



Review article

A richer and more diverse future for microglia phenotypes

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ABSTRACT

Microglia are the only resident innate immune cells derived from the mesoderm in the nerve tissue. They play a role in the development and maturation of the central nervous system (CNS). Microglia mediate the repair of CNS injury and participate in endogenous immune response induced by various diseases by exerting neuroprotective or neurotoxic effects. Traditionally, microglia are considered to be in a resting state, the M0 type, under physiological conditions. In this state, they perform immune surveillance by constantly monitoring pathological responses in the CNS. In the pathological state, microglia undergo a series of morphological and functional changes from the M0 state and eventually polarize into classically activated microglia (M1) and alternatively activated microglia (M2). M1 microglia release inflammatory factors and toxic substances to inhibit pathogens, while M2 microglia exert neuroprotective effects by promoting nerve repair and regeneration. However, in recent years, the view regarding M1/M2 polarization of microglia has gradually changed. According to some researchers, the phenomenon of microglia polarization is not yet confirmed. The M1/M2 polarization term is used for a simplified description of its phenotype and function. Other researchers believe that the microglia polarization process is rich and diverse, and consequently, the classification method of M1/M2 has limitations. This conflict hinders the academic community from establishing more meaningful microglia polarization pathways and terms, and therefore, a careful revision of the concept of microglia polarization is required. The present article briefly reviews the current consensus and controversy regarding microglial polarization typing to provide supporting materials for a more objective understanding of the functional phenotype of microglia.

1. Introduction

In the early 19th century, central nervous system (CNS) anatomists used different stains to reveal the morphology of neurons to gain insights into the anatomy of the brain at a time when glial cells were yet to be discovered. In the mid-19th century, German pathologist Rudolf Virchow used the term “glue” (the word “glue” originated from ancient Greek) to describe the entire non-neuronal compartment of the CNS [1,2]. Virchow believed that glial cells are a type of connective tissue that connects and supports various neurons; he also found that this tissue contains various cells. However, the concept of microglia was still not proposed at that time. In the early 20th century, microglia were discovered by the Spanish neuroscientist Río Hortega as a distinct cell type. Hortega [3] showed that microglia are mainly derived from primordial macrophages produced in the yolk sac and migrate from the external environment of the brain to

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the brain parenchyma in the early stage of brain development (approximately 10.5 days of embryonic development). Before the early 21st century, researchers did not recognize the critical role of microglia in the CNS. In subsequent studies, scientists found that microglia in the CNS can interact with neurons, astrocytes, and oligodendrocytes and play a persistent role in immune surveillance [4–6]. The phenotype of microglia has been a key issue while investigating its origin and function. In particular, with the advancement in sequencing technology, microglia have been found to occur from the periphery to the center of the CNS. This not only enabled researchers to achieve great milestones in the study of microglia in the past 20 years, but it also made them aware of the current challenges in this field. According to some researchers, the classification of microglia based on its polarization, that is, the strict division of microglia based on its M1/M2 polarization is a too simplified theory, and they believe that the phenotype and function of microglia are diverse. Presently, the review of microglial cell typing is mostly focused on M1/M2 phenotypes. This article initially presents the view that microglial polarization is based on M1/M2 phenotypes. In the context of the current challenges for this consideration and the several newly discovered microglial subtypes, the present article will provide support for a more comprehensive and objective understanding of microglial polarization typing and then provide novel ideas for developing new brain neural research and related treatment methods based on microglia.

