Microglia: A Central Player in Depression*

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Summary: Microglia are the major immune cells in the central nervous system and play a key role in the normal function of the brain. Microglia exhibit functional diversity, and they control the inflammation in central nervous system through releasing inflammatory cytokine, clearing apoptotic cells via phagocytosis, regulating synaptic plasticity and the formation of neural network by synapse pruning. Recent studies have strongly indicated that the microglial dysfunction is associated with a variety of neuropsychiatric diseases such as depression, which have been termed as "microgliopathy". The emergency of advanced technologies and tools has enabled us to comprehensively understand the role of microglia in physiology and pathology, and growing studies have targetted microglia to explore the treatment of neuropsychiatric diseases. Here, we describe the key progress of microglia research, and review the recent developments in the understanding of the role of microglia in physiology and etiology of depression.
Key words: microglia; central nervous system; development; adult; depression

Microglial cells are the immune cells in the central nervous system (CNS) and comprise approximately 10% of the total brain cell population. By interacting with other type of cells (including neurons, astrocytes, and oligodendrocytes) in the CNS, microglia play a vital role in the development of the brain, the formation of neural circuits and the proper function of healthy brain[1, 2]. The functional and structural characteristics of microglia are under exquisite regulation, spatially and temporally. While external or internal stimuli could cause morphological and functional alterations in microglia, which usually involve the transient adaptive response under normal circumstances, the pathological challenges including chronic infection, trauma, stroke, neurodegenerative diseases and chronic psychological stress have detrimental impacts on microglia, leading to the long-lasting changes. For example, microglia are activated, and act as a primary coordinator and executor of protection and restoration, or inflammatory and toxic effects on neurons and other brain cells[3]. Enormous body of evidence has implicated that microglia are involved in almost all brain diseases, from neurodegenerative diseases, such as Alzheimer's disease (AD), traumatic brain injury (TBI), to mental diseases such as major depressive disorder (MDD)[4]. These pathological conditions, which are mainly caused by abnormally structural and functional changes of microglia, are considered to be "microgliopathy"[5]. In this review, we describe the research advances of microglia, including their origin, development and normal physiological characteristics, and focus on the recently identified role of microglia in depression.

1 DISCOVERY OF MICROGLIA

In 1856, the famous German pathologist Rudolf Virchow defined a non-neuronal cell population as glia in the brain, which is distinct from neurons. However, in the next few decades, there has been little progress in this field. Until 1919, the Spanish neurologist Pío del Río Hortega observed and described microglia for the first time, distinguishing them from astrocytes and oligodendrocytes. In 1924, Aschoff *et al* observed phagocytosis of microglial cells in gliomas. Subsequent decades of researches on microglia were always based on the concept that microglia act as inert bystanders of the physiology of CNS. Innovative changes occurred in 2005, when scientists visualized the continuous activity of resting microglia in the brain of live mice[6], and since then, the advances in the research of microglia are progressing by leaps and bounds. In 2007, the study found that microglia are independent of circulating cells and can self-renew in the brain[7]; in 2010, synaptic

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pruning process of microglia was discovered[8]. Also, in this year, the origin of microglia was revealed and yolk nest precursors were identified[9]. The development of gene sequencing technology has greatly supported the study of microglia. In 2014, Lavin *et al* revealed the transcriptome and epigenome of microglia in adult mice[10], which promoted the understanding of the molecular characteristics of microglia at the genetic level. In 2017, Gosselin *et al* further elaborated the transcriptome and epigenome of human microglia[11]. In a recent study, by using single-cell techniques to elucidate the single-cell nature of human microglia, the presence of microglia subsets of different molecular features in the brain was revealed[12, 13]. Throughout the research history of microglia, the advances in technology have greatly facilitated their research. There are several technical nodes, which were non-negligible. In 1983, Hume *et al* firstly used immunohistochemistry to detect microglia[14]. In 1986, Giulian *et al* used flow cytometry for the first time to isolate microglia[15]. In 2000 and 2013, the chemokine c-x3-c-motif chemokine receptor 1 (CX3CR1) GFP mice and CX3CR1CreER mice were developed, respectively, for the detection and targeted intervention of microglia *in vivo*[16, 17].

