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Review

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Microglia: A Unique Versatile Cell in the Central Nervous System

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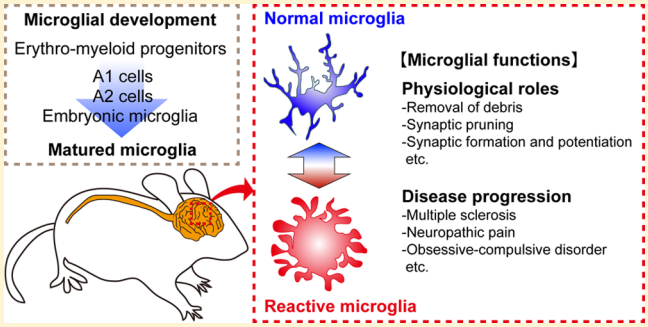
ABSTRACT: Microglia are the resident monocytic cells in the central nervous system (CNS), where they constitute the complex tissue structure together with a diverse set of cell-types, including neurons, glial cells, and vasculature. Different from other cells, microglia have distinct features, including not only their origin but functions in the CNS, and serve multiple roles within healthy and diseased CNS tissue. The present review highlights the latest advance in our understanding of the nature of microglia in the CNS, from their origin to both the physiological and pathological roles throughout their whole life.



KEYWORDS: *Microglia, brain, CNS, origin, multiple sclerosis, neuropathic pain*

THE ORIGIN AND DEVELOPMENT OF MICROGLIA

Microglia constitute ∼10% of the total cells in the adult CNS tissue.[1] Although the precise origin of microglia has been controversial for decades, recent elegant fate-mapping analysis or studies using specific gene manipulation have identified the noncommitted c-Kit$^+$ erythro-myeloid progenitors (EMPs) in extraembryonic yolk sac (YS) as primary progenitors of microglial cells,[2,3] which become YS macrophages during primitive hematopoiesis stage and migrate to the early developing brain via the primitive bloodstream[3,4] (Figure 1). These cells invade into the CNS parenchyma, subsequently giving rise to mature microglial cells. During these developing processes, acquiring a specific phenotype of microglia depends on several transcriptions factors such as PU.1 and interferon regulatory factor-8 (IRF8), which are essential for the transition from EMP to the A1 state (c-Kit^{lo} YS cells) and the differentiation from the A1 to A2 state (immature c-Kit$^-$F4/80^{hi}CX3CR1$^+$ YS macrophages), respectively.[5] Besides, runt-related transcription factor 1 (Runx1) participates in the ramification process.[6] In contrast, microglial development is independent of the transcription factor Myb,[7] which is essential for the development of circulating monocytes and tissue macrophages that originate from the fetal liver or bone marrow (BM).[7−9] In addition, signals via colony-stimulating factor 1 receptor (CSF1R) are also essential for their proliferation, maturation, and function; thus its deficiency results in the marked reduction in the number of microglia.[4,10] However, interestingly, lack of a ligand CSF1 by itself has no effect on microglial development, which is alternatively dependent on interleukin-34 (IL-34),[11,12] a second ligand for CSF1R that is principally produced by neurons.[11] Furthermore, transforming growth factor-β (TGF-β) is required for microglia persistence in the CNS but not the initial seeding from the YS.[13,14]

A recent spatial-temporal resolution fate mapping analysis in zebrafish has shown that microglia in embryonic stage are derived from the rostral blood island (RBI), the equivalent of mouse YS, whereas adult microglia originate from the ventral wall of dorsal aorta (VTA), a region crucial for definitive hematopoiesis in mouse, suggesting distinct origin for embryonic and adult microglia in zebrafish.[15] By contrast, accumulating evidence has ruled out the contribution of monocytes or BM-derived precursors, derived from hematopoietic stem cells during the definitive hematopoiesis, to the residual pool of microglia in the CNS under normal condition in mouse.[16,17] In the adult healthy CNS tissue, microglia are embedded in the heterogenic population of resident cells including neurons, astrocytes, and oligodendrocytes and maintain themselves by self-renewal throughout life with virtually no contribution from circulating or bone-marrow cells.[2−4,18,19] Following diphtheria toxin-mediated depletion of microglia that does not affect BBB integrity, the CNS tissue repopulates microglia, which originate exclusively from an internal CNS-resident pool in an IL-1receptor-dependent manner.[20] Additionally, it has recently been defined that microglia can be repopulated from nestin-positive progenitor cells, potentially the remaining microglia, in the adult brain under a specific condition, namely, after drug-mediated elimination using CSF1R inhibitors.[21]