2. M1/M2 polarization of microglia

As resident immune cells in the brain, microglia are involved in the development of brain tissue structure, maintenance of the physiological functions of the CNS, and restoration of impaired immune responses and subsequent repair. Under physiological conditions, microglia maintain a relatively quiescent neuron-specific monitoring phenotype (M0 phenotype) and play an “immune surveillance and defense” role in the microenvironment of nerve cells [7–9]. Microscopic dynamic real-time observations revealed that under physiological conditions, microglia appear to be in a “quiescent” state; however, they actually extend out of the cell body, constantly monitoring for any potential damage in the brain. Thus, microglia perform a complete assessment of the brain for any damage every few hours. To maintain this immune surveillance state, microglia need to receive a continuous “all is well” signal from the surrounding cells. The lack of this signal will immediately prompt microglia to conduct an immune status investigation [10–12]. In the state of pathological injury, microglial cells are rapidly activated and polarized, accompanied by a series of phenomena such as proliferation, chemotaxis, phagocytosis, migration, and cytokine secretion; these cells then rapidly generate complex responses depending on the degree of injury. If the injury is mild, the cells at the injury site send a “find me” signal; subsequently, microglial cells are rapidly activated to the M2 state, which is characterized by distal branches, small cell body-like changes, and secretion of anti-inflammatory factors. The M2 microglial cells can promote cell tissue repair and regeneration, enhance phagocytic activity, and play a neuroprotective role. If severe or persistent tissue damage occurs, the cells at the injury site send an “eat me” signal. Subsequently, microglial cells are completely activated into the pro-inflammatory or toxic M1 state, which is characterized by amoeboid changes with large and round cell bodies and thick protrusions [13–15]. These cells mainly exert cytotoxic effects and can quickly remove infected cells and debris of dying cells. Fig. 1 shows the morphological changes of microglia before and after polarization. Excessive activation of M1 microglia, however, can cause loss, damage, and degeneration of neuronal function, and M1 microglia have been shown to play an important role in cerebrovascular diseases and neurodegenerative diseases [8,14]. In summary, several studies have shown that M1 and M2 microglia perform different functions at different stages of inflammation and disease; in other words, M1 and M2 microglia switch between functional phenotypes according to different environmental stimuli.

2.1. M1 polarization of microglia (classically activated)

M1 microglia are considered to be pro-inflammatory cells. Following appropriate stimuli, M1 microglia serve as the first line of

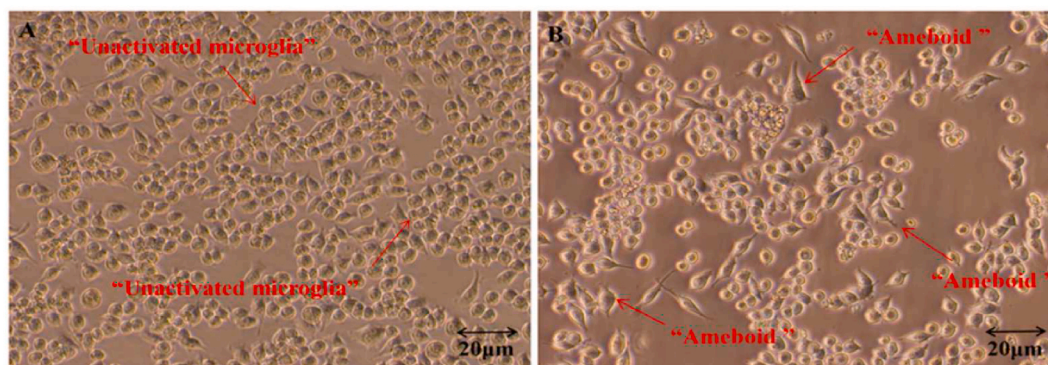


Fig. 1. Morphological changes in microglia before and after polarization. A: Microglia in the unactivated state of growth in good condition, with a clear background. The cell body is small and occasionally observed between cell synaptic connections. The microglial cell has a full three-dimensional shape with fewer branches. B: After activation, microglia cell debris increased. The background is not clear; with an increase in the size of the cell body, the emergence of “amoeba-like cells,” increased synaptic connections between the cell bodies, and increased branches.

defense of the neuroimmune system, and they usually appear within a few hours of stimulation [16–18]. Microglia use a series of immune receptors to recognize harmful stimuli, such as Toll-like receptors, nucleotide oligomerization domain receptors, and scavenger receptors. Activated microglia release high levels of pro-inflammatory cytokines such as interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α), and interleukin-1 β (IL-1 β), and a sustained inflammatory response continues to aggravate brain damage. In addition to pro-inflammatory cytokines, M1 microglia secrete a large amount of reactive oxygen species (ROS); proteolytic enzymes, including heme oxygenase 1 (HO-1), matrix metalloproteinases (MMPs), and inducible nitric oxide synthase (iNOS); and interleukin 12 (IL-12). This process causes T cells to trigger an adaptive immune response and remove foreign pathogens [19–22].

