AD in humans[126,176,177], a protective role for this receptor appears to be the predominant biologic effect. Consistent with this, it was found that TREM2 also affects microglial metabolic fitness: patients with AD carrying *TREM2* risk variants and

TREM2-deficient mice have abundant autophagic vesicles in their microglia[178]. Although it is not entirely clear how TREM2 regulates microglial signature phenotypes, TREM2 is known to recognize phosphatidylserine on the surface of apoptotic or damaged cells and to initiate ApoE signalling[52]. ApoE is a ligand for TREM2 in microglia[179–181]. It binds to apoptotic neurons and increases TREM2-mediated microglial phagocytosis of neurons[179]. ApoE also suppresses the expression of major transcription factors of homeostatic microglia[52]. Moreover, genetic ablation of *Apoe* in MGnD microglia restored their homeostatic properties in a cell-autonomous fashion. Genetic targeting of *Trem2* suppressed the ApoE pathway and restored homeostatic microglia in both AD and SOD1 mice[52]. In the tau model of neurodegeneration, genetic deletion of either *Apoe* or *Trem2* also arrested neurodegeneration, demonstrating that this pathway negatively regulates the homeostatic signature[182,183].

As outlined above, it is still a matter of debate whether MGnD or DAM microglia[63] are beneficial or detrimental in neurodegenerative disease. One report showed that microglia-derived apoptosis-associated speck-like protein containing a CARD (PYCARD) facilitates spreading of Aβ pathology in an AD mouse model[184]. MGnD microglia that are associated with Aβ significantly upregulate their expression of *Pycard*. Thus, Aβ-plaque-associated microglia may play a detrimental role in AD by facilitating Aβ plaque seeding rather than restricting Aβ plaque formation[63]. Consistent with a potential detrimental role of microglia, it was shown that ablation of microglia by a compound (PLX3397) that inhibits CSF1R did not reduce plaque burden, although it did protect synapses[185]. In addition, PLX3397 treatment was reported to reduce accumulation of intraneuronal Aβ, neuritic plaque deposition and pre-fibrillar oligomers in the same mouse model of AD[186].

Therapeutic targeting of microglia

As described above, normal microglial functions include phagocytosis, synaptic pruning, chemotaxis and regulation of neurogenesis and CNS immunity. Because microglia have such a fundamental role in brain function, the potential therapeutic landscape in targeting microglia is extraordinarily broad, including developmental diseases such as autism, acquired diseases such as MS and genetic and degenerative diseases such as AD, PD and ALS. As outlined above, it is now recognized that microglia are resident cells of the nervous system that are independent from myeloid cells that migrate into the brain from the periphery. Thus, therapeutic targeting of microglia themselves must target them in the CNS.

Broadly speaking, one would argue that the goal of microglial-targeted therapy is to maintain homeostatic microglial function and to restrain or inhibit inflammatory or disease-promoting microglia, such as those displaying the MGnD transcriptional profile described above. Thus, one would want to agonize molecular targets that enhance the homeostatic signature and antagonize those that drive MGnD. On the basis of our understanding of homeostatic and MGnD microglial signatures, a number of specific microglial molecular targets are beginning to emerge. Targets for which the goal would be to boost function include miR-124, TGFβ, MERTK, CX_3CR1, P2RY12, MEF2C, SALL1 and MAFB. Targets for antagonistic therapeutics include TREM2, ApoE4, AXL, CSF1R, complement C3, miR-155 and SPP1. As discussed above, some molecules, such as TREM2, may be either

beneficial or detrimental depending on disease stage, a characteristic that becomes important when therapeutics targeting these molecules are applied to humans.

Recent studies that have used an anti-CSF1R antibody[187] and PLX3397 to block CSF1R suggest that inhibition of CSF1R causes the depletion of microglia[188]. Treatment with PLX5622, which also targets CSF1R, prevented the association of microglia with plaques and improved cognition in the 3xTg-AD mouse model of AD[189]. Similarly, an oral tyrosine kinase inhibitor (GW2580) inhibited CSF1R in the APP/PS1 mouse model of AD, which reduced microglial proliferation and the microglial inflammatory profile[190]. Pharmacological targeting of CSF1R in this study also improved cognition and prevented synaptic degeneration[190]. These studies therefore support the potential of CSF1R inhibitory strategies in the treatment of AD.

