MICROGLIA ARE NOT BRAIN MACROPHAGES?

Marin Zhelezov* and Anton B. Tonchev

Department of Anatomy and Cell Biology, Medical University, Varna, Bulgaria

*Microglia are commonly referred to as the brain's macrophages, which leads to confusion due to the presence of several other macrophage populations in the central nervous system. The morphological, molecular and ontological differences between these cells are subtle. They need to be clearly defined in the light of the new evidence suggesting that microglia originate not in the bone marrow, but from yolk sac, or, possibly, pericyte progenitors. Recent paradigm shift redefines the specific roles of microglia during brain development, health and disease. Microglia have emerged as key players in important events such as neurogenesis, programmed cell death, elimination of synapses and remodeling of neural circuits. These novel discoveries imply a need for a better morphological and molecular differentiation of mononuclear phagocyte populations and their subtypes in the brain. This may improve our knowledge of their specific contributions and possible pharmacological manipulation in brain health and disease. **Biomed Rev 2018; 29: 99-108***

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INTRODUCTION: MACROPHAGE HETEROGENITY IN THE CENTRAL NERVOUS SYSTEM

Microglia are the main mononuclear phagocytes of the brain and are commonly referred to as “brain tissue macrophages”, similar to other tissue macrophages such as the Kupffer cells in the liver. However the central nervous system (CNS) contains several other populations of immune cells, including B-, T- and NK-cells, dendritic cells, as well as at least three separate groups of resident macrophages, namely, meningeal, perivascular and choroid plexus macrophages, which are strategically located at the interface between the parenchyma and the circulation (1–3). These other CNS macrophages were

once virtually ignored, however recent evidence (4) has shed light onto their specific functions in the brain. It is virtually impossible to distinguish microglia from other brain macrophages and monocytes, entering from the blood based on morphological criteria alone (5–7). In addition, many of the markers that these cells express are overlapping (3). This challenge was recently addressed in comparative transcriptomic studies, aimed at identifying specific molecular signatures of the diverse populations of macrophages (8–13). Currently, microglial cells can be precisely distinguished from other

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* Correspondence to: Dr Marin Zhelezov, Department of Anatomy and Cell Biology, Medical University, 55 Prof. Marin Drinov Str., BG-9002 Varna, Bulgaria. E-mail: marin.zhelezov@gmail.com

brain macrophages *via* specific expression of transmembrane protein 119 (TMEM119), P2Y purinoceptor 12 (P2RY12) and Sal-like protein 1 (SALL1) (9, 12, 14).

INSIGHTS FROM ONTOGENY

Originally it was thought that microglia arise from the neuroectoderm, a view which later changed in favor of a blood monocytic origin and dominated for decades (15). However, this was recently challenged by convincing evidence, demonstrating that microglia derive from the embryonic yolk sac (YS) (16–18). Yolk sac erythromyeloid precursors generate macrophages which migrate and colonize the embryonic brain (19, 20). It is also the site of origin of perivascular and meningeal macrophages. Interestingly, choroid plexus macrophages were shown to have dual embryonic and adult hematopoietic origins (3, 21). They are the only macrophage population in the brain that does not maintain its numbers in adulthood by self-renewal (21–24).

Despite the strong evidence for the YS origin of microglial progenitors, there was a long ongoing debate whether peripheral cells from the blood can enter the brain parenchyma and contribute to the microglial population. Originally, it was thought that, the CNS is an immune privileged site, protected by the blood-brain barrier (BBB) from cell entry from the circulation (25, 26). Indeed, experiments using parabiotic chimeric mice with shared circulation show that, under physiological conditions, there is a constant entry of monocytes to peripheral organs such as liver and spleen, but not into the brain (27). In addition, adult microglia can recover their numbers following genetic or chemical depletion (22, 28) and can maintain their population with little contribution from blood cells (16, 27, 29, 30).

It is considered that recruitment of circulating monocytes occurs only when the BBB is open, for example after irradiation or bone marrow transplantation (3, 15). Under such pathological conditions, bone marrow derived precursors can generate microglia-like cells (31–33) and, due to perceived functional similarity, were referred to as “blood-derived microglia”, which has contributed to an ongoing confusion (5, 34–36). Recent evidence demonstrated that these infiltrating cells have unique functions which cannot be provided by resident microglial cells (37–39).

Mmonocyte-macrophage system, microglia and pericytes

A very important alternative hypothesis concerning the origin of the monocyte-macrophage system (MMS) is the presumption that all members of this system originate from a

common embryonal pluripotent ancestor (3, 15, 30, 40–42). Sheng *et al* (30) presumed that all adult macrophages, resident or infiltrating, are derived from the fetal hematopoietic stem cells with the exception of microglia and partially epidermal Langerhans cells, which are yolk sac-derived. However, the hematopoietic stem cells are also epiblast derivatives, which have been migrated to the yolk sac and from there to the aorta-gonad-mesonephros region, the liver (Kupfer cells), the spleen, and the bone marrow (42, 43). Thus, the numerous variants of the microglia-macrophage system are the result of the different phases and places of development and differentiation of the cells with common embryonal origin.