A recent study has highlighted the importance of the host microbiota for shaping the phenotype of microglia in the healthy brain.[22] In mice that are not colonized by any physiological host microbiota, so-called "germ-free" (GF)

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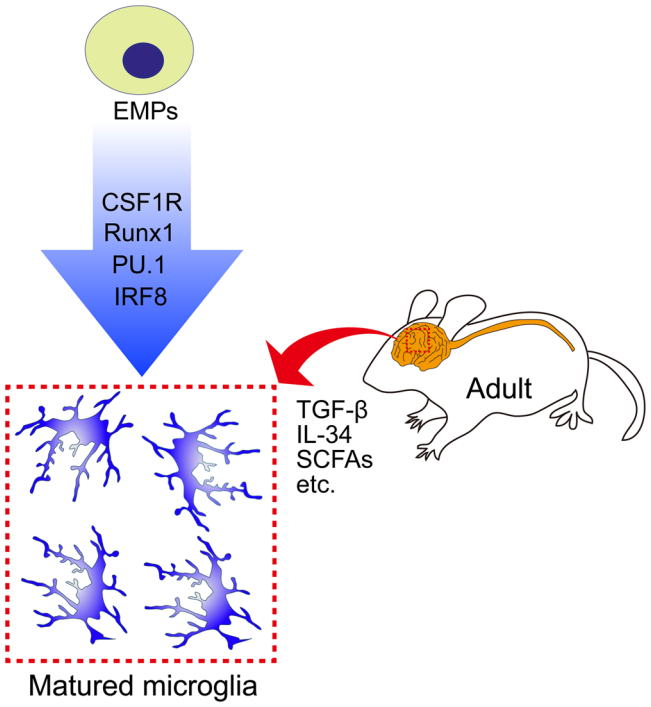

Figure 1. Microglial ontogeny and development. Microglia originate from the erythro-myeloid progenitors (EMPs) in yolk sac. The differentiation and maturation of microglia are dependent on signals via colony-stimulating factor 1 receptor (CSF1R; proliferation and maturation), runt-related transcription factor 1 (Runx1; ramification), Pu.1 (transition from EMPs to A1 state), and interferon regulatory factor-8 (IRF8; differentiation from A1 to A2 state). The persistence of adult microglia requires continuous signals of transforming growth factor-β (TGF-β), interleukin-34 (IL-34), and bacterially derived short-chain fatty acids (SCFAs).

mice, microglia exhibit markedly immature signatures with functional deficiency different from microglia in conventionally housed mice.[22] Interestingly, microglial phenotype in adult mice is highly plastic; thereby microglial immaturity can be induced by drug-mediated transient deletion of gut microbiota; conversely, microglial abnormalities in GF mice could be canceled by recolonization of the gut with complex microbiota.[22] In addition, such microglial defects are recapitulated in mice lacking a receptor for the short-chain fatty acid (SCFA), a microbiota-derived bacterial fermentation product, indicating a pivotal role of gut environment for the formation of microglial phenotype and CNS homeostasis.[22] Microbiota have also been considered to control brain functions such as mental condition and stress responses,[23,24] and thus a recent article mentions that the microbiota may bring a new type of therapeutic agent, "psychobiotics", against anxiety, depression, and other mood disorders.[25] In light of the fact that the state of microglia has a great impact on the surrounding environment, microglia might be a crucial intermediary for microbiota-mediated functional alterations in the CNS.