TAM receptors are involved in multiple aspects of microglial physiology[106]. As discussed above, microglia are damage sensors of the CNS and act as phagocytes for the routine, non-inflammatory clearance of dead brain cells. These functions are regulated by the TAM receptor tyrosine kinases MER and AXL. Animals deficient in *Mer* and *Axl* accumulate apoptotic cells[106]. Microglial expression of *Axl* is upregulated and *Mer* is downregulated in microglia exhibiting the MGnD phenotype[52]. Thus, TAM receptors may control microglial physiology and are potential targets for therapeutic intervention.

Other well-studied inhibitors of inflammation include glucocorticoids, minocycline, vitamin E, vitamin D, endocannabinoids, TGFβ1, rapamycin and other synthetic drugs[191]. However, although some anti-inflammatory drugs have been shown to diminish neuroinflammation, few have direct functional effects on microglial activity[192]. On the basis of our current understanding of microglial physiology, these treatments would be expected to mediate their effects by modulating the microglial homeostatic signature.

What then are the modalities by which one may target microglia? The most obvious and easiest to develop are monoclonal antibodies specific for microglial targets. Virtually any surface structure on microglia could serve as an antibody target. Investigators are currently developing an antibody against ApoE4 (REF.[193]) as well as antibodies against TREM2 (REF.[172]). As shown in the trial of the anti-Aβ monoclonal antibody aducanumab for AD, it appears that enough of a systemically administered monoclonal antibody reaches the CNS to have an effect[194]. Another approach involves microRNAs. miR-155 has been associated with inflammatory microglia and is a major driver of inflammation in innate immunity. Targeting miR-155 in microglia has shown to be a benefit in models of ALS[84,195] and is currently being pursued as a possible therapeutic strategy. Other microRNAs have anti-inflammatory functions themselves. For example, it has been shown that agonizing miR-124 is beneficial in models of MS[196]. Thus, it appears that microRNAs may regulate microglial transcriptional signatures. Consistent with this, in MGnD microglia from *Apoe*$^{-/-}$ mice, miR-155 expression was suppressed[52].

In addition to classical pharmacological approaches, there are other physiological methods to drive changes in microglia. One intriguing example arises from a report that environmental enrichment reduces Aβ levels and amyloid deposition in a mouse model of

AD[197]. Moreover, environmental enrichment prevents the microglial-dependent neural inflammation that is driven by human Aβ protein polymers[198]. Thus, environmental or behavioural modification could have positive effects on brain function by affecting microglia.

Environmental enrichment

Creation of complex environmental stimulation through the use of toys, ladders, tunnels and a running wheel in the environment in which animals are housed.

Finally, the microbiome plays a central role in health and disease, and the host microbiome controls maturation and function of microglia[199]. Animals raised in a germ-free environment or mice treated with broad-spectrum antibiotics develop dysfunctional microglia, an effect that can be ameliorated with short-chain fatty acids. In addition, it has been shown that the microbiome influences prenatal and adult microglia in a sex-dependent and time-dependent manner[200]. A gut-brain axis exists involving both neural and metabolic communication that could affect microglia[199]. Thus, it is possible that modulation of the microbiome could treat neurodegenerative diseases through its effects on microglia. Indeed, studies of the microbiome in neurodegenerative diseases such as PD[162], AD[201] and MS[202] are in progress.

Central to our understanding of microglial function in disease and our ability to target microglia is a requirement to be able to measure microglial function via biomarkers. The most widely used biomarkers in neurodegenerative disease involve imaging. Developing treatment for diseases such as MS has required that therapy affects the imaging measures of disease[203]. In AD, measurements of Aβ and tau are used as measures of therapeutic efficacy[204]. Methods to image microglia exist and are being used to test the effect of treatments on microglia[205–210]. With the identification of new microglial markers that are linked to homeostatic or disease-associated states, novel imaging ligands may provide a clearer window to assess microglia in disease and the effect of therapy. Other biomarkers to measure microglial function could be present in the cerebrospinal fluid. For example, soluble TREM2 has been shown to increase with time with the development of AD[211].

