

Role of microglia in the process of inflammation in the hypoxic developing brain

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1. ABSTRACT

The developing brain is susceptible to hypoxic damage because of its high oxygen and energy requirements. Hypoxia-induced inflammatory response has been recognized as one of the main culprits in the development of hypoxic brain injury. In this regard, a hallmark feature is microglial activation which results in overproduction of inflammatory cytokines, free radicals and nitric oxide. Concomitantly, activated microglia exhibit enhanced expression of ion channels such as Kv1.2, Kv1.1 and Nav which further promote the release of inflammatory cytokines, chemokines and reactive oxygen species. Through the above-mentioned inflammatory mediators,

activated microglia induce neuronal loss, axonal damage and oligodendroglial death along with myelination disturbances. Our recent studies have extended that tumor necrosis factor- α , interleukin-1 β , monocyte chemoattractant protein-1 and macrophage colony stimulating factor produced by activated microglia are linked to the pathogenesis of periventricular white matter damage in the hypoxic brain. It is envisaged that a better understanding of the interactions between microglia and neurons, axons and oligodendrocytes is key to the development of effective preventive and therapeutic strategies for mitigation of hypoxic brain injury.

2. INTRODUCTION

A regular supply of oxygen is required for the structural and functional integrity of brain (1). The brain is particularly vulnerable to hypoxic conditions because of its high energy requirements (2). The human brain represents only 2% of the body weight but accounts for 20% of the total body oxygen utilization (3, 4). Hypoxia-ischemia affects the normal development and maturation of the brain (5, 6) resulting in long-term neurological deficits (7). Although damage to selected regions of the brain is caused by hypoxia-ischemia at different ages, the white matter peripheral to the lateral ventricles called periventricular white matter (PWM) is selectively vulnerable to damage in premature infants (8, 9, 10, 11, 12). Hypoxia results in direct brain injury which occurs in the acute hypoxic phase and indirect brain injury occurring after reoxygenation which is mainly attributed to neuro-immunological activation in the central nervous system (CNS) (13). Inflammatory response induced by hypoxia plays a crucial role in indirect hypoxic brain injury (14).

Neuroinflammatory response in the CNS is a complicated process which involves numerous damage signals, cellular responses and alterations in the microenvironment (15, 16). Following the neuroinflammatory response, a complex cascade of cellular events occurs. Microglia, which are resident immune cells in the CNS and belong to the mononuclear phagocyte system, have been recognized as sensors of brain injury and main cytokine producers (17, 18). The prevailing concept is that they are mainly derived from blood monocytes and/or their hematopoietic precursors although another school of thought has maintained that they arise from the mesoderm or neuroectoderm (19). The first mentioned view states that monocytes invade the brain during early postnatal development to become amoeboid microglial cells (AMC) bearing features of monocytes or monocyte-derived macrophages (19, 20). As a consequence of pathological events such as hypoxia/ischemia, stroke or trauma, microglia are activated and increase in cell numbers through proliferation or migration (21, 22). The microglia resemble macrophages in their ability to secrete factors, scavenge, engulf and clear cellular debris in and around the injury site (21, 22, 23). In the developing brain, AMC, considered to be nascent form of microglia, are present in large numbers in the PWM (20). It is well documented that under hypoxic condition, AMC are activated and the ensuing modulated inflammatory response leads to PWM damage (PWMD) in neonatal rats (10, 24).

The pathogenesis of hypoxic brain injury is complex and is not fully elucidated. Neuronal loss, white matter damage and immune cell activation in the CNS have been described as hallmark features of hypoxic brain injury (12, 25). Over the past decade, the molecular mechanisms underlying hypoxic brain injury, especially in the developing brain, have been the focus of many studies. To this end, this review provides a brief overview on the role of microglia in the neuroinflammatory response elicited by hypoxia with special reference to the PWM in the developing brain.

3. ETIOLOGY AND RISK FACTORS OF HYPOXIC BRAIN INJURY

Hypoxia-ischemia has been reported to be a leading cause of morbidity and mortality in the perinatal period (26). The developing brain is extremely sensitive to oxygen deprivation (27) resulting in neuronal loss (28) and PWMD (29, 30, 31, 32). Seizures, mental retardation, epilepsy and cerebral palsy are some of the consequences of a cerebral hypoxic/ischemic insult suffered during the perinatal period (30, 33). Many maternal causes such as diabetes, asthma, anemia and smoking and intrapartum events such as prolonged labor are associated with fetal hypoxia (34, 35, 36). In the neonates, pulmonary or cardiac dysfunction and neonatal stroke result in hypoxia (37). Hypoxia can also occur during the antepartum or intrapartum periods due to placental insufficiency, cord prolapse, prolonged or decreased maternal oxygenation (38). Premature babies are at high risk of a variety of complications, such as respiratory distress syndrome, bronchopulmonary dysplasia (due to immaturity of lungs) and bradycardia among many others (39). These complications result in oxygen deprivation to the brain tissue.

4. PATHOLOGICAL CHANGES IN THE HYPOXIC DEVELOPING BRAIN

Following hypoxic-ischemic injuries, a significant cell death is known to occur in the neonatal brain (40, 41, 42) in many regions such as the hippocampus, cerebellum, thalamus (43, 44, 45). Neurons in the hippocampus and cerebral cortex and Purkinje cells are the most frequently injured in term infants whereas in preterm infants the oligodendrocytes in the PWM are most susceptible to hypoxic-ischemic damage (30, 46). Besides oligodendrocyte death, hypoxic-ischemic injuries in the neonatal brain are known to result in axonal degeneration (31) and disturbances in myelination (32, 47). Other changes occurring as a result of hypoxic injury in the neonatal brain are activation of microglia, astrogliosis and blood - brain barrier (BBB) damage.

4.1. Neuronal loss

Neuronal loss is known to occur following hypoxic-ischemic injuries (48, 49). Hypoxia, especially when associated with ischemia, initially results in neuronal damage due to failure of Na^+ and K^+ pumps which leads to membrane depolarization, failure of synaptic transmission and release of excess glutamate (50). Activation of calcium permeable N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy 5-methyl-4-isoxazolepropionic acid (AMPA) receptors by glutamate causes massive Ca^{2+} influx into neurons (51, 52). Excessive extracellular glutamate release and calcium influx lead to neuronal death by necrosis or apoptosis (53, 54) apoptosis being a more prominent mode of neuronal death following hypoxic-ischemia in the neonates compared to the adult brain (55, 56).

Other mechanisms of neuronal injury include free radical formation, mitochondrial dysfunction and

inflammation (25). Several pathways of free radical generation including cyclooxygenase, lipoxygenase, xanthine oxidase and nitric oxide synthase (NOS) which lead to membrane lipid peroxidation resulting in neuronal cell damage may be initiated by increased intracellular calcium in hypoxia (52). Acute hypoxic episodes result in neuronal loss in the neonatal brain. Following this, secondary brain damage, which is attributed to neuroimmunological cell activation, excess excitatory amino acid release and overproduction of free radicals occurs (31).

4.2. Oligodendrocytes injury and myelination defects

Oligodendrocytes are the myelin-forming cells in the CNS and originate from neuroepithelial cells of the ventricular zones at very early stages of embryonic life (57, 58). Oligodendrocyte progenitors migrate to the developing white matter tracts and undergo substantial proliferation before their final differentiation into myelin-forming cells (58). Mature oligodendrocyte processes spiral around the axon to form myelin sheaths (59).

Some studies have demonstrated that immature oligodendrocytes during a specific prenatal window were vulnerable to hypoxia, which is a significant underlying factor in the pathogenesis of PWMD (60). Myelination deficits have also been observed in the brains of animals following perinatal asphyxia (61). Hypoxic-ischemic injury in the neonatal brain is known to result in death of oligodendrocyte progenitors, disruption of myelin gene expression and disturbances in myelination (62, 63). Reduced myelin basic protein (MBP) immunoreactivity was observed in the PWM in hypoxic rats (31).

At present the specific mechanisms responsible for oligodendrocyte injury have not been fully elucidated. But glutamate excitotoxicity and cytokine-mediated injury are believed to be the major contributors (24, 31). In addition, developing oligodendrocytes are liable to be damaged by oxidative stress because of lack of anti-oxidant enzymes (64, 65). Likewise, activation of cytokine receptors has been involved in oligodendroglial death not only at various stages of development but also in the pathogenesis of adult demyelinating disorders (66). The activation of cytokine receptors on the surface of oligodendrocytes can cause their death through cross-talk between ligand and receptors, which activate intracellular signaling pathways related to apoptosis and energy metabolism disruption (66). Our previous study has shown that under hypoxic conditions, the AMC in the PWM in the neonatal brain produce inflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin (IL)-1 β via MAPK signaling pathway (24). The possible interaction between AMC and oligodendrocytes via the above-mentioned proinflammatory cytokines and their respective receptors might lead to damage of the latter cell type (24). This was evidenced by the common occurrence of apoptotic oligodendrocytes following hypoxia (24). It was suggested that the decrease in the number of oligodendrocytes would result in myelination deficits, a hallmark in PWMD (24).

4.3. Axon injury

The axon is an elongated fiber that extends from the neuronal body to the terminal endings and transmits the neural signal. The larger the axon, the faster it transmits information (59). Some axons are covered with a fatty substance called myelin that acts as an insulator (59). These myelinated axons transmit information much faster than other non-myelinated neurons. In normal myelinated axons, Na⁺ channels are concentrated at nodes of Ranvier, which accelerate signal transduction through allowing the action potential to rapidly jump from node to node (59). The axons are non-myelinated in the neonatal brain. In hypoxic injuries, axons are vulnerable to energy depletion, loss of myelin (59) and hypoxia-induced inflammatory response (31). In our recent study, the most conspicuous finding was the occurrence of swollen and degenerating axons when neonatal rats were subjected to a hypoxic exposure (31). Quantification of the degenerating axons in the PWM showed a significantly higher number of degenerating axons in the hypoxic rats as compared to the controls (31). Occasional axons were observed to be myelinated in 7-day-old rats, and the myelin sheaths of these axons appeared distorted (31). This phenomenon of axonal degeneration in the hypoxic rats was especially conspicuous in the vicinity of the blood vessels (31). The most striking structural alteration at 14 days after the hypoxic exposure was dilatation of a variable number of axons which appeared club shaped in sections and distortion of myelin sheaths (67). Many of the dilated axons contained a large number of vacuoles. In some sectional profiles, myelin-like figures appeared to be internalized in the axoplasm (67).

