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**Authors**

Holtman, Inge R  
Skola, Dylan  
Glass, Christopher K

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# Transcriptional control of microglia phenotypes in health and disease

Inge R. Holtman,<sup>1,2</sup> Dylan Skola,<sup>1</sup> and Christopher K. Glass<sup>1,3</sup>

<sup>1</sup>Department of Cellular and Molecular Medicine, UCSD, San Diego, California, USA. <sup>2</sup>Department of Medical Physiology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

<sup>3</sup>Department of Medicine, UCSD, San Diego, California, USA.

**Microglia are the main resident macrophage population of the CNS and perform numerous functions required for CNS development, homeostasis, immunity, and repair. Many lines of evidence also indicate that dysregulation of microglia contributes to the pathogenesis of neurodegenerative and behavioral diseases. These observations provide a compelling argument to more clearly define the mechanisms that control microglia identity and function in health and disease. In this Review, we present a conceptual framework for how different classes of transcription factors interact to select and activate regulatory elements that control microglia development and their responses to internal and external signals. We then describe functions of specific transcription factors in normal and pathological contexts and conclude with a consideration of open questions to be addressed in the future.**

## Introduction

Microglia are tissue-resident macrophages that perform CNS-specific functions (1). They derive from a unique lineage of erythromyeloid precursors (EMPs) in the yolk sac and fetal liver (2). EMPs infiltrate the brain during early development, differentiate into microglia, and maintain their population by self-renewal (3). Microglia distribute themselves throughout the CNS (4) and continuously scan their surroundings (5). Microglia share many traits with other subsets of tissue-resident macrophages, including dependence on the CSF1 receptor (CSF1R) for differentiation and survival, a requirement for PU.1 as an essential lineage-determining transcription factor (LDTF), the ability to efficiently phagocytose tissue debris, and the ability to quickly trigger an inflammatory response following detection of pathogens or tissue damage (6). In addition to responding to injury and infection, microglia carry out functions that are specific to the CNS environment, including secretion of neurotrophic factors and developmental refinement of synaptic networks (7, 8). Upon activation, microglia can acquire a range of phenotypes that can either contribute to disease progression or ameliorate it (9). Although dysregulated microglia are implicated in the pathogenesis of several neurodegenerative and psychiatric conditions (10), mechanisms controlling developmental, homeostatic, and pathogenic programs of microglia gene expression remain poorly understood.

Insights into the transcriptional regulation of cell type-specific functions can be obtained by analysis of transcriptional regulatory elements (11). Promoters provide the obligatory transcriptional start sites necessary for RNA synthesis and are often sites of signal-dependent regulation (Figure 1). However, they are primarily occupied by broadly expressed transcription factors (TFs) such as SP1 and GABP, and by themselves are insufficient to confer the specific

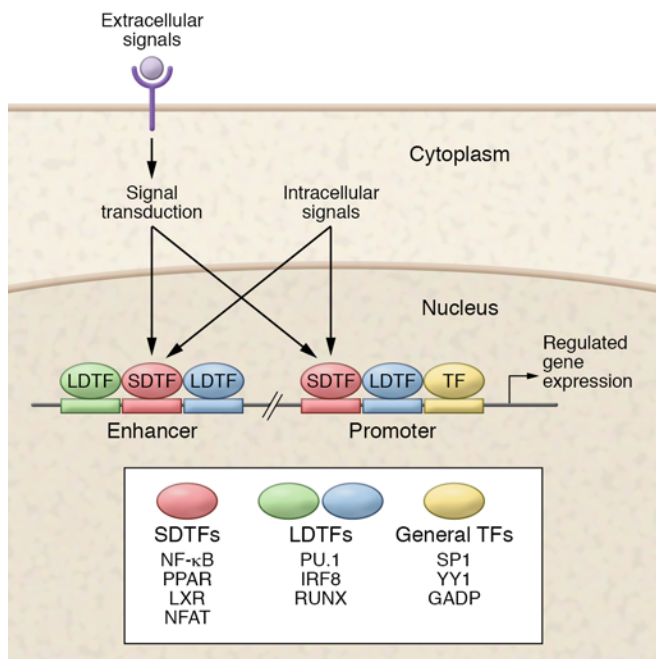
regulatory control necessary to generate cell type-specific programs of gene expression. This additional information is provided by distal regulatory elements called enhancers (12). Enhancers represent the most numerous binding sites for LDTFs and signal-dependent transcription factors (SDTFs), and are major sites for the integration of internal and external signals. Enhancers exhibit distinctive patterns of modifications to adjacent histones that can be detected by ChIP sequencing (ChIP-Seq), and these patterns can be used to putatively classify enhancers as inactive, primed, or active (13).

