Microglial subtypes: diversity within the microglial community

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Abstract

Microglia are brain-resident macrophages forming the first active immune barrier in the central nervous system. They fulfill multiple functions across development and adulthood and under disease conditions. Current understanding revolves around microglia acquiring distinct phenotypes upon exposure to extrinsic cues in their environment. However, emerging evidence suggests that microglia display differences in their functions that are not exclusively driven by their milieu, rather by the unique properties these cells possess. This microglial intrinsic heterogeneity has been largely overlooked, favoring the prevailing view that microglia are a single-cell type endowed with spectacular plasticity, allowing them to acquire multiple phenotypes and thereby fulfill their numerous functions in health and disease. Here, we review the evidence that microglia might form a community of cells in which each member (or “subtype”) displays intrinsic properties and performs unique functions. Distinctive features and functional implications of several microglial subtypes are considered, across contexts of health and disease. Finally, we suggest that microglial subtype categorization shall be based on function and we propose ways for studying them. Hence, we advocate that plasticity (reaction states) and diversity (subtypes) should both be considered when studying the multitasking microglia.

Keywords: disease, heterogeneity; homeostasis; microglia; subtypes

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Introduction

Microglia were introduced to the scientific literature a century ago (Río-Hortega, 1919a,b,c; Fig 1). During normal physiological conditions, microglial cells with a ramified morphology are regularly distributed throughout the central nervous system (CNS; Río-Hortega, 1919b). Upon pathology, microglia transform their morphology and function, leading to propose a cascade of “reaction” from ramified to hypertrophic and ameboid phenotypes that still orients research today (Flanary et al., 2007; Graéber, 2010; Fig 1). With the recent advances in genetic tools allowing for fate mapping (Ginhoux et al., 2010), microglia are now considered to be tissue-resident macrophages of the CNS that arise exclusively from the embryonic yolk sac (Alliot et al., 1999; Schulz et al., 2012; Kierdorf et al., 2013; Perdiguero et al., 2015). Microglia colonize the murine CNS from embryonic day (E)9.5 (Tay et al., 2017c) and represent a self-maintaining and long-lived cell population that persists for months, if not the entire lifespan of the organism (Lawson et al., 1992; Ajami et al., 2007, 2011; Mildner et al., 2007; Askew et al., 2017; Füger et al., 2017; Réu et al., 2017; Tay et al., 2017b). Beyond microglia functioning as mediators of injury, inflammation, and neurodegeneration, several roles in the healthy brain have been identified at an exponential rate this past decade (Cartier et al., 2014; Tremblay et al., 2015; Fig 1).

Microglia exhibit widely differing functions depending on the stage of life, CNS region, and context of health or disease. Differences in microglial number, morphology, and gene expression were also reported between sexes (Schwarz et al., 2012; Crain et al., 2013; Lenz et al., 2013; Pimentel-Coelho et al., 2013; Butovsky et al., 2015; Dorfman et al., 2017; Hanamsagar et al., 2017; Krasemann et al., 2017). Adequate microglial functions are crucial for plasticity and behavioral adaptation to the environment (Salter & Stevens, 2017; Tay et al., 2017a). Throughout life, microglia contribute to neurogenesis, neuronal circuit shaping, vascular formation and remodeling, and maintenance of homeostasis (Tay et al., 2017c). During aging and in diseases, these cells may become reactive or impaired in their surveillance and phagocytosis (Streit, 2002; Koellhoffer et al., 2017; Spittau, 2017). Microglial contribution to diseases is associated with compromised physiological roles (e.g., in synaptic maintenance and plasticity; Tay et al., 2017a) and processes that are adaptive in the healthy brain, yet leading to cell death and tissue damage in pathological settings (e.g., excitotoxicity, oxidative stress, and inflammation; Weil et al., 2008). Microglial reaction can be triggered by any kind of insults or disturbances to the CNS. Persisting microglial reaction, associated often with proliferation, is involved in pathological conditions ranging from neurodevelopmental disorders, traumatic injuries, infectious diseases, tumors, and psychiatric disorders, to neurodegenerative diseases.
Depending on the stage of the life, CNS region, and stressor or pathological insult at play, the microglial reaction process was shown to proceed differently and to result in sometimes contrasting outcomes (see Fig 2A for a classical schematic representation, depicting a ramified gray microglial cell surrounded by a palette of colorful microglia each representing a distinct reaction state). It is also now recognized that microglia display a wide range of reaction states, a tremendous shift from the M1/M2 classification still used a few years ago (Martinez & Gordon, 2014; Ransohoff, 2016). According to this view, the numerous functions of microglia would be fulfilled through their reaction toward multiple phenotypes, each associated with a distinct molecular signature (Crain et al, 2013; Hickman et al, 2013; Butovsky et al, 2014; Bennett et al, 2016; Grabert et al, 2016; Flowers et al, 2017; Galatro et al, 2017; Keren-Shaul et al, 2017; Krasemann et al, 2017; Hammond et al, 2018; Masuda et al, 2019). However, several pieces of evidence also indicate that different pools of microglia might each display distinct intrinsic properties that would be acquired during their maturation or function within the CNS. These subtypes would co-exist at steady state and undergo further modulation or phenotypic transformation in response to stimuli (Fig 2B). Indeed, beyond the view that microglia are a unique cell type in the CNS that adopts different phenotypes in response to different stimuli, we propose in this review article that microglia might constitute a community of cells in which different members display distinct properties, perform distinct physiological functions, and respond differently to stimuli (Fig 2C). We review the distinctive features of several putative microglial “subtypes”, at structural, ultrastructural, and expression levels, as well as their functional implications across contexts of health and disease. Furthermore, we propose to categorize microglial subtypes based on functions, rather than molecular signatures and markers. Finally, we suggest that microglial subtype candidates should be validated using a methodological workflow that we recommend.

Microglia: a community fulfilling the vast microglial functions

What defines a cell subtype is subject of intense debate, and it is discussed in Box 1. Accumulating evidence indicates that microglia

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**Figure 1. Historical overview of microglial subtype identification.**

Although microglial subtypes have originally been proposed by Rio-Hortega in the first report of microglia, it was only recently that this idea was revisited.
are not the naive cell type that invariably responds identically to any possible type of stimuli by assuming a predetermined phenotype. In fact, from a historical perspective, the notion of microglial subtypes had already been proposed in 1919 by Rio-Hortega in his original description of microglia (Rı́o-Hortega, 1919b; Fig 1). He noticed that some microglia that he named “satellite” microglia were found in close proximity to neuronal cell bodies. A century later, we propose that the satellite microglia, which are discussed below, might represent one of the playing cards in the deck of microglial subtypes (Fig 3). It is important to acknowledge that others, avant-garde scientists, have paved the way for the concept of microglial diversity (McCluskey & Lampson, 2000; Olah et al, 2011; Hanisch, 2013; Gertig & Hanisch, 2014).

Microglial regional heterogeneity at steady state

Although microglia are ubiquitously scattered throughout the CNS, their distribution varies across regions, also between the white matter and gray matter (Lawson et al, 1990). Microglial morphology differs with the presence of neuronal cell bodies, dendrites and axons, myelinated axons, and blood vessels. Furthermore, microglia exhibit regional differences in self-renewal and turnover rates under normal physiological conditions and upon stimuli, such as lipopolysaccharide (LPS) challenge (Lawson et al, 1992; Ajami et al, 2007, 2011; Mildner et al, 2007; Askew et al, 2017; Füeger et al, 2017; Rêu et al, 2017; Tay et al, 2017b; Furube et al, 2018). The regional microenvironment has been shown to tightly determine microglial identity at the transcriptional level, in both mouse and human (Gosselin et al, 2014, 2017). Direct evidence for microglial regional variability notably comes from studies in which microglia were isolated from wild-type, unchallenged adult mice, according to brain area, and their transcriptome was determined based on panels of pre-selected microglial markers. In one study, the expression of CD11b, CD40, CD45, CD80, CD86, F4/80, TREM2b, CX3CR1, and CCR9 was compared among microglia isolated from different CNS.
regions of young adult mice (de Haas et al., 2008). Although all of these markers were expressed across the CNS, their protein expression varied significantly between areas. In a similar study performed in adult rats, the expression levels of known microglial markers also showed region-specific profiles (Doorn et al., 2015). Similar studies performed in mice that compared microglia isolated from different brain areas additionally showed regional heterogeneity in expression pattern throughout the lifespan (Butovskiy et al., 2014; Grabert et al., 2016; De Biase et al., 2017; Masuda et al., 2019). Additionally, in an unbiased single-cell RNA sequencing (RNAseq) study, in which cerebral tissue and hippocampal tissue from unchallenged young adult mice were analyzed, 47 molecularly distinct cell subtypes were identified, including two belonging to the microglia (Zeisel et al., 2015).

These findings raise the intriguing possibility that regional differences in terms of neuronal survival, activity, growth factor release, metabolism, as well as synaptic plasticity, myelination, vascular remodeling, blood–brain barrier properties, may require distinct microglial functions, thus driving the differentiation of distinct microglial subtypes during development or function within the CNS. These microglial subtypes could be a major contributing factor to the microglial regional heterogeneity. Recently, cerebellar microglia were shown to display a unique clearance ability, defined by their expression of numerous genes supporting the engulfment and catabolism of cells or cellular debris (Ayata et al., 2018). This cerebellar microglial “type” is reminiscent of developing microglia and disease-associated microglia (DAM) that will be discussed below. By contrast, microglia from the striatum display a homo-static surveillance phenotype. This microglial differentiation in response to regional differences in the environment was shown to be driven by epigenetic mechanisms (Ayata et al., 2018). In particular, the suppression of clearance genes in striatal microglia is mediated by PRC2, which catalyzes the repressive chromatin modification histone H3 lysine 27 trimethylation (H3K27me3). The ablation of PRC2 in microglia also results in the emergence of clearance microglia even in the absence of dying neurons, among both the striatum and cerebral cortex. These aberrant clearance microglia induce impaired motor responses, decreased learning and memory, together with the development of anxiety and seizures in mice (Ayata et al., 2018). A recent study that characterized the diversity of CNS-associated macrophages (CAM) also identified three different subsets of CAM that expressed high levels of Mrc1, Ms4at, P4, Stabi, Cbr2, CD163, and Fcrls, and were associated with different CNS compartments: the leptomeninges, choroid plexus, and perivascular space (Jordão et al., 2019). Consequently, some of the regional microglial diversity described using these markers could also be partly accounted for by CAM diversity.