Mechanisms underlying axon injury are complex and remain unclear. Some studies have demonstrated that premyelinated white matter axons in isolated rodent optic nerve were highly resistant to hypoxic-ischemic injury, whereas early and late myelinating white matter axons were increasingly vulnerable (68). So the vulnerability of white matter axons to hypoxic-ischemic injury varies during development. Similarly, mechanisms of hypoxic-ischemic axon injury may also be different during development (68). Energy depletion during hypoxia-ischemia leads to failure of energy-dependent extracellular and intracellular ionic balance, resulting in axonal Ca²⁺ overload, conduction damage, and structural injury (69). Excessive glutamate receptor activation, or excitotoxicity, has been reported to result in ischemic white matter axon injury (70). This observation is supported by intracerebral injection of AMPA which induced axon injury (70). AMPA/kainate receptors participate in ischemic injury to myelinated axons *in vivo*, but not to isolated axons. Studies have shown that ionotropic glutamate receptor agonists did not cause damage to isolated axons, nor did glutamate receptors antagonists protected isolated axons from oxygen-glucose deprivation (71). Therefore, glutamate induces white matter axons injury by causing damage to myelinating oligodendrocytes. It has been reported in some studies that axonal injury in myelinated white matter results from oligodendrocyte excitotoxicity and can be prevented by blockade of oligodendrocyte AMPA/kainate receptors (71). The mechanisms linking oligodendrocyte injury to axon damage might include release of toxic substances from

injured oligodendrocytes, loss of trophic support to axons, or loss of glial homeostatic functions (72). Attenuation of oligodendrocyte-myelin-axon interactions in myelinated white matter decreases axonal injury after AMPA injection (72).

Some reports have suggested that oligodendrocyte excitotoxicity does not result in axonal damage in premyelinated white matter (71), where the axons are non-myelinated, as interaction between these axons and pre-oligodendrocytes is weak (72). Axons in premyelinated white matter are vulnerable to hypoxic-ischemic injury through a non-excitotoxic way. The mechanism of axonal injury likely includes failure of ionic homeostasis and intra-axonal Ca^{2+} overload, as mentioned above (71). The downstream pathways of Ca^{2+} -induced axonal injury remain unclear.

Activation of cytokine receptors has also been reported to be involved in axonal injury. The activation of cytokine receptors on the surface of axons can cause their death through cross-talk between ligand and receptors, which activate intracellular signaling pathways related to energy metabolism disruption (73). Our recent study has shown an upregulated TNF- R_1 and IL-1 R_1 expression on the axons in the PWM of hypoxic neonatal rats (67). This was coupled with the disruption of MBP positive processes of oligodendrocytes and associated neurofilament-200 positive axons in the PWM (67). Therefore, it was postulated that overproduction of local TNF- α and IL-1 β may damage axons and delay their myelination via binding to their respective receptors (67).

4.4. Microglial activation and astrogliosis

It is well established that microglial cells are activated in hypoxic injuries in the developing brain (74, 75, 76). Our *in vitro* study has shown that the mRNA and protein expression of TNF- α and IL-1 β in cultured microglia were upregulated significantly after hypoxic exposure for 4 hours (24). This indicated that hypoxia could activate isolated microglia to produce inflammatory mediators (24). Activated microglia can proliferate, potentiate phagocytosis, actively migrate to the site of injury and release a variety of factors including cytokines, chemokines and nitric oxide (NO) thereby causing an inflammatory response (77, 78, 79).

Under physiological conditions, astrocytes are closely associated with vascular development, formation of synapses and BBB within specific regions including PWM (10). However, astrocytes may produce reactive responses following various kinds of injury or in neurodegenerative diseases (80). This reactive process, known as astrogliosis or reactive gliosis, is characterized by cellular hypertrophy, hyperplasia, and an increase in immunodetectable glial fibrillary acidic protein (GFAP) (81). It has been reported that astrocytic reactivity occurs within the deep white matter in the hypoxic developing brain, which is coupled with widespread axonal damage and production of the pro-inflammatory cytokines (10, 82). Astrocytic responses may help to repair the injured CNS, but excessive astrogliosis may be detrimental and contribute to neuronal injury.

Reactive gliosis has the potential to overproduce free radicals and inflammatory mediators such as TNF- α and IL-1 β that are toxic to oligodendrocyte progenitors or axons (10). Reactive gliosis contributes to scar-formation at the injury site, which can form a local biochemical and physical barrier to prevent axonal regeneration and re-establishment of new synapses (83, 84).

4.5. Blood- brain barrier damage

The integrity and proper functioning of the BBB play a crucial role in maintaining the homeostasis of the brain microenvironment and so-called “immune-privileged” status of the brain by preventing the entry of T lymphocytes (74, 85). *In vitro* studies have demonstrated that hypoxia and hypoxia/reoxygenation may increase permeability of BBB and result in disruption of BBB tight junction (86, 87). Our *in vivo* studies have confirmed that permeability of the cerebral blood vessels is increased after a hypoxic injury (88). Some tracers such as rhodamine isothiocyanate (RhIC) and horseradish peroxidase (HRP) injected intravenously can extravasate from the blood vessels into the brain parenchyma following such injuries (88) suggesting that the BBB was damaged. This was further evidenced by alterations in the endothelial cells such as increased pinocytotic vesicles and derangement of the tight junction proteins (88). Overproduction of vascular endothelial growth factor (VEGF), NO and inflammatory cytokines in hypoxic conditions were reported to be responsible for the disruption and increased permeability of the BBB (85, 86). Recently increased gene and protein expression of VEGF in the PWM and its specific localization in the astrocytes in the neonatal brain was reported following a hypoxic exposure (31). Many VEGF positive astrocytes were observed in close association with the blood vessels (31). This increased VEGF expression may link hypoxia and vascular leakage which lead to the formation of cerebral oedema (31). Indeed increased leakage of tracers such as RhIC was observed in the PWM of hypoxic neonatal rats (89).

5. INFLAMMATION IN THE HYPOXIC DEVELOPING BRAIN

It is well known that hypoxia initiates an inflammatory response with enormous production of inflammatory mediators (90, 91). We reported recently a strong and persistent inflammatory response with production of inflammatory cytokines namely, TNF- α and IL-1 β in the PWM of neonatal rats after hypoxic insults (24). NO levels were also significantly higher in hypoxic rats (31). Overproduction of proinflammatory cytokines TNF- α , IL-1 β , IL-6 and IL-2 together with adhesion molecules such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 is coupled with apoptosis of oligodendrocyte progenitors, myelination delay and axonal degeneration (92, 93, 94). A concomitant expression of the corresponding receptors of above-mentioned proinflammatory cytokines was upregulated on the oligodendrocyte progenitors and axons (24) suggesting that inflammatory mediators were involved in PWMD in the hypoxic developing brain (24). Microglial

cells are present in large numbers in the developing PWM (20). Being active brain macrophages, they would be the main source of inflammatory mediators in the developing brain; other sources of proinflammatory cytokine production in the PWM may be the astrocytes. Our recent study has shown that up regulated TNF-alpha and IL-1 beta expression was specifically detected in AMC at early time points whereas in the reactive astrocytes at late time points in the PWM in hypoxic injuries (67). This suggested that microglia may be responsible for the early phase of inflammatory response and astrocytes may contribute to it at later stages.

6. ROLE OF MICROGLIA IN THE NEUROINFLAMMATORY RESPONSE IN THE HYPOXIC DEVELOPING BRAIN

Microglial cells are known to be active macrophages removing cellular debris during normal development as well as in pathological conditions. Although microglial cells are necessary for phagocytic functions in the normal developing brain, their activation can cause bystander injury to other CNS cells. There is ample evidence to suggest that microglia not only initiate PWMD, but also contribute to the growth of lesions within the white matter (10).

6.1. Distribution and morphology of microglia

Microglia constitute 5-20% of all glial cells in the CNS (95, 96, 97). In the developing brain, they are present in large numbers in the PWM (20); are more sparsely distributed in the cortex, hippocampus and thalamus (98). It is still not clear why microglial cells are distributed unevenly in the developing brain, but from a speculative point of view, it is possible that they might execute some specific physiological functions in the local environment in different brain regions.

Two microglial phenotypes have been described: AMC and ramified microglial cells (19). In the developing brain, the preponderant AMC exhibit a round cell body with some processes (19). These cells transform into ramified microglial cells with the development and maturity of the brain (19); the latter exist as resting form under normal conditions. However, under pathological conditions such as trauma, infection and hypoxia-ischemia, ramified microglia retract their processes and assume an amoeboid form (99, 100). It has been reported that AMC in the PWM begin to ramify at 7 days of age and persist as ramified cells thereafter through the adulthood (19). It is striking that all AMC remained amoeboidic in 7 days hypoxic rats (101), suggesting that hypoxia contributes to delaying the morphological transformation of AMC. A possible explanation for this may be that hypoxia being a stimulus for microglia had induced their activation which was sustained over a protracted duration.

6.2. Phagocytosis

The phagocytic nature of AMC has been shown by various methods and observations, including the localization of hydrolytic enzymes, ultrastructural features shared by tissue macrophages, uptake of exogenous

substances, and the activation of surface receptors and antigens related to phagocytosis (102). Indeed, direct phagocytosis of carbon particles (103) and the bacteria *Escheria coli* (*E coli*) by AMC was observed through ultrastructural studies (102). In the fetal and postnatal rat brains, AMC were observed to engulf dead cells after transient maternal hypoxia (104, 105). In neonatal rats exposed to hypoxia, AMC in the PWM were engaged in the phagocytosis of apoptotic cells and degenerating axons (24, 105). It is evident from these observations that AMC in the developing brain are in constant surveillance to scavenge local cellular debris and exogenous substances that may gain access into the brain tissues; hence, their protective role at a stage when the BBB is not fully established.

6.3. Antigen presentation

Although the CNS, under normal conditions, has been considered an immunologically privileged site for a long time, our studies showed for the first time that major histocompatibility class (MHC) I antigens were expressed by AMC (106, 107). MHC antigens are surface molecules required for the participation of macrophages in the activation of T lymphocytes by presenting certain antigens to them. MHC I antigens serve as restriction elements for cytotoxic/suppressor lymphocytes. The expression of these antigens on AMC was related to their phagocytic activity. The presence of MHC I antigens on AMC also suggested that these cells were ready to interact with infiltrating lymphocytes as the BBB is immature in the developing brain and a potential immune damage in early development may be present (108).