In vitro studies of elicited peritoneal macrophages provided the basis for a collaborative/hierarchical model of enhancer selection and activation involving interactions between LDTFs and SDTFs (14–16). According to this model, the initial steps of enhancer selection are driven by collaborative interactions of LDTFs and other “pioneer” factors that recognize factor-specific DNA sequences in closed chromatin and generate nucleosome-free regions (17). The selection of enhancers is thought to be a collaborative process requiring interactions between multiple factors that recognize closely spaced arrangements of corresponding recognition motifs (18). Initial occupancy of enhancers by LDTFs and their collaborative partners can result in histone modifications associated with a primed state of activity. The transition from an inactive or primed state to an active enhancer can be induced by SDTFs (19, 20) (Figure 2) that are activated in response to stimuli through cell signaling pathways that often originate at cell surface receptors. These SDTFs mainly bind to enhancers previously established by LDTFs (15, 16, 21, 22). This process is frequently hierarchical such that SDTF binding is dependent on LDTF binding, whereas loss of the SDTF does not influence the binding of the LDTF (14, 21, 23). The observation that SDTFs are directed to a predetermined set of cell-specific enhancers at least partly explains how a broadly expressed TF can regulate specific transcriptional responses in different cell types. Importantly, the distinction between LDTFs and SDTFs is not always clear cut, as some TFs that regulate the ontogeny of a cell type can also serve

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**Figure 1. Enhancers and promoters interact to confer cell type-specific expression and response profiles.** Promoters are primarily occupied by general sequence-specific transcription factors (TFs), but are also important sites of action of lineage-determining TFs (LDTFs) and signal-dependent TFs (SDTFs). Enhancers are primarily selected by LDTFs and are usually the most numerous binding sites for SDTFs. External and internal signals converge on SDTFs to regulate enhancer and promoter function.

as mediators for particular environmental stimuli. In this Review, we examine microglia development and function in the context of this general model, with a focus on roles of the major LDTFs and SDTFs that are expressed in microglia.

### Transcriptional control of microglia identity

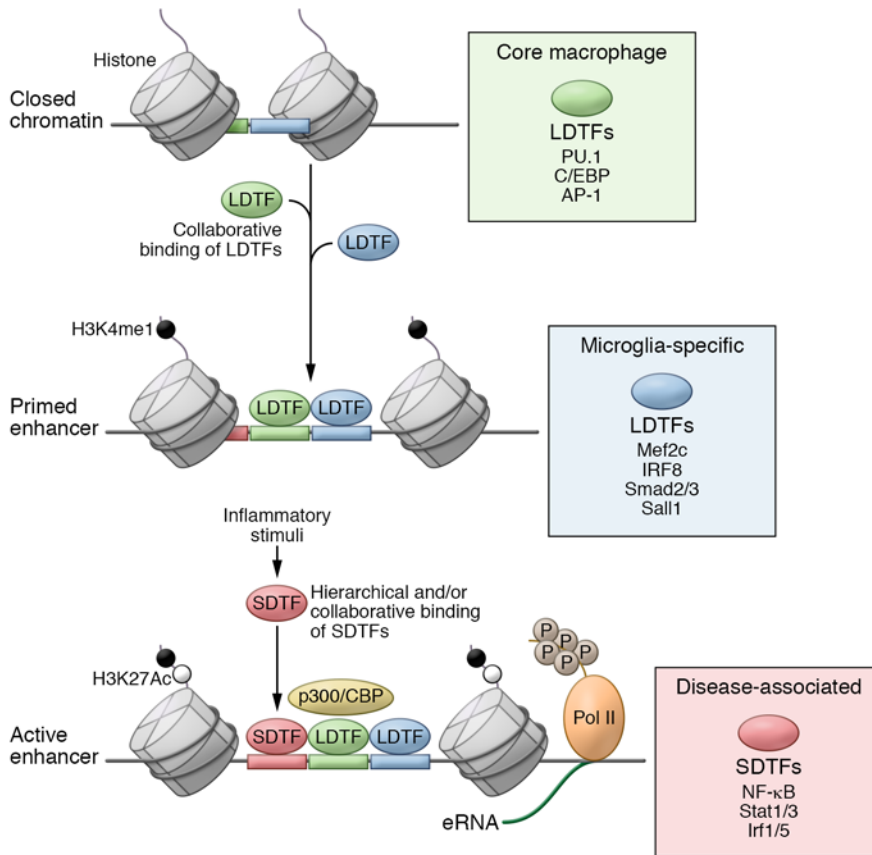
Microglia derive from a population of EMPs in the extraembryonic yolk sac during the process of primitive hematopoiesis (2, 24). In mice, EMPs exclusively express the TF runt-related TF-1 (RUNX1) between E6.5 and E8.0. Microgliogenesis is crucially dependent on the TFs PU.1 and interferon regulatory factor-8 (IRF8), but not on the TFs inhibitor of DNA binding-2 (ID2); basic leucine zipper TF, ATF-like-3 (BATF3); Krüppel-like factor-4 (KLF4); and MyB (25, 26). In the yolk sac EMPs differentiate into premacrophages (pMacs) that express a core macrophage program that includes the receptors CX3CR1 and CSF1R, as well as the TFs *Maf*, *Batf3*, *Pparg*, *Irf8*, and zinc finger E-box binding homeobox-2 (*Zeb2*) (27), a program that persists when these precursors infiltrate various organs to establish resident macrophage populations. Microglia development has been proposed to progress in three developmental stages (28): early microglia (until E14), premicroglia (from E14 to the first weeks after birth), and adult microglia (from a few weeks after birth onward). Early microglia, like EMPs, express genes associated with cell cycle signaling pathways and the TF *E2f6* and DNA methyltransferase-1 (*Dnmt1*), which may help them populate the brain. The genes expressed by premicroglia, including the TFs early growth response-1 (*Egr1*) and spalt-like TF-1 (*Sall1*), are associated with neural maturation and synaptic pruning (29, 30). Later, as the brain

matures, microglia acquire a surveilling phenotype and express the TFs *Jun*, *Fos*, *Mef2a*, and *Mafb*, as well as a set of genes that are highly expressed in microglia such as the characteristic purinergic receptor P2RY12, the transmembrane protein TMEM119, and the chemokine receptor CX3CR1 (31–34), the expression of which is largely conserved in humans (35–37) and zebrafish (38).