**Microglial subtypes as defined by differential gene expressions**

Differential gene expression is an established approach for defining distinct subpopulations of a cell type, for instance the different neuronal subtypes (e.g., GABAergic and glutamatergic) observed in the healthy brain. In various contexts, neighboring microglia were shown to display differences in gene expression at steady state. These observed differences between microglia could arise from local cues, including interactions with different subtypes of neurons (e.g., inhibitory and excitatory) and glial cells (astrocytes, oligodendrocytes, and progenitors), or slight differences in signaling thresholds.
**Figure 3. Putative microglial subtypes with unique specializations.**
Emerging data provide support to the existence of putative microglial subtypes endowed with unique genomic, spatial, morphological, and functional specializations. We anticipate that analyzing these subtypes thoroughly, with the methodological workflow proposed in Box 1, and using a similar methodology for newly discovered ones, will result in the identification of a number of different microglial subtypes with unique functional characteristics that could be targeted for disease prevention or treatment.
Similarly, differences in peripheral macrophage activation by LPS and viruses have been described, where only a subset of the population concomitantly displays a response (Ravasi et al., 2002). In addition, microglia may directly communicate with each other, which suggests that the recruitment of a specific microglial cell might lead to an inhibition of the neighboring microglia. Microglia were initially defined as occupying non-overlapping territories in the healthy brain, but this view is now changing, with improved staining methods showing direct contacts between processes and sometimes cell bodies from neighbor microglial cells (for example, see Milior et al., 2016). Furthermore, the possibility that differential marker expression among adjacent microglia results from differences in microglial...
exposure to previous challenges also has to be considered. For instance, it has been shown using non-invasive two-photon in vivo imaging that neighbor microglia respond differently to laser injury in the intact, unchallenged brain, leading to their processes converging or not toward the site of injury (Nimmerjahn et al., 2005; Paris et al., 2018). In addition, microglial cell bodies were recently shown to migrate in the cerebral cortex (Eyo et al., 2018) and cerebellum (Stowell et al., 2018) of healthy adult mice, which paints another layer of complexity. However, the existence of microglial subtypes, each endowed with intrinsic differences in gene expression, cannot be excluded and we argue that the topic deserves further investigation. Putative microglial subtypes are discussed below:

**Keratan sulfate proteoglycan (KSPG)-microglia**
A quarter of century ago, microglia were shown in the unchallenged adult rat brain to exhibit constitutive heterogeneity in their expression of KSPG (Bertolotto et al., 1993), visualized in situ using the 5D4 monoclonal antibody (Fig 3). KSPG is located in the extracellular matrix and on the cell surface. They are suggested to contribute to the control of cellular adhesion and axonal growth. In particular, 5D4-KSPG is expressed by a subpopulation of ramified microglia, contrary to ameboid microglia and peripheral macrophages (Bertolotto et al., 1993, 1998). Of note, 5D4-KSPG expression does not coincide with the expression of GFAP, NG2, or MAP2, which relate to other CNS cells. The expression of 5D4-KSPG in microglia differs significantly between strains of inbred rats (Jander & Stoll, 1996b). In mammals, a subpopulation of 5D4-KSPG-expressing microglia was also reported in the spinal cord and retina (Bertolotto et al., 1993, 1998; Jander & Stoll, 1996a; Jones & Tuszynski, 2002; Zhang et al., 2005; Foyez et al., 2015). The 5D4-KSPG-microglia exhibit a preferential regional distribution in the CNS. Indeed, whereas these cells are found in large numbers among the hippocampus, brainstem, and olfactory bulb (OB), only few of them are detected in the cerebellum and cerebral cortex (Bertolotto et al., 1993, 1998). This putative microglial subset is also observed in the neonatal rat brain (Bertolotto et al., 1998). It is of importance to mention that 5D4-KSPG-microglia were shown to co-exist with 5D4-KSPG-negative microglia in the same CNS regions (Jones & Tuszynski, 2002). Although these studies argue for the presence of two different subtypes, based on KSPG-reactivity, a systematic approach is required to confirm this possibility (Fig 4).

**HoxB8-microglia**
These microglial cells have a molecular signature that differentiates them from the canonical population, together with a unique spatial and temporal distribution (see Box 2 for distinct ontogeny of Hoxb8-microglia). Mice carrying the driver Hoxb8-Cre and the reporter ROSA26-YFP alleles were crossed to trace YFP-Hoxb8 expression. In the adult brain, the only cells showing YFP signal appeared to be microglia. YFP-positive microglia were found throughout the brain, especially in the cerebral cortex and OB (Chen et al., 2010; De et al., 2018; Fig 3). YFP-positive microglia, which represent 25–40% of the total microglial population in the adult brain, were also shown to co-exist with YFP-negative microglia (Chen et al., 2010; De et al., 2018; Nagarajan et al., 2018). Transcriptomic analyses comparing Hoxb8-positive and Hoxb8-negative microglia revealed that they are very similar at steady state, with only 21 genes differing significantly in expression between the two populations (De et al., 2018). Hoxb8-microglia express microglial signature genes, such as Trem119, Sall1, Sall3, Gpr56, and Ms4a7, and genes associated with hematopoietic ontogeny including Cle1l2a, Klra2, and Lilra5 at similar levels compared with non-Hoxb8 canonical microglia (Bennett et al., 2018; De et al., 2018). Of note, neither of the two putative microglial subtypes was found to expresses Hoxb8 in the adult brain; instead, the lineage tracer approach revealed that Hoxb8 is expressed by microglial progenitors prior to CNS infiltration (De et al., 2018). Selective inactivation of Hoxb8 in the hematopoietic system was also sufficient to induce pathological grooming behavior, as observed in constitutive Hoxb8 mutant mice (Chen et al., 2010; Nagarajan et al., 2018). The strategy for gene deletion included the use of Tie2 Cre mice that affect all hematopoietic cells and endothelial cells (Chen et al., 2010). More cell-specific deletion of Hoxb8 within microglial cells is a prerequisite to determine their selective involvement in pathological grooming behavior.

**CD11c-microglia**
Recently, evidence for an additional microglial subtype expressing the integrin CD11c was uncovered in neonatal mouse brain (Fig 3). CD11c-microglia expand during postnatal development to represent approximately one-fifth of the total microglial population at postnatal day (P)3-P5, and then drop to represent less than 3% of the population in juvenile and adult mice (Wlodarczyk et al., 2017). Whether this decrease in numbers is due to increased cell death, migration or even trans-differentiation of this putative microglial subset is yet to be determined. These microglia distribute unevenly across the developing brain, being found predominantly in primary myelinating regions, mainly the corpus callosum and cerebellar white matter. Gene ontology enrichment analysis revealed that CD11c-microglia express genes associated with neurogenic and myelino-genic processes in the neonatal brain. They are also a major source of insulin-like growth factor 1 (IGF1), while selective depletion of IGF1 in this microglial subtype leads to impaired developmental myelination (Wlodarczyk et al., 2017). Thus, CD11c-microglia in the neonatal mouse brain appear to play essential roles in neurogenesis and myelino-genesis during development.

**TREM2-microglia**
There is additional evidence that differential microglial gene expression patterns define several microglial populations in the healthy brain. For example, not all microglia express the cell surface receptor TREM2, which is known to impact on their survival, proliferation, clustering around amyloid-beta (Aβ) plaques in Alzheimer’s disease (AD) pathology, phagocytosis, and metabolism (Yeh et al., 2017). Furthermore, loss-of-function variants in the TREM2 protein increase the risk of developing late-onset AD among other forms of dementia (Colonna & Wang, 2016). However, despite the apparent essential function of TREM2 in microglia, its expression is far from being ubiquitous and homogenous in those cells throughout the brain. In the mouse brain, microglial expression of TREM2 also varies between and within individual regions (Schmid et al., 2002). The numbers of TREM2-expressing cells are highest in the cingulate cortex and lateral entorhinal cortex, and much lower in the hypothalamus and habenula, while some regions, such as the circumventricular organs, completely lack TREM2 expression. Worth notice, even in brain regions abundant in TREM2-expressing microglia, TREM2-positive and TREM2-negative cells were found in the immediate
Box 2 (with associated illustration): Revisiting the microglial origin(s)

An important question arising from the existence of microglial subtypes relates to their possible origin(s). Do microglial subtypes possess intrinsic differences prior to populating the CNS, or do they acquire their unique properties once they have assumed their regional distribution within the CNS parenchyma?

Current literature states convincingly that microglia derive from the first wave of hematopoiesis from the embryonic yolk sac in mouse (Ginhoux et al, 2010; Hoeffel et al, 2015; Perdiguerio et al, 2015; Sheng et al, 2015; Mass et al, 2016), where they follow a stepwise maturation program (Mass et al, 2016; Matcovitch-Natan et al, 2016), before populating the embryonic brain at E8.5 (Tay et al, 2017c). Based on the above literature, microglial subtypes should differentiate once they have assumed their regional distribution inside the CNS parenchyma (a). This hypothesis could explain microglial differences resulting from regional differences in microenvironments or from differences in local cues among the microenvironment such as microglial interactions with different neuronal subtypes (inhibitory, excitatory) and glial cells (astrocytes, oligodendrocytes and their progenitors), or slight differences in signaling thresholds, leading to the observed differences in adjacent microglia. The alternative hypothesis which is based on microglial cells exhibiting intrinsic differences prior to infiltrating the CNS cannot be excluded at this early stage of investigation, and should be tested (b and c). In support of the later hypothesis, Capecci et al reported that Hoxb8-microglia-progenitors already exist in the yolk sac at E8.5 (De et al, 2018). Subsequently, these cells transit through the aorta-gonad-mesonephros and fetal liver, where they expand in number, prior to their entry into the brain at E12.5 (De et al, 2018) (c). On the same lines, microglial cells found in CSF1R−/− (Ginhoux et al, 2010; Erblich et al, 2011) and in IL2−/Tgβ1;Tgβ2−/− (Keren-Shaul et al, 2017) transgenic mice are expected to exhibit intrinsic differences prior to infiltrating the brain parenchyma.