In this respect, one may hypothesize that as common precursors of the MMS come very probably the embryonic pericyte progenitors and the pluripotent pericytes in adult vertebrates. There is strong evidence that pluripotent pericyte progenitor cells arise in the epiblast of the embryonal blastocyst (21, 30, 44–46).

In the microvasculature the pericytes lie in periendothelial position within true microvascular niches as resting, reserve adult stem cells for tissue generation, maintenance, repair and regeneration (46). In fact, the pericytes can be considered as representants, respectively as the adult stem cells, of the embryonic epiblast in the adult, mature organism. Thus, it is noteworthy that the pericytes represent a highly immature form of pluripotent stem cells that maintain their phenotype throughout the whole life within the true microvascular niches.

Findings that pericytes possess macrophage properties (47–49) and are capable to build macrophages and microglia in the CNS (50–52) provide evidence that pericytes are able to transform themselves into microglial cells by virtue of an activation process in which the astrocytic neuroglia appears to play a decisive role.

The presumption that microglia may originate as progeny of activated pericytes is supported by newer studies demonstrating that microglial repopulation after experimental inhibitor cessation occurs by proliferation and differentiation of cells expressing nestin, which, interestingly, is also expressed by pericytes as well as their progeny (53, 54). The same process was observed in an experiment with regenerating testicular Leydig cells which originate from nestin-expressing pericytes (55).

In this respect, very informative are the electron microscopic results illustrating that some microglia cells lie within the expansions of basal lamina of capillaries (pericytal microglia) (56). There is evidence that these cells can break out of

their basal lamina enclosure and enter the brain neuropil and were thus termed interstitial microglia. It seems likely that these authors were unable to observe the transdifferentiation (a kind of metaplasia) of the pericytic transit amplifying cells in microglia. These results provide additional evidence that the activated pericytes, situated within the true microvascular niches, may be the ancestors of the microglia.

There is also evidence for a close resemblance between the pericytes and the microglia concerning not only their location, but also marker substances, resting and activation conditions, plasticity and origin (52). As well as their close interrelationship with the developing and differentiated vasculature reinforces the presumption that both cell types may have similar origin.

Taken together, there is a real possibility that all members of MMS, including pericytes, originate from the same ancestor, namely the embryonal epiblast. The epiblast stem cells migrate *via* the mesoderm toward different regions which are able to undergo embryonal vasculogenesis and to generate primitive vascular plexuses (e.g. yolk sac and dorsal aorta) (57, 58). These stem cells (are also pericyte progenitors) become distributed throughout the whole organism, where remain enclosed in the microvascular niches as resting adult stem cells. In the brain, *via* the process of transdifferentiation and maturation, variable forms of microglia and other cell phenotypes may arise.

The described differences in ontogeny can provide further insights for the functional differentiation of CNS myeloid cells. Importantly, in the mouse, immature microglia colonizes the embryonic brain at approximately day 9.5, which coincides with neurogenesis, before astrocytes and oligodendrocytes are generated (59). This allows microglia to participate in a number of key developmental events in the CNS, as discussed below.

MICROGLIAL ROLES DURING DEVELOPMENT AND DISEASE

Microglia constitute around 10% of all cells in the brain (60). They exhibit a number of morphological states, associated with changes in function and gene expression. During development these cells adopt an “amoeboid” morphology with large, round body and short, thick branches, showing higher levels of phagocytic activity. In contrast, adult microglia have a small body with numerous, fine, highly branched processes (61). Under pathological conditions, secretory changes in microglia are accompanied with morphological changes similar to those during development (Fig. 1). These activated cells were traditionally classified as either M1 (“toxic”) or M2 (“protective”) type. Newer evidence has challenged this view, demonstrating a continuum of microglial forms with overlapping gene expression (62).

Once microglial cells were thought to remain quiescent under physiological conditions, maintaining a “resting” state and only “activate” in pathological processes. Thus, microglia

Figure 1. Functions of microglia at different stages of activation. Ramified microglia is primarily involved in immune surveillance, as well as synaptic pruning and plasticity. Under pathological conditions it transforms to an amoeboid form with a pro-inflammatory profile, including secretion of growth factors and clearance of debris.

were thought to play a primarily passive role in brain homeostasis and were long ignored. However, a number of recent studies showed substantial microglial involvement in a large number of key events during both normal development and disease, which has led to a paradigm shift, placing microglia as “central players” in brain disease (63).

Immune surveillance

Time-lapse recordings demonstrated that microglia are highly dynamic cells, which actively survey their cell-specific territory and can scan the entire brain parenchyma in just a few hours (64). Their fine processes continuously contact neural elements, including axons and dendritic spines, being able to significantly change their motility, following extracellular stimuli such as neuronal activity (65, 66) and the release of neurotransmitters (65, 67). Indeed, it has been shown that microglia preferentially contact and neurons with higher levels of activity, thus attenuating their action (65).