MHC class II (MHC II) antigens, required for interaction with helper/inducer T lymphocytes, on the other hand, are not expressed by AMC under normal conditions. The expression of MHC II is induced under pathological and experimental conditions (106). For example, these antigens are expressed when the cells are challenged with lipopolysaccharide (LPS) or with interferon-gamma (IFN-gamma) (106). MHC II expression on AMC was also induced on the introduction of live *E coli* in their vicinity (102, 108). The expression of these antigens on AMC under pathological conditions suggests that they have the capability of interacting with helper/inducer cells to mount a potential immune response.

6.4. Proliferation

Although microglial population remains relatively static in the adult brain (57), its population has a remarkable capacity to expand in response to injury or neurological disease processes (109, 110). This expansion has been considered predominantly to be due to proliferation of activated microglia and to a lesser extent migration of microglia from adjacent brain areas (109, 110). Indeed, proliferating microglia have been implicated in the onset and/or progression of a number of CNS pathological conditions such as trauma (111), ischemia (112), Parkinson's disease (113) and demyelination disorders (114). These cells respond to injury may participate in brain repair and functional recovery by phagocytosis of debris. However, they may also contribute to glial scar formation and excess release of cytotoxic

factors (115). A variety of molecules such as IL-3, IL-6, granulocyte-macrophage colony-stimulating factor, and colony stimulating factor (CSF)-1 have been demonstrated to be potent stimuli for microglia proliferation *in vitro* (116). Very interestingly, for all of these cytokines, microglia can express the corresponding receptors (116). In addition, all of the growth factors mentioned above can be produced locally in the CNS (117). Therefore, under pathological conditions, microglial proliferation may be induced by some cytokines mentioned above through autocrine and paracrine modes. Several *in vivo* studies using cell proliferation markers such as Ki67 and BrdU, have confirmed the hypothesis that microglia can proliferate in a developing brain environment (117).

6.5. Migration

Microglia are capable of migrating toward damaged neural tissue to clear the debris of injury site when necessary (118). It has been reported that rapid increase in number of microglial cells at injury sites is partly due to recruitment from blood monocytes or migration from other CNS regions (119). The mechanisms inducing microglial migration to the injury sites are complex and poorly understood. Migration of microglia is modulated by chemokines released by themselves through autocrine or paracrine fashion during injury and infection (118). Cytoskeleton proteins such as actin and tubulin are closely related to cellular migration. Microglia have a perinuclear distribution of actin and tubulin in the resting state and lack membrane localization of these proteins (120). When cells are treated with a chemotactic stimulus, rearrangement of actin and tubulin occurs to facilitate the process of attachment, protrusion and traction that allows microglia to migrate (120). Chemokine receptors can also be redistributed when microglia acquire a migratory phenotype (121). Our studies have shown that the cell number of AMC significantly increased in the PWM of hypoxic postnatal rats (101). Very interestingly, there was no significant difference in the proliferation rate of microglia after hypoxic exposure when compared with the corresponding control rats (101). The results suggested that the increase in AMC numbers soon after hypoxic exposure was primarily due to the migration of AMC from the neighboring areas of the brain or from invasion of their precursor cells, namely, circulating monocytes into the postnatal hypoxic brain (101).

6.6. Release of cytokines and chemokines

A plethora of cytokines and chemokines is usually expressed or secreted by microglia under inflammatory conditions. They are implicated in microglial communication and effector network system with other cell types (122). Cytokines and chemokines secreted by microglia participate in innate defense mechanisms, help initiation of immune responses, modulate the recruitment of leukocytes into the CNS, and contribute to tissue repair and recovery (122).

6.6.1. Tumor necrosis factor-alpha and its receptors

TNF-alpha is one of the most important proinflammatory cytokines with pleiotropic functions, secreted by microglia as well as blood-derived

macrophages during CNS inflammation (123). In microglia culture, synthesis and release of TNF-alpha is induced by pathogens or pathogen components such as LPS and IFN-gamma (78). TNF- alpha can amplify CNS inflammatory response by inducing expression of chemokines and adhesion molecules in cerebrovascular endothelial cells and astrocytes, which help in recruitment of leukocytes into the CNS (124). In addition, TNF- alpha is a multipotent inflammatory cytokine that initiates various responses such as apoptosis in some cells and proliferation in others through activation of its different receptors (124). Therefore, it is possible that TNF- alpha plays a wide variety of roles in brain damage and repair.

All known effects of TNF- alpha are mainly completed by binding to one of two different receptors, designated TNF-R₁ (p55) and TNF-R₂ (p75), which are differentially expressed on various cell types in physiological and pathological conditions (125). The extracellular ligand-binding domains of TNF receptors show sequence homology containing cysteine-rich subdomains (126). On the contrary, the intracellular domains of TNF receptors have no sequence homology and are in lack of intrinsic enzyme activity, and transduce distinct biological signals by interaction with different cytosolic protein complexes (126). The TNF-R₁ contains death domain, but TNF-R₂ does not. The distinction in TNF-R₁ and TNF-R₂ constitution leads to their diverse functions. In general, the cell apoptotic process and pro-inflammatory response are largely mediated through TNF-R₁. Therefore, the activation of TNF-R₁ is frequently associated with tissue or organ injury (126). The consequences of TNF-R₂ activation are not fully known, but it is known to mediate cell proliferation and promote tissue repair and angiogenesis (126). It has been found that the AMC produce TNF- alpha via the MAP kinase signaling pathway and expression of TNF-R₁ in the oligodendrocytes increased significantly in the PWM in the hypoxic neonatal brain (24). This may be correlated to increased oligodendrocyte apoptosis via TNF-R₁ in hypoxic injuries (24).

6.6.2. Interleukin-1 and its receptors

IL-1 includes two mediators, IL-1 alpha and IL-1 beta, which have less than 30% sequence homology but similar three-dimensional structure and biological functions (127). The action of IL-1 is modulated by different mechanisms including a true receptor (IL-1RI), a decoy (IL-1RII), and a specific receptor antagonist (IL-1ra) (127). Although IL-1 alpha and IL-1beta can bind equally to type I and type II receptors, signal transduction across the plasma membrane has been reported to be accomplished only via the type I receptor (127). At present, there is no evidence that a signal can be transmitted through the type II receptor. In the CNS, IL-1 is expressed at high levels during prenatal and postnatal development, and its expression declines to low constitutive levels in the normal adult and markedly increases after injury (128). Astroglia and microglia possess IL-1 receptors and are the main intrinsic sources of IL-1 in the CNS, but oligodendroglial cells also produce IL-1 and express IL-1 receptors (128). IL-1 induces activation and secretion of multiple factors by

microglia and astrocytes and it has been reported by a recent study that remyelination is dramatically reduced in the absence of IL-1 β , but the effects of this cytokine on the oligodendroglial lineage remain to be elucidated (128). We have demonstrated that AMC expressed IL-1 β and the expression of IL-1RI on the oligodendrocytes was increased significantly in hypoxic rats (24). Unlike TNF- α , IL-1 β was reported as being non toxic to oligodendrocyte lineage cells as oligodendrocyte apoptosis was not induced through this receptor. However, some studies have demonstrated that IL-1 β can block oligodendrocyte proliferation at the late progenitor/pro-oligodendrocyte stage (128). Based on these results, it is postulated that IL-1 β produced by AMC in hypoxic conditions may delay the white matter development and recovery in hypoxic conditions via inhibition of oligodendrocyte progenitor proliferation (24).

6.6.3. Macrophage colony-stimulating factor

The macrophage colony-stimulating factor (M-CSF), also designated as CSF-1, is a hematopoietic cytokine that induces proliferation, migration, and activation of microglia and is released mainly by macrophages, T cells, B cells and microglia (129). M-CSF has also been identified as an important mediator of the inflammatory response and can also regulate the release of proinflammatory cytokines from macrophages (130). M-CSF exerts its pleiotropic effects by binding to its high-affinity receptor CSF-1R that is encoded by the *c-fms* proto-oncogene (131). Our recent study has shown that M-CSF produced by AMC in the early phase following hypoxic injury may activate astrocytes and regulate the release of proinflammatory cytokines from them (67). This may then sustain and exacerbate the inflammatory response in the PWM of hypoxic neonatal rats in the late phase.

6.6.4. Monocyte chemoattractant protein-1

Among various chemokines, monocyte chemoattractant protein-1 (MCP-1), (also known as CCL2), a member of beta-chemokine subfamily, mediates the migration of microglia, monocytes and lymphocytes to the inflammation sites in the CNS (132, 133, 134). It is produced mainly by microglia and astrocytes (135). MCP-1 acts on its targets by binding to its receptor, CCR2 which is a seven-transmembrane domain G-protein coupled receptor. The expression of MCP-1 and CCR2 has been shown to be induced following diverse CNS insults, including ischemia (136, 137), Alzheimer's disease (138), HIV type-1-associated dementia (139), multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis (EAE) (140, 141, 142). In EAE animal model, MCP-1 induced the recruitment and activation of endogenous microglia and blood-derived macrophages to demyelinated areas, promoting myelin phagocytosis (143). Moreover, the functional antagonism of MCP-1 attenuates leukocyte infiltration and decreases the severity of CNS injury (144, 145). In murine stroke model, MCP-1 deficiency has been shown to have a protective role in acute infarct growth (146). Our previous study has shown that under hypoxic conditions, activated microglia generate MCP-1 that would induce the migration of microglial cells themselves either in an autocrine or paracrine fashion to

PWM in the neonatal rats, since these cells express the MCP-1 receptor, namely, CCR₂ (101). It has been reported that through binding with its receptor CCR₂, MCP-1 induces changes in actin polymerization and subsequent reorganization of the actin cytoskeleton, formation of focal adhesions and pseudopod extension which contributes to the microglial migration when activated (147).

6.7. Expression of ion channels

It is well documented that microglia can express a large number of cell surface receptors and ion channels when they are activated under pathological conditions (148, 149). These ion channels or receptors can transduce extracellular stimuli into an intracellular cascade response through activation of signaling pathways (150, 151). Microglia express several types of ion channels such as Na⁺, K⁺, Cl⁻ and Ca²⁺ channels, which confer different functions on these cells.

6.7.1. Kv1.2 and Kv1.1

Microglia are known to exhibit only inwardly rectifying K⁺ channels (152). The existence of delayed rectifier channels in microglial cells has been demonstrated in cultured cells derived from different species (153). The outward potassium currents caused by delayed rectifying channels appears to be highly correlated with the activation of the microglial cells (154). We reported recently that Kv1.2 is vigorously expressed in the AMC at 1-7 postnatal days but it is barely detected in the ramified microglial cells at 14 postnatal days under physiological conditions (154). Arising from this, it was suggested that AMC are endowed with an electrophysiological characteristic distinct from the ramified microglia. It was speculated that the vigorous expression of Kv1.2 in AMC in early postnatal period may be related to their active secretion of proinflammatory cytokines and other factors such as insulin-like growth factors and endothelins (155, 156). The diminution of Kv1.2 expression in ramified microglia suggested that they may be less active in their secretory function.