2 ONTOGENY OF MICROGLIA

2.1 Origin and Development of Microglia

Like other types of brain cells, microglia have long been thought to originate in the neuroectoderm, but current studies have demonstrated that microglia are a part of the mononuclear phagocytic system[18]. Microglia originate from the yolk sac-derived primitive macrophage pool that appears on day 8.5 of the mouse embryonic stage (E), and at approximately E13, microglia precursors appear at the base of the fourth ventricle, indicating that microglia precursors migrate to the CNS at the early stage of embryo and form an independent lineage that is different from other hematopoietic stem cells by expressing lineage-specific genes (such as *Pu.1* and *Irf8*, which are required for the differentiation of microglia), and the formation of microglia is even earlier than that of other glial cells[19, 20]. Several factors, including colony stimulating factor-1 (CSF-1) receptor, TYRO protein tyrosine kinase binding protein (Tyrobp) and interferon regulatory factor-8 (IRF-8), are crucial for the development of microglia[21–23]. Microglia are essentially dependent on the continuous CSF-1 signaling that is mediated by CSF-1 receptor through interacting with the alternative ligand interleukin (IL)-34, but not CSF-1[24].

2.2 Maintenance of Microglia

An individual microglial cell has a limited average life span, and there has long been controversy about the source of microglial replenishment in adult brain. Early views suggested that supplementary microglia come from the blood system. However, recent studies indicated that the maintenance of microglia population was not dependent on the circulating monocytes, but was supported by the locally self-renewal of microglia in the brain. Microglia have a notable self-renewal capacity to maintain their densities in specific region, and the turnover rate is about 0.05% of cells per hour. The turnover of microglia regulates the balance of their apoptosis and proliferation in healthy brain[25]. It was found that microglia were eliminated by the treatment of CSF-1 receptor inhibitors in the brain of mice, and microglia repopulation following depletion depended on the internal pool of remaining microglia, but not on the peripheral macrophages, which required the role of IL-1 receptor signaling[26, 27].

3 PHYSIOLOGICAL FUNCTION OF MICROGLIA

Microglia originate in the brain at the early stage of embryonic development and span the developmental and adult stages of an individual. Due to the obvious differences in the brain circumstances between these two stages, the role of microglia in these two stages may be different. Actually, microglia in the developing brain or in the adult brain have different morphologies, roughly showing amoeba cells or branching cells respectively, which may indicate different activation states of microglia[28]. Consistent with that, the transcriptomic analyses of microglia in the embryo, early postnatal period and adult CNS have revealed that there are highly differential expression of microglial characteristics among the three stages[12, 29].

3.1 Role of Microglia in CNS Development

Microglia are the specialized phagocytes in the brain, where they rapidly and efficiently clear dead or dying cells and debris, and eliminate synapses. Furthermore, microglia recognize the cells undergoing programmed death and then migrate to the corresponding areas to play phagocytic roles[30]. In the developing brain, microglia control the fate and number of neurons, and like other phagocytes, microglia engulf adjacent cells during development, in which TNF-α and extracellular matrix factors (such as tenascin C) play an important role[31–33]. In addition, microglia control the number of neurons by regulating neural progenitor cells (NPCs) either through actively inducing the apoptosis of NPCs and eliminating excess, apoptotic or dead NPCs[34, 35], or positively promoting both proliferation/survival of NPCs, and the maturation/survival of neurons[36, 37], suggesting that activated microglia specifically interact with neurons and influence their survival either in a positive or in a negative direction during the same period. However, how the two processes are coordinated and balanced

is not yet known, and further investigation is needed.