Synaptic pruning during development

During CNS development microglia contribute to the process of synaptic pruning by engulfing synapses, thus shaping neuronal circuits (68–70). Evidence from the mouse visual system has demonstrated neuronal activity and sensory experience as important factors for this process (68, 70). Molecules of the classical-complement cascade have been recognized as key players in microglial synaptic modification (70–73). Microglial cells express a C3 receptor and are able to phagocytose immature C3-expressing synapses (71, 73). Importantly, disruption of microglial synaptic pruning during development results in defects in neuronal wiring (70).

Programmed neuronal death during development

The excess neurons produced by neurogenesis during development and in the adult die *via* programmed cell death, consequently being phagocytosed by microglial cells (74, 75). Interestingly, microglia do not simply take a passive scavenging role but can induce neuronal apoptosis themselves *via* the release of a variety of neurotoxic factors (76–79).

Learning and memory

Microglial involvement in neurogenesis and synaptic modification suggests important roles of these cells in two key processes associated with learning and memory, namely adult neurogenesis (80, 81) and activity-dependent, long-term synaptic plasticity (82) which has been supported by a growing number of studies (83–92).

Role in disease

It is now considered that microglia contribute to both neurodevelopmental and neurodegenerative disease. Experimental deletion of microglia-specific receptors in rodents leads to defects of the laminar positioning of neocortical interneurons, as well as the outgrowth of forebrain dopaminergic axons (93). Perturbed synaptic pruning and modification by microglia during development may disrupt connectivity in ways, associated with diseases such as autism and schizophrenia (94). This is supported by postmortem studies, demonstrating several microglial alterations in brains from individuals with autism, especially in regions, involved in executive function control, such as the dorsolateral prefrontal cortex (95–97).

Microglia are key players in neuroinflammation, which is associated with virtually all neurodegenerative diseases (98). A staggering amount of evidence has elucidated multiple mechanisms of microglial involvement in conditions such as Alzheimer’s disease, amyotrophic lateral sclerosis, multiple sclerosis, glaucoma, and neuropathic pain (59, 63).

FUNCTIONS OF RESIDENT MACROPHAGES IN THE CNS

The resident CNS macrophages, namely, perivascular, meningeal and choroid plexus macrophages are poorly understood. Perivascular macrophages share similarities with microglia, in terms of their transcriptional profile and marker expression, including Iba1, CD11b, CX3CR1 and others (21, 99). However, their transcriptional profile differentiates them from monocytes in blood (21).

Under normal conditions they continuously extend and retract numerous processes along blood vessels, suggesting a role in immune surveillance (2, 21, 100), whereas under inflammatory conditions they may regulate recruitment of leucocytes from the periphery (101). In addition, they may play a role in BBB establishment (102).

Yolk sac-derived resident macrophages have been described in both mouse and human meninges (21). Little is known for their physiologic functions. Peripheral macrophages play a role in regulating the proliferation of lymphatic endothelial cells and it is thought that meningeal macrophages have similar functions, although it is now known whether they are part of the newly discovered brain glymphatic system (2, 103). There is evidence that, under experimental conditions, meningeal macrophages, together with dendritic cells, are involved in antigen presentation to T cells during autoimmune diseases (2, 104).

Although choroid plexus macrophages are poorly under-

stood, their specific location close to the ependymal cells' microvilli (21) suggests involvement in cerebrospinal fluid release. Interestingly, they are present in circumventricular organs (CVOs) as well (105). These latter organs are highly vascularized special structures, located close to the brain ventricles. They contain fenestrated capillaries and lack a blood-brain barrier, thus performing both sensory and secretory roles by sampling the blood and releasing substances into the cerebrospinal fluid (105, 106).

Table 1 provides examples of microglial and brain macrophage functions.

CONCLUSION

In light of microglia's recently discovered crucial roles during development and brain disease, there is a need to elucidate the key structural and functional differences between these heterogenous populations of cells. This brings the promise of precise targeted pharmacological interventions in the future to modulate CNS function and pathology.

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CONFLICT OF INTERESTS

There are no conflicts of interests to disclose.

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Table 1. A summary of microglial and brain macrophage functions

Microglia	Perivascular macrophages	Meningeal macrophages	Choroid plexus macrophages
Development: <ul style="list-style-type: none"> • Immune surveillance • Synaptic pruning • Regulate laminar positioning of neurons • Promote cell death 	<ul style="list-style-type: none"> • Immune surveillance • Regulate recruitment of leucocytes • BBB establishment 	<ul style="list-style-type: none"> • Regulate proliferation of lymphatic endothelial cells • Antigen presentation 	<ul style="list-style-type: none"> • cerebrospinal fluid release • role in circumventricular organs
Adult: <ul style="list-style-type: none"> • Immune surveillance • Synaptic plasticity • Monitor neuronal activity • Participate in neuroinflammation • Clearance of debris 			

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