REVIEW ARTICLE

Effects of Microglia on Neurogenesis

Kaoru Sato

This review summarizes and organizes the literature concerning the effects of microglia on neurogenesis, particularly focusing on the subgranular zone (SGZ) of the hippocampus and subventricular zone (SVZ) of the lateral ventricles, in which the neurogenic potential is progressively restricted during the life of the organism. A comparison of microglial roles in neurogenesis in these two regions indicates that microglia regulate neurogenesis in a temporally and spatially specific manner. Microglia may also sense signals from the surrounding environment and have regulatory effects on neurogenesis. We speculate microglia function as a hub for the information obtained from the inner and outer brain regions for regulating neurogenesis.

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Introduction

In humans, approximately 5% of brain cells are microglia (Pelvig et al., 2008). Originally, microglia attracted attention because these cells determine the levels of inflammation in the cellular environment, which subsequently determines whether newly generated neurons survive. However, increasing evidence has demonstrated that microglia play diverse roles in neurogenesis in both the embryonic and postnatal adult stages. In this review, I summarize and organize the data concerning microglial effects on neurogenesis, particularly focusing on the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) and subventricular zone (SVZ) of the lateral ventricles, in which the neurogenic potential is progressively restricted during the life of the organism. Recent research regarding the origin of microglia (Ginhoux et al., 2010; Schlegelmilch et al., 2011; Schulz et al., 2012) has demonstrated conclusively that these cells are derived from myeloid progenitors at approximately embryonic day 7.5 (E7.5) in mice and infiltrate the brain through blood vessels between E8.5 and E9.5. This process occurs immediately before the formation of early radial progenitors for neurons and glia, which occurs at E10.5 (Dahlstrand et al., 1995). Microglial progenitors are Myb-negative and PU.1-dependent cells that express the Csf1 receptor; these cells differ from Myb-dependent hematopoietic stem cells, which differentiate into macrophages and monocytes (Ransohoff and Cardona, 2010). A recent study that combined parabiosis and myeloablation revealed that monocytes that infiltrate the brain during inflammation only contribute to a transient population of macrophages that disappear once the inflammation is resolved (Ajami et al., 2011). Therefore, most microglia that have functional roles in neurogenesis in the brain originate from embryonic microglia that differentiated from infiltrating myeloid progenitors at E7.5. Although these cells all have common ancestors, microglia have temporally and spatially specific morphologies, antigen expression profiles, proliferative potentials, and brain functions (Butovsky et al., 2014; Olah et al., 2011). The effects of microglia on neurogenesis also appear to be diverse in a temporally and spatially specific manner.

Neurogenesis in the Subgranular Zone

In mammals, the SGZ is one of the major sites for the birth of new neurons from radial glial progenitors (Eckenhoff and Rakic, 1988; Kaplan and Hinds, 1977; van Praag et al., 2002). In the hippocampus, granule and pyramidal neurons exhibit different developmental patterns (Taupin, 2008). In contrast to the cornu ammonis (CA), which begins to develop during the prenatal period, 15% of DG granule cells are generated before birth; 70% are generated during the first 2 weeks of life; and 15% are generated after postnatal day 16 (P16) in mice. Beginning at E15.5, Prox1-positive cells migrate from the dentate neuroepithelium, which is adjacent to the fimbria, to the granular layer of the DG (Nakahira

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and Yuasa, 2005). At E18, the characteristic V-shape of the DG is highlighted by Prox1 staining in the granular layer and hilar region. Within the hilar region, neuronal precursors proliferate to generate additional granule neurons. In the postnatal and adult SGZ, neural stem cells (NSCs) have been shown to be long-lived NSCs (Li et al., 2013). They initially originate from the ventral hippocampus during late gestation and then relocate into the dorsal hippocampus. These NSCs can produce not only neurons, but also stem cells, and the ratio of NSCs to neurons depends on experiences of the animal or the location of the NSCs (Dranovsky et al., 2011). The proliferating radial and nonradial precursors give rise to intermediate progenitors, which in turn generate neuroblasts (Gage et al., 1998; Ming and Song, 2011). Immature neurons migrate into the inner granule cell layer and differentiate into dentate granule cells in the hippocampus (van Praag et al., 2002). Through transcriptome analysis, neurogenesis-related transcription factors crucial for SGZ neurogenesis have been identified (Miller et al., 2013). Wnt/β-catenin signaling cascade (Lie et al., 2005; Varela-Nallar and Inestrosa, 2013) and circadian molecular clock (Bouchard-Cannon et al., 2013) are shown to be important in the adult SGZ neurogenesis. New-born functional granule cells contribute to cognitive functions (Kempermann et al., 2004) and temporal memories (Aimone et al., 2006). Aimone et al., have suggested that immature neurons provide a low specificity yet densely sampled representation of cortical inputs, whereas mature granule cells provide a highly specific yet sparse representation of an event (Aimone et al., 2011). This combined representation maximizes the information encoded by hippocampal memories, thus increasing the memory's resolution (behavioral discrimination). Kempermann et al. (1998, 2002) demonstrated that SGZ neurogenesis continues in senescent mice. Based on these back ground, the regulation of SGZ neurogenesis has received abundant attention because of the link between neurogenesis and cognitive function (Lie et al., 2004), which has been indicated by many reports.