It has been reported that Kv1 channel transcription is influenced by oxygen supply (157). Kv1.2 is an isoform of the delayed rectifier potassium channel and its α -subunit is sensitive to oxygen (158). Therefore, it was suggested that Kv1.2 in microglia is also sensitive to oxygen supply and it may take part in the hypoxia induced outward potassium rectifier especially during cell activation (158). This is supported by the fact that Kv1.2 expression in microglia was augmented in postnatal rats and in BV-2 cells (a murine microglia cell line) subjected to hypoxia (154). In this connection, increase in Kv1.2 expression was accompanied by a decline in intracellular potassium coupled by an increased expression of TNF- α and IL-1 β (154). Blockade of Kv1.2 with Tityustoxin (TsTx) reversed the above-mentioned process but remarkably it also depressed the intracellular ROS (154). It is, therefore, suggested that Kv1.2 constitutively expressed in microglia in the hypoxic developing brain may regulate the release of proinflammatory cytokines and reactive oxygen species (ROS) production through changing the intracellular potassium concentration. Likewise, a recent study demonstrated that Kv1.1, constitutively expressed by

microglia, is elicited by hypoxia and this may be linked to production of proinflammatory cytokines, endothelins and NO (159). In the light of the above, it was proposed that Kv1.2 and Kv1.1 play an important role in inflammatory response elicited by activated microglia in the hypoxic developing brain.

6.7.2. Sodium channels

Voltage-gated sodium channels are traditionally viewed as being expressed on some excitable cells such as neurons and muscle cells (160). They are mainly responsible for the initiation and propagation of action potential (160). However, it has been proven that sodium channels are also expressed in some nonexcitable cells and contribute to cellular functions (161). As mentioned above, microglia are activated under pathological conditions, which result in morphological transformation, proliferation, enhanced migration, phagocytosis, secretion of inflammatory mediators and antigen presentation (122, 162). At present, it has been found that tetrodotoxin (TTX)-sensitive Nav1.1, Nav1.6 and TTX-resistant Nav1.5 are detected in primary microglia *in vitro* (163); on the other hand, Nav1.2, Nav1.3, Nav1.7, Nav1.8 and Nav1.9 are not present in microglia. Sodium channel blockade with phenytoin (40 μ M) and TTX (0.3 μ M) significantly attenuated the phagocytic activity of microglia by 50-60% when they were activated with LPS (163). Sodium channel blockade with phenytoin significantly reduced IL-1 α , IL-1 β , TNF- α secretion by LPS-activated microglia. Phenytoin and TTX also significantly decreased adenosine triphosphate-induced migration of microglia by 50% (163). Furthermore, Nav1.6 plays a prominent role in microglial migration. These studies demonstrate that the activity of sodium channels contributes to phagocytosis, migration and secretion of inflammatory mediators by activated microglia (163). It remains to be ascertained if the expression of sodium channels in microglia is upregulated in the hypoxic developing brain and, if so, whether it would contribute to above-mentioned processes.

6.8. Syndecan-2

Syndecans (Sdcs) are transmembrane heparan sulphate proteoglycans consisting of syndecan-1,-2,-3, and -4 (Sdc-1,-2,-3 and -4). Heparan sulphate glycosaminoglycan side chains, which have affinity for a wide variety of secreted molecules and extracellular matrix components, mediate the extracellular functions of Sdcs (164, 165, 166). Sdc-2 is a cell surface heparan sulphate proteoglycan consisting of a short cytoplasmic domain, a single transmembrane domain and a large ectodomain with three covalently attached heparan sulphate chains close to the N-terminus (167). It is expressed on fibroblasts (168), endothelial cells (169), primary osteoblasts (170) and in activated macrophages (171). The various members of the Sdc family have been implicated in a number of biological processes such as cell adhesion and signal transduction (172, 173). It is well documented that heparan sulphate chains interact with many growth factors, cytokines, chemokines, and extracellular matrix molecules relevant to vascular development and repair, hypoxia, angiogenesis,

and immune cell function. It also regulates key mechanisms of the host inflammatory response (174).

A number of studies have documented that hypoxia activates macrophages and other inflammatory cells (175) and induces inflammatory responses in many tissues (176). Macrophages regulate many processes associated with injury and repair which can affect the synthesis of a wide variety of extracellular matrix proteins such as fibronectins (177) and proteoglycans (178). Both monocytes and macrophages express Sdc-1, Sdc-4, (179) and Sdc-2 (171) and secrete a variety of cytokines such as IL-1, transforming growth factor- β and TNF- α . Changes in expression of sulphated proteoglycans are associated with activation of macrophages (180). Sdcs have been implicated as key modulators of inflammation in many diseases reflecting the capacity of heparan sulphate proteoglycans to regulate a wide variety of inflammatory mediators and their processes (174). A recent study has shown that the expression of Sdc-2 was increased significantly in the AMC in the PWM of the rat developing brain following a hypoxic exposure (181). Furthermore, it has been further shown that Sdc-2 could augment the hypoxia-induced proinflammatory mediators secretion and ROS production (181). Therefore, a proinflammatory role of Sdc-2 in the hypoxic developing brain was suggested as it potentiated activated microglia in releasing more cytokines, chemokines and ROS (181).

6.9. Generation of reactive oxygen species and nitrogen intermediates

Microglia can generate ROS when activated by various stimuli such as hypoxia and LPS. Nicotinamide adenine dinucleotide phosphate hydrogenase (NADPH) oxidase, which is involved in production of microglial-derived ROS (182), is a pivotal enzyme which catalyses the production of superoxide from the oxygen. In addition to the production of ROS, NADPH oxidase is associated with microglial signaling pathway related to inflammatory response (183). NADPH oxidase-generated intracellular ROS can augment the inflammatory response through effects on kinase cascades and transcription factor activation (183). ROS can also strengthen phagocytosis by microglia by supporting the degradation of the ingested antigens and cellular debris (183). However, excessive intracellular ROS leads to microglial apoptosis. Some studies have shown that the overactivation of NADPH oxidase and the dysregulation of intracellular ROS in microglia are involved in the pathogenesis of some neurodegenerative diseases (184).

NO, an important free radical, is synthesized by the enzyme NOS from L-arginine. NOS from neurons (nNOS) and endothelial cells (eNOS) are constitutively expressed enzymes, the activities of which are stimulated by increase in intracellular calcium. Inducible NOS (iNOS) is calcium-independent, and NO generated from this isoform is known to mediate immune functions. NO possesses a diverse array of physiologic functions, such as muscle relaxation, immune modulation, and neuronal activity (185). NO directly reacts with guanylate cyclase, cytochrome P450, cyclooxygenase, and hemoglobin, to

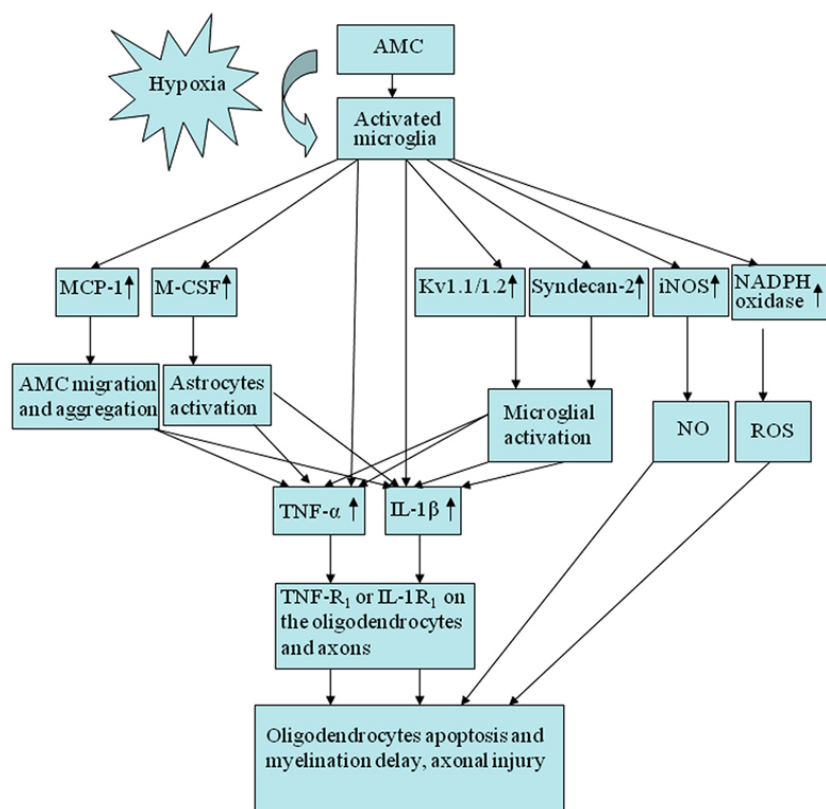


Figure 1. A schematic diagram summarizing the cellular and molecular events following hypoxic injury in the developing brain. Hypoxia activates amoeboid microglial cells (AMC) and, hence, induces severe and persistent inflammatory response with overproduction of proinflammatory cytokines, NO and ROS which cause axon injury, oligodendrocyte apoptosis and myelination delay.

modulate their functions. In addition, NO can react with membrane lipids and induce lipid peroxidation. Indirectly, the combination of NO and superoxide can form highly reactive intermediates, such as peroxynitrite, that can induce DNA strand breaks, lipid peroxidation, and protein nitration (186). In the postnatal brain, iNOS is not normally expressed but can be expressed by microglia in response to hypoxic stimuli (31, 187). As described above, activated microglial cells in the PWM may contribute to perinatal brain injury through secretion or production of proinflammatory cytokines such as TNF- α and IL-1 β . Excessive production of NO from iNOS has been reported to be toxic to the oligodendrocytes and may be of significance in the development of PWMD as it has been implicated in damaging myelin-producing oligodendrocytes (188) and, hence, delayed myelination. *In vitro* studies have shown that NO produced by the activated microglial cells is highly damaging to the oligodendrocytes causing their lysis (189).