In many model studies, the level of M1 microglia was found to increase when bacterial components such as lipopolysaccharide (LPS) or infection-related signals such as IFN- γ increase [23–25]. When the central nervous system experience a sterile inflammatory response caused by trauma or ischemic reperfusion, microglia polarize into the M1 type and secrete pro-inflammatory cytokines, including interleukin-1 α (IL-1 α), interleukin-6 (IL-6), IL-12, chemokines, reduced nicotinamide adenine dinucleotide phosphate (NADPH), surface antigen CD40, etc. [26–28]. Microglia models in rodents have shown that polarized M1 microglia induced by LPS and IFN- γ inhibit phagocytosis. The outcome of M1 microglia polarization depends on many aspects, including the production of iNOS, ROS, or NLRP3 inflammasome.

In conclusion, classically activated M1 microglia mainly secrete several pro-inflammatory factors that are involved in the process of pathogen clearance and alleviation of inflammatory injury of diseases. Microglia with M1 phenotype can inhibit phagocytosis and promote inflammation which can produce several inflammatory mediators that cause neuronal damage and further activate the release of pro-inflammatory factors, leading to a neuroinflammatory cascade. Moreover, following the overactivation of microglia with M1 phenotype, the inflammatory response of the CNS is further amplified and sustained, thereby interfering with nerve repair.

2.2. M2 polarization of microglia (alternative activation)

It is generally believed that M2 microglia are protective cells that secrete anti-inflammatory factors and upregulate neuroprotective factors. They play an active role in immune defense by providing protection against organisms, fighting infections, acting as antigen-presenting cells, killing tumor cells, performing tissue repair, providing chemotaxis orientation, and eliminating invading bacteria.

Under various microenvironment stimuli, M2 microglia can further differentiate into three subtypes: M2a, M2b, and M2c [2,29,30]. M2a and M2b play an immunoregulatory role or promote M2 immune response, while M2c inhibits immune response and tissue remodeling. M2a is activated by interleukin-4 (IL-4) or interleukin-13 (IL-13), which can promote fibrosis and repair tissues [31]. M2b type is jointly induced by Toll-like receptors or IL-1 receptors combined with immune complexes [31]. M2c type is stimulated by interleukin-10 (IL-10), transforming growth factor- β (TGF- β), and glucocorticoid release in response to specific anti-inflammatory effects [31]. Other interleukin cytokines such as interleukin-3 (IL-3), interleukin-21 (IL-21), and interleukin-33 (IL-33) and chemokines CCL2 and CXCL4 can also induce microglia to undergo M2 polarization [23,32,33]. The M2 polarization of microglia is identical to the polarization process of peripheral macrophages. This association was confirmed in *in vitro* experiments. However, in the absence

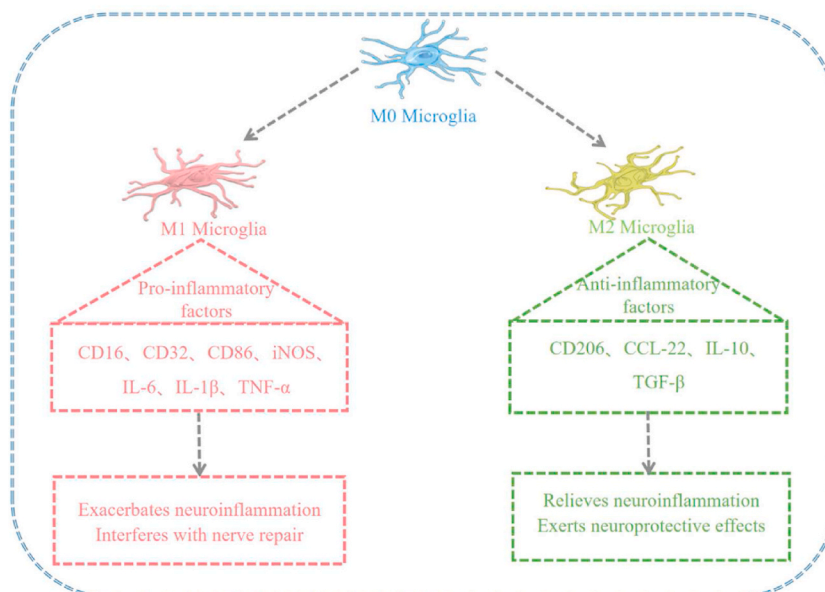


Fig. 2. Microglia polarization diagram: M1 microglia release pro-inflammatory factors and toxic substances to kill pathogens, while M2 microglia exert neuroprotective effects by promoting tissue repair and regeneration. Overactivated M1 microglia can cause neuronal disability, damage, and degeneration and play an important role in cerebrovascular diseases, neurodegenerative diseases, neurodevelopmental disorders, and mental disorders.

