Review Article

Microglia: Physiological Functions Revealed through Morphological Profiles

(central nervous system / microglia / ramified / amoeboid / morphology)

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Abstract. Microglia play key immunological roles in the central nervous system. Upon activation, resident microglial cells transform from a ramified form to an amoeboid form and acquire the ability to phagocytose and release pro-inflammatory cytokines. Here, we review microglial phenotypes that contribute to their functional roles in the central nervous system with the emphasis on their molecular profiles. Deeper understanding of the functions performed by microglia in physiological and pathological conditions can promote investigation of microglia activities in brain injury or disease and facilitate development of new treatment approaches.

Background

The central nervous system (CNS) comprises neurons and glia, which include astrocytes, oligodendrocytes, and microglia. Microglia constitute up to 12 % of the total glial cell population and are well known as key cellular contributors to post-injury and inflammatory responses in the brain (Gomez-Nicola and Perry, 2015). Microglia are morphologically heterogeneous and are broadly classified as amoeboid and ramified microglial cells based on their shape and characteristics. Comparison of amoeboid and ramified microglial cell characteristics shows that microglia constantly survey their microenvironment by producing and extending ramified processes (Ransohoff and Perry, 2009). After acute injury, ramified microglial cells are rapidly activated and change their morphology from ramified to amoeboid (Hanisch and Kettenmann, 2007). In this review, we highlight recent findings regarding microglial morphology, which advanced our understanding of microglia in the overall brain function at the molecular level in both physiological and pathological contexts. We suggest that profiling of candidate molecules associated with microglial morphology may contribute to deeper understanding of the physiological functions performed by microglia. We also discuss phenotypic and functional properties of microglia in relation to health and disease of the CNS.

The origin of microglia

Two different hypotheses of the origin of microglia have been proposed: mesodermal and monocytic (Chan et al., 2007). The mesodermal origin of microglia suggests that precursor cells developed in the yolk sac invade the brain through the mesoderm (Ginhoux et al., 2010), while the monocytic origin suggests that circulating blood monocytes transform into amoeboid microglial cells, which in turn transform into ramified microglial cells (Kaur et al., 1994).

Microglia have been found to be closely related to circulating macrophages in peripheral blood (Saijo and Glass, 2011). At the same time, monocytic genes (*HLA-C*, *CD74*, *CD302*, *LSP1*, and *RUNX3*) expressed in microglia are known to be involved in antigen presentation and lysosome-related functions (Inoue et al., 2002; Zusso et al., 2012). Microglia take up residence in the brain during early foetal development. Runt-related transcription factor 1 (RUNX1), a key regulator of proliferation and differentiation across the myeloid lineage, is expressed in microglia before the start of brain development (Ginhoux et al., 2010). Moreover, microglia dif-

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Abbreviations: AMC – amoeboid microglial cells, BDNF – brain-derived neurotrophic factor, CNS – central nervous system, CSF1 – colony-stimulating factor 1, CSF1R – CSF1 receptor, GRN – progranulin, IBA1 – ionized calcium-binding adapter molecule 1, IGF-1 – insulin-like growth factor 1, MFG-E8 – milk fat globule-EGF factor 8 protein, NGF – nerve growth factor, NMDA – N-methyl-D-aspartate, RMC – ramified microglial cells, PAMP – pathogen-associated molecular pattern, PRR – pattern recognition receptor, ROS – reactive oxygen species, RUNX1 – runt-related transcription factor 1, SIRT3 – sirtuin 3, SOD1 – superoxide dismutase 1, TREM2 – triggering receptor expressed on myeloid cells 2, TYROBP – tyrosine kinase-binding protein.

ferentiation, proliferation, and transformation into resident microglia requires CSF1, CSF1R, CD34 antigen, and transcription factor PU.1.

Development dynamics

In the mouse cerebellum, changes in the developmental profile of amoeboid microglial cells after the first postnatal week have been reported (Ashwell, 1990). Amoeboid microglial cells also express cytoskeleton-associated genes such as *CRMP1*, *DPYSL3*, *DPYSL5*, *SEPT9*, and *SEPT11* (Nagata et al., 2004; Nimmerjahn et al., 2005), and it has been proposed that cytoskeletal dynamics is important for microglial regulation (Stuart et al., 2007).