7. CONCLUSIONS

Hypoxia induces severe and persistent inflammatory response in the developing brain. Overproduction of inflammatory cytokines, free radicals

and NO has been implicated in this inflammatory response that results in neuronal loss, damage to the axons and death of oligodendrocytes along with myelination disturbances (Fig.1). Microglial cells are the main cellular source of the inflammatory mediators and culprits of hypoxia-induced injury to the oligodendrocytes and axons. Our recent studies have shed some light on the cross-talk between microglial cells, astrocytes, oligodendrocytes and axons, which may be involved in the pathogenesis of hypoxic brain injury in the neonatal period. Undoubtedly, a better understanding of the above processes will help to develop potential preventive and therapeutic strategies for mitigation of hypoxia induced brain injury.

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9. REFERENCES

1.Acker T, Acker H. Cellular oxygen sensing need in CNS function: physiological and pathological implications. *J Exp Biol* 207, 3171-3188 (2004)

2. Michiels C. Physiological and pathological responses to hypoxia. *Am J Pathol.* 164, 1875-1882 (2004)
3. Rolfe DF, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev.* 77, 731-758 (1997)
4. Attwell D, Laughlin SB. An energy budget for signaling in the grey matter of the brain. *J Cereb Blood Flow Metab.* 21, 1133-1145 (2001)
5. Perlman JM. Intervention strategies for neonatal hypoxic-ischemic cerebral injury. *Clin Ther.* 28,1353-1365 (2006)
6. Taniguchi H, Andreasson K. The hypoxic-ischemic encephalopathy model of perinatal ischemia. *J Vis Exp.* 19, pii: 955. doi: 10.3791/955 (2008)
7. Osredkar D, Derganc M, Paro-Panjan D, Neubauer D. Amplitude-integrated electroencephalography in full-term newborns without severe hypoxic-ischemic encephalopathy: case series. *Croat Med J.* 47, 285-291 (2006)
8. Johnston MV. Hypoxic and ischemic disorders of infants and children. Lecture for 38th meeting of Japanese Society of Child Neurology, Tokyo, Japan, July 1996. *Brain Dev.* 19, 235-239 (1997)
9. McQuillen PS, Ferriero DM. Selective vulnerability in the developing central nervous system. *Pediatr Neurol.* 30, 227-235 (2004)
10. Rezaie P, Dean A. Periventricular leukomalacia, inflammation and white matter lesions within the developing nervous system. *Neuropathology.* 22, 106-132 (2002)
11. Volpe JJ. Cerebral white matter injury of the premature infant-more common than you think. *Pediatrics.* 112, 176-180 (2003)
12. Folkerth RD. Periventricular leukomalacia: overview and recent findings. *Pediatr Dev Pathol.* 9, 3-13 (2006),
13. Verklan MT. The chilling details: hypoxic-ischemic encephalopathy. *J Perinat Neonatal Nurs.* 23, 59-68 (2009)
14. Kaur C, Ling EA. Antioxidants and neuroprotection in the adult and developing central nervous system. *Curr Med Chem.* 15, 3068-3080 (2008)
15. Cinat M, Waxman K, Vaziri ND, Daughters K, Yousefi S, Scannell G, Tominaga GT. Soluble cytokine receptors and receptor antagonists are sequentially released after trauma. *J Trauma.* 39, 112-118 (1995)
16. Ghirnikar RS, Lee YL, Eng LF. Inflammation in traumatic brain injury: role of cytokines and chemokines. *Neurochem Res.* 23, 329-340 (1998)
17. Martin C, Boisson C, Haccoun M, Thomachot L, Mege JL. Patterns of cytokine evolution (tumor necrosis factor-alpha and interleukin-6) after septic shock, hemorrhagic shock, and severe trauma. *Crit Care Med.* 25, 1813-1819 (1997)
18. Delgado M, Ganea D. Vasoactive intestinal peptide prevents activated microglia-induced neurodegeneration under inflammatory conditions: potential therapeutic role in brain trauma. *FASEB J.* 17, 1922-1924 (2003)
19. Kaur C, Hao AJ, Wu CH, Ling EA. Origin of microglia. *Microsc Res Tech.* 54, 2-9 (2001)
20. Ling EA, Wong WC. The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. *Glia.* 7, 9-18 (1993)
21. Giulian D, Baker TJ. Characterization of amoeboid microglia isolated from developing mammalian brain. *J Neurosci.* 6, 2163-2178 (1986)
22. Napoli I, Neumann H. Protective effects of microglia in multiple sclerosis. *Exp Neurol.* 225, 24-28 (2009)
23. Kaur C, Ling EA. Periventricular white matter damage in the hypoxic neonatal brain: role of microglial cells. *Prog Neurobiol.* 87, 264-280 (2009)
24. Deng Y, Lu J, Sivakumar V, Ling EA, Kaur C. Amoeboid microglia in the periventricular white matter induce oligodendrocyte damage through expression of proinflammatory cytokines via MAP kinase signaling pathway in hypoxic neonatal rats. *Brain Pathol.* 18, 387-400 (2008)
25. Neumar RW. Molecular mechanisms of ischemic neuronal injury. *Ann Emerg Med.* 36, 483-506 (2000)
26. Calvert JW, Zhang JH. Pathophysiology of an hypoxic-ischemic insult during the perinatal period. *Neurol Res.* 27, 246-260 (2005)
27. Valkounova I, Maresova D, Trojan S. Perinatal complication: hypoxic-ischemic encephalopathy. *Sb Lek.* 102, 455-463 (2001)
28. Cervós-Navarro J, Diemer NH. Selective vulnerability in brain hypoxia. *Crit Rev Neurobiol.* 6, 149-182 (1991)
29. Johnston MV, Trescher WH, Taylor GA. Hypoxic and ischemic central nervous system disorders in infants and children. *Adv Pediatr.* 42, 1-45 (1995)
30. Volpe JJ. Neurobiology of periventricular leukomalacia in the premature infant. *Pediatr Res.* 50, 553-562 (2001)
31. Kaur C, Sivakumar V, Ang LS, Sundaresan A. Hypoxic damage to the periventricular white matter in neonatal brain: role of vascular endothelial growth factor, nitric oxide and excitotoxicity. *J Neurochem.* 98, 1200-1216 (2006)

32. Back SA, Han BH, Luo NL, Chricton CA, Xanthoudakis S, Tam J, Arvin KL, Holtzman DM. Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia. *J Neurosci.* 22, 455-463 (2002)
33. Volpe JJ. Brain injury in the premature infant--current concepts. *Prev Med.* 23, 638-645 (1994)
34. Baud O, Li J, Zhang Y, Neve RL, Volpe JJ, Rosenberg PA. Nitric oxide-induced cell death in developing oligodendrocytes is associated with mitochondrial dysfunction and apoptosis-inducing factor translocation. *Eur J Neurosci.* 20, 1713-1726 (2004)
35. Golan H, Kashtuzki I, Hallak M, Sorokin Y, Huleihel M. Maternal hypoxia during pregnancy induces fetal neurodevelopmental brain damage: partial protection by magnesium sulfate. *J Neurosci Res.* 78, 430-441 (2004)
36. Sugai K, Ito M, Tateishi I, Funabiki T, Nishikawa M. Neonatal periventricular leukomalacia due to severe, poorly controlled asthma in the mother. *Allergol Int.* 55, 207-212 (2006)
37. Mu D, Jiang X, Sheldon RA, Fox CK, Hamrick SE, Vexler ZS, Ferriero DM. Regulation of hypoxia-inducible factor 1alpha and induction of vascular endothelial growth factor in a rat neonatal stroke model. *Neurobiol Dis.* 14, 524-534 (2003)
38. Mac Donald D. Asphyxia. *Baillieres Clin Obstet Gynaecol.* 9, 579-594 (1995)
39. As-Sanie S, Mercer B, Moore J. The association between respiratory distress and nonpulmonary morbidity at 34 to 36 weeks' gestation. *Am J Obstet Gynecol.* 189, 1053-1057 (2003)
40. Towfighi J, Mauger D, Vannucci RC, Vannucci SJ. Influence of age on the cerebral lesions in an immature rat model of cerebral hypoxia-ischemia: a light microscopic study. *Brain Res Dev Brain Res.* 100, 149-160 (1997)
41. Nakajima W, Ishida A, Lange MS, Gabrielson KL, Wilson MA, Martin LJ, Blue ME, Johnston MV. Apoptosis has a prolonged role in the neurodegeneration after hypoxic ischemia in the newborn rat. *J Neurosci.* 20, 7994-8004 (2000)
42. Sheldon RA, Hall JJ, Noble LJ, Ferriero DM. Delayed cell death in neonatal mouse hippocampus from hypoxia-ischemia is neither apoptotic nor necrotic. *Neurosci Lett.* 304, 165-168 (2001)
43. Towfighi J, Yager JY, Housman C, Vannucci RC. Neuropathology of remote hypoxic-ischemic damage in the immature rat. *Acta Neuropathol.* 81, 578-587 (1991)
44. Northington FJ, Ferriero DM, Graham EM, Traystman RJ, Martin LJ. Early neurodegeneration after hypoxia-ischemia in neonatal rat is necrosis while delayed neuronal death is apoptosis. *Neurobiol Dis.* 8, 207-219 (2001)
45. Taylor RM, Lee JP, Palacino JJ, Bower KA, Li J, Vanier MT, Wenger DA, Sidman RL, Snyder EY. Intrinsic resistance of neural stem cells to toxic metabolites may make them well suited for cell non-autonomous disorders: evidence from a mouse model of Krabbe leukodystrophy. *J Neurochem.* 97, 1585-1599 (2006)
46. Rees S, Inder T. Fetal and neonatal origins of altered brain development. *Early Hum Dev.* 81, 753-761 (2005)
47. Johnston MV, Nakajima W, Hagberg H. Mechanisms of hypoxic neurodegeneration in the developing brain. *Neuroscientist.* 8, 212-220 (2002)
48. Choi DW. Ischemia-induced neuronal apoptosis. *Curr Opin Neurobiol.* 6, 667-672 (1996)
49. Bernert G, Hoeger H, Mosgoeller W, Stolzlechner D, Lubec B. Neurodegeneration, neuronal loss, and neurotransmitter changes in the adult guinea pig with perinatal asphyxia. *Pediatr Res.* 54, 523-528 (2003)
50. Somjen GG, Aitken PG, Czeh G, Jing J, Young JN. Cellular physiology of hypoxia of the mammalian central nervous system. *Res Publ Assoc Res Nerv Ment Dis.* 71, 51-65 (1993)
51. Charriaut-Marlangue C, Remolleau S, ggoun-Zouaoui D, Ben-Ari Y. Apoptosis and programmed cell death: a role in cerebral ischemia. *Biomed Pharmacother.* 52, 264-269 (1998)
52. Zanelli SA, Numagami Y, McGowan JE, Mishra OP, ivoria-Papadopoulos M. NMDA receptor-mediated calcium influx in cerebral cortical synaptosomes of the hypoxic guinea pig fetus. *Neurochem Res.* 24, 437-446 (1999)
53. Biagas K. Hypoxic-ischemic brain injury: advancements in the understanding of mechanisms and potential avenues for therapy. *Curr Opin Pediatr.* 11, 223-228 (1999)
54. Erecinska M, Silver IA. Tissue oxygen tension and brain sensitivity to hypoxia. *Respir Physiol.* 128, 263-276 (2001)
55. Beilharz EJ, Williams CE, Dragunow M, Sirimanne ES, Gluckman PD. Mechanisms of delayed cell death following hypoxic-ischemic injury in the immature rat: evidence for apoptosis during selective neuronal loss. *Brain Res Mol Brain Res.* 29, 1-14 (1995)
56. Edwards AD, Yue X, Cox P, Hope PL, Azzopardi DV, Squier MV, Mehmet H. Apoptosis in the brains of infants suffering intrauterine cerebral injury. *Pediatr Res.* 42, 684-689 (1997)
57. Paterson JA, Privat A, Ling EA, Leblond CP. Investigation of glial cells in semithin sections. 3. Transformation of subependymal cells into glial cells, as shown by radioautography after 3 H-thymidine injection into the lateral ventricle of the brain of young rats. *J Comp Neurol.* 149, 83-102 (1973)
58. Thomas JL, Spassky N, Perez Villegas EM, Olivier C, Cobos I, Goujet-Zalc C, Martinez S, Zalc B.