of IL-4 or IL-13, microglia also polarize to M2 in aseptic wounds. This process suggests the presence of an alternative stimulus. In this process, M2 microglia are derived from M1 microglia, and they are transformed from pro-inflammatory microglia in the circulatory system to mature microglia with the ability to repair [34–36]. Therefore, the intrinsic phenotype of cells may vary due to different sources and environments.

M2 microglia promote inflammation resolution and restore homeostasis through anti-inflammatory factors such as IL-10, IL-13, TGF- β , and arginase 1 (Arg1). This includes reducing the levels of inflammatory factors by producing IL-10 and increasing the phagocytosis of apoptotic cells through IL-10-stimulated cells. In contrast, in the absence of IL-10 overexpression, the phagocytosis of macrophages is not enhanced [37–39]. M2 microglia polarization is characterized by the activation of peroxisome proliferator-activated receptors, following which M2 microglia express chemokines (CCL17, CCL22, and CCL24) [40–42]. Researchers believe that glatetomil acetate, a drug used to treat multiple sclerosis, induces the expression of insulin-like growth factor 1 (IGF-1) in microglia to exert neuroprotective effects. In IL-4-deficient model mice, the clinical symptoms of multiple sclerosis were observed to be aggravated, thus, suggesting that IL-4 deficiency is associated with reduced M2 microglial phenotype [33,43,44]. Microglial cells induced by LPS and IFN- γ damage neurons, whereas those induced by IL-4 stimulate neuronal synaptic growth. This series of studies have established a relationship between the M2 microglial phenotype and neuronal repair and damage reduction [45,46]. A schematic diagram of microglial polarization is shown in Fig. 2.

In summary, the current belief is that M2 microglia are the key cells involved in repair after CNS injury, and they possess neuroprotective effects. Compared to the pro-inflammatory effects of M1 microglia, M2 microglia promote the release of anti-inflammatory factors, thus showing an anti-inflammatory effect and inhibiting immune response. M2 microglia can also reduce local inflammation by engulfing cell debris at the injury site, thereby promoting the recovery of brain tissue and participating in damaged tissue remodeling. M2 microglia can be further divided into M2a, M2b, and M2c subtypes. Microglia can be categorized into different subtypes according to different functions, thus, suggesting the diversity of microglia phenotypes. A schematic diagram of Microglia activation and inactivation diagram is shown in Fig. 3.

3. Questioning the view that microglial polarization is an M1/M2 phenotype process

The microglial subtype was originally proposed by the Spanish neurologist Río Hortega in the early 20th century; however, it was not until 2016 that the idea was re-proposed. Following the emergence of photon imaging, genome-wide transcription analysis, and epigenetic analysis, the research on microglia entered a white-hot stage. An increasing number of researchers believe that the polarization pattern of M1 and M2 microglia is a simplified form to interpret data without the determination of the individual source and function of microglia. The description of the M1/M2 phenotype hinders the establishment of more meaningful microglia polarization pathways and terms in academic research and should therefore be abandoned [47].

3.1. Naming convention used for peripheral macrophages is not suitable for microglia

The M1/M2 polarization pattern of microglia is identical to that of macrophages; however, several lines of evidence show a large difference between macrophages and microglia in peripheral blood [34,48–50]. For example, the transcriptomes of microglia and