Synapses are specialized junctions between neurons in brain that connect neurons into millions of overlapping and interdigitated neural circuits. Synaptic pruning is essential for normal brain development, and microglia serve as the "synaptic gardener" by both forming and scavenging synapses, in which, several immune factors including classical complement components and receptors, CX3CL1/CX3CR1, major histocompatibility complex (MHC) class I, IL-33 and paired immunoglobulin-like receptor B (PirB) are involved[38–42]. Complement C3 localizes to immature synapses and mediates the developmental pruning of retinogeniculate synapses through interacting with C3 receptors[41]. Li *et al* recently identified the involvement of G protein-coupled receptor (GPR56) in microglial synaptic refinement[43]. Moreover, other studies have pointed out that microglia not only remove non-functional synapses, but also may play an active role in synaptic circuit remodeling.

In the developing brain, microglia also support the development of the vascular system and other cells such as oligodendrocytes and astrocytes[44], and in the white matter microglia have been confirmed to be essential for normal myelination[45]. In addition, it is reported that microglia are required for the formation of new blood vessels, but the specific mechanism remains to be elucidated[46, 47].

3.2 Role of Microglia in Adult Nervous System

Although previous reports suggest that microglia in the adult brain remain in a resting or quiescent state until mobilized by a threat, recent studies have found that the cell soma of microglia in the adult brain is stationary under homeostasis, its processes are dynamic and continually survey the surrounding environment, whose areas are more than 10-fold larger than that of cell body. Microglia constantly scan the surrounding brain tissue in a few hours, and directly contact neuron and synapse at a certain frequency, which is a process called "surveillance" of microglia[48, 49]. The factors that modulate this function have not been fully elucidated, but ion channels, neurotransmitters and purinergic receptors (P2X) may be involved[1]. Potassium two-pore domain halothane-inhibited potassium channel 1 (THIK-1) has been demonstrated to conduct the branching and surveillance functions of microglia[50]. Microglia continuously monitor the changes in microenvironment through surveillance function, which is achieved through the "sensome" receptors on them, so as to identify invading pathogens, misfolded proteins, chemokines and cytokines, metabolites, inorganics, as well as the changes in pH and extracellular matrix[51]. In addition to the intrinsic pathways, microglial surveillance is also driven by extracellular signals. Eyo *et al* found that neuronal activity regulated the microglial process surveillance through P2Y12 receptor signaling[52], and their recent study showed that noradrenergic signaling mediated the response of microglia to neuronal activity in a "U-shape" model[53]. Despite that microglia are highly dynamic cells that interact with neurons and non-neuronal cells via continuous process extension and retraction, the dynamic value is not constant under physiological conditions. It is found that microglial processes are less dynamic and reduced surveillance territory in awake brain, while inhibition of neuronal activity under general anesthesia dramatically increased microglial process surveillance, which is mediated by the microglial β2-adrenoceptors[53, 54]. Otherwise, the energetic requirements of microglial surveillance are supported either by glycolysis under resting conditions, or glutaminolysis when adapted to glucose deprivation[55].

The microglia in the adult brain are highly efficient in clearing dead and surplus cells, and the phagocytosis does not seem to require their activation, although microglia activation can promote phagocytosis[56]. Moreover, microglia-mediated phagocytosis of synapses seems to depend on the changes in neuronal activity. In the visual cortex, microglia directly interact with axonal terminals and dendritic spines, and preferentially phagocytose less active presynaptic inputs[4]. The role of phosphatase and tensin homologue deleted on chromosome ten (PTEN) is recently identified in the regulation of neuronal and synaptic engulfment by microglia. Activation of PTEN increases the apoptotic signal phosphatidylserine and accumulates C1q at synapse, leading to aggrandized engulfment of microglia[57].

After acute brain injury, neighboring microglial cells migrate to the damaged site within a few minutes of the insult, and after a few hours to several days, the reactive microglia shrink their processes and transform into an amoeboid form, which is called "chemotaxis" of microglia[58, 59]. Activation of purinergic receptors such as P2Y12 receptors (P2Y12R) in microglia has been previously reported to be implicated in microglial chemotaxis toward ATP that is released by injured neurons and astrocytes at early stages of the response to local brain injury[60]. However, the role of P2Y12 in microglial chemotaxis was challenged by a recent study that P2Y12 mediated the random motility of microglia induced by the activation of TLR2, but not TLR7-stimulated microglial chemotaxis[61].