Compared with monocytes and neutrophils, macrophage enhancers are enriched for a MAF binding motif with *Maf* and *Mafb* as the highest expressed corresponding TFs, raising the possibility that these TFs may act collaboratively with PU.1 to specify a general macrophage identity (39). In contrast, microglia enhancers, when compared with other tissue macrophages, were enriched for a MEF2 binding motif and exhibited high expression of the cognate TF *Mef2c* (39, 40). A comparison with peritoneal macrophages showed that PU.1-bound enhancers in microglia were enriched for PU.1-IRF, HIC2, MEF2, and SMAD binding motifs, implicating these TFs as potential collaborative partners of PU.1. However, enrichment alone cannot establish whether these factors act as LDTFs in collaboration with PU.1 or merely cobind already-established enhancers. To overcome this limitation, experiments exploiting natural genetic variation between two strains of mice were performed, in a manner analogous to a mutagenesis experiment. These experiments showed that disruption of binding sites for STAT3, MAFB, SMAD3, and upstream TF-1 (USF1) affected nearby PU.1 binding specifically in microglia, and these may therefore function as microglia-specific collaborative factors (40).

Systematic analyses of human microglia gene expression from postmortem (36) and surgical tissues (37) indicate broad similarities with mice but also a number of significant differences, including genes implicated in neurodegenerative diseases. Further, age-related changes in gene expression differed significantly between mice and humans (36). Both human and mouse microglia enhancers are enriched for motifs associated with PU.1, IRF, RUNX, MEF2, C/EBP, AP-1, SMAD, and MAF (37), indicating a conserved set of microglia LDTFs. However, *Sall2*, *Sall3*, and *Smad1* were expressed much more highly in mice, while class II MHC transactivator (*CIITA*), *PPARG*, *EGR3*, and *RUNX2* were preferentially expressed in human microglia. These findings suggest that the differences between mouse and human microglia gene expression are mainly driven by species-specific organization of regulatory elements, but some differences also result from differential expression of TFs.

The importance of the brain environment in maintaining microglia identity is suggested by the rapid and extensive changes in gene expression that occurred when they were transferred from the brain to an in vitro environment (32, 37, 40, 41). Substantial subsets of genes associated with risk alleles for neurodegenerative or behavioral diseases exhibited environment-dependent expression (37). Genes that were downregulated in vitro were highly correlated with genes that are upregulated in primitive macrophages following their migration into the fetal brain (28, 37). These changes in gene expression are associated with downregulation of numerous putative microglia LDTFs, such as *Sall1* and corresponding alterations in enhancer landscapes. Collectively, these findings provide evidence for an important role of brain-derived signals in controlling a conserved network of signal-dependent and lineage-determining TFs in mouse and human microglia.



**Figure 2. Different classes of transcription factors interact and regulate cellular identity.** The top of the figure illustrates the initial steps of enhancer selection in closed chromatin consisting of regularly positioned nucleosomes. Green and blue shading represents closely spaced binding sites for LDTFs. Below, collaborative interactions between LDTFs generate primed enhancers, characterized by a nucleosome-free region and monomethylated H3K4 (H3K4me1). At the bottom of the figure, active SDTFs localize to primed enhancers, resulting in recruitment of coactivators and RNA polymerase II and generation of enhancer RNAs (eRNAs).

## LDTFs that establish physiological microglia

Many of the TFs implicated in defining the microglia cell type during development have been shown to play roles in maintaining microglia in a ramified and surveilling state that is characteristic of healthy brain tissue. We discuss three important and well-described transcriptional regulators in more detail below.

**PU.1.** PU.1 is a well-characterized LDTF that appears to serve as a master regulator of the myeloid lineage (42). PU.1 is essential for establishing macrophage lineages, drives microgliogenesis, and is a major factor in selecting the microglia enhancer landscape. PU.1-deficient mice exhibit multiple hematopoietic abnormalities and a pronounced depletion of B cell and macrophage populations (43). PU.1 deficiency also ablates microglia in mice (26) and zebrafish (44). Moreover, PU.1 binds to most enhancers in mouse (40) and human microglia (37), highlighting its central role in establishing the microglia enhancer landscape.