Recently, it has been reported that at E14.5 two microglial subpopulations exist, based on M4a7 expression (Hammond et al, 2018). It would be of great interest to investigate the ontogeny of these two subpopulations. In zebrafish, two waves of microglial infiltration have been reported (Xu et al, 2015; Ferrero et al, 2018). Microglia of a yolk-sac-equivalent structure origin initially populate the embryonic brain. Subsequently, the microglial population is replenished by adult microglia that derive from a distinct tissue later during zebrafish development (d). This microglial diversity could result from species-specific differences between zebrafish and mouse. Nevertheless, these studies indicate that evolutionarily multiple microglial origins and maturation programs are a possibility. Recently, it has been reported that at steady state a wave of monocytes infiltrate the mouse brain parenchyma at early postnatal stages; however, these cells were rapidly depleted and did not contribute to the later microglial population (Askew et al, 2017).

Microglia supporting neurogenesis

The use of CX3CR1-EGFP reporter mice, in which one of the loci of fractalkine receptor Cx3cr1 is replaced by the gene encoding EGFP, revealed heterogeneity of the microglial cell population within the neurogenic subventricular zone (SVZ), and the adjacent rostral migratory stream (RMS) that terminates into the OB (Ribeiro Xavier et al, 2015; Xavier et al, 2015, Fig 3). First, it was noticed that microglia located along the SVZ-RMS-OB axis are significantly less ramified than microglia from adjacent areas in mice and rats (Shigemoto-Mogami et al, 2014; Ribeiro Xavier et al, 2015; Xavier et al, 2015). CX3CR1-EGFP expressing microglia were TREM2-negative, and about half of them also IBA1-negative in the SVZ and RMS of wild-type adult mice. By contrast, CX3CR1-EGFP expressing microglia expressed TREM2 in the OB of wild-type adult mice. Further adding to this diversity, a subset of the latter population additionally expressed CD68 (~35%) and/or isoclectin B4 (~15%) in the OB (Ribeiro Xavier et al, 2015). Significantly increased CD68 expression in microglia was also reported in the OB of adult wild-type rats (Doorn et al, 2015), while a regional distribution of CD68-positive microglia was reported in the brain of human midterm fetuses (gestational ages of 15–25 weeks; Cho et al, 2013). As observed in the SVZ and RMS, IBA1-negative and IBA1-positive microglia were also observed in close vicinity to another within the OB. Of note, the antigenic heterogeneity of CX3CR1-EGFP expressing microglia was reported in later development among the SVZ of newborn (P1) and early postnatal (P7) mice, suggesting persistence of these microglial cell populations beyond ontogeny (Xavier et al, 2015).
2015). In addition, at those developmental ages, CD68 expression was detected in a microglial subset only. It would be of particular interest to determine whether the CD68-expressing microglia found in the OB of adult mice originate from the ones detected in the SVZ of newborn mice and whether the microglial heterogeneity described above translates into one or more microglial subtypes during adulthood and aging. A recent study has taken advantage of single-cell RNAseq analysis to uncover regional microglial heterogeneity across different brain regions (Li et al., 2019). Contrary to expected, the authors found a remarkable homogeneity of adult homeostatic microglia (enriched in homeostatic genes such as TEMEM119 or P2ry12) regardless of the brain tissue of origin. However, a microglial population expressing Clec7a, one of the most upregulated genes in DAM (Keren-Shaul et al., 2017; Krasemann et al., 2017), was shown by immunohistochemistry to be restricted to the subgranular zone of the hippocampal dentate gyrus, another neurogenic niche, in addition to the SVZ and RMS. This restrictive pattern of localization suggests the existence of a microglial subtype that exerts a key role in adult neurogenesis. These findings illustrate the usefulness of combining different methodological strategies to uncover novel microglial populations among specific CNS regions.

These selected examples of differential gene expression among small populations of microglia argue for the existence of distinct microglial subtypes. Of importance, in the majority of these cases, the putative microglial subtypes co-existed with canonical microglia within the same microenvironment. While extrinsic factors such as local cues in the microenvironment, as discussed above, could determine this variability, microglia also appear to have intrinsic differences that warrant further investigation.

**Single-cell RNAseq data**

Recently, there has been an unprecedented influx of data from single-cell RNAseq studies in support of a significant microglial heterogeneity. Although at this point these pieces of evidence are descriptive and we suggest that they have to be coupled with functional studies to categorize the identified clusters as different microglial subtypes, it is striking to report such a plethora of different potential microglial subtypes. In particular, Stevens and colleagues performed deep single microglial cell RNAseq at different developmental stages and uncovered eight transcriptionally distinct microglial clusters co-existing in the naïve mouse brain (Hammond et al., 2018). Among them, “cluster 2” is characterized by a high expression of Ms4a family members, some of which are involved in immune cell functions (Eon Kuek et al., 2016), and partially overlaps with brain border macrophage markers (Hammond et al., 2018). The number of cells belonging to “cluster 2” decreases drastically during postnatal development. “Cluster 3” is almost exclusively found in the embryonic and early postnatal brain. It is characterized by its unique expression of Fabp5, while “cluster 6” highly expresses genes that include Cd74, Ccl24, and Arg1 and is enriched in female samples (Hammond et al., 2018). “Cluster 4” [named axonal tract-associated microglia (ATM) by the authors] has a very specific spatiotemporal expression. Microglia belonging to this cluster highly express Spp1, Gpnmmb, Lgf1, CD68, and Lgals3, and they have an ameboid morphology. Furthermore, their number is significantly enriched in the neonatal brain and they are preferentially localized in the white matter, including the corpus callosum, and cerebellum (Hammond et al., 2018).

**Proliferative-region-associated microglia (PAM)**

Similarly, Barres and colleagues performed deep single microglial cell RNAseq at different developmental stages including late embryonic, early postnatal (P7) and adult ones across different brain regions (Li et al., 2019). Three well-defined microglial clusters irrespective of cell cycle states were identified during postnatal development (Li et al., 2019) and initially named P7-C0, P7-C1, and P7-C2. The P7-C0 and P7-C1 microglial clusters both expressed homeostatic genes although at lower levels for P7-C1. This specific cluster expressed many genes recently identified in DAM including Igf1, Spp1, Gpnmmb, CD11c (also known as Itgax), and Clec7a (Keren-Shaul et al., 2017; Krasemann et al., 2017). Further analysis identified these cells to be predominantly located in the corpus callosum and cerebellar white matter, where they intermingled with Mbp” oligodendrocytes; hence, they were named the proliferative-region-associated microglia (PAM). These PAM are highly reminiscent of the CD11c-microglia described by Wlodarczyk et al. (2017) (Wlodarczyk et al., 2017) and the ATM cluster identified by Hammond et al. (2018). Indeed, PAM populate the white matter at P4, peak at P7, and almost disappear by P14. At the morphological level, PAM are ameboid with thicker primary branches and larger cell bodies, as compared with the typical microglia. They are also highly phagocytic, and contrary to the DAM, they do not depend on either triggering receptor expressed on myeloid cells 2 (TREM2) or ApoE (Keren-Shaul et al., 2017; Krasemann et al., 2017). Although it is clear that postnatal PAM may represent a specialized microglial subtype involved in the elimination of oligodendrocytes during myelination, the exact timing and appearance of this microglial population in the white matter during periods of myelination indicates that the environmental needs may be behind the polarization of homeostatic microglia toward PAM.

In a third study, Prinz and colleagues (Masuda et al., 2019) analyzed single microglial cells derived from brain areas that were previously shown to exhibit regional transcriptional differences (Grabert et al., 2016), namely cortex, cerebellum, and hippocampus, and additionally analyzed microglia from corpus callosum. Microglia from juvenile (3 weeks old) and adult (16 weeks old) animals were grouped into at least four distinct clusters which showed a variable distribution between brain regions and developmental stage (Masuda et al., 2019). Importantly, this study also identified multiple different microglial clusters in human tissue, from cortex that bears no signs of CNS pathology (Masuda et al., 2019), therefore indicating that microglial heterogeneity is also relevant to human (see also later).

**Microglial subtypes as defined by differential structural/ultrastructural properties**

Returning to the satellite microglia described by Río-Hortega (1919b), this subpopulation is currently identified based on its unique feature of having its soma being associated with neuronal cell bodies (Baalman et al., 2015; Wogram et al., 2016; Fig 3). Half of the satellite microglia extend a single process that overlaps with the portion of the axon where potentials are initiated (Baalman et al., 2015). They have been identified both during development and at adulthood in mice, while they have a preferential association with excitatory neurons (Baalman et al., 2015). In addition, this subpopulation was reported in the cerebral cortex of adult rats and adult non-human primates (Río-Hortega, 1919b; Baalman et al., 2015), hence indicating conservation across species. Whether
satellite microglia represent a transitional state of “surveilling” microglia or bear intrinsic differences compared to their neighboring microglia is yet unknown, as no specific "satellite" microglial marker has been identified and transcriptomic analysis of this microglial subpopulation was not performed. Yet, they are preferentially located in the cerebral cortex (~8% of cortical microglia) as well as hippocampus, and to a lesser degree in the thalamus and striatum (Baalman et al., 2015; Wogram et al., 2016), suggesting that they perform a specialized function. Interestingly, in the adult mouse cerebellum, a dynamically unique population of “satellite” microglia was recently described using non-invasive two-photon in vivo imaging (Stowell et al., 2018). These cells display migration of their soma, which interact with both Purkinje neuronal cell bodies and proximal dendrites. These cerebellar microglia are also less ramified than their cortical counterparts and show reduced surveillance. This finding indicates that satellite microglia are heterogeneous in their dynamics under steady-state conditions.