Spatiotemporal development of oligodendrocytes in the embryonic brain. *J Neurosci Res.* 59, 471-476 (2000)

59. Baumann N, Pham-Dinh D. Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiol Rev.* 81, 871-927 (2001)

60. Kinney HC, Back SA. Human oligodendroglial development: relationship to periventricular leukomalacia. *Semin Pediatr Neurol.* 5, 180-189 (1998)

61. Kohlhauser C, Mosgoller W, Hoyer H, Lubec B. Myelination deficits in brain of rats following perinatal asphyxia. *Life Sci.* 67, 2355-2368 (2000)

62. Skoff RP, Bessert DA, Barks JD, Song D, Cerghet M, Silverstein FS. Hypoxic-ischemic injury results in acute disruption of myelin gene expression and death of oligodendroglial precursors in neonatal mice. *Int J Dev Neurosci.* 19, 197-208 (2001)

63. Back SA. Perinatal white matter injury: the changing spectrum of pathology and emerging insights into pathogenetic mechanisms. *Ment Retard Dev Disabil Res Rev.* 12, 129-140 (2006)

64. Mitrovic B, Parkinson J, Merrill JE. An *in vitro* Model of Oligodendrocyte Destruction by Nitric Oxide and Its Relevance to Multiple Sclerosis. *Methods.* 10, 501-513 (1996)

65. Ludwin SK. The pathobiology of the oligodendrocyte. *J Neuropathol Exp Neurol.* 56, 111-124 (1997)

66. Casaccia-Bonnet P. Cell death in the oligodendrocyte lineage: a molecular perspective of life/death decisions in development and disease. *Glia.* 29, 124-135 (2000)

67. Deng YY, Lu J, Ling EA, Kaur C. Microglia-derived macrophage colony stimulating factor promotes generation of proinflammatory cytokines by astrocytes in the periventricular white matter in the hypoxic neonatal brain. *Brain Pathol.* 20, 909-925 (2010)

68. Fern R, Davis P, Waxman SG, Ransom BR. Axon conduction and survival in CNS white matter during energy deprivation: a developmental study. *J Neurophysiol.* 79, 95-105 (1998)

69. Stys PK. General mechanisms of axonal damage and its prevention. *J Neurol Sci.* 233, 3-13 (2005)

70. Cuthill DJ, Fowler JH, McCulloch J, Dewar D. Different patterns of axonal damage after intracerebral injection of malonate or AMPA. *Exp Neurol.* 200, 509-520 (2006)

71. McCarran WJ, Goldberg MP. White matter axon vulnerability to AMPA/kainate receptor-mediated ischemic injury is developmentally regulated. *J Neurosci.* 27, 4220-4229 (2007)

72. Fowler JH, Edgar JM, Pringle A, McLaughlin M, McCulloch J, Griffiths IR, Garbern JY, Nave KA, Dewar D. Alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid-mediated excitotoxic axonal damage is attenuated in the absence of myelin proteolipid protein. *J Neurosci Res.* 84, 68-77 (2006)

73. Tezel G. TNF-alpha signaling in glaucomatous neurodegeneration. *Prog Brain Res.* 173, 409-421 (2008)

74. Kaur C, Ling EA. Blood brain barrier in hypoxic-ischemic conditions. *Curr Neurovasc Res.* 5, 71-81 (2008)

75. Li JJ, Lu J, Kaur C, Sivakumar V, Wu CY, Ling EA. Effects of hypoxia on expression of transforming growth factor-beta1 and its receptors I and II in the amoeboid microglial cells and murine BV-2 cells. *Neuroscience.* 156, 662-672 (2008)

76. Li JJ, Lu J, Kaur C, Sivakumar V, Wu CY, Ling EA. Expression of angiotensin II and its receptors in the normal and hypoxic amoeboid microglial cells and murine BV-2 cells. *Neuroscience.* 158, 1488-1499 (2009)

77. Farber K, Kettenmann H. Functional role of calcium signals for microglial function. *Glia.* 54, 656-665 (2006)

78. Dheen ST, Jun Y, Yan Z, Tay SS, Ling EA. Retinoic acid inhibits expression of TNF-alpha and iNOS in activated rat microglia. *Glia.* 50, 21-31 (2005)

79. Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci.* 10, 1387-1394 (2007)

80. Panicker KS, Norenberg MD. Astrocytes in cerebral ischemic injury: morphological and general considerations. *Glia.* 50, 287-298 (2005)

81. Yu AC, Lee YL, Eng LF. Astroglialosis in culture: I. The model and the effect of antisense oligonucleotides on glial fibrillary acidic protein synthesis. *J Neurosci Res.* 34, 295-303 (1993)

82. Deguchi K, Oguchi K, Takashima S. Characteristic neuropathology of leukomalacia in extremely low birth weight infants. *Pediatr Neurol.* 16, 296-300 (1997)

83. Davies SJ, Goucher DR, Doller C, Silver J. Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord. *J Neurosci.* 19, 5810-5822 (1999)

84. Di GS, Movsesyan V, Ahmed F, Cernak I, Schinelli S, Stoica B, Faden AI. Cell cycle inhibition provides neuroprotection and reduces glial proliferation and scar formation after traumatic brain injury. *Proc Natl Acad Sci U S A.* 102, 8333-8338 (2005)

85. Brown RC, Mark KS, Egleton RD, Davis TP. Protection against hypoxia-induced blood-brain barrier

disruption: changes in intracellular calcium. *Am J Physiol Cell Physiol*. 286, C1045-C1052 (2004)

86. Fischer S, Clauss M, Wiesnet M, Renz D, Schaper W, Karliczek GF. Hypoxia induces permeability in brain microvessel endothelial cells via VEGF and NO. *Am J Physiol*. 276, C812-C820 (1999)

87. Mark KS, Davis TP. Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. *Am J Physiol Heart Circ Physiol*. 282, H1485-H1494 (2002)

88. Kaur C, Sivakumar V, Zhang Y, Ling EA. Hypoxia-induced astrocytic reaction and increased vascular permeability in the rat cerebellum. *Glia*. 54, 826-839 (2006)

89. Kaur C, Sivakumar V, Ling EA. Melatonin protects periventricular white matter from damage due to hypoxia. *J Pineal Res*. 48, 185-193 (2010)

90. Saed GM, Munkarah AR, bu-Soud HM, Diamond MP. Hypoxia upregulates cyclooxygenase-2 and prostaglandin E(2) levels in human peritoneal fibroblasts. *Fertil Steril*. 83 Suppl 1, 1216-1219 (2005)

91. Varez-Diaz A, Hilario E, de Cerio FG, Soler A, varez-Diaz FJ. Hypoxic-ischemic injury in the immature brain--key vascular and cellular players. *Neonatology*. 92, 227-235 (2007)

92. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. *Pediatr Res*. 42, 1-8 (1997)

93. Yoon BH, Romero R, Kim CJ, Koo JN, Choe G, Syn HC, Chi JG. High expression of tumor necrosis factor-alpha and interleukin-6 in periventricular leukomalacia. *Am J Obstet Gynecol*. 177, 406-411 (1997)

94. Kadhim H, Tabarki B, De PC, Sebire G. Cytokine immunoreactivity in cortical and subcortical neurons in periventricular leukomalacia: are cytokines implicated in neuronal dysfunction in cerebral palsy? *Acta Neuropathol*. 105, 209-216 (2003)

95. Ling EA, Leblond CP. Investigation of glial cells in semithin sections. II. Variation with age in the numbers of the various glial cell types in rat cortex and corpus callosum. *J Comp Neurol*. 149, 73-81 (1973)

96. Lawson LJ, Perry VH, Gordon S. Turnover of resident microglia in the normal adult mouse brain. *Neuroscience*. 48, 405-415 (1992)

97. Benveniste EN. Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. *J Mol Med*. 75, 165-173 (1997)

98. Perry VH, Hume DA, Gordon S. Immunohistochemical localization of macrophages and microglia in the adult and

developing mouse brain. *Neuroscience*. 15, 313-326 (1985)

99. Ling EA, Ng YK, Wu CH, Kaur C. Microglia: its development and role as a neuropathology sensor. *Prog Brain Res*. 132, 61-79 (2001)

100. Dheen ST, Kaur C, Ling EA. Microglial activation and its implications in the brain diseases. *Curr Med Chem*. 14, 1189-1197 (2007)

101. Deng YY, Lu J, Ling EA, Kaur C. Monocyte chemoattractant protein-1 (MCP-1) produced via NF-kappaB signaling pathway mediates migration of amoeboid microglia in the periventricular white matter in hypoxic neonatal rats. *Glia*. 57, 604-621 (2009)

102. Kaur C, Too HF, Ling EA. Phagocytosis of Escherichia coli by amoeboid microglial cells in the developing brain. *Acta Neuropathol*. 107, 204-208 (2004)