Regarding the effects on neurogenesis, microglia originally gained attention because these cells determine the inflammation levels in the cellular environment, which then determines whether newly generated neurons survive. Monje et al. (2003) showed that inflammation caused by LPS inhibited neurogenesis in the adult rat hippocampus via the microglial release of IL-6 and TNFα. The *in vitro* data of these authors indicated that the neurogenic lineage has greater sensitivity to inflammation than does the gliogenic lineage (Monje et al., 2003). Ekdahl et al. (2003) also demonstrated that inflammation-associated microglial activation impairs both basal and insult-induced hippocampal neurogenesis in adult rats. Studies that are more recent have clarified that TNFα signaling via TNFR2 is required for basal neurogenesis, whereas signaling via TNFR1 impairs neurogenesis (Chen and Palmer, 2013). Aging is associated with a substantial decrease in hippocampal neurogenesis (Kempermann et al., 1998; Kohman et al., 2012; Rao et al., 2006; van Praag et al., 2005; Walter et al., 2011), which is associated with cognitive deficits (Bizon et al., 2004; Drapeau et al., 2003; Merrill et al., 2003). Aging also alters microglial activity and drives microglia toward an inflammatory phenotype (Dilger and Johnson, 2008). Gemma et al. (2007) reported that IL-1β released from microglia reduced neurogenesis in aged rats. Age-related priming of microglia contributes to a prolonged neuroinflammatory response following an immune challenge, resulting in the exaggerated expression of sickness behaviors and cognitive deficits (Dilger and Johnson, 2008; Godbout et al., 2005; Kohman et al., 2007).

Some models in which aging-induced neurogenesis is ameliorated have suggested that microglia contribute to SGZ neurogenesis. An enriched environment (EE) is a housing manipulation that increases physical and social stimuli (Diamond et al, 1976) and that is reported to stimulate neurogenesis in the aged brain, leading to better performance in a water maze (Kempermann et al., 1998). Ziv et al. (2006) demonstrated that EE-induced SGZ neurogenesis is associated with T cell recruitment and microglial activation with increased MHCII expression. An EE increased microglial Iba1+ expression only in the DG but not in the CA1 or CA3 and blunted the proinflammatory hippocampal response to LPS (Williamson et al., 2012), suggesting that the effects of an EE on microglia may be specific to the DG. Wheel running, i.e., voluntary physical exercise, also ameliorated several of the behavioral consequences of aging and reversed the decrease in neurogenesis to 50% of that in the young control animals (van Praag et al., 2005). In this case, despite increased levels of neural precursors and newborn neurons, microglia remain in a resting state morphologically and antigenically, and T cells and MHCII-expressing microglia are not present in the DG in the wheel running model (Olah et al., 2009). Voluntary wheel running suppressed the age-associated increase in the number of microglia, increased the proportion of microglia that express IGF-1, and enhanced the survival of new neurons simultaneously (Kohman et al., 2012). Running induces a neuroprotective microglia phenotype and promotes neurogenesis by reducing the expression of proinflammatory cytokines such as TNF-α (Vukovic et al., 2012), and by increasing the expression of anti-inflammatory cytokines such as IL-1ra or the chemokine CX3CL1 (Pervaiz and Hoffman-Goetz, 2011). Wheel running has also been reported to prevent the infection-induced reduction in hippocampal BDNF expression in sedentary rats (Barrientos et al., 2011) and increased neurogenesis in healthy adult mice (van Praag et al., 1999). Gebara et al. (2013) monitored microglia