Microglia are immunocompetent cells that are activated in the CNS in response to inflammation by releasing immune mediators. Microglia respond to damage-associated molecular patterns (DAMPs) stimulation, such as pathogenic activated molecular patterns, misfolded proteins, by releasing pro-inflammatory factors, such as IL-6[62]. Microglia activation is typically classified into a pro-inflammatory

neurotoxic (M1) pattern, which could be induced by the stimulation of lipopolysaccharides (LPS) and IFN-γ with the following release of pro-inflammatory factors such as TNF-α, IL-6 and IL-lβ, and an anti-inflammatory or neuroprotective (M2) pattern, which could be induced by IL-4 and IL-13 stimulation with neuroprotective effect[63], although this dichotomous division is oversimplified, and now, the concept of M1/M2 polarization is challenged[63–65]. The current findings reveal that microglia reactivity is a highly dynamic process that varies in different pathological processes and even in the same pathological process, which was also confirmed by the results of genome sequencing[66].

3.3 Heterogeneity of Microglia

The temporal heterogeneity of microglia in the developing and adult brain has been well illuminated, but its spatial heterogeneity remains largely unknown. It is found that microglia display a region-specific density and morphology, and simultaneously, microglia in different brain regions also differ in function[67, 68]. More specifically, the regional heterogeneity of microglia in the brain is revealed by density distribution, surface immune molecules, electrophysiological features, the response to the modulators (i.e. IL-34), and clearance activity[24, 28, 69–71]. Compared with the striatum and cortex, microglia in the cerebellum contribute to the growing appreciation of the clearance activity of microglia during postnatal development[71], which is driven by the CSF-1R ligand CSF-1, and independent of the alternative CSF-1R ligand IL-34[72]. A unique microglia subpopulation with low expression of purine receptors and lack of chemotaxis has been identified in the subventricular zone in which adult neurogenesis occurs[73]. A recent study found that there are two functionally distinct subpopulations of microglia in the retina[73]. Furthermore, a latest comprehensively analysis of a single-cell resolution has categorized the subclasses of microglia in multiple regions in detail[12]. The above evidence confirms the spatial heterogeneity of microglia, but further studies are needed to illuminate the underlying mechanism.

In addition to spatial heterogeneity, a large number of studies have shown that microglia density and phenotype vary between male and female rodents in several brain areas, indicating the gender heterogeneity of microglia. The males have a higher density of microglia that show an activated morphological phenotype characterized by an increased cell body size and decreased branching pattern and length. Moreover, various functional consequences resulting from microglial gender heterogeneity have been elucidated. For example, only the microglia in male affect social behavior in adolescent rats via eliminating the spines that express dopamine D1 receptors in nucleus accumbens[74]. Transcriptome sequencing analysis excavates more than 500 differentially expressed genes (DEGs) in microglia between male and female mice, in which, NF-κB as the transcription factor primarily regulates the genes that are highly expressed in male[75]. The molecular mechanisms underlying gender difference remain elucidated, but the role of estradiol during development is involved, since treating females with estradiol in the early postnatal period may lead to an increased microglial phenotype which was seen in males[76, 77]. However, female microglia retain their phenotype when the cells were transplanted into the male brain, and play their neuroprotective role even in male brain, suggesting a hormonal cue in an independent manner[75].

4 MICROGLIA IN DEPRESSION

MDD is the most common mood disorder in the world and has a lifetime prevalence of about 17% in the United States. Due to its complex pathological mechanism, the diagnosis of MDD is mainly carried out by psychiatrists through structured interviews based on diagnostic manuals (e.g., DSM-IV)[78]. Growing studies have shown that the impairment of the normal structure and function of microglia in both development and adult brain contributes to the etiology of MDD, and therefore, MDD can be regarded as a microgliopathy.