PHYSIOLOGICAL ROLES OF MICROGLIA

Microglia in the normal physiological brain show a complex morphology with a compact cell body and highly branched processes, with which microglia routinely scan the surrounding territory to sense pathological alterations or disturbances.[1,26−28] When focal tissue damage occurs in the CNS, a dense and rapid extension of microglial processes toward the site of lesion takes place within several minutes,[29] by which microglia prevent the spread of the lesion as a "protector". These early responses are mediated by purinergic receptors, such as $P2Y_{12}$ receptor, stimulated by extracellular adenosine triphosphate (ATP) and adenosine diphosphate (ADP), which are leaked or released from dying or injured cells.[29,30]

In addition, as an expert on phagocytosis, it has long been assumed that their primary role in the CNS tissue is to engulf and clear foreign substances or apoptotic neurons that die as a result of programmed cell death,[31] through which microglia destroy infectious agents, remove cell debris, and promote tissue repair.[32] However, beyond such a classical idea, it has recently been revealed that microglial processes transiently contact synapses for monitoring neuronal activity and synaptic function with receptors for multiple types of neurotransmitters[26,27,33,34] and eventually prune them via phagocytosis in a neuronal-activity dependent manner[33,34] (Figure 2); thereby

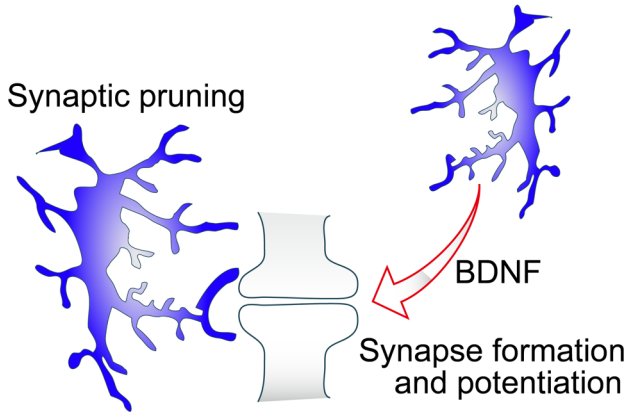

Figure 2. Interaction between microglia and neurons under physiological conditions. Microglial processes transiently contact synapses for monitoring neuronal activity and synaptic function and in some cases prune them in an activity-dependent manner. Microglia-derived BDNF contributes to synaptic formation and potentiation.

microglia control the number of synaptic connections properly. These phenomena are thought to play a key role in shaping proper neural circuits both at early postnatal stages[35,36] and in adult neurogenic niches.[37,38] In addition, microglia may actively engulf viable neurons by phagocytosis, leading to neuronal death.[39] The question then arises as to how microglia recognize and interact with neuronal components. A recent paper has shown the involvement of complement factors including complement receptor 3 (CR3) for microglia-mediated synaptic remodeling in the postnatal retinogeniculate system.[36] In the CNS, CR3 is expressed only by microglia, and its ligand, complement component C3, is localized to synaptically enriched regions.[36] Of note, the synaptic elimination by microglial phagocytosis is lost in mice lacking CR3,[36] indicating that microglia utilize the complement-dependent signaling to eliminate synapses.

Recently, it was shown that microglia are also responsible for synaptic plasticity and learning (Figure 2). Mice in which microglia are specifically depleted with a diphtheria toxin-mediated system[40] showed deficits in motor learning-dependent synapse formation following multiple learning tasks.[41] These phenomena are recapitulated when brain-derived neurotrophic factor (BDNF), a neurotrophin that is known to regulate synaptic plasticity,[42] is deleted from microglia,

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indicating that microglia participate in contextual fear conditioning and motor learning and in motor-learning-associated spine formation by supplying BDNF.[41] In addition, microglia also play a role in supporting neuronal development in cortical lamina V via neurotrophic insulin-like growth factor 1 (IGF-1) and fraktalkine (CX3CL1)−CX3CR1 signals.[43]

Overall, considering the recent findings that demonstrate the crucial function of microglia by virtue of highly dynamic processes in synaptic elimination and plasticity, learning, and neurogenesis, microglia are absolutely essential for the normal physiological functioning of the adult CNS. Thus, dysregulated microglia might be responsible for the pathogenesis of various CNS diseases.