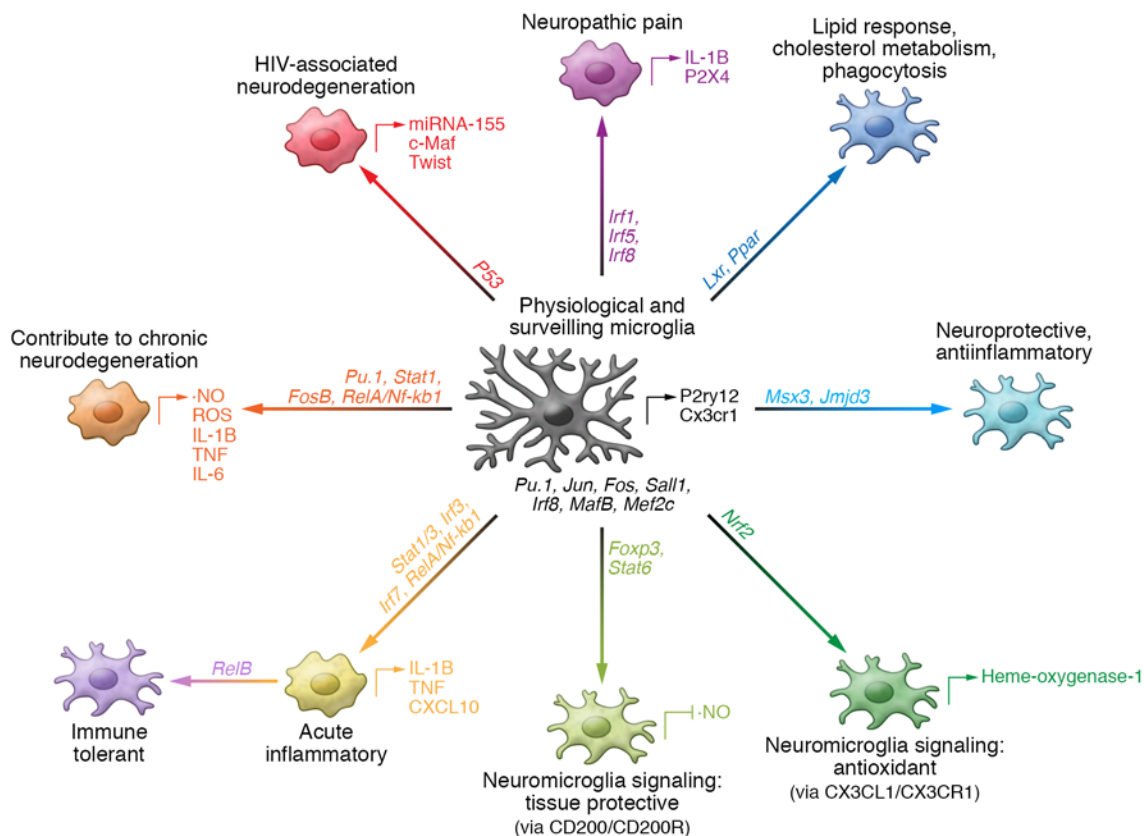
**SALL1.** SALL1 is a zinc finger transcriptional repressor that belongs to the SALL-like family of TFs that function in tissue morphogenesis (45). SALL1 regulates cortical neurogenesis and laminar fate specification in mice (46). Mutations in *SALL1* are associated with Townes-Brocks syndrome, an autosomal dominant developmental disorder in which roughly 10% of patients exhibit neural or behavioral abnormalities (46). Human and mouse microglia uniquely and highly express SALL1 as compared with other macrophage populations, suggesting that it functions as an LDTF (34, 35, 37). *Sall1*-deficient animals have normal microglia colonization in the brain, but the microglia exhibit an abnormal amoeboid morphology (30). Moreover, in the absence of *Sall1*,

microglia are more proinflammatory and phagocytic, and show downregulation of the microglia-specific gene expression signature (29). This altered microglia phenotype has detrimental consequences for neurogenesis and tissue homeostasis. Taken together, these studies suggest that SALL1 inhibits a reactive microglia phenotype, and promotes a physiological surveilling phenotype.

**MAFB.** MAFB is a member of the large MAF subfamily of TFs that binds to the MAF recognition element and forms heterodimers with cMAF, Jun, and Fos. In macrophages, MAFB promotes an anti-inflammatory phenotype, and its deletion exacerbates inflammatory and pathological conditions (47, 48). In microglia, expression of MAFB increases during microglia development, and its deletion disrupts gene expression in adult microglia more severely than in premature microglia (28). Motif mutation analysis suggests that MAFB functions as an LDTF in collaboration with PU.1 (40). In microglia, MAFB regulates the expression of immune and viral genes (28), and its expression is downregulated in the SOD1 mouse model of amyotrophic lateral sclerosis (49). In primary microglia, MAFB regulates cell division by dampening the response to granulocyte-macrophage CSF (50). These findings suggest that MAFB helps to maintain a healthy adult microglia phenotype by inhibiting inflammatory and proliferative responses.

