Biology of Microglia in the Developing Brain
Charanjit Kaur, PhD, Gurugirijha Rathnasamy, PhD and Eng-Ang Ling, PhD, DSc

Abstract
Microglia exist in different morphological forms in the developing brain. They show a small cell body with scanty cytoplasm with many branching processes in the grey matter of the developing brain. However, in the white matter such as the corpus callosum where the unmyelinated axons are loosely organized, they appear in an amoeboid form having a round cell body endowed with copious cytoplasm rich in organelles. The amoeboid cells eventually transform into ramified microglia in the second postnatal week when the tissue becomes more compact with the onset of myelination. Microglia serve as immunocompetent macrophages that act as neuropharmacology sensors to detect and respond swiftly to subtle changes in the brain tissues in pathological conditions. Microglial functions are broadly considered as protective in the normal brain development as they phagocytose dead cells and sculpt neuronal connections by pruning excess axons and synapses. They also secrete a number of trophic factors such as insulin-like growth factor-1 and transforming growth factor-β among many others that are involved in neuronal and oligodendrocyte survival. On the other hand, microglial cells when activated produce a plethora of molecules such as proinflammatory cytokines, chemokines, reactive oxygen species, and nitric oxide that are implicated in the pathogenesis of many pathological conditions such as epilepsy, cerebral palsy, autism, and perinatal hypoxic-ischemic brain injury. Although many studies have investigated the origin and functions of the microglia in the developing brain, in-depth in vivo studies along with analysis of their transcriptome and epigenetic changes need to be undertaken to elucidate their full potential be it protective or neurotoxic. This would lead to a better understanding of their roles in the healthy and diseased developing brain and advancement of therapeutic strategies to target microglia-mediated neurotoxicity.

Key Words: Antigen presentation, Developing brain, Hypoxia-ischemia, Inflammatory cytokines, Microglia, Phagocytosis.

INTRODUCTION
Microglia are gaining tremendous recognition in recent years because they are implicated in different neurodegenerative diseases and neurological disorders (1, 2). In the mature rodent brain, they contribute 5%–12% of the total glial population, and in reference to other glial cells, the frequency appears to vary with regions, for example, in the cerebral cortex or corpus callosum (3). The prevailing view regarding roles of microglia is that they act as resident immunocompetent phagocytic cells in the disease processes in infectious, traumatic, inflammatory, ischemic, and degenerative conditions of the central nervous system (CNS) (4). As far as can be ascertained, the existence of microglia as a separate cellular entity in the normal brain or in pathological conditions was first documented by Del Rio-Hortega (5) in the early part of the last century; yet compared with other glial types in the brain, it would appear that microglia had attracted little attention by the contemporary authors until decades later (6, 7). It is possible that this might have been hindered by the lack of a reliable staining method for identification of microglia during that period. The weak silver carbonate staining method routinely used then for this purpose was described to be capricious, often yielding unsatisfactory results. Despite the technical limitations, some early authors had attempted to investigate the origin, development and roles of microglia. The study was later extended to the ultrastructural identification of microglia in which the silver carbonate staining was adapted for transmission electron microscopy (6, 7). The seminal report on the characterization of microglia by electron microscopy was documented in a comprehensive review by Ling (8). Adding to this was the first ultrastructural evidence indicating that microglia shared features of phagocytes (9).

Another step forward was a subsequent experimental study that demonstrated that microglia in the developing brain represent nascent brain macrophages; more importantly, they were derived from infiltrated blood monocytes (8, 10). The view that microglia in the developing brain were monocyte derived had since opened a new vista on the roles of microglia in the normal and diseased brain (11). This was followed by the immunophenotypic characterization of microglia using specific markers common to tissue macrophages (12–15). Since then, studies on microglia specifically their roles in neuropathology have been extensively explored using different experimental models or paradigms (16–18). The past 3 decades have seen an upsurge in microglia research and, indeed, an exponential growth in our knowledge on microglia especially in connection with their roles in various
neurodegenerative diseases and neurological disorders. Undoubtedly, the roles of microglia are now better clarified and amplified, although they appear to be extremely complex either as neuroprotective or neurotoxic (4, 19).

**ORIGIN OF MICROGLIA**

Microglial cells have been reported to originate from different mesodermal sources such as the embryonic mesenchymal cells in the pia, fetal macrophages of the yolk sac or the fetal liver depending on the embryonic age (20). The concept of mesodermal origin was first postulated by Del Rio-Hortega (3) based on congregation of pial cells stained with silver carbonate in the superior tela choroidea, pia covering the cerebral peduncles and inferior tela choroidea in the embryonic brain. From these concentrations the cells then invaded the brain and transformed into the round amoeboid microglial cells distributed throughout the brain. The pial (mesodermal) origin of microglial cells was supported by many authors (21–25). Later studies supported further the mesodermal origin of microglia using histochemical staining with isoelectin Griffonia simplicifolia (GSA I-B4) and Ricinus communis agglutinin-1 (RCA-1) (26–28). Other studies have suggested that precursor cells in the yolk sac give origin to microglia during the embryonic period (29). In the embryos of chick and quail, macrophages in the CNS were reported to originate from the yolk sac before the circulation was established (30). It has been demonstrated that microglia in the avian embryonic brain were derived by invasion and proliferation of macrophages from the pial surface (31).

As opposed to the above views some authors, however, had argued in favor of a neuroectodermal lineage of microglia (32–35). Thus, the subependymal cells (glioblasts) in the subventricular zone of lateral ventricles were thought to be the precursors of microglia (32, 36, 37). With the use of RCA-1 and monoclonal antibodies that recognize tissue macrophages and microglia, it was proposed that microglia originate in the germinal matrix rather than in the pial mesenchyme. However, others authors had contended that microglial precursors within the neuroepithelium were cells traversing the neuroepithelium from the cerebral ventricles to enter the nervous parenchyma and settle as microglia (38).

The hypothesis that microglia are derived from circulating monocytes was proposed by other workers including the bone marrow or the myeloid tissue (43), cannot be excluded.

**MICROGLIA IN THE DEVELOPING HUMAN BRAIN**

There is only a modicum of information addressing the issue of origin of microglia in the human brain. Almost all studies regarding microglial origin and differentiation in humans have been carried out in the fetal CNS tissue collected at the time of elective or spontaneous pregnancy terminations (44–46). Microglia have been reported to colonize the human brain and spinal cord before 12th week of gestation (47, 48), with limited numbers of amoeboid microglia being present in these regions (45, 49). By using RCA-1 or antibodies such as CD 68 (EBM-11) that recognize human tissue macrophages and microglia, it had been shown that the greatest number of labeled cells between 13 and 18 weeks of gestation was observed in the germinatal matrix (44) and in the white matter (50). During this period of development, the morphology of microglia was of the amoeboid type in the germinal matrix, whereas they progressively differentiate into ramified cells with the growth of the brain (44, 50) and disperse throughout the CNS. Andjelkovic et al had used immunoperoxidase, RCA-1, Lycopersicon esculentum (tomato lectin) and CD68 to label microglia in the brain tissue of human embryos and fetuses ranging from 4.5 to 13.5 gestational weeks of age (45).