Postnatal amoeboid microglial cells are mitotic and motile after birth and then gradually progress toward ramified microglial cells. At postnatal day 17, there is a significant increase in the round structures of actin characteristic for phagocytosis in amoeboid microglial cells (Perez-Pouchoulen et al., 2015). The round structures have been identified as apoptotic bodies in the CNS (Kaur and Ling, 2009; Sierra et al., 2010). Early evidence of microglial phagocytosis in the developing cerebellum was obtained in amoeboid microglial cells (Lawson et al., 1992). Thus, amoeboid microglia are required for efficient phagocytosis of neurites (axons and myelin debris) and apoptotic bodies in physiological conditions. It was demonstrated that unchallenged microglia phagocytose apoptotic cells during development, suggesting a function of microglia for brain remodelling in the normal physiologic condition (Sierra et al., 2013).

In the adult rat brain, ramified microglial cells are quiescent and have a low turnover rate (Lawson et al., 1992), while in the developing rat brain, amoeboid microglial cells have high proliferative activity during the first two postnatal weeks (Imamoto and Leblond, 1978). No detectable expression of RUNX1 was observed in ramified microglial cells, which express ionized calcium-binding adapter molecule 1 (IBA1), CD11B, and F4/80. In contrast, RUNX1 expression was detected in response to microglia activation in the adult CNS (Berger et al., 2011; Sanagi et al., 2010).

Steady-state microglia in CNS

In the steady state, microglia are presented by a ramified phenotype, which can secrete neurotrophic factors such as insulin-like growth factor 1 (IGF1), brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) (Bessis et al., 2007; Sanagi et al., 2010). The resting phenotype under the steady state is maintained by CX3C-chemokine ligand 1 through its surface receptor CX3CR1 expressed on microglia (Saijo and Glass, 2011). In a mouse model of Alzheimer's disease, CX3CR1 loss in microglia reduced neuronal death (Fleisher-Berkovich et al., 2010). Microglial cell receptors CD172, CD200R and CD45 following interaction with the neuronal cell-surface proteins CD47, CD200 and CD22, respectively, have also been reported in the resting state (Fig. 1).

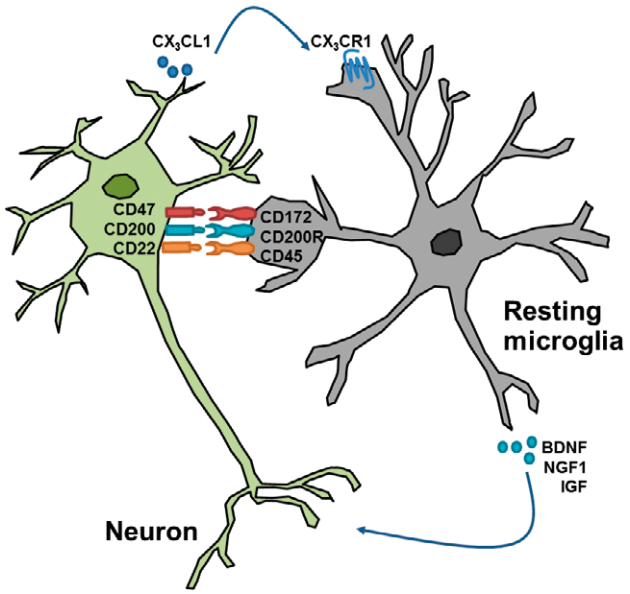

Fig. 1. Steady-state microglia cell phenotype. In steady-state conditions, microglia exhibit a ramified phenotype. This morphology is maintained through the corresponding receptors (CD172, CD200R, and CD45) and CX_3C-chemokine ligand 1 (CX_3CL1).

Classically activated microglia

In many pathological conditions, microglia can induce release of cytokines and chemokines from the neighbouring cells, which in turn can target microglia (Fig. 2) (Hanisch, 2002; Zhang et al., 2010). Microglia-derived TNF-α mediates neuronal death and upregulates expression of IL-1β (Van Ginderachter et al., 2006; Takeuchi et al., 2006). Upon pro-inflammatory challenge, microglia showed increased expression of MHC-II and CD11c and enhanced antigen-presenting activity (Neher et al., 2011). Anti-inflammatory cytokines, such as TGF-β, secreted by activated microglia can reduce inflammation (Holmans et al., 2013). Chemokines released by microglia promote recruitment of peripheral immune cells during inflammation in the CNS (Guerreiro et al., 2013). Increased microglial activity can lead to microglial apoptosis (Hooper and Pocock, 2007) caused by nitric oxide-dependent mitochondrial depolarization and activation of caspases such as CASP2, CASP3, and APAF1 (Kingham and Pocock, 2000).