In addition to the distinct microglial morphological properties that were revealed by light or fluorescence microscopy, the use of high spatial-resolution electron microscopy (EM) uncovered alongside the typical microglia the occurrence of microglia filled by cellular debris, akin to the fat granule or gitter cells initially described by Río-Hortega (1920), during aging, age-related loss of sensory function, and Werner syndrome in mice (Tremblay et al., 2012; Hui et al., 2018b). Recently, EM also allowed to identify an ultrastructurally distinct microglial population, the “dark” microglia, in the adult and aged mouse hippocampus (CA1 region and dentate gyrus), cerebral cortex, amygdala, and hypothalamus. These cells co-existing with the canonical population display several ultrastructural features of microglia, particularly their size, morphology, long stretches of endoplasmic reticulum, interactions with neurons and synapses, and association with the extracellular space. However, contrary to the typical microglia, they also display markers of oxidative stress like a condensed, electron-dense cytoplasm and nucleoplasm, giving them a dark appearance in EM, accompanied by Golgi apparatus/endoplasmic reticulum dilation, mitochondrial alteration, and a partial to complete loss of heterochromatin pattern (Bisht et al., 2016; Hui et al., 2018a). In neurons, alterations to the heterochromatin pattern are linked to cellular stress, aging, and brain disorders such as schizophrenia and AD (Medrano-Fernández & Barco, 2016).

The physiological significance of these dark microglia has yet to be elucidated, but they appear to be extremely active. The hundreds of dark microglia examined with EM contacted several synaptic regions, but not in the cerebral cortex. Thus, microglia respond differentially to one stimulus/pathological changes.

Microglial response to stimuli/pathological changes

Microglial subtypes can respond differently to one stimulus

Different subpopulations, and potentially subtypes, of microglia in the CNS, have been reported to respond differently to an identical stimulus, or to be differently affected by injuries or diseases affecting the CNS. As an illustration, LPS stimulation results in the induction of TNF-α or inducible nitric oxide (NO) synthase (NOS2) expression only in a subpopulation of microglia ex vivo and in vivo (Schefel et al., 2012; Kiyofuji et al., 2015). Besides, only a subset of cultured primary rat microglia survive to an LPS challenge, an effect that is related to the self-production of granulocyte-macrophage colony-stimulating factor (GM-CSF) and upregulation of its receptor GM-CSFR (Kamigaki et al., 2016). Recently, Furube et al. (2018) further reported that the administration of LPS in the circumventricular organs affects microglial proliferation locally and in neighboring regions, but not in the cerebral cortex. Thus, microglia respond unequally to an LPS challenge.

In vivo experiments in rodents have shown that various peripheral inflammatory stimuli can affect microglial morphology, gene expression, and/or function in the brain (Hoogland et al., 2015). Hence, it is reasonable to speculate that microglial heterogeneity could also be influenced by the periphery. Neher and coworkers recently demonstrated that peripherally applied inflammatory stimuli which induce either acute immune training (e.g., single intraperitoneal injection of LPS) or tolerance (e.g., daily injections of low-dose LPS on 4 consecutive days) in the brain lead to differential epigenetic reprogramming, associated with unique transcriptome profiles of microglia that persist for at least half a year (Wendeln et al., 2018). Epigenetic changes, including histone modifications or DNA methylation as well as microRNA expression, are important modifiers of gene expression, and have been involved in cell phenotype regulation and reprogramming and are therefore part of the mechanisms regulating cellular plasticity including microglia (Cheray & Joseph, 2018). These two types of immunological...
imprinting, i.e., training and tolerance, were shown to impact on the microglial responses to subsequent disease-associated stimuli, such as β-amyloidosis or ischemia (Wendeln et al., 2018). Worth a note, the authors noted that in that context the global epigenetic and transcriptional changes were relatively modest, and proposed that they might originate from a small number of microglia. Whether this restricted responsive microglial population reflects a limited number of microglial cells that received the required secondary stimulus or the response of microglial subtype(s) showing a different response threshold remains to be explored.

**Microglial subtypes can respond differently to signal withdrawal and depletion attempts**

The class III transmembrane receptor tyrosine kinase, CSF1R, is a key regulator of myeloid cell proliferation, survival, and functions (Dai et al., 2002; Ginhoux et al., 2010; Erblich et al., 2011; Elmore et al., 2014). CSF1R can be activated by ligation of colony-stimulating factor 1 (CSF1) and interleukin (IL)-34. Despite the fact that CSF1 and IL-34 share affinity for the same receptor, their biological functions do not strictly overlap, as revealed by their distinct spatiotemporal brain expression patterns, the different impact of their gene deletion, and the cellular responses observed upon their binding to CSF1R (Chihara et al., 2010; Nandi et al., 2012; Wang et al., 2012).

In the brain, although CSF1R is primarily expressed by microglia, a subset of hippocampal and cortical neurons were reported to also express this receptor (Wang et al., 1999; Luo et al., 2013). Homozygous null mutation of the CSF1R gene in mice results in a reproducible and robust, yet not complete, loss of myeloid cells defined by their expression of CD45/CD11b/F4/80 markers (Ginhoux et al., 2010), and IBA1- and F4/80-double-positive cells displaying an ameboid morphology (Erblich et al., 2011). Similarly, recent post-mortem human brain examination of a 10-month-old individual carrying a homozygous missense mutation in the CSF1R gene revealed a dramatic reduction in IBA1-positive cells in the brain. Nonetheless, there was a residual IBA1-positive cell population with an ameboid morphology that was located mainly around blood vessels (Oosterhof et al., 2019). Whether this residual population is conclusively microglia requires further investigation.

CSF1R inhibitors (e.g., PLX5622 and AFS98) have been used to deplete microglia (Han et al., 2017). These inhibitors have proved very efficient at robustly reducing microglial numbers in adult mice; however, upon treatment cessation microglia rapidly repopulate the brain parenchyma. Although the mechanisms underlying this repopulation are still under investigation, it was recently shown that residual microglia are the only source of repopulation upon PLX5622 treatment (Huang et al., 2018b). Similarly, administration of AFS98 at E8.5 and E7.5 (when yolk-sac erythro-myeloid progenitors are generated) is sufficient to deplete macrophages in the E10.5 yolk sac, as well as microglia at E10.5 and E14.5. The depletion is transient with microglia repopulating the brain parenchyma partially around E17.5 (Hoeftel et al., 2015), and fully during the first postnatal weeks (Squarzoni et al., 2014). The above findings argue for a subset of microglia that might not fully depend on CSF1R stimulation for its maintenance. As further illustration, a recent report indicates that upon microglial depletion with PLX5622, the central cornea is repopulated by a subpopulation of microglia which resides in the optic nerve (Huang et al., 2018a).

In addition to CSF1R signaling, TGFβ1 has been identified as a major differentiation and survival factor for the microglial cell population. In particular, IL2-Tgfβ1/Tgfβ1−/− transgenic mice, lacking TGFβ1 expression in the CNS, while retaining its expression in T lymphocytes, show a robust loss of microglia, although a microglial subpopulation exhibiting an ameboid morphology, contrary to microglia in age-matched wild-type mice, persisted upon this CNS TGFβ1 deficiency (Butovsky et al., 2014).

**Microglial heterogeneity in disease conditions**

The 5D4-KSPG-microglial population described above is influenced by pathology. In particular, 5D4-KSPG expression is increased selectively in a subset of IBA1/CD11b-positive microglia in the SOD1G93A mouse model and human cases of amyotrophic lateral sclerosis (ALS; Hirano et al., 2013; Foyez et al., 2015), and in a Wallerian degeneration mouse model of spinal cord injury (Shinjo et al., 2014), while it decreases in a Guillain–Barré syndrome rat model of experimental autoimmune neuritis (Matsui et al., 2013). Mice deficient in N-acetylgalactosamine 6-O-sulfotransferase-1 (GlCNAc6ST-1), an enzyme involved in 5D4-KSPG biosynthesis, also show reduced 5D4-KSPG expression in the CNS (Zhang et al., 2006). Remarkably, SOD1G93A/GlCNAc6ST1−/− mice exhibit a significantly reduced lifespan and accelerated clinical symptoms as compared to SOD1G93A mice (Hirano et al., 2013; Foyez et al., 2015), suggesting that 5D4-KSPG-microglia which expand in SOD1G93A mice may play a beneficial suppressive role in the early pathogenic phases of ALS (Hirano et al., 2013).

In addition, the dark microglia which are rarely observed in healthy young adult mice became highly prevalent upon chronic stress, aging, fractalkine signaling deficiency (in CX3CR1 knockout mice), and AD pathology (APP/PS1 model). Signaling between the neuronal fractalkine (CX3CL1) and its unique receptor, CX3CR1, expressed by microglia, is a main mode of neuron–microglia communication in the brain (Paolicelli et al., 2014; Arnoux & Audinat, 2015). The number of dark microglia in the hippocampus quadruples in young adult mice after 2 weeks of chronic unpredictable stress as compared to counterparts housed under control conditions. In the CX3CR1 knockout exposed to stress, the dark microglial population reached approximately half of the typical microglial population. These cells also became more prevalent during normal aging, at 14 months which corresponds to middle age in mice, where their density tripled compared with young adulthood (3 months of age). In age-matched APP/PS1 littermates, the number of dark microglia corresponded to almost two-thirds of the typical microglial population (Bisht et al., 2016).

In the APP/PS1 mice, the dark microglia associated with Aβ or neuronal dystrophy were TREM2 positive. These dark microglia frequently contained fibrillary Aβ and encircled synaptic elements displaying signs of dystrophy, including an accumulation of autophagic vacuoles. In adult offspring exposed to maternal immune activation and displaying schizophrenia-like behavior, dark microglial numbers tripled those in 14-month-old APP/PS1 mice (Hui et al., 2018a). Interestingly, both the schizophrenia-like behavior and prevalence of dark microglia were exacerbated in males mice (Hui et al., 2018a), suggesting sexual dimorphism in these cells. These overall findings suggest that dark microglia could represent a microglial subtype that exerts specialized functions across development, stress-induced plasticity, and disease. The physiological roles and molecular determinants of these microglial cells warrant further investigation. Fate-mapping strategies will be required to determine, using non-invasive longitudinal two-photon in vivo imaging,
whether typical microglia can transform into dark microglia under adaptive pressure, or whether the typical and dark microglia represent distinct subsets already from the developmental stages.