Based on the staining patterns, these authors believed that 2 populations of microglia exist in the human brain during early development and these may arise from 2 different sources—monocytes and the yolk sac—and progressively develop typical microglia morphology. Ramified microglial cells have been reported to be the predominant type in the infant brain (48).

**AMOEBOID MICROGLIA AND RAMIFIED MICROGLIA**

In the search of the origin and mode of formation of microglia in the corpus callosum in the mature brain (32), in which the cells were first identified and characterized ultrastructurally (6, 7), attention was first drawn to the investigation of microglia in the same region of the pre (41) and postnatal (9, 33, 51) rat brain (Fig. 1). Interestingly, microglia as identified by their small cell body and scanty cytoplasm with branching processes in the mature brain and termed “ramified microglia” were absent in the corpus callosum; instead, the same area composed of loosely organized and unmyelinated axons was occupied by a large number of round and amoeboid cells that were also stained by the weak silver carbonate staining (Fig. 2) (33). These cells termed “amoeboid microglia” exhibited cytochemical characteristics such as staining with acid phosphatase and nonspecific esterase shared by tissue macrophages (51, 52), thus alluding to the possibility of them being monocytic in nature as in other tissue macrophages. With the progress of brain maturation, the amoeboid microglia transformed into ramified microglia (Fig. 2) within 2 weeks postnatally as evidenced by using a fluorescent tracer, rhodamine isothiocyanate (53), and specific microglia markers such as the antibody OX42 (which recognizes complement type 3 receptors) (14) and lectins (GSA I-B4, RCA-1, and tomato lectin) (54, 55) (Fig. 2). The transformation of amoeboid microglia into ramified microglia is a process that is coincident with the onset of myelination of axons in the corpus callosum (11, 56, 57). Electron microscopic studies have shown that amoeboid cells in 1- to 5-day-old animals possess a round nucleus with marginal chromatin clumps and abundant cytoplasm displaying lysosomal dense granules, vacuoles and a well-developed Golgi apparatus (58). Most of the cells in older animals were elongated and branched, showing a flattened nucleus and scanty cytoplasm containing a few lysosomal granules (58) (Fig. 3). These cells were immunoreactive with the antibody OX42 and showed

Kaur et al. J Neuropathol Exp Neurol • Volume 0, Number 0, Month 2017

MICROGLIA IN THE DEVELOPING HUMAN BRAIN

There is only a modicum of information addressing the issue of origin of microglia in the human brain. Almost all studies regarding microglial origin and differentiation in humans have been carried out in the fetal CNS tissue collected at the time of elective or spontaneous pregnancy terminations (44–46). Microglia have been reported to colonize the human brain and spinal cord before 12th week of gestation (47, 48), with limited numbers of amoeboid microglia being present in these regions (45, 49). By using RCA-1 or antibodies such as CD 68 (EBM-11) that recognize human tissue macrophages and microglia, it had been shown that the greatest number of labeled cells between 13 and 18 weeks of gestation was observed in the germinatal matrix (44) and in the white matter (50). During this period of development, the morphology of microglia was of the amoeboid type in the germinal matrix, whereas they progressively differentiate into ramified cells with the growth of the brain (44, 50) and disperse throughout the CNS. Andjelkovic et al had used immunoperoxidase, RCA-1, Lycopersicon esculentum (tomato lectin) and CD68 to label microglia in the brain tissue of human embryos and fetuses ranging from 4.5 to 13.5 gestational weeks of age (45). Based on the staining patterns, these authors believed that 2 populations of microglia exist in the human brain during early development and these may arise from 2 different sources—monocytes and the yolk sac—and progressively develop typical microglia morphology. Ramified microglial cells have been reported to be the predominant type in the infant brain (48).
labeling with lectin (Fig. 4) It is to be noted that most of the in vitro studies of primary microglia in later years by us as well as by others have been based on microglia harvested from neonatal rats, which presumably would represent predominantly the amoeboid microglia (59, 60). Notwithstanding, in view of their developmental relationship of having the same cell lineage and the existence of transitional forms, they are regarded as and simply referred to as microglia in the present description.

MICROGLIA AS A NEUROPATHOLOGY SENSOR

In the developing brain, microglia notably those preponderant in the white matter, respond vigorously to lipopolysaccharide (LPS) and interferon-γ (IFN-γ) (61–63) injected intraperitoneally into postnatal rats. They were induced to express major histocompatibility complex II (MHC II) antigens with some of the cells closely associated with the blood capillaries, indicating that they serve as immunocompetent cells in the developing brain, which might interact with the circulating lymphocytes. Microglia in the postnatal brain are also extremely sensitive to hypoxic exposure. It was argued that they may contribute to the periventricular white matter damage in the developing brain in hypoxia (64). Either in the developing or mature brain, microglia act as a neuropathology sensor that can detect and respond swiftly to subtle changes in the brain tissues, be it acute or chronic (11).

FUNCTIONS OF MICROGLIA

Phagocytosis

Microglia are active phagocytes that help to eliminate degenerating axons and cells during early CNS development as well as during infections, injuries and other pathological conditions. They share many common features of peripheral tissue macrophages. The phagocytic nature microglia especially on activation was evidenced in the developing brain as many of these cells were found to contain phagosomes at the ultrastructural level (Fig. 3). Additional evidence of their macrophagic nature was provided by the demonstration of hydrolytic enzymes including acid phosphatase, aryl phosphatase, nonspecific esterase and 5'-nucleotidase localized in them (52, 69). Remarkably, microglia in the developing brain exhibit nonspecific esterase known to be specific to blood monocytes (52), thus alluding to for the first time their cytochemical link to monocytes. The above-mentioned enzymes were found to be localized in the lysosomes. These cells avidly engulfed exogenous substances such as rhodamine isothiocyanate and horseradish peroxidase (HRP) that leaked into the brain tissue when administered intraperitoneally or intravenously (70, 71) and biotinylated dextran administered directly into the brain (72). Ingestion of HRP by the microglial cells occurred following injection of HRP in the lumbosacral region of the spi-
nal cord (73). *E. coli* injected intracerebrally into the neonatal brain were internalized by the microglial cells in <3 hours following the injection (74). Recent investigations in the developing cerebellum have provided further evidence for the phagocytic nature of these cells. Microglial cells were found to engulf varying amounts of cellular debris in the neonatal cerebellum until postnatal day 17 (75). Microglial cells were shown to limit the production of cortical neurons in the cerebral cortex of prenatal and postnatal macaques and rats by phagocytosing the neural precursor cells (76). In our own studies, we have encountered phagocytosis of dead cells and non-myelinated axons in the normal developing corpus callosum by electron microscopy (33, 58).