MDD patients usually suffer from chronic inflammation with changes in the central inflammatory state. Furthermore, MDD is significantly more common in people with inflammatory disease than in healthy individuals[79]. Microglia, the brain-resident immune cells, are emerging as a central player in inflammation in the brain of MDD patients. Previous studies have found that significant activation of microglia in depression-related brain regions such as prefrontal cortex and anterior cingulate cortex has been observed during severe episodes of MDD, and activation of microglia in anterior cingulate is positively correlated with the severity of depressive episode[80]. Correspondingly, positron emission tomography (PET) scan shows that the decrease in microglia activation is along with the improvement of depressive symptoms under cognitive-behavioral therapy and supportive psychotherapy[81]. Furthermore, in a post-mortem study for MDD, the density of microglial cells producing quinolinic acid is increased in the subgenual anterior cingulate cortex and anterior midcingulate cortex of suicide victims who suffered from MDD, indicating that microglia have an enhanced response to cytokine signals[82]. These clinical studies confirm that the occurrence of depression is strongly correlated with the increase in microglia-mediated inflammation. Nevertheless, another hypothesis indicates that the low levels of inflammatory status in the brain may promote depressive symptoms in patients[83]. Several clinical

evidence supports this hypothesis, including the decreased density and number of glial cells in multiple brain regions in MDD[84–86], the pro-depression effect of non-steroidal anti-inflammatory drugs[87], and the induction of depressive or suicidal behaviors by TNF-α inhibitor in individuals with enteropathy[88, 89]. Therefore, there seems to be a microglial balance in the inflammatory status of brain to influence the depressive symptoms, but the mechanism remains elucidated.

Relative to clinical data, the consequences from preclinical studies have provided more abundant evidence. Overactivation of microglia with high level of pro-inflammatory cytokines in multiple brain regions is observed in various animal models of depression[90–94], and the inhibition of microglia-mediated neuroinflammation alleviates depressive-like behaviors[95]. Microglia have been recognized as a critical component of stress sensitization and innate immune challenge[96]. LPS-induced inflammation results in depression-like behavior of mice while anti-inflammation agents effectively correct the depression-like behaviors by restoring levels of inflammatory cytokines[97]. It has been found that activation of indoleamine 2,3-dioxygenase (IDO) in microglia is indispensable for LPS-induced depression-like behaviors, which can be reversed by the microglial inhibitor minocycline, suggesting that microglia activation and inflammatory responses are crucial in LPS-induced depressive-like behaviors[98, 99]. In addition, TBI-induced hyperreactivity in microglia significantly aggravated the depressive-like symptoms induced by LPS, heightening that the microglial activity was associated with the development of LPS-induced anhedonia and despair behavior in mice[100]. Convincingly, innovative antidepressant drug ketamine has been reported to improve depressive-like symptoms in mice induced by LPS via targeting microglial production of quinolinic acid, which also is a promising biomarker of ketamine response in treatment-resistant depression[101]. Another animal model of depression is established by chronic social defeat stress, which induces the hypertrophy of microglia, increases the expression of pro-inflammatory microglia markers, and elevates the level of IL-1β in the hypothalamus, hippocampus and pituitary gland. The innate immune receptors TLR2/4 in microglia have been identified as an important mediator of repeated social defeat stress-induced social avoidance through the activation of microglia in mice[102]. Glucocorticoid signaling is also involved in the inflammatory response of microglia to stress[103–105], and the inhibition of β-adrenergic receptor signaling suppresses the effects of chronic social defeat stress. Minocycline inhibits the activation of microglia and the expression of IL-1β in the hippocampus of mice after chronic social defeat stress, thereby improving chronic social defeat stress-induced cognitive and memory impairment[106]. It is worth noting that the alteration of microglia including the unique immune-related transcriptomic signature induced by repeated social defeat stress is persistent, which could maintain a long-time period after stress[96], resulting in the following changes in other cell types. Typically, repeated social defeat stress induces increased neuronal excitability and robust inflammatory activation of microglia in lateral habenula and basolateral amygdala. Blockade of the microglia activation prevents the increase in the neuronal excitability and reverses the behavioral consequences of stress[107, 108]. Other studies show that inhibition of NLR family pyrin domain containing protein 3 (NLRP3) in microglia could improve the depression-like behavior induced by chronic mild stress and chronic restraint stress[109–111].