Plaque-associated microglia illustrate how a specific disease-associated microenvironment can result in the emergence of different microglial phenotypes (or subtypes). In addition to the dark microglia, an age-dependent increase in CD11c-labeled microglia is associated with Aβ plaques burden in different models of AD, including CVN-AD (Kan et al., 2015) and APP/PS1 (Kamphuis et al., 2016) mice. A characteristic feature of these microglial “types” is their high expression levels of Igf1 (Kan et al., 2015; Kamphuis et al., 2016), which is reminiscent of the recently defined CD11c-microglia involved in myelination and neurogenesis during development (Wlodarczyk et al., 2017), the ATM (Hammond et al., 2018), and PAM (Li et al., 2019). These observations suggest the existence of distinct microglial subtypes that emerge to exert specialized physiological functions during specific stages of development and that can also be recruited during pathological conditions when there is a need for such functions. Given the role that CD11c-microglia play in myelination in the healthy brain, their presence in demyelinating diseases was expected. In different studies, indeed, the occurrence of a similar CD11c-microglial population was demonstrated in mouse models of demyelination, such as cuprizone (Remington et al., 2007; Wlodarczyk et al., 2015), experimental autoimmune encephalomyelitis (EAE; Wlodarczyk et al., 2014, 2015), and neumyelitis optica-like pathology (Wlodarczyk et al., 2015). It is important to note that there is no definite proof that disease-associated CD11c-microglia arise from the population of CD11c-positive microglia found in the neonatal mouse brain. When technically possible, performing lineage tracing of the initial CD11c-microglial subtype into adulthood, at steady state and upon demyelination, would be very informative.

However, some clues about the origin of these cells may come from recent investigations that identified a microglial signal regulatory protein α (SIRPα)-dependent signaling pathway that controls the expansion of CD11c-microglia in adult mice (Sato-Hashimoto et al., 2019). Genetic ablation of this membrane protein SIRPα (including selectively in microglia) or of CD47, a physiological ligand of SIRPα, results in the emergence of CD11c-microglia in the white matter of adult mice, thus suggesting that the CD47-SIRPα signaling pathway normally represses the activation of CD11c-microglia. A small resident CD11c-microglial population was also reported in the normal adult mouse brain (Bulloch et al., 2008; Sato-Hashimoto et al., 2019). However, genetic ablation of SIRPs in this small subset of resident CD11c-microglia only, which was obtained by crossing SIRPα-flox mice and CD11c-Cre mice, does not promote the expansion of the CD11c-microglial population in adult mice. These data suggest that the increased number of CD11c-microglia, at least in mice deficient for the CD47–SIRPα-signaling pathway, is not due to the expansion of the resident CD11c-microglial subset, but instead likely results from the acquisition of CD11c expression in microglia that were initially negative for it. Nevertheless, it is important to notice that multiple signaling pathways can control the expansion or emergence of CD11c-microglia. Indeed, in the CNS, CSF1R stimulation by its ligands, i.e., IL-34 or CSF1, coincides with the expansion of CD11c-microglial population. Treatments with these CSF1R ligands also appear to reduce both demyelination and symptoms in EAE mouse model (Wlodarczyk et al., 2018). However, as it was performed for the CD47–SIRPα-signaling pathways, it remains to be explored whether this CSF1R-dependent signaling pathway could impact on the expansion of the resident CD11c-microglial population observed at adulthood.

Developing and demyelination-associated CD11c-microglia share transcriptomic similarity, but they are far from being identical (Kan et al., 2015; Kamphuis et al., 2016; Keren-Shaul et al., 2017; Kremmer et al., 2017; Wlodarczyk et al., 2017; Hammond et al., 2018; Li et al., 2019). Whether these transcriptomic differences define two distinct populations of CD11c-microglia, or differences due to the stage of the lifespan and the particular context of health or injury, for a unique CD11c-microglial subtype, remains unanswered. Furthermore, recently Stevens and colleagues identified and reported the expansion of a Ccl4-microglial subset upon aging and injury (Hammond et al., 2018).

Recent studies have exploited massive transcriptomic analyses to uncover microglial reaction states associated with homeostatic (Butovsky et al., 2014; Bennett et al., 2016), aging (Hickman et al., 2013; Grabert et al., 2016; Galatro et al., 2017), or disease-associated (Orte et al., 2014; Keren-Shaul et al., 2017; Kremmer et al., 2017; Mathys et al., 2017) conditions. Transcriptional profile studies of microglia isolated from models of aging and different neurodegenerative diseases demonstrated a strikingly similar transcriptional network across these conditions. For instance, two recent studies performing single-cell RNaseq of microglia (isolated as discussed below) in mouse aging and models of AD, multiple sclerosis, and ALS, defined a distinctive microglial molecular signature of disease defined as (DAM; Keren-Shaul et al., 2017) or “microglia neurodegenerative phenotype” (MgND; Kremmer et al., 2017), suggested to be driven by TREM2. This apparent simplicity in the switch from homeostatic microglia to a common disease-associated phenotype, even in response to varied conditions of altered microenvironment, suggests that the disease-associated phenotype program may represent a priming response against more specific challenges. Using mass cytometry which allows to investigate changes in microglial proteome, at the single-cell level, a similar “disease-associated” microglial phenotype was recently described in aging and AD pathology (APP/PS1 mice; Mrdjen et al., 2018). These cells displayed increased expression of CD14, a co-receptor of the Toll-like receptor 4, that is activated by Aβ and pro-inflammatory cytokines such as TNF-α (Mrdjen et al., 2018).

While the disease-associated program is inherently linked to AD pathology (derived from the use of 5xFAD or APP/PS1 transgenic mice), a recent report by Mathys et al. (2017) also revealed that this microglial molecular signature may proceed in a heterogeneous fashion. CK-p25 mice rapidly develop AD-associated features ranging from neuronal DNA damage and Aβ accumulation (2 weeks after p25 induction) to progressive neuronal and synaptic loss, as well as cognitive impairment (already severe 6 weeks after p25 induction). The short time window allowed to identify microglial populations associated with early-stage and late-stage responses. Analysis of the transcriptional dynamics of late response microglia identified small subsets of single cells upregulating specific gene sets, including antiviral and interferon response genes and components of the MHC class II pathway, which displayed extensive variability (Mathys et al., 2017). The authors concluded on the existence of two distinct disease-associated microglial subpopulations. Since the DAM program, described by Keren-Shaul et al. (2017), was not associated with changes in antiviral and interferon response genes, different disease-associated microglial “subtypes” might be behind these different findings. However, CK-p25 mouse is an AD model of severe neurodegeneration...
in contrast to the 5xFAD and APP/PS1 models originally used for the identification of DAM (Keren-Shaul et al., 2017; Krasmann et al., 2017; Mathys et al., 2017). As a model of severe neurodegeneration, the CK-p25 mouse exhibits important cell-associated DNA damage, a well-known inducer of type I interferons (Hårtlová et al., 2015), which may underlie the specific changes in antiviral and interferon response seen by Mathys et al. (2017).

Further evidence was recently provided by Tay et al. (2018) performing single-cell RNAseq analysis of microglia following facial nerve axotomy at different temporal stages of the disease (i.e., peak and recovery) in adult mice. The authors identified a unique TREM2-independent microglial subtype at the onset of recovery, which upregulated ApoE and Ccl5, in addition to showing ameboid and anucleated morphologies. Additionally, mass cytometry was used to investigate the changes in signaling and cytokine molecular signatures of microglia in three disease contexts: Huntington’s disease (HD), EAE, and ALS (Ajami et al., 2018). Although defined as relatively homogeneous by common cell surface markers, microglia in fact contain heterogeneous functional subsets based on their cytokine secretion profile. In particular, two distinct cytokine-secreting microglial subsets that represent the signature of neuroinflammatory conditions, compared with neurodegeneration models, were identified. Although both neuroinflammatory and neurodegenerative models developed double-positive TNF-α- and GM-CSF-producing cells, this subset abundance correlated best with the height of neuroinflammatory conditions in EAE—peak and onset, whereas it was extremely rare or absent in both models of HD and ALS. Conversely, both neurodegenerative conditions showed a unique subset of cells expressing IL-10, often considered an anti-inflammatory cytokine, across all three CNS-resident myeloid populations (i.e., microglia, meningeal macrophages, and perivascular macrophages) before late-stage disease symptoms (Ajami et al., 2018). Using single-cell RNAseq of CD45-positive cells, Prinz and colleagues have recently demonstrated an apparent microglia and CAM diversity under different disease conditions including EAE, cuprizone-induced demyelination, and unilateral facial nerve axotomy (Jordão et al., 2019; Masuda et al., 2019). Especially relevant was the microglial heterogeneity associated with multiple sclerosis, a view that was confirmed in both experimental mice and humans (Jordão et al., 2019; Masuda et al., 2019). Thus, three different microglial subsets linked with the EAE-associated lesion sites were identified in mice and termed daMG2, daMG3, and daMG4 (Jordão et al., 2019). A common feature in the three subtypes differing in the expression of specific chemokines, cytokines, and cytochrome proteases was their downregulation of the homeostatic markers P2RY12 and TMEM119. In particular, daMG2 showed strong upregulation of CD74, Ctsb, and Apoe, whereas daMG3 showed high levels of Cxcl10, Tnf, and Ccl4 and daMG4 of Ccl5, Cts, and Itm2b (Jordão et al., 2019). Remarkably, the Prinz team performed scRNAseq from brains of patients with multiple sclerosis and analyzed together with healthy human brains (Masuda et al., 2019). This study identified three different clusters associated with homeostatic microglia highly expressing TMEM119 and P2RY12. Three microglial clusters were associated with multiple sclerosis (Hu-C2, Hu-C3, and Hu-C8), thus mimicking and validating the data obtained using the mouse models. Common features of the three clusters were increased expression of Apoe and MAFB along with downregulation or even absence of microglial core genes (Jordão et al., 2019). These studies support the challenging view that different microglial subtypes may change their phenotypes to generate context- and time-dependent subtypes (as depicted in Fig 2).