In fetal and postnatal rat brains, the microglial cells were found to phagocytose necrotic and apoptotic cells as well as degenerating axons following a hypoxic insult (77, 78). They tend to accumulate near dead neurons and clear dead or dying cells from the neonatal hippocampus following injury, thereby helping to limit secondary injury during the critical early time point following excision of hippocampal slices (79, 80). In inflammatory lesions and neonatal stroke, microglial cells were found to be engaged in phagocytosis (81).

Microglial cells in the developing brain express complement type 3 receptors (CR3), which are known to be involved in endocytosis (14), similar to tissue macrophages. CR3-expressing microglial cells appeared to phagocytose a

**FIGURE 2.** (A) Amoeboid microglia in the corpus callosum of a 1-day-old rat are evidently labeled by the weak silver carbonate staining. The cells are rich in cytoplasm, which appears to be vacuolated. The cells appear round or amoeboidic bearing short processes. (B) Two ramified microglia in the cerebral cortex of a 5-day-old rat brain stained by weak silver carbonate stain. Note the long branching processes. (C) OX42-labeled round amoeboid microglial cells in the corpus callosum of a 2-day old rat. (D) OX 42-labeled ramified cells in the cerebral cortex of a 2-day-old rat. (E) Lectin-labeled round, amoeboid microglial cells in the corpus callosum of a 1-day-old rat. Blood vessels (asterisks) are also labeled. (F) Lectin-labeled ramified microglial cells in the cerebral cortex of 7-day-old rat.
A large number of apoptotic cells following an X-ray induced injury in the neonatal rat brain (82). Upregulation of CR3 was observed on the microglial cells following intracerebral *E. coli* administration in postnatal rats (74). Besides CR3, microglia are known to express triggering receptor expressed on myeloid cells-2 (TREM2), which is thought to be involved in phagocytosis (83, 84). Recent studies in the developing brain have reported that TREM2 was expressed on a subpopulation of microglial cells during first postnatal week in grey and white matter (85), suggesting that it may be related to phagocytosis of apoptotic cells. In the white matter of the developing cerebellum, microglia express ganglioside GD3 (86), which has been reported to play a part in phagocytosis of oligodendrocytes during CNS development. Toll-like receptors (TLRs) have also been shown to be involved in microglial clearance of axonal debris of degenerating axons in microglial-axon coculture (87), and

**FIGURE 3.** (A) Electron micrograph of an amoeboid microglia in the loosely structured corpus callosum of a 5-day-old rat. Note the widely spaced unmyelinated axons. The abundant cytoplasm shows profiles of rough endoplasmic reticulum (rER), Golgi apparatus (G), lysosomes (Ly), a large phagosome (P) and some vacuoles (V). (B) Electron micrograph of a ramified microglia in the corpus callosum of a 20-day-old rat. Note the closely packed axons some of them are evidently myelinated (Ax). The cell shows a small amount of cytoplasm with a paucity of organelles and inclusions, which include a few lysosomes (Ly), Golgi apparatus (G) and profiles of rough endoplasmic reticulum (arrow).

**FIGURE 4.** (A) Electron micrograph of an amoeboid microglial cell showing OX42 immunostaining reaction products on the plasma membrane (arrows) in the corpus callosum of a 5-day-old rat. The cell shows a reniform nucleus (N), Golgi apparatus (G) and a phagosome (P). (B) Electron micrograph shows an elongated microglial cell whose plasma membrane is outlined by the lectin GSA I-B4 staining (arrows) in the corpus callosum of a 10-day old rat. The cell contains a nucleus (N) bearing coarse chromatin masses and some lysosomes (Ly).
their deficiency impairs phagocytosis of degenerating axons by microglial cells. We have reported enhanced TLR4 expression in microglia in neonatal rat brain following a hypoxic injury (88). Although the expression was related to neuroinflammation in response to hypoxia, it may also be involved in phagocytosis of degenerating axons and apoptotic cells which are seen frequently in different parts of the developing brain such as the hippocampus, cerebellum, and the corpus callosum (78, 89) (Fig. 5).

Antigen Presentation

Macrophages, dendritic, and other cells belonging to the immune system capture foreign antigens, process them and present them to T lymphocytes during initial stages of an immune response. Major histocompatibility complex I (MHC I) and MHC II molecules on the surface of macrophages and other cells (90–92) mediate antigen presentation.

The CNS has been considered historically as an immunologically privileged site for a long time based on the presence of blood-brain barrier (BBB), lack of lymphatics and an absence of antigen presenting cells. This concept has been changed in recent years in view of the expression of MHC I antigens, involved in presentation of foreign antigens to cytotoxic T lymphocytes, in microglial cells in the developing brain (15). Expression of MHC II antigens responsible for presentation of a foreign antigen to helper T lymphocytes, however, was not found on these cells under normal conditions. When stimulated with LPS, IFN-γ, or E coli, the expression of MHC II antigens was induced on microglial cells in the developing brain (61, 62, 74). Expression of MHC II has also been reported on microglia in the normal and pathological human fetal spinal cord (93). MHC I/II antigen expression on microglial cells has also been reported following perinatal hypoxia (94). The expression of these molecules provides evidence for the protective role of microglial cells in the developing brain as BBB is immature and leaky and, indeed, vulnerability to a potential immune threat is feasible. Disruption of the BBB has been reported in neonatal meningitis (95), hypoxic-ischemic injuries (96) and in excitotoxic lesions (97), and increased numbers of CD4-positive T cells in the brain have been noted in the white matter of brains of mice suffering from chronic perinatal hypoxia (98).

Synaptic Pruning and Remodeling

It has been suggested that microglia play an important role in regulating synaptic function through elimination of synaptic connections and their involvement in maturation of synapses in the developing brain. Studies have shown that microglia are involved in synaptic pruning and remodeling during postnatal brain development (99, 100) and impaired microglia function results in delayed maturation of synaptic circuits in certain regions of the brain such as the hippocampus (99). Complement 3 (C3) molecules are localized on the developing synapses and microglia specific phagocytic pathway, CR3/C3-dependent signaling, is believed to be one of the underlying mechanisms in synaptic pruning and remodeling of developing synapses by microglia (100, 101). In vitro studies suggested that interleukin-10 signaling may be involved in promoting synapse formation by microglia (102). Depletion of microglia from the mouse brain had resulted in alterations in synaptic protein levels and glutamatergic synaptic function (103). Besides CR3/C3 cascade, MHC class I molecules may also be involved in developmental microglia-mediated pruning or refinement of synapses (104–107).