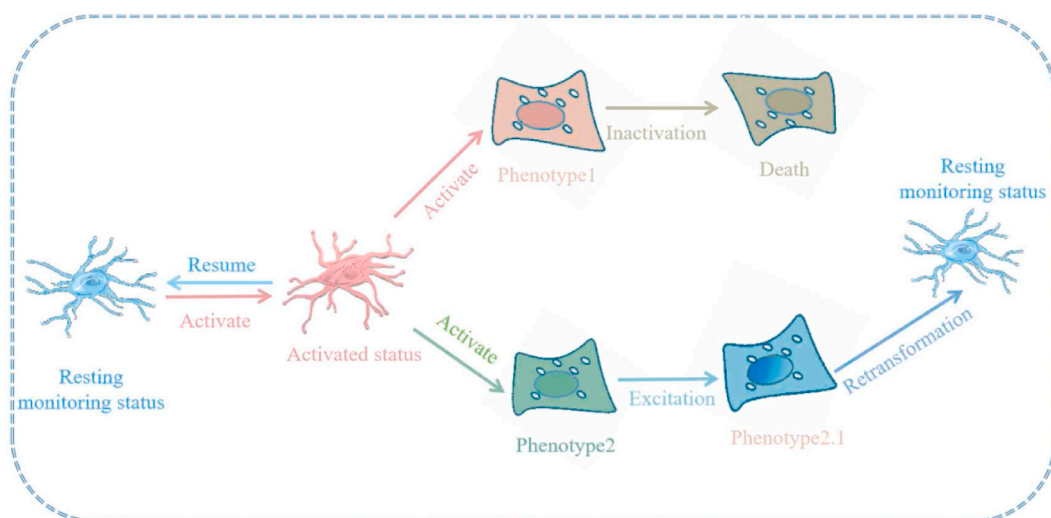


Fig. 3. Microglia activation and inactivation diagram: Resting microglia are constantly in an “immune surveillance” state. After stimulation, microglia convert to an activated state and then return again to the resting state. Following stimulation, microglial cells change their morphological appearance and become “amoeba-like” glial cells, which respond to external stimuli, play an immunological role, and then return to the resting state. Some microglial cells are inactivated and die during the immune response.

macrophages from blood and tissues are significantly different; moreover, microglia and mononuclear macrophages show significant differences in expression profile, function, and structural characteristics. These studies showed that activated microglia and macrophages derived from monocytes are completely different cells. Infiltrating macrophages after tissue damage or infection do not follow the M1/M2 polarization pattern. If the nomenclature of peripheral macrophage typing is applied to microglia, we will have an insufficient understanding of the concept of microglial typing. Furthermore, peripheral macrophages themselves include dozens of different types of cells. The nomenclature of M1/M2 is only a macroscopic and broad concept [51–53]. Considering the irreplaceable role of microglia in the CNS, this nomenclature may not be appropriate for microglia.

Researchers also consider that if this model is still used, it will seriously hinder the progress of research on microglia. The specific reasons include the following aspects [54]. First, the classification of M1/M2 is made to enable *in vitro* culture of microglia by using a single stimulation mode, which is inconsistent with the highly complex environment of the human CNS [55]. Second, according to recent studies, the phenomenon that microglia polarize only to the M1 or M2 phenotype does not occur exclusively in the body [56]. Finally, the M1/M2 polarization of macrophages is an inflammatory response that occurs when infiltrating monocytes or bone marrow-derived macrophages encounter pathogens, dead cells, bacterial debris, or tumor cells in diseased tissues. In contrast, microglia are resident macrophages in the body that are different from the macrophages occurring in the hematopoietic circulatory system [57,58]. On the basis of these reasons, the simple division of microglia into the M1/M2 phenotype may not be applicable.

3.2. Microglia subtypes should not be classified solely on the basis of molecular characteristics and markers

Vassilis Stratoulis et al. believed that [59] microglia may constitute a cell community in which different subtypes exhibit varying properties, perform different physiological functions, and respond differently to stimuli. Stratoulis suggested that microglia subtypes should be classified according to function rather than molecular characteristics and markers. There is also new evidence that different phenotypes of microglia exhibit varying functions, which are determined not only by their environment but also by their intrinsic characteristics. That is, such cells are a highly plastic single-cell phenotype with various different functional phenotypes, thus exerting different physiological and pathological effects. This study also recognized that the multiple activation states of microglia are not entirely consistent with the M1/M2 classification currently used at this stage. Microglia perform multiple functions through various phenotypes, each of which corresponds to a different phenotype.