Microglia-neuron communication is reciprocal, in which microglia greatly influence multi-aspect of neurons, neurons also regulate microglia function through soluble factors, including chemokines, cytokines, and neurotransmitters. Neuron-derived fractalkine (CX3CL1) regulates microglia activation through binding to the receptor CX3CR1, which is enriched on microglia[112]. In the CX3CR1-deficient mice, synapse maturation defects and impairments in functional connectivity between the prefrontal cortex and hippocampus are accompanied by decreased social interactions and induce depressive-like behaviors[113, 114]. Besides, microRNAs, the small non-coding RNAs, have been reported to mediate the influence of neurons on microglia. It is found that neuron-derived miR-21-5p, miR-124 and miR-9 transport to microglia and trigger microglial alterations via exosome[115, 116]. Microglial miR-9 has been recently considered as a target for depression[117]. The direct effects of microglia on neurons in the development of depression have been revealed. Recent study has shown that abnormalities in microglia-mediated structural remodeling of neurons promote synaptic plasticity impairment and behavioral deficits induced by repeated stress. Exposure to chronic unpredictable stress results in an increase in the proportion of engulfed synapses and dendritic structures by hippocampal microglia, suggesting that microglia act as phagocytic cells, engaging in inappropriate phagocytosis of neurons, which may be associated with the depressive-like behaviors[118]. Moreover, glucocorticoid receptor has been found to promote the chronic unpredictable stress-induced depressive behaviors by enhancing microglia-mediated neuronal remodeling[119]. These studies show that in addition to mediating CNS inflammation, microglia also increase the risk of depressive-like behavior by causing defects in neural plasticity and neurotransmission.

Particularly, there is a handful of evidence supporting a new hypothesis on the mechanism

of depression, that is, microglial decline in the hippocampus is an important inducement of depression pathogenesis. Treatment of chronic stress-exposed mice with either LPS, endotoxin, macrophage colony-stimulating factor or granulocyte-macrophage colony-stimulating factor or amphotericin B liposome, all of which stimulate hippocampal microglial proliferation, partially or completely reverse the depressive-like behavior and dramatically increase hippocampal neurogenesis[120–122], suggesting that microglia stimulators could serve as fast-acting anti-depressants in some forms of depressive and stress-related conditions. In addition, the gender heterogeneity of microglia contributes to the sex differences in depression. Seney *et al* conducted a cell type-specific analysis of DEGs in MDD patients, and found that men with MDD exhibited the elevated expression of oligodendrocyte- and microglia-related genes, while women with MDD exhibited the decreased expression in markers of these cell types[123].

5 CONCLUSION

In the past two decades, great progress has been made in elucidating the function of microglia, and great interest has been generated surrounding microglia as indicators of mood disorders. However, there are still some questions to be resolved. The interactions of microglia with other types of nerve cells, as well as their role in depression, are complex and dynamic. With the development of *in vivo* real-time imaging and CRISPR-Cas9 technologies and the advent of single-cell sequencing technologies, we would make new insights into the much more complex and fascinating biology of microglia, and their interactions with other brain cells. In addition, by far the majority of studies on microglia are conducted on animals, and microglia derived from animals and humans exhibit obvious differences in multiple aspects, which may adversely impact our understanding of their role in depression. However, the emergence of multifunctional stem cell induction technology[124], 3D brain tissue culture[125] and other technologies will greatly solve the limitations of current microglia studies in animal models, providing us with the possibility to fully analyze the complex role of microglia in human depression.

Conflict of Interest Statement

The authors declare that they have no conflict of interests.

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