and the proliferation of adult hippocampal stem/progenitor cells in young adult and aged mice with or without wheel running and demonstrated that the number of microglia in the DG correlated inversely with the stem/progenitor cell number and the cell proliferation rate in the granule cell layer. The interesting data from this study have indicated that both the aging-induced decrease and voluntary running-induced increase in the radial glia number highly correlate with the number of microglia, suggesting that the regulation of radial glia number correlates with the regulation of microglia number. In the case of the DCX+ or Tbr2+ cell number, the correlation coefficients remained high but lower than were those of RGLs, suggesting that other mechanisms that are independent of the regulation of the microglia number are involved in neuronal differentiation. In contrast, some studies have demonstrated that running induces the transcription of genes involved in inflammation, including genes related to MHCI (β2-microglobulin, H2-D1) and elements of the complement system (C4A, C3, and C1q) or the inflammatory response (COX-2 and CX3C; Kohman et al., 2011; Tong et al., 2001). The endpoint of running may depend on the exercise intensity. The largest difference between the embryonic and postnatal adult brains may be the degree of neuronal network completion. Data from status epilepticus (SE) models have suggested that excitatory inputs influence both hippocampal neurogenesis and microglial effects on neurogenesis. Granule cell generation is induced in electrically evoked SE models, and the cells that do not die during the first month after SE induction survive for 6 months despite chronic inflammation (Bonde et al., 2006). Pilocarpine-induced SE can trigger the activation of CRE-mediated gene expression (Lee et al., 2007), including IGF-1 expression predominantly in activated microglia near the SGZ (Choi et al., 2008). Recent studies have indicated that microglia express a variety of neurotransmitter receptors, i.e., alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type receptors, metabotropic glutamate receptors (mGluRs), gamma-aminobutyric acid (GABA) B receptors, purinergic receptors, and adenosine receptors, as well as adrenergic, dopaminergic, cannabinoid, and opioid receptors (Pocock and Kettenmann, 2007). The neurogenic effects of microglia may be modified by the signalings of these neurotransmitters. *In vitro* data have suggested that VIP, a neuropeptide released by DG interneurons, enhances the proliferative and proneurogenic effects of microglia via the VPAC1 receptor and that these effects are mediated by IL-4 release from microglia (Nunan et al., 2014). Notably, SE-induced neurogenesis differs from physiologically coordinated neurogenesis. Aberrant neurogenesis following seizure activity has been reported to contribute to cognitive impairment (Jessberger et al., 2007). More recently, Parkhust et al. (2013) demonstrated that microglia promote the formation of synapses and the achievement of multiple learning tasks in young adult mice that carry CXCR1CreER to drive the diphtheria toxin receptor allowing cell ablation after diphtheria toxin administration. Therefore, the effects of microglia on learning should be interpreted as the composite results of the effects on neurogenesis and synaptogenesis.

In addition to regulating the proliferation and differentiation of NSCs/neural progenitor cells (NPCs), microglia also control the resulting number of newborn neurons via phagocytosis. Sierra et al. (2010) reported that unchallenged microglia (CD11b-low and CD68-low) maintain the homeostasis of the baseline neurogenic cascade in the young adult SGZ in a microglial activation-independent manner. The primary critical period occurs in the first 4 days of a cell's life, specifically during the transition from late amplifying neuroprogenitors to neuroblasts. The cells that are committed to apoptosis in the first 4 days of cell life interact with unchallenged microglia to induce phagocytosis (Sierra et al., 2010). Interestingly, although the numbers of newly generated neurons and apoptotic cells decrease with age and acute inflammation, the phagocytosis index (the percentage of apoptotic cells that undergo microglial phagocytosis) remains constant, suggesting that once microglia become ramified, their phagocytic activity is unaffected by the surrounding environment (Sierra et al., 2010). Two distinct functional types of phagocytic receptors have been characterized in microglia (Neumann et al., 2009). The first group, including TLRs, recognizes microbes; these receptors support the removal of pathogens and simultaneously stimulate a proinflammatory response in phagocytes. The second group of receptors recognizes apoptotic cellular materials, such as phosphatidylserine (PS); these receptors are important for ingesting apoptotic cell corpses and for stimulating an anti-inflammatory response in phagocytes (Ravichandran, 2003). The latter pathway may be involved in the microglial phagocytosis of newborn cells in the SGZ. Furthermore, *in vivo* studies have shown that DAP12-CD11b-ROS signaling actively contributes to the developmental death of postnatal hippocampal neurons (Wakselman et al., 2008).

Mechanisms That Underlie the Neurogenic Effects of Microglia in the Postnatal and Adult SGZ

The interaction between T cells and microglia is important for the EE-induced neurogenic effects of microglia (Ziv et al., 2006). An *in vitro* study demonstrated that microglia activated by T helper cell type 2 (Th2)-derived cytokines, interleukin-4 (IL-4) and a low level of IFN-γ differentially induce neurogenesis and oligodendrogenesis in adult NSCs/NPCs. NPCs co-cultured with IL-4-activated microglia are

biased toward oligodendrogenesis, whereas NPCs co-cultured with IFN-γ-activated microglia are biased toward neurogenesis. The effect of IL-4-activated microglia is mediated by IGF-1 release (Butovsky et al., 2006). Increased IGF-1 expression has also been observed in a voluntary wheel running model (Kohman et al., 2012). In addition to the antiapoptotic effects of IGF-1 during development (Chrysis et al., 2001), IGF-1 reduces the G1 phase length and total cell cycle length but increases NPC cell cycle reentry (Hodge et al., 2004). The interaction of CNS-specific autoimmune T cells (T cells directed to myelin basic protein, MBP) with resident microglia is important for the effects of an EE on spatial learning and memory, as well as for BDNF expression (Ziv et al., 2006). However, whether this effect is mediated by direct cell-cell contact between T cells and microglia remains unclear. Because T cells can barely be detected in healthy CNS parenchyma, T cells likely interact with microglia via secreted factors (Ziv and Schwartz, 2008b).