MICROGLIA IN THE PATHOGENESIS OF THE CNS DISEASES

Multiple Sclerosis and Microglia. Multiple sclerosis (MS) is an autoimmune disease that is characterized by a progressive axonal damage as a consequence of loss of oligodendrocytes and demyelination neurodegeneration.[44] Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model for MS, which is generated by peripheral immunization with myelin peptides in an adjuvant context.[45] The characteristic features of this model are massive infiltration of peripheral immune cells, such as T lymphocytes, monocytes, and dendritic cells (DCs), into the CNS tissue associated with prominent blood−brain barrier (BBB) disruption.[44,46] Within such a complex environment, microglia get quickly activated and interact with several types of cells including infiltrating cells, damaged neurons, and oligodendrocytes, which may produce diversity in microglial function.

Despite recent advances in our knowledge about MS and EAE, the contributions of microglia to the generation, the perpetuation, and the resolution of this inflammatory CNS disease still remain a matter of controversy. For instance, it has been shown that following immunization the E3 ubiquitin ligase Peli1, which is abundantly expressed in microglia, promotes microglial activation concomitantly with the induction of chemokines and proinflammatory cytokines, thereby encouraging recruitment of T lymphocytes into the CNS during the course of EAE induction.[47] Mice lacking Peli1 show less severe EAE, whereas the induction of inflammatory T cells occurs in the peripheral lymphoid organs.[47] These data support the hypothesis of significant microglial involvement in the induction of EAE. In contrast, using CCR2−RFP:CX_3CR1−GFP double-reporter mice in which $CCR2^+$ monocytes and $CX3CR1^+$ microglia express RFP or GFP, respectively, it has shown that resident microglia exhibit a distinct gene expression profile from that of monocyte-derived macrophages,[48] with which the authors conclude that microglia display a rather inert signature for the development of EAE symptoms.

To discriminate the functions of microglia from those of other monocytic cells (e.g., macrophages and monocytes) in the body is still challenging because they share a series of marker proteins.[49] However, recently, a novel Cre−LoxP-based methodology that specifically targets microglia without affecting monocyte-derived macrophages was developed using a mouse line in which a tamoxifen (TAM)-inducible Cre-recombinase is expressed under the control of the *Cx3cr1* promoter (*Cx3cr1-Cre*^{ERT2} mice).[8,50] When activated by TAM, Cre-mediated recombination occurs only for a limited period in cells expressing CX3CR1, and at a later time point, only long-lived microglia still carry Cre-mediated mutations, whereas other short-lived cells will be replaced by nonmutated progenitors. By taking advantage of this system, Goldmann et al. reported that microglia-specific deletion of TGF-β-activated kinase 1 (TAK1), a signal regulator required for the activation of various kinases, causes significantly decreased production of the proinflammatory mediators and confers mice resistant to EAE.[50] Consistently, those mice that had received immunization showed a significant reduction in both infiltration of immune cells and demyelination.[50] Thus, these findings highlight the detrimental role of microglia in EAE.

A cuprizone model that also captures several aspects of MS pathology is generated by administering a copper chelating toxin cuprizone, which causes cell death of oligodendrocytes by disrupting metabolic processes.[51] In contrast to the EAE model, the BBB in this model remains intact with no recruitment of peripheral immune cells to the CNS.[17,52] Interestingly, deletion of the voltage-gated proton channel Hv1, which is specifically expressed in microglia and participates in NADPH oxidase-dependent reactive oxygen species (ROS) production in the CNS,[53] partially prevents demyelination following cuprizone exposure,[54] indicating that microglia may play a role for the disease progression.