103. Ling EA, Tseng CY, Voon FC, Wong WC. Isolation and culture of amoeboid microglial cells from the corpus callosum and cavum septum pellucidum in postnatal rats. *J Anat*. 137 (Pt 2), 223-233 (1983)

104. Li YB, Kaur C, Ling EA. Neuronal degeneration and microglial reaction in the fetal and postnatal rat brain after transient maternal hypoxia. *Neurosci Res*. 32, 137-148 (1998)

105. Kaur C, You Y. Ultrastructure and function of the amoeboid microglial cells in the periventricular white matter in postnatal rat brain following a hypoxic exposure. *Neurosci Lett*. 290, 17-20 (2000)

106. Xu J, Ling EA. Upregulation and induction of major histocompatibility complex class I and II antigens on microglial cells in early postnatal rat brain following intraperitoneal injections of recombinant interferon-gamma. *Neuroscience*. 60, 959-967 (1994)

107. Xu J, Ling EA. Expression of major histocompatibility complex class II antigen on amoeboid microglial cells in early postnatal rat brain following intraperitoneal injections of lipopolysaccharide. *Exp Brain Res*. 100, 287-292 (1994)

108. Kaur C, Dheen ST, Ling EA. From blood to brain: amoeboid microglial cell, a nascent macrophage and its functions in developing brain. *Acta Pharmacol Sin*. 28, 1087-1096 (2007)

109. Ladeby R, Wrenfeldt M, Dalmau I, Gregersen R, Garcia-Ovejero D, Babcock A, Owens T, Finsen B. Proliferating resident microglia express the stem cell antigen CD34 in response to acute neural injury. *Glia*. 50, 121-131 (2005)

110. Ladeby R, Wrenfeldt M, Garcia-Ovejero D, Fenger C, ssing-Olesen L, Dalmau I, Finsen B. Microglial cell population dynamics in the injured adult central nervous system. *Brain Res Brain Res Rev*. 48, 196-206 (2005)

111. Urrea C, Castellanos DA, Sagen J, Tsoulfas P, Bramlett HM, Dietrich WD. Widespread cellular

proliferation and focal neurogenesis after traumatic brain injury in the rat. *Restor Neurol Neurosci.* 25, 65-76 (2007)

112. Denes A, Vidyasagar R, Feng J, Narvainen J, McColl BW, Kauppinen RA, Allan SM. Proliferating resident microglia after focal cerebral ischaemia in mice. *J Cereb Blood Flow Metab.* 27, 1941-1953 (2007)

113. Henze C, Hartmann A, Lescot T, Hirsch EC, Michel PP. Proliferation of microglial cells induced by 1-methyl-4-phenylpyridinium in mesencephalic cultures results from an astrocyte-dependent mechanism: role of granulocyte macrophage colony-stimulating factor. *J Neurochem.* 95, 1069-1077 (2005)

114. Remington LT, Babcock AA, Zehntner SP, Owens T. Microglial recruitment, activation, and proliferation in response to primary demyelination. *Am J Pathol.* 170, 1713-1724 (2007)

115. Byrnes KR, Faden AI. Role of cell cycle proteins in CNS injury. *Neurochem Res.* 32, 1799-1807 (2007)

116. Sawada M, Suzumura A, Yamamoto H, Marunouchi T. Activation and proliferation of the isolated microglia by colony stimulating factor-1 and possible involvement of protein kinase C. *Brain Res.* 509, 119-124 (1990)

117. Schobitz B, de Kloet ER, Sutanto W, Holsboer F. Cellular localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. *Eur J Neurosci.* 5, 1426-1435 (1993)

118. Zhou Y, Ling EA, Dheen ST. Dexamethasone suppresses monocyte chemoattractant protein-1 production via mitogen activated protein kinase phosphatase-1 dependent inhibition of Jun N-terminal kinase and p38 mitogen-activated protein kinase in activated rat microglia. *J Neurochem.* 102, 667-678 (2007)

119. Brockhaus J, Moller T, Kettenmann H. Phagocytosing amoeboid microglial cells studied in a mouse corpus callosum slice preparation. *Glia.* 16, 81-90 (1996)

120. Lazar G, Pal E. Removal of cobalt-labeled neurons and nerve fibers by microglia from the frog's brain and spinal cord. *Glia.* 16, 101-107 (1996)

121. Eugenin EA, Dyer G, Calderon TM, Berman JW. HIV-1 tat protein induces a migratory phenotype in human fetal microglia by a CCL2 (MCP-1)-dependent mechanism: possible role in NeuroAIDS. *Glia.* 49, 501-510 (2005)

122. Hanisch UK. Microglia as a source and target of cytokines. *Glia.* 40, 140-155 (2002)

123. Arnett HA, Mason J, Marino M, Suzuki K, Matsushima GK, Ting JP. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat Neurosci.* 4, 1116-1122 (2001)

124. Andrews T, Zhang P, Bhat NR. TNFalpha potentiates IFNgamma-induced cell death in oligodendrocyte progenitors. *J Neurosci Res.* 54, 574-583 (1998)

125. Nakazawa T, Nakazawa C, Matsubara A, Noda K, Hisatomi T, She H, Michaud N, Hafezi-Moghadam A, Miller JW, Benowitz LI. Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. *J Neurosci.* 26, 12633-12641 (2006)

126. Ledgerwood EC, Pober JS, Bradley JR. Recent advances in the molecular basis of TNF signal transduction. *Lab Invest.* 79, 1041-1050 (1999)

127. Grehan S, Uhlar CM, Sim RB, Herbert J, Whitehead AS. Expression of a biologically active recombinant mouse IL-1 receptor antagonist and its use *in vivo* to modulate aspects of the acute phase response. *J Immunol.* 159, 369-378 (1997)

128. Vela JM, Molina-Holgado E, revalo-Martin A, Almazan G, Guaza C. Interleukin-1 regulates proliferation and differentiation of oligodendrocyte progenitor cells. *Mol Cell Neurosci.* 20, 489-502 (2002)

129. Stanley ER, Berg KL, Einstein DB, Lee PS, Pixley FJ, Wang Y, Yeung YG. Biology and action of colony-stimulating factor-1. *Mol Reprod Dev.* 46, 4-10 (1997)

130. Hao AJ, Dheen ST, Ling EA. Expression of macrophage colony-stimulating factor and its receptor in microglia activation is linked to teratogen-induced neuronal damage. *Neuroscience.* 112, 889-900 (2002)

131. Rovida E, Spinelli E, Sdelci S, Barbetti V, Morandi A, Giuntoli S, Dello SP. ERK5/BMK1 is indispensable for optimal colony-stimulating factor 1 (CSF-1)-induced proliferation in macrophages in a Src-dependent fashion. *J Immunol.* 180, 4166-4172 (2008)

132. Taub DD, Proost P, Murphy WJ, Anver M, Longo DL, van DJ, Oppenheim JJ. Monocyte chemotactic protein-1 (MCP-1), -2, and -3 are chemotactic for human T lymphocytes. *J Clin Invest.* 95, 1370-1376 (1995)

133. Gunn MD, Nelken NA, Liao X, Williams LT. Monocyte chemoattractant protein-1 is sufficient for the chemotaxis of monocytes and lymphocytes in transgenic mice but requires an additional stimulus for inflammatory activation. *J Immunol.* 158, 376-383 (1997)

134. Babcock AA, Kuziel WA, Rivest S, Owens T. Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. *J Neurosci.* 23, 7922-7930 (2003)

135. Hayashi M, Luo Y, Laning J, Strieter RM, Dorf ME. Production and function of monocyte chemoattractant protein-1 and other beta-chemokines in murine glial cells. *J Neuroimmunol.* 60, 143-150 (1995)