Role in Development of Various Cells in the Brain

Microglia can regulate the number of neurons in the developing brain by phagocytosis of dead or dying cells and provide trophic support to the neural progenitor cells for their proliferation and maturation (108–110). In vitro studies have
shown that depletion of microglia or a deficiency of the chemokine receptor CX3C chemokine receptor 1 (CX3CR1), also known as fractalkine receptor, in microglia increases the number of apoptotic neurons in the cerebral cortex in normal brain (111). Microglia-derived insulin-like growth factor-1 (IGF)-1 was also identified as a trophic factor involved in maintenance of neuronal survival. Production of factors such as IGF-1, nuclear factor-kappaB (NF-κB), interleukin (IL)-1β, and IL-6 by microglia is thought to play a part in the survival, differentiation and maturation of oligodendrocytes (112–114). Using cultures of neural progenitor/stem cells obtained from rat embryonic day 16 subventricular zone and microglial cells from 1 day old rat cortex, Nakamichi et al (115) have shown that microglia-derived IL-6 and leukemia inhibitory factor are essential molecules for differentiation of neural progenitor cells into astrocytes.

In addition to the above, microglial cells in the developing CNS have also been suggested to regulate vascularization (110, 116) and influence myelination (117).

Angiotensin and Angiotensin Receptors

The renin angiotensin system (RAS) regulates blood pressure and electrolyte homeostasis. Renin produced by the juxtaglomerular cells of the kidney converts angiotensinogen to angiotension I, which is then converted to angiotensin II (Ang II) by the angiotensin-converting enzyme. Ang II is the most active peptide of the RAS and binds to 2 receptors, Ang II type 1 (AT1R) and type 2 (AT2R). Most of the physiological or pathophysiological functions of Ang II are carried out through its binding with these receptors (118, 119). Several studies have reported the existence of various components of the RAS in the brain (120) and have also reported that this system is involved in the regulation of fetal cardiovascular responses, body fluid balance, and neuroendocrine regulation (121). We have reported that Ang II, AT1R, and AT2R are expressed on microglial cells in the neonatal rat and the expression is sustained in the mature brain (122). The expression of Ang II and AT1R was reduced following a hypoxic injury whereas the expression of AT2R was increased (122). Since hypoxic insults to the brain are known to reduce the blood flow to the various regions of the brain and induce BBB dysfunction (123, 124), decreased Ang II and AT1R expression may be helpful in restoring the blood flow in such conditions. AT1R has also been reported to play a part in in apoptosis, oxidative stress, and neuroinflammation, and its suppression with AT1R blockers reduces inflammation (125) and suppresses apoptosis (126). An enhanced expression of AT2R in the microglial cells following a hypoxic injury was suggested to be neuroprotective (122).

Production of Neuroprotective Factors

Endothelins

The family of endothelins (ETs), considered traditionally as potent vasoconstrictors, consists of 3 isopeptides, ie endothelin-1 (ET-1), endothelin-2, and endothelin-3 (127, 128), which bind to 2 specific G-protein-coupled receptors subtypes, ET-A and ET-B receptors (129, 130), to carry out their actions. Endothelial cells, monocytes and macrophages are important sources of circulating ET-1 (131). In addition to its vasoconstrictor action, ET-1 exerts mitogenic and anti-apoptotic actions (132, 133). Microglia in the periventricular white matter in the developing brain express ET-1 (134), which was suggested to be involved in the proliferation and differentiation of glial precursors under normal conditions. Hypoxic injury resulted in a decreased production of ET-1 by the microglial cells with implications that its reduction may adversely affect the development of glial cells (134). However, ET-1 appears to be a double-edged sword with several studies reporting its beneficial effects as mentioned above whereas others argued that its increased expression is correlated with vasogenic edema formation via BBB disruption (135).

Insulin-Like Growth Factors

Microglial cells produce IGF-1 and -2 (136, 137) that are known to regulate the development of the nervous system (138) by promoting cell proliferation and differentiation (139) especially oligodendrocyte survival and myelination (140–142). In a coculture of primary microglia and neurons, microglia-derived IGF-1 favored the survival of neurons (111). IGF-1 is known to play a significant role in recovery from hypoxic-ischemic insults (143) by blocking tumor necrosis factor (TNF)-α-induced apoptosis and promoting proliferation/differentiation and survival of oligodendrocyte precursors (144, 145). However, when proinflammatory cytokines are overexpressed the secretion of IGF-1 by microglia decreases, which could cause deleterious effects (146).

Transforming Growth Factor-β

Transforming growth factor-β (TGF-β) family plays an important role in embryogenesis determining the right-left organization, axis formation and tissue patterning. All 3 isoforms of TGF-β, (TGF-β1-3), are expressed in the brain and are essential for neuronal and glial differentiation (147, 148). Following hypoxic exposure, TGF-β1 mRNA expression was increased in the periventricular white matter of neonatal rats. In these animals, TGF-β1 expression was localized in the microglial cells (149). In TGF-β1 knockout mice there was increased neuronal loss and microglial activation (150), suggesting a role for TGF-β1 in regulating microglial activation status. Interestingly the microglial cells also expressed receptors for TGF-β (149), which supports the idea of TGF-β-mediated regulation of microglial functions. This could include the ability of TGF-β to decrease the expression of MHCII, intercellular cell adhesion molecule 1, vascular cell adhesion molecule and TNF-α (151), which are known to be expressed in activated microglia. TGF-β is known to induce the production of nerve growth factors by acting through its receptors expressed by the brain parenchymal cells (152). It is highly possible that TGF-β could do the same in microglia by acting in an autocrine manner. Moreover, TGF-β1 was recently dem-
onstrated to have a role in the synapse formation in developing cerebellum (153).

Neurotrophins

Microglia also serve as a source for neurotrophins such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) (154–157). While unstimulated microglia could express mRNAs for all these neurotrophic factors mentioned above, stimulation with LPS up-regulates the mRNA expression of these factors (158). These neurotrophins are essential for the survival, differentiation and proliferation of the brain parenchymal cells during postnatal development (159), and they exhibit their action by either activating the tyrosine kinase B (TrkB) receptors or the p75 neurotrophin receptors (160–162). While activation of TrkB receptors could result in enhanced survival, activation of p75 neurotrophin receptor has been linked to neuronal apoptosis (163). In cocultures of microglia and dorsal root ganglion neurons, microglia-derived BDNF promotes neurite outgrowth and terminal contacts of dorsal root ganglion neurons (164). BDNF derived from ethanol treated microglia was shown to prevent apoptosis of hypothalamic neurons through mechanisms modulating reactive oxygen species (ROS) and cAMP responsive element binding protein pathway (165). Addition of conditioned medium from neurons to the microglial cultures favors the production of neurotrophins in microglia (157). In microglia cell lines, BV2 and N9, BDNF and NGF enhance their proliferation in a concentration dependent manner (166). Consistent with this, under inflammatory conditions, intranasal administration of BDNF was found to increase the number of activated and phagocytic microglia at the site of injury (167). Similar to TGF-β, these neurotrophins could inhibit the expression of MHC II in the activated microglial cells via the p75 neurotrophin receptor (160). Taken together these studies put forward the premise that brain injury could trigger neurotrophin production in microglia in order to enhance the survival of neurons. In turn, the neurotrophins could act in an autocrine fashion on microglia and inhibit inflammation providing a conducive environment for the survival of neurons at the injured site.