In summary, the expanding molecular characterization of homeostatic and disease associated microglia provides an avenue for understanding and treating human nervous system disease.

Conclusions

As described in this article, microglia have now been redefined as having either a homeostatic or a disease-associated phenotype on the basis of their molecular signature, and many of the pathways that regulate these signatures have been elucidated. Nonetheless, the distinction between homeostatic and disease-associated signatures is not absolute, particularly in terms of their impact on disease: some microglial disease signatures that are not homeostatic may in fact have beneficial roles in halting the disease process, as illustrated by the paradoxical findings with TREM2. Future directions in this field will relate to the development of strategies to manipulate microglia to treat disease states. The microglial

signatures that have been identified to date will provide a basis to identify new therapeutic targets and to measure the effect of microglial-directed therapies.

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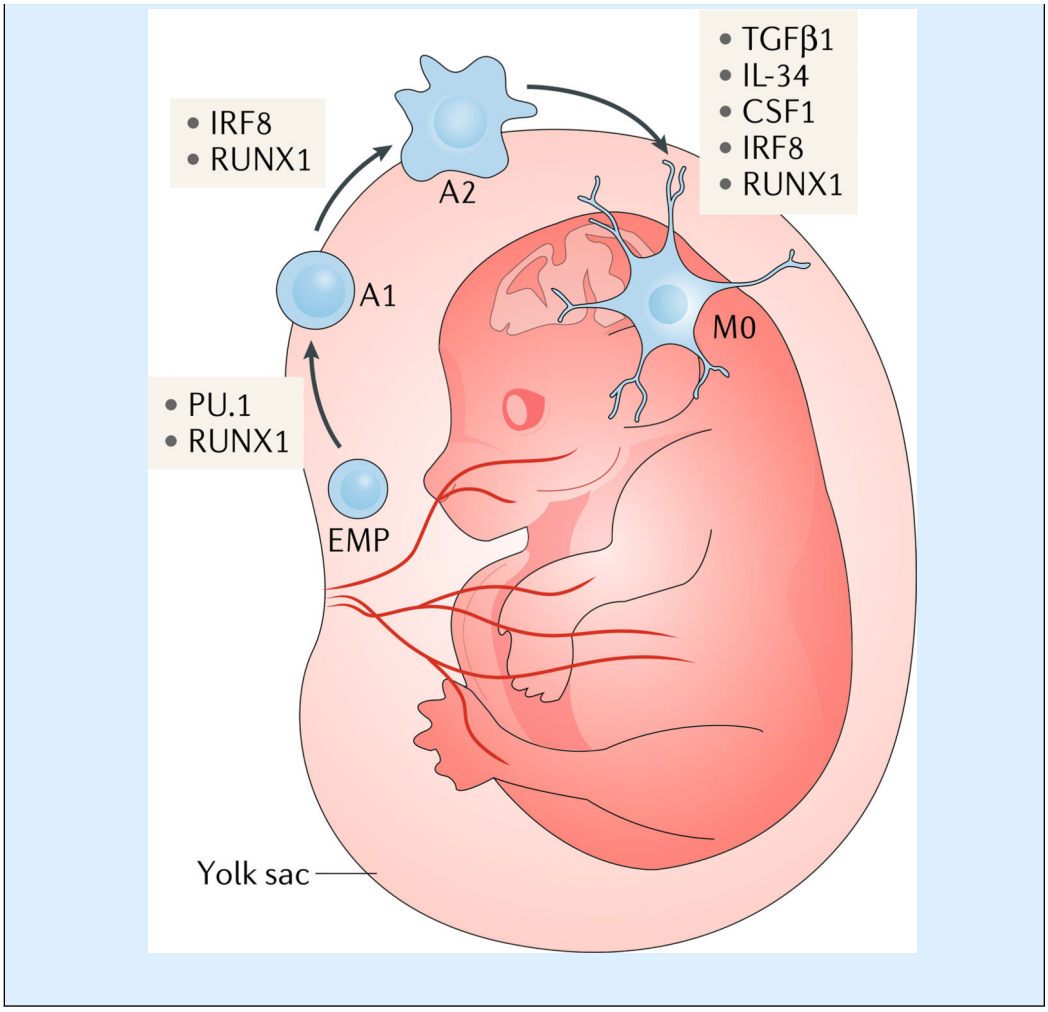
Box 1 |