Microglial phagocytosis following activation with toll-like receptor agonists such as lipopolysaccharide or amyloid β caused neuronal loss (Fleisher-Berkovich et al., 2010; Neher et al., 2011). In mice, lipopolysaccharide injection induced microglia-mediated inflammation and neuronal loss, which was markedly diminished by blocking MFG-E8 or vitronectin receptor (Fricker et al., 2012). MFG-E8 is upregulated by brain ischaemia, sug-

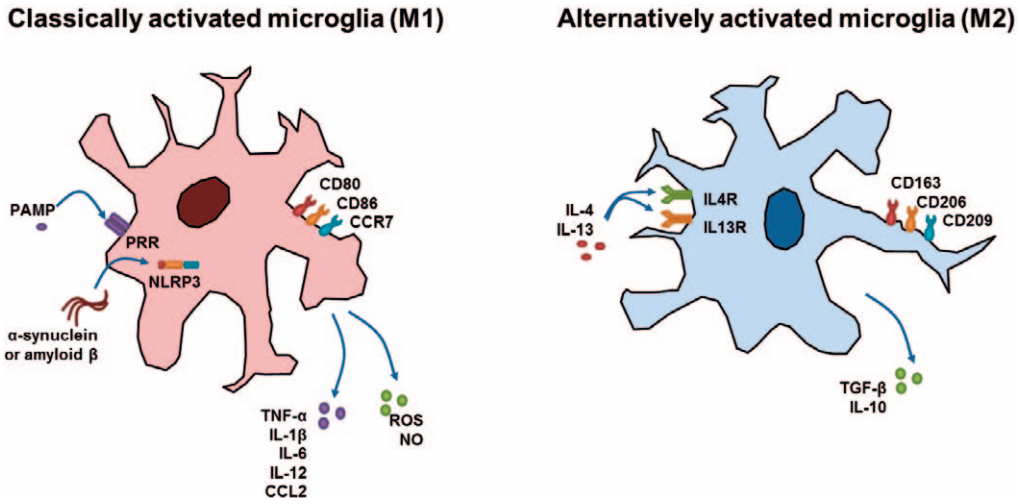

Fig. 2. Classical microglial activation. Microglia express pattern recognition receptors (PRRs) that recognize various pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) as well as neurodegenerative disease-specific protein aggregates (such as α-synuclein and amyloid β). Activated microglia produce pro-inflammatory cytokines (such as TNF-α, IL-1β, IL-12 and IL-23) and TGF-β. In addition, activated microglia upregulate expression of MHC class II molecules and CD11c.

gesting that in ischaemic conditions, microglia retain the ramified phenotype and have reduced ability to phagocytose (Mari et al., 2004). TREM2 is thought to increase phagocytic activity and suppress cytokine production (Holmans et al., 2013). The upregulation of *TREM2* variants has recently been shown to increase the risk of Alzheimer's disease (Guerreiro et al., 2013; Jonsson et al., 2013), while *TREM2* gene deletion resulted in microglia dysfunction and cognitive disorder. It is also known that *TREM2*/*TYROBP* mutations lead to Nasu-Hakola disease (Ohgidani et al., 2014).

In case of direct stress in microglia, mutations in phagocytosis-related genes are risk factors for neurodegeneration (Kao et al., 2011); these include mutations in *SOD1*, *TREM2*, *DAP12*/*TYROBP*, and *GRN* (Xie et al., 2005; Zhu et al., 2013). In amyotrophic lateral sclerosis (ALS), *SOD1* mutation was found associated with the increased expression of *TREM2* and its downstream target *TYROBP*. Moreover, progranulin is a pro-survival factor released by microglia, which acts on neurons, and mutations in the encoding *GRN* gene are linked to frontotemporal dementia (Thrash et al., 2009; Melchior et al., 2010).