Some evidence also shows that the different phenotypes of microglia exhibit different intrinsic characteristics acquired during the development and maturation of the CNS. Under physiological conditions, these subtypes coexist in the CNS. Furthermore, under pathological conditions, these cell subtypes undergo different phenotypic transformations according to different stimuli to perform varying physiological functions. Therefore, researchers suggest that microglia subtypes should be classified according to the function of microglia rather than according to their molecular characteristics [60–62].

4. Possible microglial subtypes

Researchers believe that microglia exhibit varying levels of response to different stimuli. Although microglia are widely distributed in the CNS, their distribution varies in different regions. In an unbiased single-cell RNA sequencing study [56], 47 different cell subtypes were identified by analyzing the brain and hippocampus of unstimulated adult mice, and two of these cell subtypes belonged to microglia. Microglia may constitute a cell community in which different subtypes exhibit different properties, perform varying physiological functions, and respond differently to stimuli.

4.1. “Satellite” microglia

In 1919, Rio-Hortega found that the cell body of a type of microglia was associated and interacted with the initial segment of a neuronal axon. The researcher named this type of microglia as “satellite” microglia, which was located on the axon side of the neuron cell body and overlapped with the initial segment of the neuronal axon (i.e., the part of the neuron potential initiation). “Satellite” microglia are found in the development stage of mice and during the adult stage, and they also occur in the cerebral cortex of primates; thus, they are closely related to neuronal excitation [63,64]. Researchers believe that “satellite” microglia may be a transitional state in which microglia exert immune surveillance [65]. Because no markers of “satellite” microglia have been found, it is not yet possible to determine the intrinsic differences between them and microglia. Wogram et al., however, confirmed that “satellite” microglia mainly exist in the cerebral cortex, thalamus, and hippocampus [66]. Stowell et al. observed the dynamic migration process of “satellite” microglia through two-photon *in vivo* imaging and observed their interaction with the proximal dendrites of neurons [67,68]. These microglia also show differences in self-renewal and transformation under normal physiological conditions and LPS stimulation. Presently, there are a limited number of studies on the function of “satellite” microglia; thus, resulting in an insufficient understanding of many aspects of these microglia. We expect that further exploration of these cells will enable us to elucidate more new functions of microglia.

4.2. KSPG microglia

Keratan sulfate proteoglycan (KSPG) is an extracellular matrix molecule that forms boundaries for axon growth in the developing brain and spinal cord. KSPG microglia, located on the extracellular matrix and cell surface, are keratin sulfate-positive microglia detected around motor neurons in amyotrophic lateral sclerosis [69,70]. Tuszyński et al. [71] used 5D4 monoclonal antibodies in the

brain of healthy adult rats for *in situ* observation and found that KSPG microglia showed heterogeneity, which contributed to axonal growth and cell adhesion. In particular, 5D4-KSPG microglia are expressed by branch microglia subsets, which completely contrasts with amoeboid microglia. The expression of 5D4-KSPG microglia significantly varied in different varieties of inbred rats. A subset of microglia expressing 5D4-KSPG was also detected in the mammalian spinal cord [72]. Although 5D4-KSPG microglia are abundantly found in the hippocampus and brainstem, only a few of them are detected in the cerebellum and cerebral cortex. Previous studies have shown that 5D4-KSPG microglia and 5D4-KSPG-negative microglia coexist in the same region of the CNS; however, these studies suggest the existence of two different subtypes of KSPG microglia. On the basis of the reactivity of KSPG microglia, a comprehensive and systematic approach is needed.

4.3. *Hoxb8* microglia

Compared to typical microglial subtypes, *Hoxb8* microglial cells differ in molecular characteristics and spatial distribution. Mice carrying the *Hoxb8*-Cre and ROSA26-YFP genes were hybridized to track the expression of YFP-*Hoxb8* [73]. In the adult brain, microglial cells were the lone cells that showed the YFP signal, i.e., the localization of the YFP signal revealed the presence of microglial cells throughout the brain, especially in the cerebral cortex. Transcriptional analysis of *Hoxb8*-positive and *Hoxb8*-negative microglia revealed significant differences in the expression of only 21 genes [74]. Compared to non-*Hoxb8* microglia, *Hoxb8* microglia express microglia-specific genes such as *Tmem119*, *Sall3*, and *Ms4a7* and also express hematopoietic-related characteristic genes such as *Cle112a*, *Klra2*, and *Lilra5*. In *Hoxb8* mutant mice, selective inactivation of *Hoxb8* is sufficient to cause pathological phenomena in the hematopoietic system [75]. Previous studies have also shown that the loss of *Hoxb8* function in microglia affects corticostriatal neuronal circuits, leading to anxiety and impaired social behavior [76–78]. The emergence of *Hoxb8* microglia reveals that microglia play an important role in the hematopoietic system and behavior, thereby further enriching the function of microglia.