Key features of mammalian microglial development

Fate mapping of runt-related transcription factor 1 (RuNX1)-expressing haematopoietic stem cell (HSC)-derived precursors in mice showed that microglia do not arise from these cells but instead originate between embryonic day (E)7 and E7.5 (REF.[8]) from early, uncommitted erythromyeloid progenitor (EmP)-derived yolk sac macrophages that express mast/stem cell growth factor receptor Kit (KIT)[10]. The EmP-derived yolk sac macrophages enter the embryonic brain and take up residence before the differentiation of other cell types. HSCs are detected in bone marrow only at E10.5, whereas microglial precursors expressing the markers adhesion G protein-coupled receptor E1 (ADGRE1) and αm integrin (ITGAm) begin to appear in the brain at E9.5 (REFS[2,212,213]). Thus, microglial precursors appear in the developing brain earlier than HSCs, indicating different origins of these two cell types[213]. murine induced pluripotent stem cell (iPSC)-derived yolk sac macrophage-like cells adopt a microglia-like morphology during co-culture with neurons and can engraft into the brain and differentiate into microglia-like cells[214], showing that they have microglial potential that is determined by the brain milieu.

Microglial development is independent of the transcription factor MYB[215], which controls HSC differentiation, but is dependent on the transcription factors RUNX1, PU.1 and interferon regulatory factor 8 (IRF8)[8,10,216]. During development, PU.1 and RUNX1 drive the transition of EMPs to the immature A1 state (cells that express CD45 and low levels of KIT but do not express CX_3C chemokine receptor 1 (CX_3CR1) or ADGRE1) at E8 in mice (see the figure). IRF8 and RUNX1 are required for the maturation of A1 cells to A2 cells, which start to express CX3CR1 and ADGRE1 and lose KIT expression at E9 (REF.[10]). The cytokines interleukin-34 (IL-34), colony-stimulating factor 1 (CSF1) and transforming growth factor-β1 (TGFβ1) are also essential for microglial development[25,68]. Human pluripotent stem cells can be differentiated into microglia-like cell subsets, including those resembling homeostatic (M0) microglia, via the induction of CSF1, IL-34 and TGFβ1 signalling[37,38]. Thus, brain environmental cues and transcriptional regulation define microglia as a unique population separate from myeloid cells.

Once microglia establish themselves in the brain, they sustain the microglial pool via local clonal expansion throughout life[12–14,217]. However, peripherally derived macrophages can replace dying microglia (while maintaining their own unique identity)[17,18] (FIG. 1).