CX3CR1 is expressed primarily in microglia (Cardona et al., 2006; Harrison et al., 1998) and is located in hippocampal neurons (Sheridan and Murphy, 2013). Its ligand, CX3CL1/fractalkine, can act as a signaling molecule when cleaved (Chapman et al., 2000). Wheel running increases the expression of CX3CL1 (Pervaiz and Hoffman-Goetz, 2011) and the phenotypic transition of microglia from non-neurogenic to neurogenic fates by facilitating the CX3CL1/CX3CR1 signaling axis, which underlies the exercise-induced reversal of age-related decreases in neurogenesis (Vukovic et al., 2012). Furthermore, CX3CL1 administration resulted in the recovery of the age-related decrease in hippocampal neurogenesis and an increase in the number of microglia with ramified morphology (Bachstetter et al., 2009). Generally, CX3CL1 limits the activation of microglia and the expression of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α 2009 (Bachstetter et al., 2009; Rogers et al., 2011), which act directly on neural progenitors (Koo and Duman, 2008; Monje et al., 2003). Therefore, CX3CL1 likely contributes to the preservation of a neuronal niche that is optimal for SGZ neurogenesis by maintaining a milieu skewed toward quiescence. Hippocampal neurogenesis is decreased in mice that lack CX3CR1; these mice have significant deficits in cognitive functions and LTP induction (Rogers et al., 2011). In contrast, another report demonstrated that CX3CR1−/− mice exhibited enhanced neurogenesis and better hippocampus-dependent memory, with increased numbers and soma sizes of hippocampal microglia (Reshef et al., 2014). CX3CL1/CX3CR1 signaling is multifunctional in various processes of neuronal development, including the synaptic pruning process during postnatal synaptogenic development (Paolicelli et al., 2011). The effects of CX3CL1/CX3CR1 signaling manipulation may vary in a manner dependent on the developmental stage.

Neurogenesis in the SVZ

The ventricular zone (VZ) and SVZ are germinal zones during the embryonic period. Although several major classes of neocortical neural precursor cells have been identified in these areas, the lineal relationships and molecular profiles of these cells remain largely unknown. Using a novel fate-mapping approach, neural precursors have been divided into distinct subtypes based on their lineage profile, morphology, and transcription factor expression *in vivo*. These results have suggested that neocortical neurons are produced via multiple indirect routes during embryonic development (Tyler and Haydar, 2013). Until the report by Cunningham et al. (2013), few reports had been published regarding the role of microglia in embryonic SVZ neurogenesis. Cunningham et al. (2013) demonstrated that microglia regulate the size of the neuronal precursor pool via phagocytosis of Tbr2+ and Pax6+ cells during the late stages of cortical neurogenesis, as analyzed during embryonic stages in these experiments. Notably, most precursor cells that were targeted by microglia did not exhibit signs of cell death or apoptosis. These authors also demonstrated that maternal immune activation reduces the size of the neural precursor cell pool via microglial phagocytosis. The phagocytosis that occurs in the embryonic SVZ differs from that in the adult SGZ, in which unchallenged microglia phagocytose apoptotic cells as discussed previously (Sierra et al., 2010). The precise mechanisms for embryonic SVZ phagocytosis remain to be clarified. Sultan et al. (2013) used doxycycline (Dox) and liposomal clodronate to deactivate microglia and demonstrated that Dox directly increased neurogenesis in the adult mouse SGZ (6 w), emphasizing that multiple tools should be used to suppress microglial activation and the reproducibility of the results should be confirmed to study about the contribution of microglial activation to the actions. More recently, using multiple mouse models, including cell-depletion approaches and cx3cr1−/−, CR3−/−, and DAP12−/− mutants, embryonic microglia in the forebrain were shown to act as modulators of dopaminergic axon outgrowth and neocortical interneuron positioning (Squarzoni et al., 2014). The microglial functions during brain development have increasingly attracted more attention to provide a framework for understanding the etiology of neuropsychiatric diseases.