Microglia and Neuropathic Pain. Neuropathic pain is one of the most debilitating pain conditions, which develops concomitantly with neuronal injury and degeneration caused by invasive cancer, infection, autoimmune disease, or traumatic injury.[55] A hallmark symptom of neuropathic pain is tactile allodynia, characterized by pain hypersensitivity to innocuous stimuli, which is can be assessed using animal models subjected to peripheral nerve injury (PNI). When injuries occur in peripheral nerves, microglia undertake a series of changes in morphology and gene expression in spinal dorsal horn (SDH),[56] where the terminus of the injured nerve exists. During this process, SDH microglia undergo proliferation,[57] thereby markedly increasing their number.[57−59] In addition, it was shown that BM-derived cells injected intravenously infiltrate into the SDH parenchyma ipsilateral to the injury and colonize as microglia-like CNS macrophages.[60] However, it remains controversial whether these BM cells infiltrate into the parenchyma of the spinal cord after PNI because these experiments are accomplished under specific conditions such as whole-body irradiation,[60] which is known to damage the BBB and could produce artificial effects.[19] Activated spinal microglia exhibit dramatic changes in the expression of various genes including purinergic P2X4 receptor (P2X4R).[58,61,62] Following PNI, mice in which this gene is deleted do not express pain behaviors, whereas the characteristic microgliosis is not affected.[62,63] Therefore, such an increase in number of microglia the SDH is a direct consequence of peripheral nerve damage but is not *per se* sufficient to cause pain hypersensitivity.[64]

What mechanism controls a reactive state of microglia? Recently, IRF8 was shown to be increased in spinal microglia after PNI.[65] Forced IRF8 expression in cultured microglia promotes the transcription of genes associated with reactive states, which mimics the gene profile of spinal microglia after PNI.[65] Furthermore, microglia lacking IRF8 failed to increase the expression of a variety of genes, suggesting that IRF8 is a crucial player for reactive microglia.[65] In addition, with stimuli of fibronectin upregulated in the spinal cord of the PNI-animals,[66] IRF5 further activates P2X4R expression through direct binding to its promoter in microglia.[67] Stimulating these

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receptors with extracellular ATP, which is supplied through an as-yet-unknown mechanism in the spinal cord, results in the release of bioactive factors including BDNF,[62,68] which causes neurotransmission alterations such as the inhibition of the inhibitory system or the enhancement of excitatory synaptic transmission in the dorsal horn neurons of the spinal cord.[61,68] These pathological alterations convert innocuous inputs to a nociceptive signal output to the brain, thereby contributing to the abnormal sensory perception related to pain hypersensitivity (Figure 3). However, such a microglia-mediated