## Transcriptional control of disease phenotypes

In disease conditions, microglia can adopt various phenotypes that exert beneficial and deleterious effects, including tissue support, excessive pruning of synapses, induction of neuropathic pain, and exacerbation of neurodegeneration (51). New studies are begin-



**Figure 3. Transcription factors that regulate different microglia phenotypes.** Microglia ontogeny is crucially dependent on LDTFs such as PU.1, MAFB, and SALL1 that are essential for the development of the ramified phenotype depicted in the center. Microglia are diverse cells that acquire different functional phenotypes in response to the environment in which they reside. Several transcriptional regulators have been identified that regulate different microglia phenotypes. For example, proinflammatory microglia are regulated by IRF7, RelA, STAT1/3, and FosB. Microglia that mediate neuropathic pain are induced by expression of *Irf1*, *Irf5*, and *Irf8* in microglia. Microglia that contribute to HIV-associated neurodegeneration are characterized by an induction of p53. Figure adapted with permission from *Nature Reviews Neurology* (128).

ning to shed light on how these phenotypes are transcriptionally regulated (Figure 3). The collaborative/hierarchical model suggests that, in disease, a unique combination of pathological and inflammatory factors activates SDTFs that regulate the expression of specific microglia response programs. A major obstacle to understanding the relationships among the different classes of TFs is that the environmental factors and pathological signals are complex and interact on multiple levels. The observation that culturing microglia so that they no longer receive brain-derived signals results in dramatic changes to their enhancer landscape (32, 37, 40) calls into question the extent to which findings about SDTFs obtained *in vitro* are translatable to microglia *in vivo*. Furthermore, the frequently used immortalized murine microglia cell line, BV2, only partially recapitulates the response to a simple LPS stimulus observed in primary microglia (52). Given these differences, findings regarding the roles of SDTFs from *in vitro* studies should be interpreted with caution until they can be confirmed in *in vivo* conditions. Nevertheless, an increasing number of studies have identified transcriptional regulators of microglia activation and microglia phenotypes in pathological conditions.

The dynamic interaction between LDTFs and SDTFs in human disease is illustrated by the response of microglia to the mutant form of the huntingtin protein (HTT) responsible for

Huntington’s disease (HD) (53). Multiple studies demonstrated increased microglial activation in HD patients, but it remained an open question whether this resulted from the action of mutated HTT (mHTT) in the microglia or represented the response of microglia to mHTT-induced changes in neurons. This was addressed by the expressing of mHTT in BV2 cells, resulting in increased expression of the SDTF NF-κB1 and the proinflammatory cytokines IL-6 and TNF, as well as increased expression and binding of the macrophage LDTFs PU.1 and C/EBP. Neurons that were cocultured with LPS-stimulated microglia expressing mHTT had increased apoptosis compared with neurons cocultured with LPS-stimulated WT microglia.

### SDTFs that mediate inflammation

As the resident macrophages of the CNS, microglia are the first to respond to inflammatory insults in the brain, which they do by expressing a wide array of cytokines, chemokines, nitric oxide (NO), and various reactive oxygen species (ROS). Several transcriptional families have been identified whose members regulate the expression of proinflammatory mediators in macrophages and microglia.

**NF-κB family.** Members of the NF-κB family are pleiotropic regulators that play key roles in apoptosis and inflammation (54). NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelA (p65), RelB, and c-Rel form



homodimers and heterodimers. Without stimulation, NF- $\kappa$ B is sequestered in the cytoplasm. Once activated, it translocates to the nucleus, where it binds to NF- $\kappa$ B binding sites exposed in open chromatin regions and induces the expression of target genes (55). Many studies have examined the role of NF- $\kappa$ B in brain aging and neurodegenerative conditions (54), but, since expression of NF- $\kappa$ B is not limited to microglia, most of these studies have focused on its role in other cell types or its generic expression in the brain.

In microglia, NF- $\kappa$ B is activated by signal transduction pathways responding to the presence of ROS (56), saturated fatty acids (57),  $\alpha$ -synuclein (58, 59), and amyloid- $\beta$  (60). The induced response can be either protective or deleterious depending on the context of the stimulation (61). A small number of studies have experimentally assessed the functional role of NF- $\kappa$ B in microglia. Bacterial LPS is a TLR4 ligand that induces a strong immune response mediated by several TFs, including the NF- $\kappa$ B1/RelA complex. A single intraperitoneal injection of LPS has long-term consequences in microglia and results in blunted expression of proinflammatory cytokines upon LPS restimulation; this attenuation is mediated in part by RelB (62). These observations exemplify how different members of the same NF- $\kappa$ B family can play antagonistic roles in inflammation and mediate changes in the microglia phenotype, and highlight the need for a more thorough understanding of how NF- $\kappa$ B family members regulate microglia phenotypes.

**Activator protein-1 family.** The activator protein-1 (AP-1) family of TFs is involved in numerous processes including cell growth, differentiation, apoptosis, and immune activation (63). This family consists of Jun, Fos, activating TF (ATF), and Jun dimerization protein (JDP) subfamilies, and its members can form both homo- and heterodimers. In macrophages, AP-1 members have been described to function as both LDTFs (15) and SDTFs (64). Similarly, in microglia, Jun and Fos are upregulated during microglia development, suggesting that they play roles as LDTFs that regulate microglia identity (28). However, FosB has been suggested to function as an SDTF that regulates excitotoxic microglia activation (65). Constitutive *Fosb*-knockout mice were relatively resistant to kainate-induced excitotoxicity, with fewer amoeboid microglia, and less expression of the proinflammatory cytokines IL-6 and TNF compared with their WT counterparts. Since *FosB* expression is similar between hippocampal neurons and microglia, it is currently unclear whether FosB ablation directly affects microglia, neurons, or both. More research into the role of AP-1 members in microglia is needed for a better understanding of how these factors regulate both microglia identity and pathological phenotypes.