The SVZ is a niche of life-long neurogenesis and oligodendrogenesis. Neurogenesis is initiated in quiescent type B stem cells that, upon activation to their proliferative state (activated type-B cells), give rise to type C transit amplifying progenitors, which subsequently generate type A neuroblasts (Doetsch et al., 1999; Garcia-Verdugo et al., 1998; Ihrie and Alvarez-Buylla, 2011). Adult-born SVZ type A cells migrate along the rostral migratory stream (RMS) to the OB, where they differentiate into GABA- and dopamine (DA)-releasing interneurons (Lledo et al., 2006; Luskin, 1993). In contrast,

newly generated oligodendrocytes migrate toward the corpus callosum, the striatal white matter tracts and the fimbria fornix (Gonzalez-Perez and Alvarez-Buylla, 2011; Hack et al., 2005; Menn et al., 2006). The SVZ NSCs are therefore important in terms of cell lineage determination and their potential application in clinical therapy. Although it has been shown that microglia play bidirectional roles for the maintenance of proper circuitry, i.e., microglia eliminate unnecessary cells, axons, and synapses, while support the neighboring ones (Ueno and Yamashita, 2014), limited information is available especially regarding the interaction between microglia and postnatal-adult SVZ neurogenesis. During the first few days after birth, a marked increase in the number of microglia is observed in the rodent brain (Sminia et al., 1987). As brain development progresses, amoeboid microglia become less abundant, with a concomitant increase in the number of ramified microglia, which acquire a surveillance role (Ginhoux et al., 2010; Prinz and Mildner, 2011; Schlegelmilch et al., 2011). However, the microglia in the neurogenic zone, such as the SVZ, have different functions compared with those in the non-neurogenic regions in terms of constitutive and postlesion levels of microglial activation (Goings et al., 2006).

Recently, we demonstrated that activated microglia first accumulate in the SVZ during the early postnatal period and then disperse to white matter where they became more ramified. In addition, the number of activated microglia was highest in the medial SVZ throughout the study period (P1–P30). Using a combination of *in vivo* and *in vitro* approaches, we demonstrated that these activated microglia in the early postnatal SVZ enhanced neurogenesis and oligodendrogenesis via cytokine release (Shigemoto-Mogami et al., 2014). The addition of microglia-conditioned medium to cultured P8 mouse SVZ cells increased neuroblast production (Walton et al., 2006), which is consistent with our data. Despite the lifelong presence of NSCs, a progressive reduction in neurogenesis is also observed in the aging SVZ (Tropepe et al., 1997). Our data also demonstrated that microglia adopt a more ramified shape by P30 (Shigemoto-Mogami et al., 2014). Walton et al. (2006) demonstrated that conditioned medium from adult-derived microglia is less effective in reconstituting inducible neurogenesis; however, this medium is markedly more effective in promoting the rapid morphological development of axonal processes. Taken together, these data suggest that the SVZ microglia undergo phenotypic changes during aging.

The effects of microglia on SVZ neurogenesis appear to differ in the rostrocaudal and dorsoventral locations. In our study, the number of activated microglia in the early postnatal SVZ was highest in the center plane along the rostrocaudal axis (Shigemoto-Mogami et al., 2014). Blood vessels may be related to this rostrocaudal distribution. The vasculature may be a critical niche compartment for stem cells in the adult SVZ (Goldberg and Hirschi, 2009; Quaegebeur et al., 2011). The SVZ is extensively vascularized by a rich plexus of blood vessels (Ihrie and Alvarez-Buylla, 2011; Shen et al., 2008; Tavazoie et al., 2008), and the central SVZ has large blood vessels that originate from the ventral aspect (Dorr et al., 2007; Shen et al., 2008). Among the soluble factors released from blood vessels (Goldberg and Hirschi, 2009; Shen et al., 2004), CXCL12/CXCR4 signaling may be involved in the accumulation of microglia in the center plane because microglia express CXCR4 and are recruited in the developing cerebral cortex by CXCL12/CXCR4 signaling (Arno et al., 2014). The adult SEZ (subependymal zone to discriminate from the embryonic SVZ when no ependymal cells are present) is highly regionalized, with neuronal progeny of distinct identities being generated in different areas along its dorsoventral and rostrocaudal axes (Brill et al., 2009; Merkle et al., 2007). The dorsal SEZ has an increased rate of oligodendrogenesis compared with the lateral SEZ (Ortega et al., 2013). The contribution of SVZ microglia to each progeny can be clarified if we specifically focus on the subregions in the SVZ along the dorsoventral and rostrocaudal axes.

Mechanisms That Underlie the Neurogenic Effects of Microglia in the Postnatal and Adult SVZ

As mentioned previously, the interaction between microglia and T cells is important for SGZ neurogenesis (Ziv et al., 2006). In an *in vitro* study, NSCs/NSPs were collected from the adult SVZ to demonstrate that neurogenesis and oligodendrogenesis are enhanced by microglia stimulated by Th2-derived cytokines (Butovsky et al., 2006). In our study (in the early postnatal SVZ), although the small population of activated microglia produced IGF-1, IGF-1 did not play a primary role in the neurogenic effects of activated microglia (Shigemoto-Mogami et al., 2014). We demonstrated that the neurogenic effects of early postnatal SVZ microglia were mediated by a combination of cytokines. We determined that the levels of IL-1β, IL-6, TNF-α, and IFN-γ in the SVZ cytosol during the early postnatal period were increased transiently compared with other periods (P1–P30) and that minocycline suppressed both neurogenesis and the cytokine levels significantly. Given that we could reproduce the neurogenic effects of activated microglia and the suppressive effects of minocycline in an *in vitro* neurosphere assay, we further examined the contribution of each cytokine to the effects of activated microglia. Interestingly, in our *in vitro* co-culture experiments, the enhancement of neurogenesis was suppressed by a mixture of function-blocking antibodies (anti-IL-1β, anti-IL-6, anti-TNF-α, and anti-IFN-γ) but not by any single