4.4. *CD11c* microglia

In 2017, Kohno et al. reported another subtype of microglia, *CD11c*, in the brain of neonatal mice, which showed a positive result for *CD11c* expression [79]. The number of *CD11c* microglia first increased to 20% of the total number of microglia after birth and then decreased to 3% of the total microglia number. The reasons for this alteration in the microglial population need to be determined, which may be related to cell death, cell migration, or differentiation of microglia subsets [80]. *CD11c*-positive microglia are unevenly distributed during brain development, and they are concentrated in the corpus callosum and cerebellar white matter. *CD11c*-positive microglia are also the main source of IGF1, and this microglia subtype selectively consumes IGF1 and affects the development of myelin [79,81]. Therefore, *CD11c*-positive microglia in the brain of neonatal mice play an important role in myelin development and neurogenesis. Kamphuis et al. suggested that the number of *CD11c* microglia is positively correlated with age and is also associated with A β protein deposition in different Alzheimer's disease models [81]. This clarifies that the close correlation between the subtype and age should not be ignored when focusing on the number and function of microglia.

Two specific subtypes of microglia exist, namely “microglial cells supporting neurogenesis,” which are critical for the survival and migration of nerve cells [82], and “dark microglia,” which interact with blood vessels and synapses and are characterized by a dark

Table 1
Different types of glial cells.

Number	Types of microglia	Concept	Function	Reference
1	“Satellite” microglia	“Satellite” microglia are located on the axon side of the neuronal cell body and overlap with the site where the neuronal potential starts, mainly in the cerebral cortex, thalamus, and hippocampus	“Satellite” glial cells are the most abundant in sensory ganglia and play a major role in sensory function; they are closely related to neuronal excitation	[15,23,44,45,62]
2	KSPG microglia	KSPG microglia are located in the extracellular matrix and cell surface; they are positive for keratin sulfate	Help cell axon growth and adhesion, establish boundaries for axonal growth in the developing brain and spinal cord and are a potential inhibitor of axon growth	[30,31,33,34]
3	<i>Hoxb8</i> microglia	<i>Hoxb8</i> glial cells are derived from the <i>Hoxb8</i> lineage in the second wave (E8.5) hematopoiesis of the yolk sac	Loss of <i>Hoxb8</i> glial function causes behaviors such as chronic anxiety and obsessive-compulsive disorder	[10,17,29,53,69,73]
4	<i>CD11c</i> microglia	<i>CD11c</i> expression is positive in these glial cells	Play an important role in myelin development and neurogenesis and are associated with A β protein deposition	[36,42,52]
5	Support neurons to form microglia	Glial cells associated with neurogenesis contribute to embryonic neurogenesis and neuronal differentiation and development	Critical for adult neurogenesis, removal of apoptotic neurons, learning-dependent synapse formation, synaptic and spinal remodeling, axon guidance, and survival and migration of neuroblasts	[32]
6	“Dark” microglia	Immunohistochemically appear as “dark” glial cells in transmission electron microscopy, which are often in contact with the synaptic cleft while extensively surrounding axon terminals	Interactions with blood vessels and synapses; they may be linked with the pathological remodeling in neural circuits that can impair learning, memory, and other basic cognitive functions	[4,63,64]

color in electron microscopy [83–85]. “Microglia supporting neurogenesis” are critical for neuroblast survival and migration. On the other hand, dark microglial cells are rarely observed in healthy adult mice; however, they are commonly detected in aged mice, CX3CR1 knockout mice, and Alzheimer’s disease model mice. These findings suggest that dark microglial cells may represent a subtype of microglia and play a special role in development, stress, and disease [83].