A successful isolation of microglia appears critical to identify microglial subtypes. Recent studies aimed at characterizing the molecular signature of microglia at the single-cell levels under disease conditions using FACS, with different sorting strategies based on either CD45 or CD11c (Keren-Shaul et al., 2017), FCRLS/CD11b or FCRLS/Clec7a (Krasemann et al., 2017) or CD11b/CD45 (Mathys et al., 2017; Li et al., 2019) or CD45, CD11b, and Cx3cr1 (Hammond et al., 2018) or CD45, CD11b, Ly6C, and CD206 (Masuda et al., 2019). However, positive selection based on “pan-microglial” markers accepts that such markers exist. Of note, the widely used “pan-neuronal” marker NEUN and the “pan-astrocytic” marker GFAP are not expressed in all neurons (Gusel’nikova & Korzhnevskiy, 2015) and astrocytes (Walz & Lang, 1998), respectively. Recent studies have successfully isolated microglia by non-invasive methods such as RiboTag, single nuclei sequencing, and TRAP (Ayata et al., 2018; Haimon et al., 2018). These methods preclude non-specific microglial reactivity, associated notably with the induction of immediate early and pro-inflammatory genes, during FACS-based whole single-cell isolation (Ayata et al., 2018; Haimon et al., 2018; Li et al., 2019). The RiboTag and TRAP approaches require the usage of mice that express Cre recombinase under the control of a microglia-specific gene promoter. Thus, Cx3cr1Cre has been shown to target neurons and tamoxifen-based Cx3cr1CreER mice target not only microglia, but also non-parenchymal macrophages (Goldmann et al., 2016). This limitation is especially relevant under disease conditions associated with blood–brain barrier disruption. However, whole-cell transcriptomes are usually contaminated by artifacts inherent to tissue dissociation, by phagocytosed material, and by transcripts sequenced away from ribosomes. These limitations of FACS-based cell strategy are, on the other hand, a strength when performing RiboTag, single nuclei sequencing, or TRAP. Consequently, each technology has its strengths and weaknesses that should be carefully evaluated when facing the experimental design. Each of them along with complementary technology like mass cytometry, successfully employed for identification of signaling and cytokine molecular signatures of microglia under disease conditions (Ajami et al., 2018), will help in the better understanding of the microglial diversity.

We conclude this section with the importance of unraveling the functional roles exerted by microglia in diseases and aging, and to develop for this purpose efficient microglial isolation procedures, as required for cutting-edge single-cell RNAseq and mass cytometry. At present, there is even uncertainty as to whether the roles of microglia are protective or deleterious, which may depend on subtype, and vary along CNS regions, stages of the lifespan, and contexts of disease. Interestingly, single-cell RNAseq has been shown, depending on the analysis depth, to detect only abundant transcripts (Petersson et al., 2017). Several transcripts and proteins of the DAM did not overlap (Keren-Shaul et al., 2017; Mrdjen et al., 2018), while a marked dissociation of mRNA and protein networks was described in microglia exposed to LPS challenge (Boutej et al., 2017). It should be noted also, on a positive note, that mass cytometry data could be useful for instructing isolation procedures, through the identification of cell surface markers that can be used (Mrdjen et al., 2018). Complementary techniques that should be considered for investigating microglial subtypes, in order to reduce the influence of microglial isolation procedures on their phenotypic transformation, are
RiboTag, TRAP, and single nuclei sequencing. Here, changes in translating ribosomes—thus preventing artifacts introduced by tissue dissociation, cargo contamination, and transcripts sequenced from ribosomes (Haimon et al., 2018)—and nuclei—which does not require tissue dissociation and can be performed on fresh frozen or lightly fixed samples (Habit et al., 2017)—are selectively investigated among microglia. Multiple approaches are indeed needed to reveal changes in gene and protein expression, and ultimately function, to solve this exciting mystery.

Summary and conclusion

The existing literature provides compelling evidence that several microglial subtypes could co-exist within the CNS. Microglial heterogeneity in terms of regional differences directed by the microenvironment has been proposed before (McCluskey & Lampson, 2000; Olah et al., 2011; Hansich, 2013; Gertig & Hansich, 2014), but was overshadowed by a more restrictive one-microglial population view. Here, we propose the existence of microglial subtypes that bear intrinsic differences and we suggest methodological tools that could lead to the identification, classification, and study of putative microglial subtypes. Our recommendations for the investigation of microglial subtypes are presented in Fig 4.

In summary, we should aim at playing with the full deck of microglial subtypes’ cards (Fig 3), which will tremendously help us in our understanding of microglial biological functions and provide us with the tricks required to selectively act on selected subtype(s), and thereby obtain the desired effect from manipulating selected card(s) and not the whole deck. Considering that different microglial cells can exert various functions under normal physiological conditions, depending on their regional distribution, gene, and protein expression, a better understanding of microglial diversity (made of phenotypes and perhaps subtypes also) is required for the future development of better-targeted therapies for a variety of disease conditions in which these cells were shown to be involved.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM (2011) Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. Nat Neurosci 14: 1142 – 1149

Ajami B, Samusik N, Wieghofer P, Ho PP, Crotti A, Bjornzon Z, Prinz M, Fanti WJ, Nolan GP, Steinman L (2018) Single-cell mass cytometry reveals distinct populations of brain myeloid cells in mouse neuroinflammation and neurodegeneration models. Nat Neurosci 21: 541 – 551

Alliot F, Godin I, Pessac B (1999) Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. Brain Res Dev Brain Res 117: 145 – 152

Arnoux I, Audinat E (2015) Fractalkine signaling and microglia functions in the developing brain. Neural Plast 2015: 689404

Askew K, Li K, Olmos-Alonso A, Garcia-Moreno F, Liang Y, Richardson P, Tipton T, Chapman MA, Riecken K, Beccari S et al (2017) Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. Cell Rep 18: 391 – 405

Ayata P, Badimon A, Strasburger HJ, Duff MK, Montgomery SE, Loh YE, Ebert A, Pimenova AA, Ramirez BR, Chan AT et al (2018) Epigenetic regulation of brain region-specific microglia clearance activity. Nat Neurosci 21: 1049 – 1060

Baalman K, Marin MA, Ho TS, Godoy M, Cherian L, Robertson C, Raszban MN (2015) Axon initial segment-associated microglia. J Neurosci 35: 2283 – 2292

Bennett ML, Bennett FC, Liddelow SA, Ajami B, Zamanian JL, Fernhoff NB, Mulinyawe SB, Bohlen CJ, Adil A, Tucker A et al (2016) New tools for studying microglia in the mouse and human CNS. Proc Natl Acad Sci USA 113: E1738 – E1746

Bennett FC, Bennett JL, Yaqoob F, Mulinyawe SB, Grant GA, Hayden Gephart M, Plowey ED, Barres BA (2018) A combination of ontology and CNS environment establishes microglial identity. Neuron 98: 1170 – 1183 e8

Bertolotto A, Caterson B, Canavese G, Migheli A, Schiffer D (1993) Monoclonal antibodies to keratan sulfate immunolocalize ramified microglia in paraffin and cryostat sections of rat brain. J Histochem Cytochem 41: 481 – 487

Bertolotto A, Agresti C, Castello A, Manzardo E, Riccio A (1998) SD4 keratan sulfate epitope identifies a subset of ramified microglia in normal central nervous system parenchyma. J Neuroimmunol 85: 69 – 77

Bisht K, Picard K, Vernoux N, Sharma K, Grinberg Y, Faustino J, Carson M, Vexler Z, Tremblay M. Dark microglia: A follow-up study across the lifespan. Program No. 295.07/NS. 2017 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2017. Online

Bisht K, Sharma KP, Lecours C, Gabriela Sánchez M, El Haj J, Millior G, Olmos-Alonso A, Gómez-Nicola D, Luheni G, Vallières L et al (2016) Dark microglia: a new phenotype predominantly associated with pathological states. Cilia 64: 826 – 839

Boutej H, Rahimian R, Thammissetty SS, Bélancourt MA, Lalancette-Hébert M, Kri J (2017) Diverging mRNA and protein networks in activated microglia reveal SRSF3 suppresses translation of highly upregulated innate immune transcripts. Cell Rep 21: 3220 – 3233

Bulloch K, Miller MM, Gal-Toth J, Milner TA, Gottfried-Blackmore A, Waters EM, Kaunzner UW, Liu K, Lindquist R, Nussenzweig MC et al (2008) CD11c/EYFP transgene illuminates a discrete network of dendritic cells within the embryonic, neonatal, adult, and injured mouse brain. J Comp Neurol 508: 687 – 710

Butovsky O, Jedrychowski MP, Moore CS, Cialric R, Lancer AJ, Gabriely G, Koeglspberger T, Dake B, Wu PM, Doykan CE et al (2014) Identification of a unique TGF-β-dependent molecular and functional signature in microglia. Nat Neurosci 17: 131 – 143