**Interferon regulatory factors.** The interferon regulatory factor (IRF) family consists of nine members that bind to genomic loci known as interferon-sensitive response elements. IRF3, IRF5, and IRF7 mediate type I interferon responses induced by various TLR ligands (66), while IRF1 is essential for the type II interferon response (63). In microglia, IRF8 is a key regulator that appears to function as both an LDTF and an SDTF. IRF8 was recently identified as an essential regulator of tissue macrophage maturation, including microglia (27, 67). *Irf8* deletion substantially reduces microglia numbers in mice (26). Microglia-specific disruption of *Irf8* resulted in a pronounced reduction in microglia ramification and surface area as well as altered expression of several cell surface markers, indicating increased microglia immune activation (68). In zebrafish, *Irf8*-null

mutants showed a complete absence of microglia (69). IRF8 has also been shown to regulate microglia motility (70).

In spinal cord microglia, IRF8, in conjunction with IRF1 and IRF5, contributes to the induction of neuropathic pain after peripheral nerve injury (PNI) (71–74). IRF8 expression is markedly upregulated by PNI, which results in hypersensitivity to pain (71). IRF8 is upstream of IRF5, and *Irf5*-deficient mice exhibited substantial resistance to neuropathic pain (74). In microglia, IRF8 is also upstream of IRF1 and thereby regulates IL-1 $\beta$  expression after PNI (73). Collectively, these studies indicate that IRF8 is both a key regulator of microglia identity and a modulator of a reactive microglia phenotype associated with neuropathic pain.

IRF7 in microglia has been shown to promote both an anti-inflammatory phenotype (75) and a proinflammatory phenotype (76). Downregulation of IRF7 by long-term exposure to TGF- $\beta$ 1 or inhibition of IRF7 impairs the ability of microglia to acquire an anti-inflammatory phenotype (75), while IFN- $\beta$ -mediated activation of IRF7 restores the anti-inflammatory phenotype. In spinal cord injury, microglia overexpressing IRF7 have decreased proinflammatory activity (75). In contrast, IRF7 promotes LPS-induced inflammation via phosphorylation of STAT1 (76). Reconciliation of these two opposing observations concerning a single TF deserves further attention.

**p53 tumor suppressor.** p53 is a well-studied tumor suppressor gene that functions as a complex integrator of signals that regulate DNA damage repair, cell cycle progression, and apoptosis (77). The expression and activity of p53 increases in microglia in response to cellular stress, DNA damage, and oxidative stress (78–80). In post-mortem brain tissues from patients with HIV-associated dementia (HAD) elevated levels of p53 were particularly prominent in the microglia (78, 81). HIV-infected p53-deficient neuron/microglia cocultures showed increased neuronal survival compared with WT (78). Microglial p53 activation is detrimental to neuronal synapses during inflammation (82). *P53*<sup>-/-</sup> microglia had lower expression of proinflammatory genes and demonstrated increased phagocytosis and expression of tissue support genes in response to IFN- $\gamma$  (81). p53 may act to downregulate the anti-inflammatory transcription factor cMAF through a transcriptional circuit involving TWIST2 and miR-155 (83). Collectively these studies suggest that p53 regulates microglia activity and induces a more reactive inflammatory profile with decreased tissue-supportive functions and phagocytic activity in HAD that might also play a more generic role in regulating microglia in disease.

**STAT family.** STAT TFs are pleiotropically expressed and mediate diverse functions such as proliferation, apoptosis, and differentiation in response to cytokines (63). STAT proteins form both homo- and heterodimers and become activated when phosphorylated STAT dimers translocate to the nucleus and bind to genomic elements. The binding pattern of STAT TFs is largely cell type-specific, and individual STAT members often bind in the proximity of cell type-specific TFs (84). Aberrant activation of JAK/STAT signaling in innate immune cells is associated with both multiple sclerosis (MS) and Alzheimer's disease (AD) (85).

In mice, alterations in the STAT3 binding site affect nearby PU.1 binding in microglia, suggesting that STAT3 functions as an LDTF (40). STAT1 and STAT3 also appear to function as SDTFs that regulate LPS-induced activation (86). In primary

mouse microglia, changes in the binding of STAT1, STAT3, and STAT5 in response to LPS were assessed, and a group of STAT-regulated inflammatory genes was identified including the histone 3 lysine-27 (H3K27) demethylase JMJD3. The expression of these putative target genes was only affected if both STAT1 and STAT3 were inhibited simultaneously using siRNA in BV2 cells, suggesting a functional redundancy between these two factors. Knockdown of both STAT1 and STAT3 inhibited the expression of JMJD3 and other inflammatory genes. This evidence suggests that STAT1/STAT3 signaling regulates expression of inflammatory mediators in microglia, possibly via JMJD3.