The abovementioned studies confirmed the presence of several different subtypes of microglia that can be differentiated on the basis of their function. It should be noted that in most cases, microglial subtypes and typical microglial cells exist simultaneously in the same environment, and this state will change according to the internal and external environment; this aspect should be investigated in detail in further studies. Single-cell RNA sequencing technology also supports the existence of various subtypes of microglia. For example, Hammond et al. [86] reported the presence of eight transcriptomes of microglial clusters in the mouse brain, some of which are involved in cellular immune function, some microglial clusters decrease dramatically during postnatal development, and some microglial clusters show gender differences. RNA sequencing revealed that different subtypes of microglial cells correspond to varying functions, thus, providing strong evidence for the presence of microglial subtypes. Many scientists also classify microglia on the basis of their functions and propose the concept of microglial diversity, as shown in Table 1.

5. Summary

Significant advancements have been made in the study of microglia polarization typing. RNA sequencing expression profiles, complementary techniques, and epigenomic analysis provide broad prospects for studying the activity of microglia in the healthy and pathological CNS and for developing appropriate therapeutic strategies for these cells. However, regarding the use of the nomenclature of peripheral macrophages to study microglia, our understanding of the concept of microglia typing is insufficient, and it is difficult to continue to conduct more systematic and in-depth research without an appropriate approach for microglia typing. Researchers have also found that microglia have richer neurobiological functions during development, including regulating overactive neurons, pruning synapses, and promoting neuronal survival. M1/M2 microglia typing does not show the complete biological functions of microglia. Therefore, on the basis of the source, we need a new nomenclature system to redefine microglia and their subtypes. Similarly, if the M1/M2 microglia polarization typing method is no longer used, it also implies that researchers cannot determine the activation status of microglia based only on the typical markers of M1/M2 (iNOS, Arg1, CD206, TGF- β , TNF- α , and CD11b). These components have been widely used to identify the activation status of microglia. Thus, the complete expression of reactive microglia can be determined by monitoring one or several gene expression markers. Reactive microglia express only two polarization products and are thus not comprehensive.

Summarizing previous studies, RNA transcriptomics, proteomics, and other computational biology and transcriptomics techniques should be used to investigate the CNS from the embryonic development stage to the aging stage and to reclassify the subtypes of microglia in different pathological states such as trauma, infection, ischemia, inflammation, and neurodegeneration. The repeated use of the M1/M2 typing method cannot positively promote the transcriptional expression profile analysis of microglia. Existing studies have revealed that the subtypes of microglia may coexist in the CNS and that microglia exhibit varying characteristics in different environments. Therefore, researchers should use different methods to recognize microglia and classify their subtypes in detail. In summary, a complete microglial subtype structure should be established that could help understand the biological functions of microglia through whole-genome transcriptomics, epigenomics, and proteomics. Considering that different microglia play various functions under normal physiological condition, we can better understand the diversity of microglia according to their regional distribution and gene expression. The classification of microglia should not be limited to its division into M1/M2 phenotypes based on cell surface markers. Although sufficient progress has been made in the study of microglia phenotypic polarization, the complete picture remains to be further discovered.

5. Outlook

The development and evolution of microglia in biology have undergone a revolution, resulting in significant progress in the understanding of their roles and types. However, many questions about microglia and their role in human diseases remain unanswered, including the different functions of the various subtypes of microglia in the CNS. Microglia are generally considered to be associated with the entire process of development, aging, disease, trauma, and infection of the CNS. Substantial progress has been made in understanding the phenotype and function of microglia by using their whole genome expression profile. However, in recent years, there has been no consensus on the topic of the polarization of microglia, and many obstacles and unsolved problems remain in the study of microglia phenotype. In the context of these controversies, future research needs to use more highly sensitive methods for evaluation, especially with advances in epigenetics, single-cell sequencing techniques, and imaging techniques in recent years. Significant advances have been reported in the extraction of whole-genome expression profiles of microglia. In future studies, other techniques such as multi-omics methods at the single-cell level, new animal models, patient-derived microglia, mass spectrometry cell analysis, immunohistochemical methods with high-resolution laser ablation, and other innovative techniques should be combined to help us clarify the unresolved issues in microglial phenotypes. This will enable a more in-depth understanding of microglia.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article. </p>

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Data availability statement

No data was used for the research described in the article.

6. Declaration of interest's statement

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article..

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