Neurotoxic Roles

Besides their neuroprotective functions, microglial cells are also known to play detrimental roles under various pathological conditions when they produce a plethora of molecules such as proinflammatory cytokines, chemokines, reactive oxygen species (ROS), and nitric oxide (NO), thus causing neurotoxicity.

Role in Neuroinflammation

Proinflammatory molecules are expressed at higher levels in the developing than in the mature brain in the absence of any pathology. Thus, it is suggested that they may have an important role in CNS development. Involvement of cytokines such as TNF-α and interleukin (IL)-1β in developmental processes such as neural cell migration, proliferation, differentiation, and death has been reported (168), whereas IL-6 has been shown to contribute to development of the vasculature (169, 170). In CNS injury, microglial cells release augmented amounts of proinflammatory cytokines which when sustained over a long period can cause damage to the developing brain cells. TNF-α and IL-1β are released in varying amounts by microglial cells in different areas of the developing brain following a hypoxic injury (67, 68, 171, 172). Upregulation of TNF-α and IL-1β in microglial cells was coupled with expression of their respective receptors TNF-R (1) and IL-1R (1) on oligodendrocytes in the periventricular white matter and the Purkinje neurons in the developing cerebellum (67, 68). The binding of these cytokines to their respective receptors was suggested as a mechanism leading to cell death. In neonatal rats subjected to a hypoxic exposure accumulation was increased in the microglial cells and this was shown to mediate augmented production of TNF-α, IL-1β, and ROS (172, 173). In addition to the above, cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), microsomal prostaglandin-E synthase, E-prostanoid receptor 2, and prostaglandin E2 (PGE2) expression was increased in microglia in the hypoxic developing brain (174). In connection with this, PGE2 was found to be involved in the regulation of TNF-α and IL-1β production. Increased accumulation of microglial cells occurred in the periventricular white matter in hypoxic neonatal rats through expression of monocyte chemoattractant protein (MCP)-1 and augmented the inflammatory response (175). Macrophage colony-stimulating factor (M-CSF) is another cytokine derived from microglia that promoted proinflammatory cytokine production by other glial cells such as the astrocytes in the periventricular white matter following hypoxic exposure, which added to the white matter damage induced by microglia-derived inflammatory cytokines (176). TLRs have been reported to play a role in immune responses. Microglia also express TLR4 whose expression is enhanced in hypoxic injuries and mediates neuroinflammation via NF-κB signaling pathway through production of TNF-α, IL-1β, inducible nitric oxide synthase (iNOS), ROS, and NO (88).

Microglia in the developing brain have also been demonstrated to be activated by alcohol in conditions such as the fetal alcohol syndrome. In this regard, ethanol triggers production of ROS and inflammatory cytokines as well as phagocytosis through activation of TLR2 and TLR4 signaling in microglia (177–179). Although alcohol-induced abnormalities in glial cells have been implicated in the adverse effects of alcohol on the developing brain (180), more work is clearly desirable to unravel the underlying molecular mechanisms.

Following a maternal injection of the teratogen cyclophosphamide to induce neural tube defects, the microglial cells in the fetal brain showed a marked increase in the levels of TNF-α and TGF-β expression (181, 182). It was suggested that upregulation of proinflammatory cytokines caused by cyclophosphamide may be the underlying cause of increased rate of neural tube defects. Increased expression of M-CSF by the microglia and a concomitant increased expression of M-CSF receptor in these cells following the teratogen-induced neuronal injury suggested that microglia are capable of responding to self-derived M-CSF in an autocrine fashion that results in cell proliferation and a proinflammatory response (183). This observation is supported by several studies that
have reported that M-CSF receptor expression induces microglial proliferation, cytokine expression, and a paracrine inflammatory response in brain pathologies (184, 185).

Accumulation of glutamate in hypoxic conditions in the developing brain results in activation of N-methyl D-aspartate receptor (NMDAR) and γ-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) on microglial cells (137, 186). Increased accumulation of glutamate enhanced the production of TNF-α and IL-1β by microglia (137). Furthermore, NMDAR expression in microglia resulted in TNF-α and IL-1β release via NF-κB signaling pathway in the periventricular white matter of neonatal rats following hypoxia (186). In addition, activation of glutamate receptors also led to the production of excessive amounts of NO through the iNOS isoform in the microglial cells (186). Activation of the microglial NMDAR triggers inflammation and plays a pivotal role in neuronal death in the perinatal and mature brain (187).

Notch signaling pathway is a key regulator of neurogenesis in the CNS (188) and administration of Notch ligands in the brain after ischemic injury has been reported to expand stem cell numbers and improve motor skills (189, 190). On the other hand, activation of Notch signaling and Notch pathway has been reported to be associated with exaggerated inflammation in many tissues (191), and inhibition of this pathway has been shown to ameliorate the severity of conditions such as autoimmune encephalomyelitis (192) and experimental autoimmune uveoretinitis (193). We have shown that microglia in the developing brain express Notch-1 receptor, which was colocalized with its ligands, Jagged-1, and Delta-1 (194). Notch-1 receptor expression was increased in the microglial cells in postnatal rats challenged with LPS and its suppression resulted in increased levels of TNF-α released from microglial cells, suggesting that Notch signaling pathway plays an important role in neuroinflammation (194).

