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Bidirectional tuning of microglia in the developing brain: from neurogenesis to neural circuit formation

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The developing brain employs multi-step processes to construct neural circuitry. Recent studies have highlighted that microglia, traditionally known to be the resident immune cells in the brain, have essential roles in these processes, which range from neurogenesis to establishing synaptic connections. Microglia play bidirectional roles for maintaining proper circuitry: eliminating unnecessary cells, axons, and synapses, while supporting the neighboring ones. Although these processes are performed in different parts of the neuron, similar molecular mechanisms are possibly involved. This paper reviews recent progress on the knowledge of the roles of microglia in brain development, and further discusses the application of this knowledge in therapies for brain disorders and injuries.

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Introduction

Microglia account for 3–12% of the cells in the central nervous system (CNS) and are commonly referred to as the brain's resident immune cells or tissue macrophages [1,2]. Microglia play diverse roles in pathological conditions, owing to their ability to dramatically change morphology and engage in inflammatory/repair responses [3,4]. Recent technological advances have revealed additional physiological functions of microglia in the healthy brain, particularly, during development where dramatic changes in neuronal circuitry occur daily. Here, we review the newly discovered role of microglia on brain development, and further discuss whether understanding these functions will aid in therapeutic treatment of diseases that target the CNS.

Origin of microglia and their distribution

Although the majority of cells in the brain are generated from neural progenitor cells, microglia are considered to be of monocyte/macrophage lineage because of their shared features. However, whether their origin is distinctly defined during development or recruited from monocytes in circulating blood has been a subject of debate [5,6]. This topic has recently been addressed by a study that used parabiosis to demonstrate that blood-derived monocytes are not provided to the adult brain and microglia can 'self-renew' [7]. This implies that there is a specific, predetermined origin of microglia during development. Indeed, expression of microglial markers suggest that they are derived from the embryonic yolk sac [8], and more recent studies using genetic mice have shown direct evidence of this (Fig. 1a) [9,10•,11•]. In these studies, taking advantage of early markers such as RUNX1, a transcription factor, and CSF1R, a receptor for CSF1 (M-CSF), the lineage of microglia was traced using *Runx1-CreER* and *Csf1r-CreER*; *YFP* reporter mice with tamoxifen injection to label yolk sac progenitor cells in a restricted developmental time window. It was demonstrated that microglia were derived from myeloid progenitors at around E7.5 and the cells were Myb-negative and PU.1-dependent Csf1-receptor — expressing progenitors, which are different from Myb-dependent hematopoietic stem cells that differentiate into other macrophages and monocytes [9,10•]. The molecular profiles of these progenitors have been further identified [11•].

In mice, cells migrate out of the yolk sac and infiltrate the brain at E9.5 (Fig. 1a and b) [5,8,9,10•,12,13]. Although the route to infiltration is not yet completely understood, histological studies imply that these cells enter from the meninges, ventricles, and intracerebral blood vessels. Once in the brain, microglia gradually increase in number and colonize areas such as subcortical and cerebellar white matter in early postnatal days (Fig. 1d). These colonized regions are referred to as 'fountains of microglia' in rodents [14••,15,16] and humans [17,18] and are ultimately distributed to most brain regions presumably through migration and proliferation [12,14••,16].

The timely and locally invasive nature of microglia implicates that these cells play a critical role in brain

Table 1

Molecules involved in developmental functions of microglia.

Process	Paradigm	Region or target cells	Molecules	Reference	Notes
Initial differentiation/proliferation of microglia	*in vivo*	Yolk sac	PU.1, CSF1R, IRF8	[9,10•,11•,19,21]	
Migration into the CNS	*in vivo*	CNS	CSF1R?	[9,13,21]	
	in vivo	CNS	MMPs	[11•]	
	in vivo	Hippocampus, Sensory cortex	CX3CR1	[58,59]	
	in vivo	Lateral ventricle — SVZ	NOX2, VEGFR	[65•]	
	in vivo	Optic tectum	Pannexin-1 hemichannels — ATP?	[62••]	Process direction to neurons
Cell death induction	*in vitro*	Retina	proNGF — P75 NTR	[25]	
	in vitro	Motor neurons in spinal cord	TNFα	[26]	Macrophage
	in vitro	Purkinje cells in cerebellum	NADPH oxidase	[32]	
	in vivo	Hippocampus	DAP12 — CD11b — ROS	[33]	
Cell survival	*in vivo*	Layer V neurons in cortex	IGF1	[14••]	
Phagocytosis of neurons	*in vivo*	Striatum	PS — MFG-E8 — vitronectin receptor	[34]	In inflammatory condition
	in vitro	Dorsal root ganglion	Jedi-1, MEGF10	[38,39]	Phagocitizing glial cell precursors
Axonal growth	*in vitro*	Subcortical white matter	Thrombospondin	[47,48]	
Phagocytosis of axons	*in vivo*	Mushroom body axons	Draper, Ced-6	[44]	Glial cells in Drosophila
Synapse elimination	*in vivo*	Retinogeniculate circuit	C3–CR3	[60]	
	in vivo	Hippocampus, Sensory cortex	CX3CR1?	[58,59]	

development. In fact, recent reports have revealed that microglia are involved in the construction of neural circuits, which are assembled in multiple stages: neural progenitors proliferating around the ventricular zone (VZ), differentiated neurons migrating to specific areas, and neurons projecting axons to target areas and forming synapses (Fig. 1).

Bidirectional roles of microglia in neurogenesis

During the course of brain development, significant numbers of neurons and glial cells (astrocytes and oligodendrocytes) must be precisely generated from neural progenitor cells. Interestingly, microglia have been observed in the neuroepithelium where progenitor cells proliferate [5,6,8,9,19], implying a role for microglia in neurogenesis (Fig. 1b and c). Microglia have been shown to exert trophic effects *in vitro*. For example, cultured embryonic cortical cells of PU.1$^{-/-}$ mice lacking microglia demonstrate no change in cell survival and neurogenesis of progenitor cells, but exhibit reduced proliferation and astrogenesis [19]. A number of factors known to be expressed in microglia, such as BDNF, FGF2, and IGF1 [14••,20], may be involved in this process. *In vivo*, a decline in microglia is correlated with reduced histogenesis of the brain in *Csf1r*$^{-/-}$ mice [21]. This study implies a role for microglia in neurogenesis; however, whether this is a direct effect of microglia remains unclear.

Conversely, it was found an opposite role of microglia in that they can restrict the number of neural progenitor cells *in vivo* [22•]. This group observed prominent numbers of microglia in the VZ and subventricular zone (SVZ). In these zones, non-dying progenitors phagocytosed by microglia gradually increased, and this was correlated with reduction in the number of progenitor cells. Deactivating or eliminating microglia by tetracycline or liposomal clodronate, respectively, significantly increased the number of progenitor cells, whereas activating microglia by lipopolysaccharide (LPS) decreased the number of these cells. Therefore, the authors concluded that microglia regulate the number of progenitor cells mainly through phagocytosis. This can be related to the phagocytosis of new born cells in the hippocampal proliferative zone [23].

From these results, it has been postulated that microglia play bidirectional roles in neurogenesis by supporting cell generation and removing unnecessary cells.

Microglia control the survival and death of developing neurons

The regulation of neuronal number is essential to the construction of neural circuitry. In the developing CNS, cell death occurs extensively to eliminate unhealthy and misconnected neurons [24]. Survival of neurons is presumably maintained by factors derived from target or connected neurons and glial cells. While cell death was