### Antiinflammatory and tissue-supportive mediators

Excessive inflammation in the CNS is detrimental for neuronal health (87). Not surprisingly, several transcriptional mechanisms limit the inflammatory responses of microglia and induce neuroprotective behavior.

**MSX family.** The Msh-like homeobox (MSX) genes are part of the homeobox family and are implicated in brain development and neurogenesis (88). In microglia, MSX3 is normally lowly expressed but upregulated during experimental autoimmune encephalomyelitis (EAE) in mice (89). Transplantation of MSX3-overexpressing microglia to EAE mice resulted in milder EAE clinical scores, increased remyelination, and decreased inflammation. ChIP analysis indicated that MSX3 bound at the promoters of *Pparg*, *Stat6*, and *Jak3*, suggesting that it might exert its effect by regulating these genes. This study suggests that MSX3 might play a role in maintaining a tissue-supportive phenotype in microglia.

**NR4A2.** Nuclear receptor subfamily 4, group A, member 2 (NR4A2, also known as NURR1) plays an essential role in the generation and maintenance of dopaminergic neurons in the brain (90). Mutations in *NR4A2* are associated with familial Parkinson's disease (PD) (91). In microglia, NR4A2 inhibits LPS-induced expression of proinflammatory cytokines (92). Reduced NR4A2 expression results in exaggerated inflammatory responses in microglia and increases dopaminergic neuron death. NR4A2 exerts an antiinflammatory effect by binding to RelA on inflammatory gene promoters and recruits the CoREST corepressor complex, which clears RelA and represses transcription. Additionally, pharmacological activation of NR4A2 in BV2 cells blocks inflammatory gene expression by inhibiting RelA (93), suggesting that NR4A2 is an antiinflammatory regulator that counteracts RelA-based inflammation in microglia.

**Estrogen receptors.** Estrogen receptor- $\alpha$  (ER $\alpha$ ) and ER $\beta$  are a subclass of the nuclear receptor superfamily that function as estrogen-dependent TFs. Estrogens exert neuroprotective actions by signaling through ERs that are widely distributed in the male and female brain (94). Estradiol is not only a reproductive hormone but also a brain-derived neuroprotective factor in both males and females that enables ERs to coordinate multiple signaling mechanisms that protect the brain from neurodegenerative diseases. In microglia, estrogen signaling was also shown to exert antiinflammatory effects (95). Androstenediol (or 5-androsten-3 $\beta$ ,17 $\beta$ -diol) is an estrogenic steroid produced in the brain that binds to ER $\beta$ . Androstenediol activation of ER $\beta$  suppresses the inflammatory responses of microglia by recruitment of C-terminal binding protein (CtBP) corepressor

complexes to AP-1-dependent promoters (96). Reduction of androstenediol or ER $\beta$  expression results in exaggerated LPS responses, and administration of androstenediol *in vivo* prevents EAE. These findings provide evidence for an ER $\beta$  signaling pathway that controls inflammatory responses in microglia.

**Nuclear respiratory factor 2.** Nuclear respiratory factor 2 (NRF2, also referred to as nuclear factor 2 erythroid 2 like 2 [NFE2L2]) regulates an antioxidant response to the oxidative damage that is characteristic of many neurodegenerative conditions (97). In macrophages, NRF2 expression represses inflammation not only by regulating the antioxidative defense (98), but also by directly inhibiting the recruitment of RNA polymerase II to *Il6* and *Tnf* (99). Many studies suggest that NRF2 activation is involved in neuroprotection (97) by regulating antioxidative proteins such as heme oxygenase-1 (HO-1). In microglia, NRF2 has been demonstrated to counteract inflammation and neurodegeneration in the tau pathology model for AD (100), the MPTP model for PD (101), hematoma (102), and LPS-induced inflammation (103). Stressed neurons signal microglia through fractalkine to induce the NRF2-mediated antioxidative response (100). These findings were corroborated in human AD brain tissues, suggesting that this mechanism might be an adaptive tissue-restorative response to pathological conditions. Besides regulating the antioxidative response, NRF2 has also been implicated in regulation of phagocytic clearance, a process that is essential for the uptake of blood cells in hematoma, via upregulation of CD36 (102). Collectively, these results indicate that NRF2 dampens inflammation through mediating the antioxidative response and facilitating phagocytosis, thereby positioning it as a crucial regulator of a microglia tissue-restorative phenotype.

### SDFs that regulate cholesterol and lipid metabolism

Lipids and cholesterol derivatives are essential for the CNS, where they function as key components of myelin sheets, synapses, dendrites, and intercellular signaling molecules (104, 105). Defects in cholesterol metabolism are clearly described in neurodegenerative diseases (106). The PPAR and liver X receptor/retinoid X receptor (LXR/RXR) families play key roles at the crossroads of lipid and cholesterol metabolism with immune responses in tissue macrophages (107). In microglia, much less is known about these factors since, to date, no studies have experimentally targeted members of the LXR/RXR or the PPAR family to microglia *in vivo*.