Apart from cytokines, activated microglia also secrete chemokines that mediate the infiltration of leukocytes to the site of injury. LPS treatment increases the levels of chemokines such as interferon-γ-inducible protein-10, regulated on activation normal T expressed and secreted (RANTES), MCP-1, macrophage inflammatory protein (MIP)-1α (MIP-1α, also called CCL3), and MIP-1β in human fetal microglia (195, 196). In neonatal rat, following hypoxic ischemic injury, there was increased expression of MIP-1α/CCL3 in microglia (197). Hypoxic microglia in the presence of syndecan-2 released excessive amounts of CCL2/MCP-1, CXCL12 (also called stromal derived factor-1 [SDF-1]) (198). Infection of the prenatal rodent brains in utero with cytomegalovirus also upregulated the expression of chemokines such as CCL2/MCP1, CCL3, CCL4/MIP-1β, CCL7/MCP-3, and CCL12/MCP-5 in microglia (199), and all of the above-mentioned chemokines have been demonstrated to attract peripheral monocytes into the brain and exacerbate the injury. In addition to secreting chemokines, microglia also express chemokine receptors that influence their activation. One of the chemokine receptors expressed by microglia during development include CX3CR1, a receptor for neuronal chemokine fractalkine (200). The fractalkine-CX3CR1 signaling is implicated in the developmental events carried out by microglia, such as phagocytosis and synaptic pruning (201). Similarly, microglial migration during cortical development is also dependent on the chemokine CXCL12/SDF-1. This is facilitated in microglia through the expression of SDF-1 receptor, CXCR4. Chemokine signaling in an injured brain could also be detrimental. For instance, following hypoxic-ischemic injury in neonatal rodents, the neurons at the site of injury express MCP-1 that is reported to facilitate the migration of microglial cells to the injury location (202), which might exaggerate the injury by producing inflammatory mediators. In these animals, the expression of MIP-1α and MIP-1β in the infarcted region of the brain, preceded the microglia/monocyte accumulation (203).

Role in Oxidative Stress

Microglia-derived free radicals or ROS is a common observation following an injury to the brain. Free radicals such as superoxide, hydrogen peroxide, etc. are unstable molecules that could initiate oxidative stress. Under physiological conditions, they are neutralized by the antioxidant defense system. However, in the immature brains there is a lack of antioxidant system to combat the excessive oxidants (204, 205). Hence, excessive generation of free radicals in the developing brain could cause damage to the susceptible cells (ie oligodendrocytes [206] and neurons) by causing lipid peroxidation (207) and by damaging the myelin sheath (208). As aforementioned, microglial activation could also lead to excess production of iNOS and subsequent NO, which is also a free radical. NO has multiple roles depending on the cellular source of origin, and NO produced by iNOS is considered detrimental. The most harmful effect of NO is observed when it reacts with superoxide to produce the highly reactive peroxynitrite, which could result in DNA strand breaks, lipid peroxidation, and protein nitration. In developing periventricular white matter, following hypoxic exposure the expression of iNOS was predominant in the microglia, suggesting them to be the source of NO (78). In cultured microglia, hypoxic exposure increases the generation of reactive oxygen as well as reactive nitrogen species (172), including NO (186), and their subsequent release into the culture medium. In primary oligodendrocytes treated with conditioned medium from hypoxic microglia there was increased lipid peroxidation with a parallel reduction in the glutathione content (172). However, antioxidant drugs such as edaravone could inhibit free radical production by microglia and render protection to the immature brains (198). Taken together, all of these studies provide converging evidence for the damaging role of activated microglia in an injured immature brain.

SIGNALLING PATHWAYS IN MICROGLIA

Associated with the production of various proinflammatory mediators, activated microglia showed upregulated expression of JNK and p38 MAPK pathways (209). Moreover, increased NF-κB/p65 expression was a consistent feature (210), which was to be expected given the fact that the cells produce significant amount of iNOS and NO. JAK-STAT pathway is also implicated in the production of proinflammatory molecules, iNOS and NO in N9 microglial cells (211). In addition, activation of JAK/STAT1 pathway was reported to favor migration of microglia towards the
injury site in a stab wound mouse model (212). Hence, it is possible that depending upon the STAT subunit that is activated, it might have an influence on the functions of microglia. The most unexpected finding was the detection of Notch-1 signaling in activated amoeboid microglia including its downstream elements when challenged with LPS both in vivo and in vitro (88, 193, 213, 214). Even more striking was the demonstration of a reciprocal transactivation between Notch-1 and NF-κB in activated amoeboid microglia in regulation of production of proinflammatory mediators (209, 215). Microglia also produce pro-inflammatory molecules through activation of receptors for recognizing molecular patterns that are associated with pathogens or any type of danger. TLRs are one of such receptors expressed by amoeboid microglia and activation of TLRs might lead to inflammation either via the NF-κB pathway (88) or the interferon regulatory-3 (IRF3) signaling pathway (216). The other pattern recognition receptors that are not well studied in amoeboid microglia include the nucleotide-binding oligomerization domain (nod)-like receptors, and the retinoic acid-inducible gene-1-like receptors. Similar to TLR, activation of these pathways could also lead to inflammation by converging at either the NF-κB or IRF3 signaling pathways; however, further studies are required to confirm this in microglia in the developing brain. TREM2 signaling pathway is a pathway through which microglial innate immune response is controlled in the postnatal brain (85) and a favorable environment is ensured for myelination and normal development (217). Suffice it to say that the above may represent only a few of the many signaling pathways that regulate microglial activation (4).

**RELEASE OF GLUTAMATE AND GLUTAMATE RECEPTOR EXPRESSION**

Glutamate is the most predominant and vital excitatory neurotransmitter in the immature brain, that exerts its action by binding through its receptors, which are either ionotropic (AMPAR, NMDAR, kainate receptors) or metabotropic receptors (mGluR) (218–220). In the developing brain, it aids in the early development by signaling for proliferation, differentiation and migration of neurons. However, in the injured immature brains, excessive levels of glutamate are detrimental and activated microglia seem to be one of the sources of glutamate (137, 221–223). For instance, production of glutamate was apparently increased in cultured microglia treated with either LPS or sodium arsenite or infected with virus (222, 224). The same was observed even in microglial cultures subjected to hypoxia (137). In addition to releasing glutamate, microglial cells express receptors for glutamate (185, 225). In the white matter of neonatal rat brain following hypoxic injury, microglial cells were found to concomitantly overexpress both AMPAR (GluR2-4) and NMDAR subunits (NR1, NR2A-D) (137, 186). Overactivation of these receptors instigates the production of pro-inflammatory cytokines such as TNF-α and IL-1β along with other toxic factors such as NO and Fas L, which are known to cause oligodendrocyte and neuronal apoptosis. Addition of glutamate or kainate to the microglial cultures enhances the release of TNF-α through activation of either AMPAR or kainate receptors (226, 227). Along with TNF-α and IL-1β, hypoxia-induced activation of microglial NMDAR increases the expression of iNOS and subsequent production of NO (186). Even activation of mGluR2 expressed in microglia could enhance the release of cytokines and Fas ligand. All of these toxic factors act upon the highly susceptible cells of the brain, such as the oligodendrocytes and neurons, and cause their death by activating caspase-3 (172, 186, 228). While activated microglia could also produce neurotrophic factors such as IGF-I and IGF-II, excess glutamate suppressed IGF-I levels and delayed the repair mechanisms (137). The proinflammatory cytokines released from microglia could inhibit the glutamate reuptake by astrocytes (229), further complicating the entire injury mechanism. Thus excess glutamate released by activated microglia could be detrimental to oligodendrocytes and neurons, either by direct binding to their receptors expressed on these cells, or indirectly by enhancing the release of toxic factors and suppressing the release of neurotrophic factors from microglia.