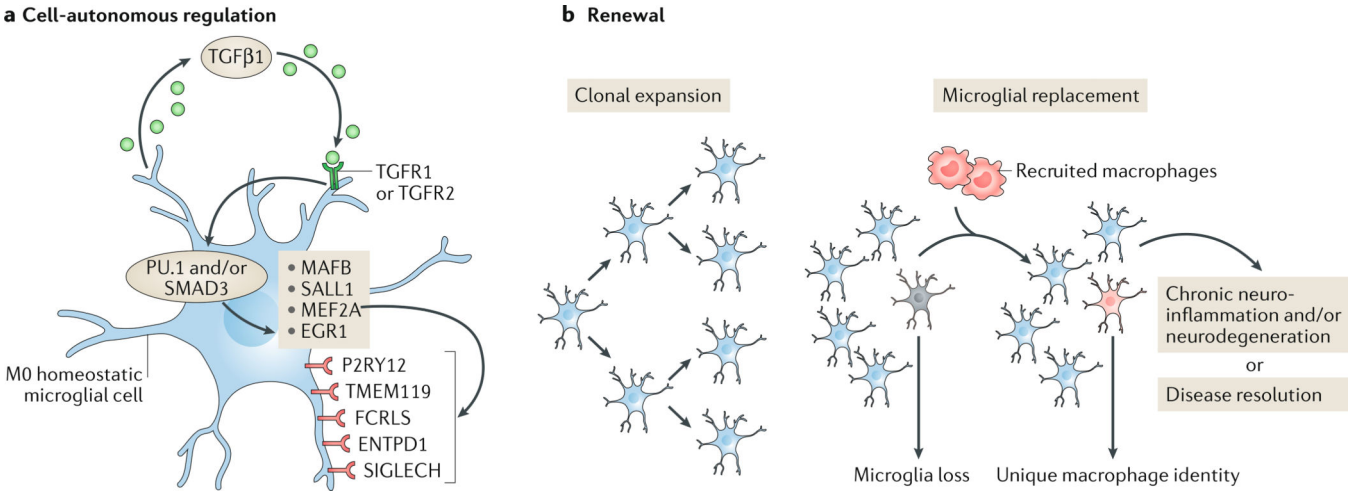


Fig. 1 |. Regulation of microglia homeostasis.

a | Microglia are maintained in a cell-autonomous fashion via transforming growth factor-β1 (TGFβ1) signalling, which drives the transcriptional regulation (via the transcription factors PU.1 and/or mothers against decapentaplegic homologue 3 (SMAD3)) of genes that include those encoding the transcription factor MafB (MAFB), Sal-like protein 1 (SALL1), myocyte-specific enhancer factor 2A (MEF2A) and early growth response protein 1 (EGR1)[25,75]. Several unique surface receptors — P2Y purinoceptor 12 (P2RY12), transmembrane protein 119 (TMEM119), Fc receptor-like S, scavenger receptor (FCRLS), ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1; also known as CD39) and sialic acid binding Ig-like lectin H, isoform CRA_a (SIGLECH) — identify microglia. P2RY12 senses damaged tissue through the detection of extracellular nucleotides[53], whereas ENTPD1 controls microglial process ramification[218] and its loss is associated with handling-induced seizures in mice[219]. The role of the other receptors is as yet unknown. **b** | Microglial renewal occurs by local clonal expansion[13]. Furthermore, under certain conditions, lost microglial populations can be replaced with peripherally derived macrophages that are recruited to the CNS. These cells maintain a unique molecular identity and have distinct functional roles as compared with microglia[17], although TMEM119 and other microglial markers may also be expressed by these macrophages[220]. Loss of TGFβ1 signalling in these macrophages has been shown to lead to a progressive and fatal demyelinating motor disease[18], suggesting that the manipulation of engrafted myeloid cells has therapeutic potential in neurodegenerative disease. TGFR1 and TGFR2, TGFβ receptors type 1 and 2, respectively.