antibody. These results suggest that these cytokines enhance neurogenesis and oligodendrogenesis cooperatively. In support of this model, among these four cytokines, we confirmed that only IL-1β and IFN-γ enhanced neurogenesis, whereas only IL-1β and IL-6 exhibited the potential to enhance oligodendrogenesis. Previous reports have demonstrated that neural progenitor cells express IL-1β, IL-1RI, and IL-1RII and that IL-1β regulates the proliferation and differentiation of neural progenitor cells (Wang et al., 2007). Furthermore, IL-6 and IL-6R have been reported to promote neurogenesis (Islam et al., 2009). Li et al. (2010) showed that the effects of IFN-γ are modified in the presence of microglia, supporting a model of complementary interactions between cytokines. IL-1β, IL-6, TNF-α, and IFN-γ are proinflammatory cytokines that have been reported to suppress neurogenesis in pathological conditions, such as chronic LPS stimulation (as described in the SGZ chapter; Monje et al., 2003), allergic encephalomyelitis (EAE; Ben-Hur et al., 2003), and SE (Iosif et al., 2006; Koo and Duman, 2008). However, recent reports have indicated that a slight modification of the LPS application protocol induces a phenotypic change in microglia (Cacci et al., 2008). The microenvironment and ambient conditions may regulate the combination and concentrations of the cytokines that are released by microglia. In fact, some reports have suggested that the effects of cytokines change in a concentration- and context-dependent manner (Bernardino et al., 2008; Cacci et al., 2008; Das and Basu, 2008; Russo et al., 2011). Bernardino et al. (2008) demonstrated that TNFα results in NSC proliferation at 1 ng/mL but causes apoptosis at 10–100 ng/mL. A more recent study has confirmed that TNFα signaling via TNFR2 is required for basal neurogenesis, whereas signaling via TNFR1 impairs neurogenesis (Chen and Palmer, 2013). Our *in vivo* data indicate that when microglial activation is suppressed by minocycline, the number of neural progenitors decreases to fewer than half of the number observed under control conditions; however, the decrease in each cytokine level was uniform and mild. Cytokines are released by not only microglia but also other glia. Cytokine release from microglia may stimulate cytokine release from other cell types in a manner akin to "cytokine drizzling" (a milder effect than a "cytokine storm"; Clark, 2007).

The next question to be resolved is to determine what activates SVZ microglia in the early postnatal SVZ. A valuable clue is that the role of microglia in postnatal SVZ neurogenesis differs from that in embryonic neurogenesis (Cunningham et al., 2013). Circulating mediators and hormones are altered after birth (Spencer et al., 2008). During pregnancy, maternal proinflammatory responses are suppressed (Aguilar-Valles et al., 2007) and anti-inflammatory responses are increased (Ashdown et al., 2007). The levels of circulating estrogen and progesterone increase and progesterone rapidly decreases immediately before birth (Mesiano and Welsh, 2007). We speculate that SVZ microglia function as a "hub" that may trigger an optimal "cytokine nest" for neurogenesis in response to peripheral signals. Interestingly, neural progenitors contact blood vessels at sites devoid of the blood brain barrier (BBB) in the SVZ (Tavazoie et al., 2008), and neural progenitors regulate microglia, proliferation, migration, and phagocytosis via the secretion of immunomodulatory proteins (Mosher et al., 2012). The interaction between neural progenitors and microglia may also be important in terms of peripheral signal delivery to the brain.