**MICROGLIAL ACTIVATION IN VARIOUS DISORDERS OF THE DEVELOPING BRAIN**

**Periventricular White Matter Damage**

Microglia in the amoeboid form accumulate preferentially in the periventricular region in the corpus callosum above the lateral ventricles and the subependyma (33, 53), cavum septum pellucidum (230, 231), and also the subependymal cysts associated with the ventricular system (231, 232). The significance of this remains speculative, but it was suggested that microglia in these areas may be involved in clearance of cellular debris resulting from spontaneous cell death or remodeling of callosal axons (33) or early physical expansion of the developing brain (231). In view of their close association with the fiber tracts, it is conceivable that when activated by hypoxia (64), ischemia or septicemia (233), excess amounts of proinflammatory mediators released by them would affect the structural and functional integrity of the fiber tracts. There is evidence supporting the involvement of activated microglia in aggravating death of immature oligodendrocytes and degeneration of axons in the periventricular white matter in neonatal rats following a hypoxic insult (64, 78). We have demonstrated that the pathogenesis of periventricular white matter damage is multifactorial involving factors such as inflammation, excitotoxicity, excess production of NO, iron related oxidative stress, and vascular changes among many others (64, 67, 78, 172, 175, 186). The involvement of some of the above factors in periventricular white matter damage in hypoxic injuries has received support from other studies (223, 234–238).

**Cerebral Palsy**

Cerebral palsy is a motor disorder due to brain white matter damage that appears in infancy or in early childhood and affects movement, muscle tone, coordination, and balance. Most cases are congenital as the damage to the brain white matter occurs before birth. Intrauterine infection/inflammation, premature birth, and low birth weight have been suggested as the underlying causes leading to cerebral palsy. Pre-
clinical studies subjecting animals to hypoxia–ischemia or LPS have shown axonal damage and oligodendrocyte death in the cerebral white matter as well as neuronal damage in many regions of the developing brain (78, 239–241). Microglia are present in large numbers in the developing white matter tracts and are believed to regulate the development of the white matter by removing excessive axons in the developing brain (242). Postmortem studies in the human newborns have implicated cytokines such as TNF-α and its receptors TNFR1 and TNFR2 as the major players in causing damage (243, 244). Activation of microglial cells in the white matter and other regions of the brain and enhanced release of TNF-α, IL-1β, and other proinflammatory molecules such as release of NO through iNOS by them has been reported in various animal models (67, 172, 186, 245, 246). Besides their inflammatory role, microglia may also have a protective role in early stages by phagocytizing dead cells and degenerating axons (78). Microglial activation and accompanying COX-1 enzyme expression have also been implicated in overproduction of proinflammatory mediators resulting in white matter damage and development of cerebral palsy (247). Excitotoxic mechanisms have also been suggested to play a role in neuronal death (248) and in axonal damage in the developing brain and this is supported by expression of NMDAR on premyelinated axons in human mid-term fetuses (249) and on the microglial cells in the neonatal rat brains (186).

Epilepsy

Epilepsy is a disorder in which seizures develop due to an imbalance between cerebral excitability and inhibition resulting in uncontrolled excitability. Seizures have been reported to develop more readily in the immature brain than in the mature brain. Many developmental processes such as cell division, migration, expression of receptors, formation, and stabilization of synapses are affected by seizures (250, 251). Temporal and frontal lobes are frequently involved. Premature birth or low birth weight, birth trauma, fever or infections are some of the underlying causes of epilepsy in newborns and infants. Glial activation and neuronal damage occurring in seizures has been reported in brain regions such as the hippocampus (252). Several studies have shown that proinflammatory molecules influence susceptibility to seizures (253–255). Enhanced levels of TNF-α and IL-1β were reported to play a role in the pathophysiology of epilepsy (256). Microglial activation in the postnatal mouse hippocampus was linked to heightened seizure susceptibility (257). Upregulation of TNF-α and IL-1β in microglia was readily detected after the induction of acute seizures (257, 258). Binding of IL-1β to its receptor in the hippocampus has been suggested to result in increased synaptic excitability (259). TNF-α affects neural circuits by preventing refinement and elimination of synapses occurring during normal brain development that may lead to increased connectivity and epilepsy (260).

Autism Spectrum Disorders

Autism spectrum disorders (ASD) are neurodevelopmental disorders with disabilities in communication and social skills and repetitive behaviors. Genetic factors, prenatal drugs, or chemical exposures are some of the risk factors for development of autism. Involvement of microglia due to their role in synaptic refinement during development has been suggested in these disorders (261). Deficient synaptic pruning due to a reduction in microglial numbers has been associated with weak synaptic transmission and decreased functional brain connectivity (262) that may be involved in autism and other neuropsychiatric disorders. Cerebellar dysfunction and subsequent thalamic hyperactivation in early childhood may be related to the development of ASD (263). Thalamic hyperactivation is thought to be induced by microglia-mediated neuroinflammation (263), and neuroinflammation is considered as an important factor in the pathogenesis of neuropsychiatric disorders (264, 265). Microglia activation and proliferation, along with increased expression of TNF-α, IL-1β, IL-6, and IL-17 in the brain and cerebrospinal fluid of ASD patients, has been observed (266, 267). Focal inflammation due to abnormal microglial activation and proliferation seems to affect normal synaptic activity in brains of patients with ASD (268–270). Along with increased microglia activation, decreased neuronal activity was observed in human brain cortical tissue samples from ASD patients (267). The above findings point towards the important role that microglia play in the pathogenesis of ASD (271, 272). Interactions between mast cells and microglia in the brain have been reported to occur as evidenced by induction of microglia activation and proliferation by mast cell-derived histamine (273, 274) that ameliorated with suppression of mast cells (275).

Perinatal Stroke

Perinatal stroke occurs in newborns in the first few days after birth due to a disturbance in the blood supply to the brain resulting in hypoxia and leads to significant morbidity and long-term neurological and cognitive deficits. Blood clotting disorders, maternal infection, preeclampsia, maternal diabetes and smoking are some of the risk factors that can lead to a perinatal stroke. In the rodent model of neonatal stroke after transient middle cerebral artery occlusion, microglial cells have been reported to have a neuroprotective function. Depletion of microglia by intracerebral injection of liposome-encapsulated cladronate in rats at postnatal day 5 has been shown to trigger hemorrhages at 24 hours after transient middle cerebral artery occlusion (276). Along with this, increased levels of several cytokines and chemokines already elevated by ischemia-reperfusion and increase in the severity and volume of injury were demonstrated in animal models of neonatal stroke following depletion of microglia (81). These findings point towards a protective role exerted by microglia during the subacute injury phase. However, other studies have reported that infiltration and activation of microglia in the brain of postnatal rats after neonatal stroke occurs over several days resulting in neuroinflammation and cell death (277).