**PPARs.** The PPAR family of nuclear receptor TFs consists of three members: PPAR $\alpha$ , PPAR $\delta$ , and PPAR $\gamma$ . PPARs function as lipid sensors and are involved in fatty acid metabolism (108). In adipose-resident macrophages, members of the PPAR family are implicated in suppressing fatty acid-induced inflammation associated with obesity and metabolic syndrome by increasing fatty acid oxidation (109). PPAR $\gamma$  is a central switch that inhibits the expression of proinflammatory mediators (110) and induces a wound healing phenotype (111, 112). However, the role of PPARs in microglia and neuroinflammation is still poorly understood. A rapidly growing number of studies have investigated the role of PPAR $\gamma$  agonists in neurodegenerative conditions such as AD, PD, HD, and amyotrophic lateral sclerosis, but these findings have not conclusively shown a protective effect (113). However, in AD mice, a PPAR $\gamma$  agonist did

show beneficial effects by counteracting neurodegeneration, lowering amyloid- $\beta$  deposits, and inhibiting neuroinflammation (114). It has been suggested that the beneficial effects of PPAR $\gamma$  agonist are produced by inhibition of the proinflammatory activity of microglia and promotion of their phagocytic activity (115–117).

**LXRs and RXRs.** Liver X receptors (LXRs) and retinoid X receptors (RXRs) are nonsteroid nuclear receptors that play key roles in cholesterol homeostasis and inflammation (118, 119). The LXR isoforms LXR $\alpha$  and LXR $\beta$  can form dimers with any of the three RXR isoforms (RXR $\alpha$ , RXR $\beta$ , and RXR $\gamma$ ) and are activated in the presence of cholesterol derivatives such as oxysterols. In macrophages, the majority of LXR binding sites are found near PU.1-bound primed enhancers (15). LXRs have antiinflammatory effects and modify macrophage phagocytic capability (120). Age-related neurodegenerative conditions have been linked to the disruption of cholesterol metabolism and LXR signaling (118). Likewise, LXR agonists have been shown to produce cognitive improvement in AD mouse models (121–123), but the mechanisms are not well understood. It has been suggested that the protective effects of LXR agonists are achieved by promotion of an increase in phagocytosis of amyloid- $\beta$  by microglia (117), by inhibition of microglia nitric oxide synthetase (NOS) activity (124), or by attenuation of the microglia inflammatory response (125).

## Conclusions and future perspectives

Many transcriptional regulators that mediate microglia in health and disease have been identified, but much remains unknown about their functional consequences, how they guide microglia phenotypic diversity, the effects of natural genetic variation, and their responses to specific signals in the local microenvironment.

One challenge is that many studies are purely descriptive or are performed on cultured microglia. The recent development of effective and inducible drivers of Cre-recombinase such as *Cx3cr1-CreER* (126) and *Sall1-CreER* (29) enables microglia-targeted knockout and overexpression of genes of interest. These tools could not only provide stronger and more detailed experimental evidence but also give qualitatively new insights into the functional roles of TFs in regulating microglia in vivo.

A further challenge is to untangle the interrelationships between transcriptional regulators and the microglia enhancer landscape, which is required to understand the molecular mechanisms underlying their functional roles in microglia. With the increasing adoption of high-throughput technologies, it is now possible to map the binding patterns of different TFs in the context of the enhancer landscape. Additionally, new techniques that unravel the 3D structure of chromatin, such as Hi-C, can be used to determine enhancer-promoter interactions, and help to address whether particular TFs regulate specific classes of enhancers.

Naturally occurring variation in noncoding regions of the genome can have modulatory effects on gene regulation or enhancer formation and could have considerable importance for understanding the involvement of such genetic variation in the roles of microglia in neurodegenerative diseases. Relatively large numbers of human microglia samples are necessary to determine whether disease-associated SNPs act on microglia expression and/or enhancer profiles. Therefore, the effect of natural genetic variation on microglia expression and enhancer landscapes remains largely unexplored.

Another major aim for future research will be to identify the specific factors of the local CNS microenvironment, such as TGF- $\beta$  or amyloid- $\beta$ , that either help to maintain microglial physiological identity or are responsible for disease-specific responses. Additionally, further identification of the TFs through which these signals are transduced to produce the microglia-specific patterns of gene expression remains an ongoing subject of investigation.

Microglia can exhibit a diverse range of phenotypes in response to their surroundings, but these phenotypes are much more complex than the simple M1/M2 dichotomy (127) originally proposed for macrophages. Nor are these phenotypic states easily classified on a one-disease/one-phenotype basis. The proper framework for cataloging microglia diversity across pathological conditions, brain regions, local microenvironment, and developmental stages remains elusive. New technologies such as single-cell RNA sequencing and mass cytometry should facilitate a framework for microglia phenotype classification.

Finally, the degree to which findings about transcriptional regulation in mice and zebrafish translate to humans is critical for insight into the pathophysiology of neurodegenerative and neuropsychiatric diseases and is still largely unaddressed. Further studies of human microglia are essential to identify the strengths and weaknesses of animal and cell culture models.

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Address correspondence to: Christopher K. Glass, University of California, San Diego, Department of Cellular and Molecular Medicine, School of Medicine, GPL Room 210, 9500 Gilman Drive, Mail Code 0651, La Jolla, California 92093, USA. Phone: 858.534.6011; E-mail: ckg@ucsd.edu.

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