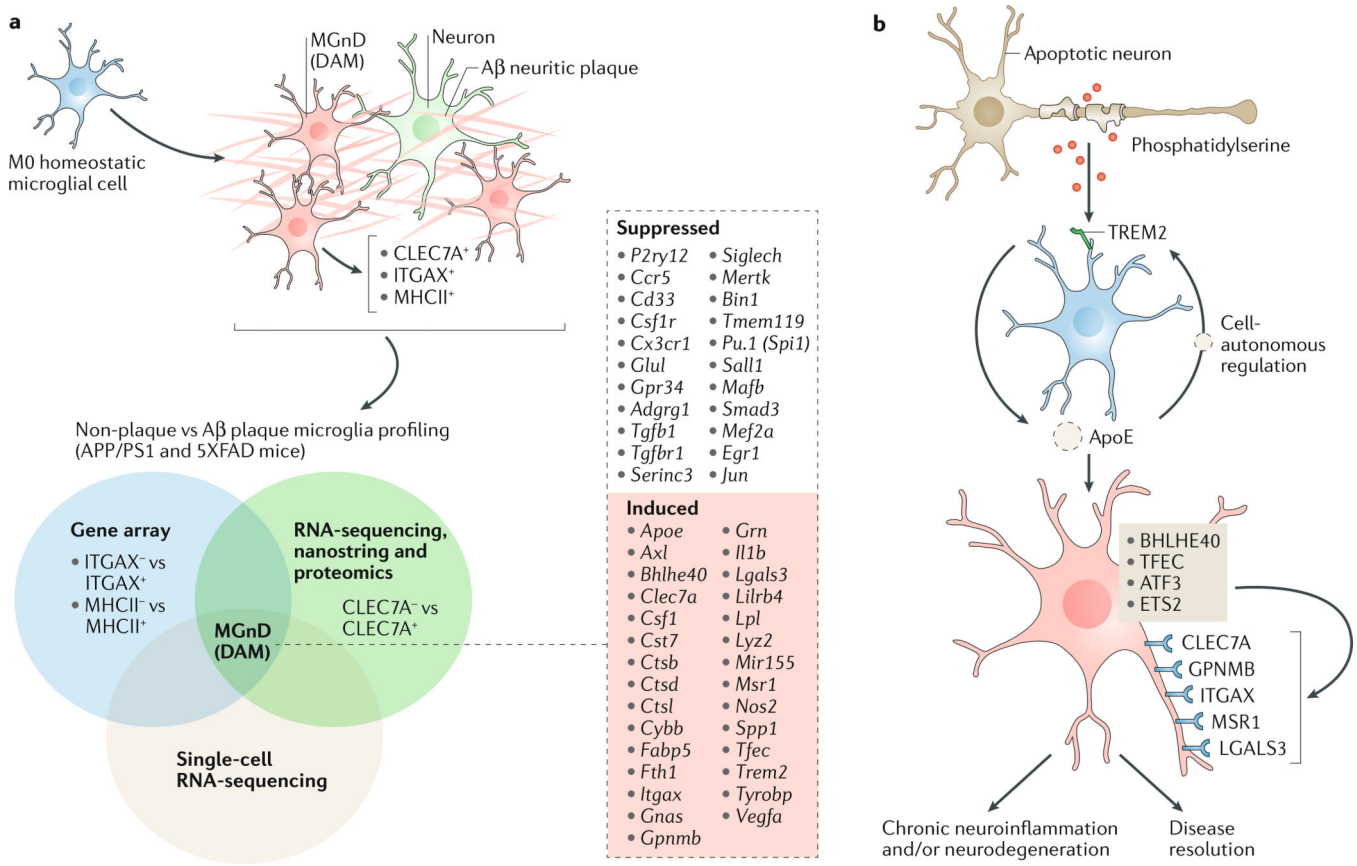


Fig. 2 |. Regulation of the neurodegenerative microglial phenotype.

a | Several laboratories profiled microglia gene and protein expression through microglial isolation using αX integrin (ITGAX, also known as CD11c)[139] major histocompatibility complex class II (MHCII) antibodies[140], C-type lectin domain containing 7A (CLEC7A) antibodies[52] and single-cell RNA-sequencing[111] (see Venn diagram). Comparison of these findings shows that microglia associated with amyloid-β (Aβ) plaques acquire a specific phenotype: these have been defined as MGnD (microglial neurodegenerative phenotype)[52] or disease-associated microglia (DAM microglia)[111]. This phenotype is associated with expression of CLEC7A, ITGAX and MHCII molecules[52,111]. The middle box lists the top suppressed and induced genes that are common to both MGnD and DAM microglia. **b** | The schematic depicts triggering receptor expressed on myeloid cells 2 (TREM2)-apolipoprotein E (ApoE) signalling, which induces a microglial phenotype switch from homeostatic to the MGnD phenotype. TREM2 present on M0 homeostatic microglia senses phosphatidylserine on apoptotic or damaged cells and activates ApoE signalling, which induces an MGnD neurodegenerative phenotype in a cell-autonomous manner. This phenotype is associated with the suppression of the M0 homeostatic signature and induction of molecules associated with inflammation, including CC-chemokine ligand 2 (CCL2), CLEC7A, macrophage colony-stimulating factor 1 (CSF1), secreted phosphoprotein 1 (SPP1), miR-155 and nitric oxide synthase, inducible (NOS2). This phenotype may have dual roles. ATF3, cAMP-dependent transcription factor ATF3; BHLHE40, class E basic helix-loop-helix protein 40;

ETS2, protein C-ets-2; GPNMB, transmembrane glycoprotein NMB; LGALS3, galectin 3; MSR1, macrophage scavenger receptor types I and II; TFEC, transcription factor EC.