Alternatively activated microglia

Microglia are polarized into M1 and M2 lineages (Fig. 3). Classically activated peripheral myeloid cells

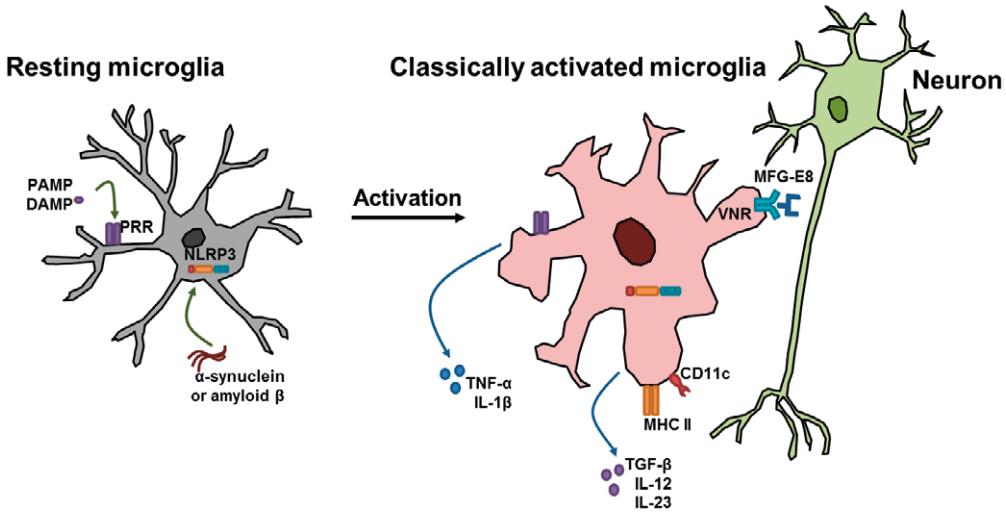

Fig. 3. M1/M2 polarization of microglia and their immune response. M1 cells express surface markers such as CD80, CD86, and CCR7, and produce chemokine CCL2 and pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-12. M2 cells express cell surface markers such as scavenger receptor CD163, CD206, and CD209 and produce anti-inflammatory cytokines, including TGF-β1 and IL-10.

exhibit the M1 phenotype. M1 cells are characterized by rounded morphology, which is consistent with the state of hyperactivity; they express surface markers such as CD80, CD86, and CCR7, and produce pro-inflammatory cytokines such as TNF-α, IL-6, and IL-12 (Van Ginderachter et al., 2006; Fairweather and Cihakova, 2009). In acute neuroinflammatory conditions, including spinal cord injury and stroke, M1-polarized microglia predominate (Cherry et al., 2014). In contrast, M2 cells have typical elongated morphology, express cell surface markers such as scavenger receptor CD163 and C-type lectins CD206 and CD209, and produce anti-inflammatory cytokines, including TGF-β1 and IL-10 (Mantovani et al., 2004; Martinez et al., 2006). M2 cells are important for resolving the inflammatory response in an animal model of stroke (Desestret et al., 2013), spinal cord injury (Shechter et al., 2013), and demyelination (Miron et al., 2013). M2 cells contribute to repair processes in the injured CNS (Michell-Robinson et al., 2015). Interestingly, glioma cells are reported to secrete cytokines such as IL-4, IL-10, IL-6 and TGF-β and these promote an M2-like phenotype (Charles et al., 2011).

Conclusions

The past few years have established considerable progress in the understanding of microglia. Relationships between specific states of microglia and specific pathological processes remain less well defined. However, there is the association between activation states and pathology to consider the regulation of microglial cell phenotype as a potential therapeutic approach for intervention. Here, we summarized the current knowledge regarding morphological transformation of microglia accompanied by modifications of their molecular profiles. Morphological transformations in microglia may have functional importance and, therefore, warrant further investigation of the underlying changes in gene expression. It is evident that this approach could be used to determine specific molecular profiles of cell phenotypes in microglia. We also described how microglia respond to a variety of external stimuli, underscoring the crucial importance of microglial function for the survival of neurons under pathological conditions. Furthermore, a holistic understanding of various physiological roles played by microglia may be useful to identify therapeutic targets in neuropathological processes occurring in the CNS, in which microglia are characteristically implicated.

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