136. Che X, Ye W, Panga L, Wu DC, Yang GY. Monocyte chemoattractant protein-1 expressed in neurons and astrocytes during focal ischemia in mice. *Brain Res.* 902, 171-177 (2001)
137. Minami M, Satoh M. Chemokines and their receptors in the brain: pathophysiological roles in ischemic brain injury. *Life Sci.* 74, 321-327 (2003)
138. Fenoglio C, Galimberti D, Lovati C, Guidi I, Gatti A, Fogliarino S, Tiriticco M, Mariani C, Forloni G, Pettenati C, Baron P, Conti G, Bresolin N, Scarpini E. MCP-1 in Alzheimer's disease patients: A-2518G polymorphism and serum levels. *Neurobiol Aging.* 25, 1169-1173 (2004)
139. Conant K, Garzino-Demo A, Nath A, McArthur JC, Halliday W, Power C, Gallo RC, Major EO. Induction of monocyte chemoattractant protein-1 in HIV-1 Tat-stimulated astrocytes and elevation in AIDS dementia. *Proc Natl Acad Sci U S A.* 95, 3117-3121 (1998)
140. Simpson JE, Newcombe J, Cuzner ML, Woodroffe MN. Expression of monocyte chemoattractant protein-1 and other beta-chemokines by resident glia and inflammatory cells in multiple sclerosis lesions. *J Neuroimmunol.* 84, 238-249 (1998)
141. Sorensen TL, Tani M, Jensen J, Pierce V, Lucchinetti C, Folcik VA, Qin S, Rottman J, Sellebjerg F, Strieter RM, Frederiksen JL, Ransohoff RM. Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J Clin Invest.* 103, 807-815 (1999)
142. Glabinski AR, Bielecki B, Ransohoff RM. Chemokine upregulation follows cytokine expression in chronic relapsing experimental autoimmune encephalomyelitis. *Scand J Immunol.* 58, 81-88 (2003)
143. Ambrosini E, Aloisi F. Chemokines and glial cells: a complex network in the central nervous system. *Neurochem Res.* 29, 1017-1038 (2004)
144. Calvo CF, Yoshimura T, Gelman M, Mallat M. Production of monocyte chemotactic protein-1 by rat brain macrophages. *Eur J Neurosci* 8:1725-1734 (1996)
145. Muessel MJ, Klein RM, Wilson AM, Berman NE. Ablation of the chemokine monocyte chemoattractant protein-1 delays retrograde neuronal degeneration, attenuates microglial activation, and alters expression of cell death molecules. *Brain Res Mol Brain Res.* 103, 12-27 (2002)
146. Hughes PM, Allegrini PR, Rudin M, Perry VH, Mir AK, Wiessner C. Monocyte chemoattractant protein-1 deficiency is protective in a murine stroke model. *J Cereb Blood Flow Metab.* 22, 308-317 (2002)
147. Cross AK, Woodroffe MN. Chemokines induce migration and changes in actin polymerization in adult rat brain microglia and a human fetal microglial cell line *in vitro*. *J Neurosci Res.* 55, 17-23 (1999)
148. Husemann J, Loike JD, Anankov R, Febbraio M, Silverstein SC. Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. *Glia.* 40, 195-205 (2002)
149. Cartier L, Hartley O, Dubois-Dauphin M, Krause KH. Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases. *Brain Res Brain Res Rev.* 48, 16-42 (2005)
150. Imai Y, Kohsaka S. Intracellular signaling in M-CSF-induced microglia activation: role of Iba1. *Glia.* 40, 164-174 (2002)
151. Sperlagh B, Zsilla G, Baranyi M, Illes P, Vizi ES. Purinergic modulation of glutamate release under ischemic-like conditions in the hippocampus. *Neuroscience.* 149, 99-111 (2007)
152. Kettenmann H, Hoppe D, Gottmann K, Banati R, Kreutzberg G. Cultured microglial cells have a distinct pattern of membrane channels different from peritoneal macrophages. *J Neurosci Res.* 26, 278-287 (1990)
153. Eder C. Ion channels in microglia (brain macrophages) *Am J Physiol.* 275, C327-C342 (1998)
154. Li F, Lu J, Wu CY, Kaur C, Sivakumar V, Sun J, Li S, Ling EA. Expression of Kv1.2 in microglia and its putative roles in modulating production of proinflammatory cytokines and reactive oxygen species. *J Neurochem.* 106, 2093-2105 (2008)
155. Kaur C, Sivakumar V, Dheen ST, Ling EA. Insulin-like growth factor I and II expression and modulation in amoeboid microglial cells by lipopolysaccharide and retinoic acid. *Neuroscience.* 138, 1233-1244 (2006)
156. Wu CY, Kaur C, Lu J, Cao Q, Guo CH, Zhou Y, Sivakumar V, Ling EA. Transient expression of endothelins in the amoeboid microglial cells in the developing rat brain. *Glia.* 54, 513-525 (2006)
157. Prentice HM, Milton SL, Scheurle D, Lutz PL. Gene transcription of brain voltage-gated potassium channels is reversibly regulated by oxygen supply. *Am J Physiol Regul Integr Comp Physiol.* 285, R1317-R1321 (2003)
158. Conforti L, Bodi I, Nisbet JW, Millhorn DE. O₂-sensitive K⁺ channels: role of the Kv1.2 -subunit in mediating the hypoxic response. *J Physiol.* 524 Pt 3, 783-793 (2000)
159. Wu CY, Kaur C, Sivakumar V, Lu J, Ling EA. Kv1.1 expression in microglia regulates production and release of proinflammatory cytokines, endothelins and nitric oxide. *Neuroscience.* 158, 1500-1508 (2009)
160. Hodgkin AL, Huxley AF, Katz B. Measurement of current-voltage relations in the membrane of the giant axon of Loligo. *J Physiol.* 116, 424-448 (1952)
161. Sontheimer H, Waxman SG. Ion channels in spinal cord astrocytes *in vitro*. II. Biophysical and pharmacological

- analysis of two Na⁺ current types. *J Neurophysiol.* 68, 1001-1011 (1992)
162. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* 19, 312-318 (1996)
163. Black JA, Liu S, Waxman SG. Sodium channel activity modulates multiple functions in microglia. *Glia.* 57, 1072-1081 (2009)
164. Hsueh YP, Sheng M. Regulated expression and subcellular localization of syndecan heparan sulfate proteoglycans and the syndecan-binding protein CASK/LIN-2 during rat brain development. *J Neurosci.* 19, 7415-7425 (1999)
165. Gotte M, Yip GW. Heparanase, hyaluronan, and CD44 in cancers: a breast carcinoma perspective. *Cancer Res.* 66, 10233-10237 (2006)
166. Yip GW, Smollich M, Gotte M. Therapeutic value of glycosaminoglycans in cancer. *Mol Cancer Ther.* 5, 2139-2148 (2006)
167. Tumova S, Woods A, Couchman JR. Heparan sulfate proteoglycans on the cell surface: versatile coordinators of cellular functions. *Int J Biochem Cell Biol.* 32, 269-288 (2000)
168. Lories V, Cassiman JJ, Van den BH, David G. Differential expression of cell surface heparan sulfate proteoglycans in human mammary epithelial cells and lung fibroblasts. *J Biol Chem.* 267, 1116-1122 (1992)
169. Mertens G, Cassiman JJ, Van den BH, Vermynen J, David G. Cell surface heparan sulfate proteoglycans from human vascular endothelial cells. Core protein characterization and antithrombin III binding properties. *J Biol Chem.* 267, 20435-20443 (1992)
170. Modrowski D, Basle M, Lomri A, Marie PJ. Syndecan-2 is involved in the mitogenic activity and signaling of granulocyte-macrophage colony-stimulating factor in osteoblasts. *J Biol Chem.* 275, 9178-9185 (2000)
171. Clasper S, Vekemans S, Fiore M, Plebanski M, Wordsworth P, David G, Jackson DG. Inducible expression of the cell surface heparan sulfate proteoglycan syndecan-2 (fibroglycan) on human activated macrophages can regulate fibroblast growth factor action. *J Biol Chem.* 274, 24113-24123 (1999)
172. Jalkanen M, Elenius K, Salmivirta M. Syndecan--a cell surface proteoglycan that selectively binds extracellular effector molecules. *Adv Exp Med Biol.* 313, 79-85 (1992)
173. Rosenberg RD, Shworak NW, Liu J, Schwartz JJ, Zhang L. Heparan sulfate proteoglycans of the cardiovascular system. Specific structures emerge but how is synthesis regulated? *J Clin Invest.* 100, S67-S75 (1997)
174. Bartlett AH, Hayashida K, Park PW. Molecular and cellular mechanisms of syndecans in tissue injury and inflammation. *Mol Cells.* 24, 153-166 (2007)
175. Lewis C, Murdoch C. Macrophage responses to hypoxia: implications for tumor progression and anti-cancer therapies. *Am J Pathol.* 167, 627-635 (2005)
176. Taylor CT, Colgan SP. Therapeutic targets for hypoxia-elicited pathways. *Pharm Res.* 16, 1498-1505 (1999)
177. Brown LF, Dubin D, Lavigne L, Logan B, Dvorak HF, Van de WL. Macrophages and fibroblasts express embryonic fibronectins during cutaneous wound healing. *Am J Pathol.* 142, 793-801 (1993)
178. Edwards IJ, Wagner WD, Owens RT. Macrophage secretory products selectively stimulate dermatan sulfate proteoglycan production in cultured arterial smooth muscle cells. *Am J Pathol.* 136, 609-621 (1990)
179. Yeaman C, Rapraeger AC. Membrane-anchored proteoglycans of mouse macrophages: P388D1 cells express a syndecan-4-like heparan sulfate proteoglycan and a distinct chondroitin sulfate form. *J Cell Physiol.* 157, 413-425 (1993)
180. Edwards IJ, Xu H, Obunike JC, Goldberg IJ, Wagner WD. Differentiated macrophages synthesize a heparan sulfate proteoglycan and an oversulfated chondroitin sulfate proteoglycan that bind lipoprotein lipase. *Arterioscler Thromb Vasc Biol.* 15, 400-409 (1995)
181. Kaur C, Sivakumar V, Yip GW, Ling EA. Expression of syndecan-2 in the amoeboid microglial cells and its involvement in inflammation in the hypoxic developing brain. *Glia.* 57, 336-349 (2009)
182. Babior BM. Phagocytes and oxidative stress. *Am J Med.* 109, 33-44 (2000)
183. Block ML, Hong JS. Chronic microglial activation and progressive dopaminergic neurotoxicity. *Biochem Soc Trans.* 35, 1127-1132 (2007)
184. Shimohama S, Tanino H, Kawakami N, Okamura N, Kodama H, Yamaguchi T, Hayakawa T, Nunomura A, Chiba S, Perry G, Smith MA, Fujimoto S. Activation of NADPH oxidase in Alzheimer's disease brains. *Biochem Biophys Res Commun.* 273, 5-9 (2000)
185. Grisham MB, Jour'dHeuil D, Wink DA. Nitric oxide. I. Physiological chemistry of nitric oxide and its metabolites: implications in inflammation. *Am J Physiol.* 276, G315-G321 (1999)
186. Beckman JS. Oxidative damage and tyrosine nitration from peroxynitrite. *Chem Res Toxicol.* 9, 836-844 (1996)
187. You Y, Kaur C. Expression of induced nitric oxide synthase in amoeboid microglia in postnatal rats following

an exposure to hypoxia. *Neurosci Lett.* 279, 101-104 (2000)

188. Kaur C, Sivakumar V, Lu J, Tang FR, Ling EA. Melatonin attenuates hypoxia-induced ultrastructural changes and increased vascular permeability in the developing hippocampus. *Brain Pathol.* 18: 533-547 (2008)

189. Merrill JE, Ignarro LJ, Sherman MP, Melinek J, Lane TE. Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. *J Immunol.* 151, 2132-2141 (1993)

Abbreviations: AMC: amoeboid microglial cells, AMPA: alpha-amino-3-hydroxy 5-methyl-4-isoxazolepropionic acid, BBB: blood - brain barrier, CNS: central nervous system, CSF-1: colony stimulating factor-1, CSF-1R: colony stimulating factor-1 receptor, EAE: experimental autoimmune encephalomyelitis, eNOS: endothelial nitric oxide synthase, GFAP: glial fibrillary acidic protein, GM-CSF: granulocyte-macrophage colony stimulating factor, IFN- gamma: interferon- gamma, IL-1beta: interleukin-1 β , IL-1R1: IL-1 receptor 1, IL-2: interleukin-2, IL-6: interleukin-6, iNOS: inducible nitric oxide synthase, LPS: lipopolysaccharide, MBP: myelin basic protein, MHC: major histocompatibility class, MCP-1: monocyte chemoattractant protein-1, M-CSF: macrophage colony-stimulating factor, NADPH: nicotinamide adenine dinucleotide phosphate hydrogenase, NMDA: N-methyl-d-aspartate, nNOS: neuronal nitric oxide synthase, NO: nitric oxide, PWM: periventricular white matter, PWMD: periventricular white matter damage, ROS: reactive oxygen species, Sdcs: syndecans, TNF-alpha: tumor necrosis factor-alpha, TNF-R1: TNF receptor 1, VEGF: vascular endothelial growth factor

Key Words: Hypoxia, Periventricular White Matter Damage, Microglia, Inflammation, Review

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