Butovsky O, Jedrychowski MP, Cialric R, Krasemann S, Murugaiyan G, Fanek Z, Greco DJ, Wu PM, Doykan CE, Kiner O et al (2015) Targeting miR-155
restores abnormal microglia and attenuates disease in SOD1 mice. Ann Neur 77: 75–99
Cartier N, Lewis CA, Zhang R, Rossi FM (2014) The role of microglia in human disease: therapeutic tool or target? Acta Neuropathol 128: 363–380
Chen SK, Tvdrik P, Peden E, Cho S, Wu S, Spangrude G, Capecki MR (2010) Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. Cell 141: 775–785
Chery M, Joseph B (2018) Epigenetics control microglia plasticity. Front Cell Neurosci 12: 243
Chiara T, Suzu S, Hassan R, Chutiswitoonchai N, Hiyoshi M, Motoyoshi K, Kimura F, Okada S (2010) IL-34 and M-CSF share the receptor Fms but are not identical in biological activity and signal activation. Cell Death Differ 17: 1917–1927
Cho KH, Cheong JS, Kim JH, Abe H, Murakami G, Cho BH (2013) Site-specific distribution of CD68-positive microglial cells in the brains of human midterm fetuses: a topographical relationship with growing axons. Biomed Res Int 2013: 762303
Cleverson H, Rafaelski S, Elowitz M, Klein A, Shendure J, Trapnell C, Lein E, Lundberg E, Uhlen M, Martinez-Arias A et al. (2017) What is your conceptual definition of “cell type” in the context of a mature organism? Cell Syst 4: 255–259
Colonna M, Wang Y (2016) TREM2 variants: new keys to decipher Alzheimer disease pathogenesis. Nat Rev Neurosci 17: 201–207
Crain JM, Nikodemova M, Watters JJ (2013) Microglia express distinct M1 and M2 phenotypic markers in the postnatal and adult central nervous system in male and female mice. J Neurosci Res 91: 1143–1151
Dai XM, Ryan GR, Hapel AJ, Dominguez MG, Russell RG, Kapp S, Sylvestre V, Stanley ER (2002) Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reparative defects. Blood 99: 111–120
De Biase LM, Schuebel KE, Fusfeld ZH, Jair K, Hawes IA, Cimbro R, Zhang HY (2017) Microglial turnover with aging and in an Alzheimer’s model via long-term in vivo single-cell imaging. Nat Neurosci 20: 1371–1376
Furube E, Kawai S, Inagaki H, Takagi S, Miyata S (2018) Brain region-dependent heterogeneity and dose-dependent difference in transient microglia population increase during Lipopolysaccharide-induced inflammation. Sci Rep 8: 2203
Galatro TF, Holtmann IR, Lerario AM, Vainchtein ID, Brouwer N, Sola PR, Veras MM, Pereira TF, Leite RP, Möller T et al. (2017) Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. Nat Neurosci 20: 1162–1171
Gertig U, Hanisch UK (2014) Microglial diversity by responses and responders. Front Cell Neurosci 8: 101
Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER et al. (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330: 841–845
Goldmann T, Wieghofer P, Jordão MJ, Prutek F, Hagemeyer N, Frenzel K, Uchimura K, Sajti E, Jaeger K (2015) Colony-stimulating factor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. Neuron 82: 380–397
Eon Kuek L, Leffler M, Mackay GA, Hulett MD (2016) The M54A family: counting past 1, 2 and 3. Immunol Cell Biol 94: 11–23
Erblich B, Zhu L, Etgen AM, Dobrenis K, Pollard JW (2011) Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. PLoS One 6: e26317
Eyo UB, Mo M, Yi MH, Murugan M, Liu J, Yarlagadda R, Margolis DJ, Xu P, Wu L (2018) P2Y12R-dependent translocation mechanisms gate the changing microglial landscape. Cell Rep 23: 959–966
Ferrero G, Mahony CB, Dupuis E, Yvernegeau L, Di Ruggiero E, Miserocchi M, Caron M, Robin C, Traver D, Bertrand JY et al. (2018) Embryonic microglia derive from primitive macrophages and are replaced by cmyb-dependent definitive microglia in zebrasfish. Cell Rep 22: 130–141
Flanary BE, Sammons NW, Nguyen C, Walker D, Streit WJ (2007) Evidence that aging and amyloid promote microglial cell senescence. Rejuvenation Res 10: 61–74
Flowers A, Bell-Temin H, Jalloh A, Stevens SM, Bickford PC (2017) Proteomic analysis of aged microglia: shifts in transcription, bioenergetics, and nutrient response. J Neuroinflammation 14: 96
Forabosco P, Ramasamy A, Trabzuni D, Walker R, Smith C, Bras J, Levine AP, Hardy J, Pocock JM, Guerreiro R et al. (2013) Insights into TREM2 biology by network analysis of human brain gene expression data. Neurobiol Aging 34: 2699–2714
Foyez T, Takeda-Uchimura Y, Ishigaki S, Narentuya ZZ, Sobue G, Kadomatsu K, Uchimura K (2015) Microglial keratin sulfate epitope elicits in central nervous tissues of transgenic model mice and patients with amyotrophic lateral sclerosis. Am J Pathol 185: 3035–3065
Füger P, Hefendehl JK, Veeraraghavulu K, Wendeln AC, Schlosser C, Obermüller U, Wegenast-Braun BM, Neher JJ, Martus P, Kohsaka S et al. (2017) Microglia turnover with aging and in an Alzheimer’s model via long-term in vivo single-cell imaging. Nat Neurosci 20: 1371–1376
Graeber MB, Schuebel KE, Pollard JW, Elkon RB, Prutek F, Hagemeyer N, Frenzel K, Uchimura K, Sajti E, Jaeger K (2015) Colony-stimulating factor-1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reparative defects. Blood 99: 111–120
De Biase LM, Schuebel KE, Fusfeld ZH, Jair K, Hawes IA, Cimbro R, Zhang HY, Liu QR, Shen H, Xi ZX et al. (2017) Local cues establish and maintain region-specific phenotypes of basal ganglia microglia. Neuron 95: 341–356 e6
Habib N, Avraham-David I, Basu A, Burks T, Shekhar K, Hoeffme R, Choudhury SR, Aguet F, Celfand E, Arldike K et al (2017) Massively parallel single-nucleus RNA-seq with DroNc-seq. Nat Methods 14: 955–958

Haimon Z, Volaski A, Orthgiess J, Boura-Halfon S, Varol D, Shemer A, Yona S, Zuckerman B, David E, Chappell-Maar L et al (2018) Re-evaluating microglia expression profiles using RiboTag and cell isolation strategies. Nat Immunol 19: 636–644

Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Wysoker A, Walker AJ, Gergits F, Segel M, Nemesh J et al (2018) Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. Immunity 50: 253–271

Han J, Harris RA, Zhang XM (2017) An updated assessment of microglia depletion: current concepts and future directions. Mol Brain 10: 25

Hanamsagar R, Alter MD, Block CS, Sullivan H, Bolton JL, Bilbo SD (1996) sulfate proteoglycans in the normal rat central nervous system: inverse correlation with constitutive expression of major histocompatibility complex class II antigens. Glia 18: 255–260

Jongs L, Tuszyński MH (2002) Spinal cord injury elicits expression of keratan sulfate proteoglycans by macrophages, reactive microglia, and oligodendrocyte progenitors. J Neurosci 22: 4611–4624

Joost E, Jordão MJ, Mages B, Prinz M, Bechmann I, Krueger M (2019) Microglia contribute to the glia limits around arteries, capillaries and veins under physiological conditions, in a model of neuroinflammation and in human brain tissue. Brain Struct Funct 224: 1301–1314

Jordão MJ, Sankowski R, Brendedee SM, Sagar Locatelli G, Tai YH, Tay TL, Schramm E, Armbruster S, Hagemeyer N, Groß O et al (2019) Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation. Science 363: eaat7554

Kamigaki M, Hide I, Yanase Y, Shiraki H, Harada K, Tanaka Y, Seki T, Shirafuji T, Tanaka S, Hide M et al (2016) The Toll-like receptor 4-activated neuroprotective microglia subpopulation survives via granulocyte macrophage colony-stimulating factor and JAK2/STAT5 signaling. Neurochem Int 93: 82–94

Kamphuis W, Kooijman L, Schetters S, Orre M, Hol EM (2016) Transcriptional profiling of CD11c-positive microglia accumulating around amyloid plaques in a mouse model for Alzheimer’s disease. Biochim Biophys Acta 1862: 1847–1860

Kaji M, Lee JE, Wilson JG, Everhart AL, Brown CM, Hoofnagle AN, Jansen M, Vitek MP, Gunn MD, Colton CA (2015) Argininer deprivation and immune suppression in a mouse model of Alzheimer’s disease. J Neurosci 35: 5969–5982

Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astasio D, Toth B et al (2017) A unique microglia type associated with restricting development of Alzheimer’s disease. Cell 169: 1276–1290 e17

Kierdorf K, Emy D, Goldmann T, Sander V, Schulz C, Perdiguero EG, Wiegahofre P, Heinrich A, Riemke P, Hölscher C et al (2013) Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat Neurosci 16: 273–280

Kiyosu K, Kurauchi Y, Hisatsune A, Seki T, Mishima S, Katsuki H (2015) A novel compound macelgin protects midbrain dopaminergic neurons from inflammatory degeneration via microglial arginase-1 expression. Eur J Pharmacol 760: 129–135

Koellhofer EC, McCullough LD, Ritzel RM (2017) Old maids: aging and its impact on microglia function. Int J Mol Sci 18: E769

Krasemann S, Madore C, Cialici R, Baufeld C, Calcagno N, El Fatimi R, Beckers L, O’Loughlin E, Xu Y, Fanek Z et al (2017) The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. Immunity 47: 566–581 e9

Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39: 151–170

Lawson LJ, Perry VH, Gordon S (1992) Turnover of resident microglia in the normal adult mouse brain. Neuroscience 48: 405–415

Lenz KM, Nugent BM, Haliyur R, McCarthy MM (2013) Microglia are essential for microglia-dependent pathways. Science 337: 273–277