Molecular Signaling Regulates the Activation State of Microglia

The development of microglia, including their renewal, during neuronal development has been largely unstudied. However, this information is important for understanding the temporally and spatially specific effects of microglia on neurogenesis during brain development. Runt-related transcription factor 1 (Runx1), a key regulator of the proliferation and differentiation of hematopoietic stem cells (HSCs; Burns et al., 2005), is expressed in forebrain microglia during late embryogenesis and the first 2 weeks of postnatal development (Zusso et al., 2012). Runx1 regulates microglial proliferation throughout the neurogenic regions (Logan et al., 2013) during development (Ginhoux et al., 2010). Runx1 has also been demonstrated to be necessary for the transition from the proliferative activated amoeboid state to the deactivated ramified phenotype in the postnatal mouse SVZ (Zusso et al., 2012). Runx1 is a major genetic target of Notch signaling (Burns et al., 2005), and amoeboid microglia express Notch1 together with its ligands Jagged-1 and Delta-1 from P1 to P10 (Cao et al., 2008). During this period, microglial populations supporting neurogenesis or oligodendrogliogenesis acquire gene expression of adult microglia (after P4; Butovsky et al., 2014). TGFβ1 signaling is also essential for microglia to adapt to the adult brain environment. TGFβ signaling induces the quiescent microglial phenotypic characteristics of adult microglia *in vitro*, and most microglia are lost in Tgfb1−/− mice. Similar to tissue macrophages, microglia are dependent on Csf-1R for their development (Erblich et al., 2011; Hamilton, 2008). Recently, a new cytokine, IL-34, has been identified in mice and humans and shown to bind Csf-1R with high affinity (Lin et al., 2008). The expression of IL-34 rescued the phenotype of Csf-1-deficient mice in a Csf-1R-dependent manner (Wei et al., 2010), suggesting that Csf-1 and IL-34 both regulate Csf-1R signaling. In specific areas of the adult brain, including the cortex, olfactory bulb, ventral striatum, and hippocampus, microglia rely on IL-34/Csf-1R signaling for their own maintenance (Greter et al.,

2012). Whether IL-34 and Csf-1 have complimentary or redundant roles remains to be established.

Heterogeneity of Microglia and Neurogenesis

As described above, microglia have diverse effects on neurogenesis in a spatially and temporally specific manner. In general, we use the term "activated" to describe the microglial phenotype in contrast to the ramified phenotype. However, recent studies have demonstrated that ramified microglia are not only quiescent, i.e., ramified microglia survey their territory actively with fine processes and receive environmental stimuli as sensory cells (Kettenmann et al., 2011). Currently, the definitions of microglial "activation" are ambiguous. Microglial phenotypes are determined by the microenvironment, including the myelin content, vascular and BBB features, cellular neighborhood, extracellular matrix constituents, prevalent neurotransmitters, and neurochemical milieu (Hanisch and Kettenmann, 2007; Kettenmann et al., 2011; Lawson et al., 1990). Hippocampal microglia express increased levels of mRNA for TNFα, CD4, and FcγRII compared with microglia in the diencephalon, tegmentum, cerebellum, or cerebral cortex (Ren et al., 1999). NT3 expression is identified selectively in microglia from the cerebral cortex, globus pallidus, and medulla but not from other brain regions (Elkabes et al., 1996). Even within a given region, the reactive phenotypes in response to infection or injury appear to be represented by subsets rather than by a uniform population of microglia (Scheffel et al., 2012). A single population of microglia can potentially adopt various phenotypes; however, whether these phenotypic differences can be accurately classified as "subtypes" remains unclear. Therefore, categorizing the neurogenic microglia present in the SGZ and SVZ may be difficult at present.

Furthermore, the M0, M1, and M2 stages are considered the functional phenotypes of microglia at present. These delineations are based on data regarding monocyte and macrophage biology (Sica and Mantovani, 2012). Macrophages that have been exposed to different stimuli are designated M1, M2a, M2b, and M2c depending on the stimulus and context (Geissmann et al., 2010; Mantovani et al., 2002; Mills, 2012). Approximately 50 surface markers that are characteristic of the mononuclear phagocyte lineage have been identified for macrophages, and 35 of these markers are considered useful for discriminating subsets of macrophages and dendritic cells (Chan et al., 2007). M1 microglia are proinflammatory and cause neurogenesis failure. However, whether anti-inflammatory M2 microglia represent the active neurogenic phenotype remains unknown. The characteristics of M2 microglia are not necessarily associated with positive outcomes; they can induce an inappropriate downregulation of the inflammatory response (Mantovani et al., 2002). Moreover, stimulus-induced transcriptional plasticity of microglia has been described. Because this type of plasticity does not correspond to M1 or M2 plasticity and is distinct from the resting state transcriptome, this stage has been referred to as M0 (Butovsky et al., 2014). Furthermore, the existence of intermediate phenotypes is possible. P3 mouse brain microglia express increased levels of both M1 (iNOS and TNFα) and M2 (Arginase-1) genes compared with adult microglia (Crain et al., 2013). The systemic intraperitoneal administration of LPS (1 mg/kg) to P5 mice increased the proliferation of microglia/microglial precursor cells and caused a transient inhibition of neuronal differentiation in the SVZ (Smith et al., 2014). The Cd86 (M1) and Ym1 (M2) genes were both upregulated at 48 h, whereas iNOS (M1) was upregulated, and IL-6 (M1) was downregulated. These data suggest that multiple microglial phenotypes, which cannot be simply categorized as M1 or M2, may exist. Recent comparative analyses have demonstrated that microglia exhibit distinct phenotypic and functional properties compared with peripheral macrophages regarding their responsiveness to M1-M2 polarization conditions (Durafourt et al., 2012). In addition to the M1-M2 classification, HoxB8 expression also divides microglia into positive and negative groups. HoxB8-expressing cells represent approximately 15% of the CD11b+ cells in the adult brain and may be ontogenically distinct from the yolk sac-derived dominant population of microglia (De et al., 2013). The characterization of this population is important because mice with a complete loss of HoxB8 exhibit excessive grooming, leading to hair loss and skin lesions; these phenotypes are similar to the human phenotype of the OCD spectrum disorder trichotillomania (Chen et al., 2010). The relationship between HoxB8 expression and neurogenic potential remains unknown.