EPIGENETICS AND TRANSCRIPTOME ANALYSIS

A recent search for the interacting noncoding RNAs, genes, and their epigenetic changes, (epigenetics refers to the heritable changes in the mechanisms that regulate the gene expression without altering the underlying genetic code), has
brought into light several unknown aspects of microglia, including their involvement in disorders that were thought to be microglia independent. By cDNA microarray analysis, amoeboid microglia were found to express proliferation- and differentiation-related genes, Sox4, Sox11, and Runx1t1 and also those genes involved in cell cycle process and migration (278). Interestingly, with the ramification of the cells with age, the expression of various genes was altered and this may be related to the specific functions of the 2 phenotypes. Microglia also express a neuroprotective and inflammatory phenotype, which depends on the environmental cues. For instance, transcriptional analysis in postmortem brain samples from autistic children revealed the transformation of microglia towards the inflammatory phenotype (279). Genes of immune pathway such as C1qA, C3, CR3, and TNF-α, which are expressed by microglia and transcription factors such as IRF8 and SPI1, that are essential for development of microglia were found to be methylated in autistic brains (280). Additional evidence for the epigenetic regulation in microglia and its influence in the childhood disorders comes from microglia specific methyl cytosine binding protein 2 (MeCP2) knockout mice. Of note, MeCP2 regulates gene transcription by binding to the methylated cytosine and guanine rich sites (CpG islands), and its reduced expression has a role in the etiology of autism (281). Deletion of MeCP2 in mice contributed to the dysregulation of extracellular glutamate levels and aberrant neuronal dendrites (282). In primary cultures of microglia from neonatal rodents, hypoxia mediated reduction in the microRNA21 expression accounted for the increased production of Fas ligand that induces neuronal injury (283). microRNA124 is specifically expressed in microglia and it suppresses the expression of MHCII antigens. Loss of microRNA124 precedes the onset of experimental autoimmune encephalitis (284), which explains the enhanced autoimmunity observed. Prenatal stress has been shown to increase the methylation of genes such as those for glucocorticoid receptors elsewhere (285); however, at this juncture there is no clear evidence if the same is seen in microglia of prenatal brains, which could be linked to the increased inflammatory phenotype that is observed under injury conditions. All these studies converge to a point, that epigenetic regulations occurring in microglia could have a greater impact on the normal brain development as well as various disorders of the developing brain.

Concluding Remarks

Microglia in the developing brain exist in 2 different phenotypes: amoeboid microglia and ramified microglia. Amoeboid microglia are monocyte-derived brain macrophages, that evolve to become the ramified microglia in the course of brain development. Microglia play an indispensable role in building the normal brain histotarchitecture including phagocytosis of apoptotic neurons and axons, and pruning of unwanted synapses in brain remodeling. They secrete neurotrophic factors such as IGF-1, NT-3, BDNF, and NGF; hence are neuroprotective. Overt microglia activation due to perturbation of the microenvironment can lead to increased release of glutamate and proinflammatory mediators including TNF-α, IL-1β, NO, ROS, etc., which in turn would exacerbate brain damage. Microglia-mediated neuroinflammation is implicated in different neurological diseases and disorders such as periventricular white matter damage, cerebral palsy, autism spectrum disorders, epilepsy, and perinatal stroke. Microglia activation in the developing brain is controlled through a complex regulatory mechanism and multiple signaling pathways such as NF-kB, Notch-1, JNK, and p38 MARK that are linked to production of proinflammatory mediators. While the functional roles of microglia in the developing brain are greatly amplified in recent years, there remain many issues to be fully explored such as transcriptome and epigenetics changes in microglial activation. This may pave way for designing appropriate therapeutic strategies in which the neurotoxic effects of microglial activation may be eliminated to mitigate the injury in the developing brain.

ACKNOWLEDGMENT

The technical assistance provided by Ms. Suat Hoon Tan, Ms. Yee Gek Chan, Mrs. Eng Siang Yong, Mrs. Geok Lan Ng, Mr. Tuck Yong Yick, Dr. Qiong Cao, and Dr. Yajun Wu is gratefully acknowledged.

REFERENCES

14


74. Perez-Pouchoulen M, VanRyzin JW, McCarthy MM. Morphological and phagocytic profile of microglia in the developing rat cerebellum(1,2,3). eNeuro 2015;2:doi: 10.1523/ENEURO.0036-15.2015

75. Cunningham CL, Martinez-Cerdeño V, Noctor SC. Microglia regulate the number of neural precursor cells in the developing cerebral cortex. J Neurosci 2013;33:4216–33


85. Wolswijk G. Strongly GD3+ cells in the developing and adult rat cerebellum belong to the microglial lineage rather than to the oligodendrocyte lineage. Glia 1995;13:13–26


89. Rocha N, Neckejes J. MHC class II molecules on the move for successful antigen presentation. EMBO J 2008;27:1–5


91. Roche PA, Furuta K. The ins and outs of MHC class II-mediated antigen processing and presentation. Nat Rev Immunol 2015;15:203–16


103. Goddard CA, Butts DA, Shatz CJ. Regulation of CNS synapses by neuronal MHC class I. Proc Natl Acad Sci USA 2007;104:6828–33


116. Arnold T, Betsholtz C. The importance of microglia in the development of the vasculature in the central nervous system. Vasc Cell 2013;5:4
118. Fitzsimons JT. Angiotensin, thirst, and sodium appetite. Physiol Rev 1998;78:583–686
158. Davis MI. Ethanol-BDNF interactions: still more questions than answers. Pharmacol Ther 2008;118:36–57
159. Zucca S, Valenzuela CF. Low concentrations of alcohol inhibit BDNF-dependent GABAAergic plasticity via L-type Ca2+ channel inhibition in developing CA3 hippocampal pyramidal neurons. J Neurosci 2010;30:6776–81


170. Gadient RA, Otten U. Expression of interleukin-6 (IL-6) and interleukin-6 receptor (IL-6R) mRNAs in rat brain during postnatal development. Brain Res 1994;637:10–4


181. Limatola C, Ransohoff RM. Modulating neurotoxicity through the fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. J Immunol 2012;188:29–36


199. Limatola C, Ransohoff RM. Modulating neurotoxicity through the fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. J Immunol 2012;188:29–36


263. Nakagawa Y, Chiba K. Involvement of neuroinflammation during brain development in social cognitive deficits in autism spectrum disorder and schizophrenia. J Pharmacol Exp Ther 2016;358:504–15


283. Zhang L, Dong LY, Li YJ, et al. miR-21 represses FasL in microglia and protects against microglia-mediated neuronal cell death following hypoxia/ischemia. Glia 2012;60:1888–95