Luo J, Elwood F, Britschgi M, Villeda S, Zhang H, Ding Z, Zhu L, Alabsi H, Getachew R, Narasimhan R et al (2013) Colony-stimulating factor 1 receptor (CSF1R) signaling in injured neurons facilitates protection and survival. J Exp Med 210: 157–172
Martinez FO, Gordon S (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 6: 13
Mass E, Ballesteros I, Farlik M, Halbritter F, Günther P, Crozet L, Jacome-Galarza CE, Händler K, Klughammer J, Kobayashi Y et al (2016) Specification of tissue-resident macrophages during organogenesis. Science 353: aaf4238
Masuda T, Sankowski R, Staszewski O, Böttcher C, Amann L, Scheiwe C, Nessler S, Kunz P, van Loo G, Coenen VA et al (2019) Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. Nature 566: 388 – 392
Matcovich-Natan O, Winter DR, Giladi A, Vargas Aguilar S, Spinrad A, Sarrazin S, Ben-Yehuda H, David E, Zelada González F, Perrin P et al (2016) Microglia development follows a stepwise program to regulate brain homeostasis. Science 353: aad8670
Mathys H, Adaikkan C, Gao F, Young JZ, Manet E, Hemberg M, De Jager PL, Nagarajan N, Jones BW, West PJ, Marc RE, Capecchi MR (2015) The origin of tissue-resident macrophages: when an erythro-myeloid progenitor is an erythro-myeloid progenitor. Immunology 43: 1023 – 1024
Peterson VM, Zhang XX, Kumar N, Wong J, Li L, Wilson DC, Moore R, McClanahan TK, Sadekova S, Klappenbach JA (2017) Multiplexed quantification of proteins and transcripts in single cells. Nat Biotechnol 35: 936 – 939
Pimentel-Coelho PM, Michaud JP, Rivest S (2013) Evidence for a gender-specific protective role of innate immune receptors in a model of perinatal brain injury. J Neurosci 33: 11556 – 11572
Ransohoff RM (2016) A polarizing question: do M1 and M2 microglia exist? Nat Neurosci 19: 987 – 991
Ravasi T, Wells C, Forest A, Underhill DM, Wainwright BJ, Aderem A, Grimmond S, Hume DA (2002) Generation of diversity in the innate immune system: macrophage heterogeneity arises from gene-autonomous transcriptional probability of individual inducible genes. J Immunol 168: 44 – 50
Remington LT, Babcock AA, Zehntner SP, Owens T (2007) Microglial recruitment, activation, and proliferation in response to primary demyelination. Am J Pathol 170: 1713 – 1724
Rêu P, Khosravi A, Bernard S, Mold JE, Salehpour M, Alkass K, Perl S, Tisdale J, Possnert G, Druid H et al (2017) The lifespan and turnover of microglia in the human brain. Cell Rep 20: 779 – 784
Ribeiro Xavier AL, Kress BT, Goldman SA, Lacerda de Menezes JR, Nedergaard M (2015) A distinct population of microglia supports adult neurogenesis in the subventricular zone. J Neurosci 35: 11848 – 11861
Río-Hortega P (1919a) El “tercer elemento de los centros nerviosos”. IV. Poder fagocitario y movilidad de la microglía. Bol Soc Esp Biol VIII: 155 – 166
Río-Hortega P (1919b) El “tercer elemento” de los centros nerviosos. I. La microglía en estado normal. II. Intervención de la microglía en los procesos patológicos (células en bastoncito y cuerpos gránulos-adiposos). Bol Soc Esp Biol VIII: 69 – 109
Río-Hortega P (1919c) El “tercer elemento” de los centros nerviosos. III. Naturaleza probable de la microglía. Bol Soc Esp Biol VIII: 108 – 115
Río-Hortega P (1920) Estudios sobre la neuroglía. La microglía y su transformación en células en bastoncito y cuerpos gránulos-adiposos. Trab Lab Invest Biol Univ Madrid XVII: 37 – 82
Saltz MW, Stevens B (2017) Microglia emerge as central players in brain disease. Nat Med 23: 1018 – 1027
Sato-Hashimoto M, Nozu T, Toriba R, Horikoshi A, Akaie M, Kawamoto K, Hirose A, Hayashi Y, Nagai H, Shimizu W et al (2019) Microglial SIRPα regulates the emergence of CD11c+ Eilef 8: e44025
Schafra DP, Lehrman EK, Kautzmann AC, Koyama R, Mardini AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barnes BA, Stevens B (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74: 691 – 705
Schellff J, Regen T, Van Rossum D, Seifert S, Ribes S, Nau R, Parsa R, Harris RA, Boddeke HW, Chuang HN et al (2012) Toll-like receptor activation
reveals developmental reorganization and unmask responder subsets of microglia. Glia 60: 1930 –1943
Schmid CD, Sautkulis LN, Danielson PE, Cooper J, Hasel KW, Hilbush BS, Sutcliffe JG, Carson MJ (2002) Heterogeneous expression of the triggering receptor expressed on myeloid cells-2 on adult murine microglia. J Neurochem 83: 1309 –1320
Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SE, Pollard JW et al (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 336: 86 –90
Schwarz JM, Sholar PW, Bilbo SD (2012) Sex differences in microglial colonization of the developing rat brain. J Neurochem 120: 948 –963
Sheng J, Ruedl C, Karjalainen K (2015) Most tissue-resident macrophages except microglia are derived from fetal hematopoietic stem cells. Immunity 43: 382 –393
Shigemoto-Mogami Y, Hoshikawa K, Goldman JE, Sekino Y, Sato K (2014) Microglia enhance neurogenesis and oligodenogreation in the early postnatal subventricular zone. J Neurosci 34: 2231 –2243
Shinjo R, Imagama S, Ito Z, Ando K, Nishida Y, Ishiguro N, Kadomatsu K (2014) Keratin sulfate expression is associated with activation of a subpopulation of microglia/macrophages in Wallerian degeneration. Neurosci Lett 579: 80 –85
Spitau B (2017) Aging microglia-phenotypes, functions and implications for age-related neurodegenerative diseases. Front Aging Neurosci 9: 194
Squarzoni P, Oller G, Hoefell G, Pont-Lezica L, Rostaing P, Low D, Bessis A, Ginhoux F, Garel S (2014) Microglia modulate wiring of the embryonic forebrain. Cell Rep 8: 1271 –1279
Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B et al (2007) The classical complement cascade mediates CNS synapse elimination. Cell 131: 1164 –1178
Stowell RD, Wong EL, Batchelor HN, Mendes MS, Lamantia CE, Whitelaw BS, Majewski AK (2018) Cerebellar microglia are dynamically unique and survey Purkinje neurons in vivo. Dev Neurobiol 78: 627 –644
Streit WJ (2002) Microglia as neuroprotective, immunocompetent cells of the CNS. Glia 40: 133 –139
Tay TL, Béchade C, D’Andrea I, St-Pierre MK, Henry MS, Roumier A, Tremblay ME (2017a) Microglia gone rogue: impacts on psychiatric disorders across the lifespan. Front Mol Neurosci 10: 421
Tay TL, Mai D, Dautzenberg J, Fernández-Klett F, Lin G, Sagar Datta M, Drougard A, Stempfl T, Ardura-Fabregat A, Staszewski O et al (2017b) A new fate mapping system reveals context-dependent random or clonal expansion of microglia. Nat Neurosci 20: 793 –803
Tay TL, Savage JC, Hui CW, Bishk T, Tremblay M (2017c) Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. J Physiol 595: 1929 –1945
Tay TL, Sagar DJ, Grün D, Prinz M (2018) Unique microglia recovery population revealed by single-cell RNAseq following neurodegeneration. Acta Neuropathol Commun 6: 87
Trapnell C (2015) Defining cell types and states with single-cell genomics. Genome Res 25: 1491 –1498
Tremblay M, Lowery RL, Majewskas AK (2010) Microglial interactions with synapses are modulated by visual experience. PLoS Biol 8: e1000527
Tremblay M, Zettel ML, Ison JR, Allen PD, Majewski AK (2012) Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. Glia 60: 541 –558
Tremblay M, Lecours C, Samson L, Sánchez-Zafría V, Sierra A (2015) From the Cajal alumni Achúcarro and Río-Hortega to the rediscovery of never-resting microglia. Front Neuronat 9: 45
Walz W, Lang MK (1998) Immunocytochemical evidence for a distinct GFAP-negative subpopulation of astrocytes in the adult rat hippocampus. Neurosci Lett 257: 127 –130
Wang Y, Berezovska O, Fedoroff S (1999) Expression of colony stimulating factor-1 receptor (CSF-1R) by CNS neurons in mice. J Neurosci Res 57: 616 –632
Wang Y, Szretter KJ, Vermi W, Gilfillan S, Rossini C, Cella M, Barrow AD, Diamond MS, Colonna M (2012) IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. Nat Immunol 13: 753 –760
Weil ZM, Norman GJ, DeVries AC, Nelson RJ (2008) The injured nervous system: a Darwinian perspective. Prog Neurobiol 86: 48 –59
Wendeln AC, Degenhardt K, Kaurani L, Gertig M, Ulas T, Jain G, Wagner J, Häsl er LM, Wild K, Skodras A et al (2018) Innate immune memory in the brain shapes neurological disease hallmarks. Nature 556: 332 –338
Wlodarczyk A, Labner M, Cédile O, Owens T (2014) Comparison of microglia and infiltrating CD11c+ cells as antigen presenting cells for T cell proliferation and cytokine response. J Neuroinflammation 11: 57
Wlodarczyk A, Cédile O, Jensen KN, Jasson A, Monte JY, Khorooshi R, Owens T (2015) Pathologic and protective roles for microglial subsets and bone marrow- and blood-derived myeloid cells in central nervous system inflammation. Front Immunol 6: 463
Wlodarczyk A, Holtman IR, Krueger M, Yogev N, Bruttiger J, Khoroshii R, Benmamar-Badel A, de Boer-Bergsma JJJ, Martin NA, Karram K et al (2017) A novel microglial subset plays a key role in myelinogenesis in developing brain. EMBO J 36: 3292 –3308
Wlodarczyk A, Benmamar-Badel A, Cédile O, Jensen KN, Kramer I, Elsborg NB, Owens T (2018) CSF1R stimulation promotes increased neuroprotection by CD11c+ microglia in EAE. Front Cell Neurosci 12: 523
Wogram E, Wendt S, Matyash M, Pinvea T, Draguhn A, Kettenmann H (2016) Satellite microglia show spontaneous electrical activity that is uncorrelated with activity of the attached neuron. Eur J Neurosci 43: 1523 –1534
Xavier AL, Lima FR, Nedergaard M, Menezes JR (2015) Ontogeny of Cxcr3/Cxcr1-EFGP expressing cells unveil microglia as an integral component of the postnatal subventricular zone. Front Cell Neurosci 9: 37
Xu J, Zhu L, He S, Wu Y, Jin W, Yu T, Qu Y, Wen Z (2015) Temporal-spatial resolution fate mapping reveals distinct origins for embryonic and adult microglia in zebrafish. Dev Cell 34: 632 –641
Yeh FL, Hansen DV, Sheng M (2017) TREM2, microglia, and neurodegenerative diseases. Trends Mol Med 23: 512 –533
Zeisel A, Muñoz-Manchado AB, Codeluppi S, Lönnerberg P, La Manno G, Juréus A, Marques S, Munguba H, He L, Betsholtz C et al. (2015) Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science 347: 1138 –1142
Zhang C, Lam TT, Tso MO (2005) Heterogeneous populations of microglia/macrophages in the retina and their activation after retinal ischemia and reperfusion injury. Exp Eye Res 81: 700 –709
Zhang H, Muramatsu T, Murase A, Yusa S, Uchimura K, Kadomatsu K (2006) N-Acetylglucosamine 6-O-sulfotransferase-1 is required for brain keratan sulfate biosynthesis and glial scar formation after brain injury. Glycobiology 16: 702 –710

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