A Note regarding the Experimental Design

An instructive role of microglia in the regulation of neuronal differentiation was first demonstrated by *in vitro* studies. *In vitro* microglial systems can never faithfully reproduce the complex characteristics of the *in vivo* environment; however, these systems allow us to investigate specific aspects of microglia that are masked by surrounding factors. Aarum et al. (2003) demonstrated that microglia can guide the differentiation of precursor cells isolated from the embryonic brain, as well as adult mouse neural precursor cells toward a neuronal phenotype. Reports that are more recent have demonstrated that microglia possess intrinsic, spatially restricted characteristics that are independent of their *in vitro* environment and that they represent unique and functionally distinct populations. SGZ microglia *in vitro* are uniquely capable of providing sustained levels of inducible neurogenesis (Marshall et al., 2014). Furthermore, the neurogenic

phenotype acquired from wheel running is sustained *in vitro* (Ziv and Schwartz, 2008a). However, isolated microglia do not exhibit the highly ramified structure that is typically observed in the healthy brain (Neiva et al., 2014). One of the major reasons for differences between *in vivo* and *in vitro* microglia is serum. In living organisms, microglia reside behind the BBB and are shielded from plasma proteins (Bechmann et al., 2007). *In vitro* cultures include high concentrations of serum (derived from plasma, to which microglia are never exposed in the healthy brain) (Ransohoff and Perry, 2009). Gene expression analysis has indicated that microglial cultures stimulated with cytokines and bacterial cell-wall components expressed genes in a pattern that was more similar to macrophages (challenged with the same stimuli) from the abdominal cavity compared with microglia isolated from animals that received the same cocktail of cytokines and bacterial components injected into the brain (Schmid et al., 2009). Plasma fibrinogen may be a key component of serum (Adams et al., 2007). Fibrinogen-stimulated microglia exhibit increased phagocytic capacity, an effect that is mediated via AKT- and Rho-dependent pathways (Ryu et al., 2009). *In vitro* microglial cultures are useful as long as the differences between *in vivo* and *in vitro* systems are acknowledged. To achieve convincing significance from an *in vitro* system, confirming that the observed phenomena are reproduced *in vivo* and demonstrating that the effect in question is mediated via the same signaling pathways are essential.

At P4, male rats have a significantly greater number of microglia compared with females in many brain regions critical for cognition, learning, and memory, including the hippocampus, parietal cortex, and amygdala (Schwarz and Bilbo, 2012). The same authors also demonstrated that females have a greater number of microglia later in development (P30-60). Most microglia in P4 males have been demonstrated to have an activated/amoeboid morphology, whereas microglia in P30-60 females exhibited a more ramified morphology. Furthermore, the hippocampal and cortical expression profiles of numerous cytokines, chemokines and their receptors shift dramatically during development and are highly sex dependent (Schwarz et al., 2011). In human females, the number of brain microglia has been demonstrated to increase with age (Pelvig et al., 2008). A strong sex bias is known to exist with respect to the prevalence of many neuropsychiatric developmental disorders that exhibit differences in the latency to onset, as well as to strong dysregulation of the immune system. For example, males are more likely to be diagnosed with early-onset developmental neurological disorders, such as autism, dyslexia, and schizophrenia (Bao and Swaab, 2010). Therefore, sex differences should be considered in animal experiments, particularly for studies that investigate animal models of developmental disorders.

Closing Remarks

In this review, I have summarized and organized the data regarding the effects of microglia on neurogenesis, particularly focusing on the SGZ and the SVZ, in which the neurogenic potential is progressively restricted during the life of the organism. As described above, microglia regulate neurogenesis in a temporally and spatially specific manner. To further understand the physiological significance of the role of microglia in neurogenesis, the development of microglia in the brain should also be clarified. The data introduced here raise the possibility that microglia sense signals from the surrounding environment and have regulatory effects on neurogenesis. We speculate that microglia function as a “hub” for information from the inner and outer brain regions during neurogenesis regulation. When the precise mechanisms for the enhancement of neurogenesis, interaction between microglia and NSCs, and delivery of peripheral signals into the brain are clarified, these new findings will be helpful in the clinical therapy of neuronal disorders, including developmental disorders.

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