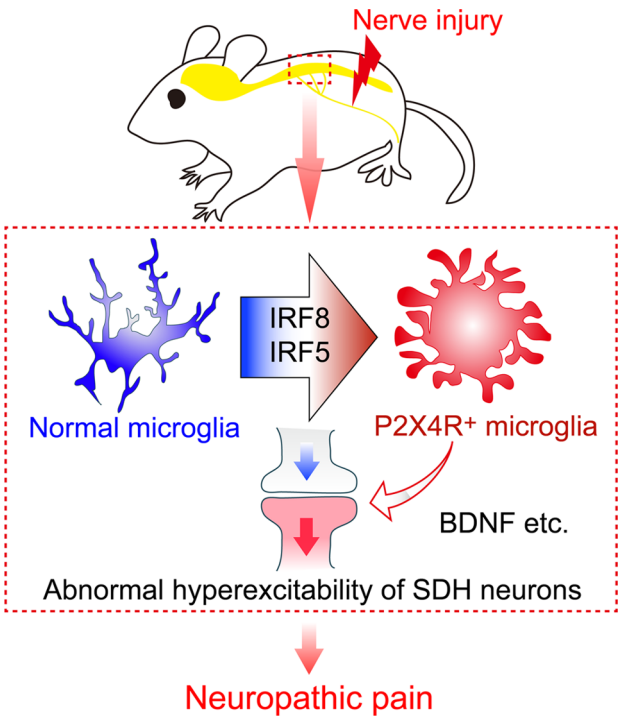

Figure 3. Reactive microglia are crucial for the pathogenesis of neuropathic pain. Following nerve injury, microglia transform into a P2X4R-expressing reactive phenotype in an IRF8- and IRF5-dependent manner and release bioactive factors including BDNF, which elicits abnormal excitability of spinal dorsal horn (SDH) neurons, resulting in the production of neuropathic pain.

pathological system for neuropathic pain may be functioning only in males. A recent paper published by Sorge et al. provided a novel concept for the generation of neuropathic pain in which microglia are not required for neuropathic pain in females.[69] Rather adaptive immune cells including T lymphocytes were shown to fulfill a similar role to microglia for the production of pain hypersensitivity.[69] In fact, although spinal microgliosis is indistinguishable between the two genders, inhibiting microglial P2X4R or BDNF is insufficient to reverse pain hypersensitivity in female mice.[69] Furthermore, minocycline, an inhibitor of microglial activation, has no effect on pain behavior,[69] indicating that a gender difference occurs in the spinal cord.

Such a strong pain symptom frequently appears concomitantly with the disease progression of MS. In addition, robust microglial activation accompanied with P2X4R upregulation was evident in the SDH of EAE animal models.[70,71] Although the underlying mechanism of this pain associated with MS remains poorly understood, it is most likely that activated microglia may also contribute to this pain pathology.

A Specific Microglia Phenotype Expressing Hoxb8 Is Related to a Compulsive Disorder. Recent genome-wide expression profiling technology has provided detailed transcriptome data for microglia,[13,72−74] in which microglia are compared with other cells in the CNS or other monocytic cells, and these data have suggested that there may be several types of microglia with distinct transcriptomes in the CNS. Chen et al. showed a unique subpopulation of microglia that express the transcription factor Hoxb8.[75] These Hoxb8$^+$ microglia represent ∼30% of all microglial cells in the adult brain. Mice lacking Hoxb8 function or with the specific deletion of Hoxb8 in Tie2$^+$ myeloid cells show a specific behavior manifested by excessive grooming and hair removal,[75] which is very similar to that described for humans with the obsessive-compulsive disorder trichotillomania.[76] Interestingly, such behavioral phenotypes are reversed by transplanting BM cells taken from mice expressing Hoxb8 following irradiation, which allows Hoxb8$^+$ myeloid cells to replace Hoxb8-deficient microglia in the CNS tissue. However, it remains to be investigated how the irradiation changed microglia phenotypes in chimeric animals. Thus, defects in the normal function of Hoxb8 in microglia may play a role in this disorder.

■ CONCLUDING REMARKS

The recent substantial evidence demonstrates that microglia are a highly unique and plastic cell population in the CNS that serve multiple roles not only in development and homeostasis of the CNS but in also deterioration of or recovery from pathological conditions though inflammatory as well as noninflammatory responses. Thus, defining specific microglial molecules or subpopulations within anatomically distinct CNS regions associated with specific vital phenomena will be necessary for deepening our understanding about the nature of the CNS, which may provide further exciting insights into the functions of microglia and clues to develop novel therapeutics for the management of CNS diseases in the future.

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Notes

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■ ABBREVIATIONS

CNS, central nervous system; EMP, erythro-myeloid progenitors; YS, yolk sac; CSF1R, colony-stimulating factor 1 receptor; TGF-β, transforming growth factor-β; RBI, rostral blood island; VTA, ventral wall of dorsal aorta; ATP, adenosine triphosphate; ADP, adenosine diphosphate; CR3, complement receptor 3; BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor 1; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; BBB, blood−brain barrier; ROS, reactive oxygen species; PNI, peripheral nerve injury; SDH, spinal dorsal horn

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