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Brain-immune interactions in perinatal hypoxic-ischemic brain injury

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Abstract

Perinatal hypoxia-ischemia remains the primary cause of acute neonatal brain injury, leading to a high mortality rate and long-term neurological deficits, such as behavioral, social, attentional, cognitive and functional motor deficits. An ever-increasing body of evidence shows that the immune response to acute cerebral hypoxia-ischemia is a major contributor to the pathophysiology of neonatal brain injury. Hypoxia-ischemia provokes an intravascular inflammatory cascade that is further augmented by the activation of resident immune cells and the cerebral infiltration of peripheral immune cells response to cellular damages in the brain parenchyma. This prolonged and/or inappropriate neuroinflammation leads to secondary brain tissue injury. Yet, the long-term effects of immune activation, especially the adaptive immune response, on the hypoxic-ischemic brain still remain unclear. The focus of this review is to summarize recent advances in the understanding of post-hypoxic-ischemic neuroinflammation triggered by the innate and adaptive immune responses and to discuss how these mechanisms modulate the brain vulnerability to injury. A greater understanding of the reciprocal interactions between the hypoxic-ischemic brain and the immune system will open new avenues for potential immunomodulatory therapy in the treatment of neonatal brain injury.

1. Introduction

Perinatal hypoxia-ischemia induced brain injury is the most common form of neonatal brain injury that occurs in 3 per 1000 term newborns (>36 weeks of gestation) (Hagberg *et al.*, 2015) and about 7 per 1000 preterm newborns (≤36 weeks of gestation) (Chalak *et al.*, 2012). Approximately 40% of newborns suffering hypoxic-ischemic (HI) brain injury do not survive in the neonatal period and another 30% develop long-term neurological disorders, such as cerebral palsy, visual impairment, seizures, epilepsy, mental retardation, and learning disabilities (Higgins *et al.*, 2011; Rocha-Ferreira and Hristova, 2015). In developing

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Conflict of Interests

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countries, the incidence increases up to 26 per 1000 term newborns, with fewer survivors (Douglas-Escobar and Weiss, 2015). Perinatal HI brain injury has been causing a global public health problem and major burdens to the society.

Inflammation has been considered as an important contributor in the pathophysiology of cerebral HI injury. HI triggers immediate and robust activation of brain resident cells and subsequent infiltration of circulating peripheral leukocytes. The initial inflammatory response to hypoxia-ischemia leads to secondary neuronal injury that can last for several days, followed by an anti-inflammatory response toward resolution of inflammation. Numerous experimental studies have depicted the important functions of resident immune cells and peripheral innate immune cells in promoting brain injury and ensuing tissue repair and remodeling in the various stages of the hypoxic-ischemic cascade (Bhalala *et al.*, 2014; Hagberg *et al.*, 2012; Hagberg *et al.*, 2015). However, the exact mechanisms of their activation and interaction with brain cells in the immature brain following hypoxia-ischemia remain largely unknown. Furthermore, T and B lymphocytes have been recently implicated in delayed neuroinflammation in neonatal HI injury. Advancing our understanding of the interactions between the immature hypoxic-ischemic brain and the immune system may provide the new insights for manipulating specific subsets of immune cells to achieve the therapeutic benefit.

In this review, we aim (a) to summarize the development of innate and adaptive immune responses in human and animal neonates with HI brain injury, (b) to highlight their contributions to neonatal HI brain injury, and (c) to assess the therapeutic potential of immunomodulatory targets in neonatal HI brain injury.

2. Animal models of neonatal HI brain injury

Neonatal hypoxic-ischemic encephalopathy (HIE) in the term newborns often results from perinatal asphyxia. It directly leads to 23–25% of neonatal death worldwide, and also causes a high risk of developing long-term devastating impairments in the neonates who survive perinatal asphyxia (Black *et al.*, 2010; Rocha-Ferreira and Hristova, 2016). Perinatal asphyxia is mainly caused by systemic hypoxemia and/or reduced cerebral blood flow. A variety of risk factors have been recognized, including maternal hypotension, maternal cardiac arrest, clotting of placental arteries, placental abruption, uterine rupture, and inflammation (Locatelli et al., 2008). In term newborns, severe asphyxia globally affects the cerebral cortex and selectively damages the sensorimotor cortex, basal ganglia, and thalamus, which results in prominent cortical and deep gray matter injuries (Folkerth, 2005; Gopagondanahalli *et al.*, 2016).

To study the underlying mechanisms of neonatal HIE at term, the neonatal rodent animal models have been widely used to mimic human condition. Postnatal day (PND) 7–10 rodents have brain maturity equivalent to that of 36–40 week old human fetus (term/near term infant) (Rumajogee *et al.*, 2016). The Rice-Vannucci model of neonatal HIE in the PND7–10 Sprague-Dawley rat is an extensively studied pre-clinical model, in which the unilateral ligation of common carotid artery is followed by at least 90 min of exposure to 8% hypoxia (Goren *et al.*, 2017; Northington, 2006; Patel *et al.*, 2015; Rice *et al.*, 1981; Wood *et*

al., 2016). The ligated carotid artery alone does not cause brain damage since the circle of Willis compensates the cerebral blood flow. However, hypoxia lowers oxygen tension and reduces blood flow in the ipsilateral hemisphere, which promotes brain damage. The cerebral cortex, the hippocampus, the deep gray matter and the periventricular white matter, suffer the most HI injury. This model shows many clinical features that occur in human HIE patients, such as the extent of brain injury, the epilepsy development, and the learning and memory disabilities. More research has adapted this model to the term-equivalent mice (PND7–10). However, it should be noted that different strains of mice have the prominent difference in susceptibility to hypoxia-ischemia insult. Compared to 129Sv (HI resistance) and C57BL/6 mice (high mortality), CD1 mice display maximum neonatal HI brain injury with very low mortality rate (Sheldon *et al.*, 1998). Adapting this model to the neonatal mice has also revealed that the duration of hypoxic exposure producing a moderate to severe injury in mice is significantly shorter (20–60 min) (Burnsed *et al.*, 2015; Reinboth *et al.*, 2016; Ten *et al.*, 2004).

The incidence of neonatal HI brain injury is higher in the preterm newborns than in the term newborns. Although the risk factors of perinatal asphyxia in the preterm newborns are similar to those observed in the term newborns, the immature brain of the preterm newborns, particularly those with a very low birth weight (born before 32 weeks), is highly vulnerable to HI injury. Two major reasons have been documented: 1) The poorly functioning hearts and lungs typically seen in the preterm newborns can cause hypoperfusion; 2) The immature brains possess poor auto-regulatory capacity (Huang and Castillo, 2008). In preterm infants, cerebral white matter is poorly vascularized compared with cerebral cortex. Thus, HI insult predominantly induces the white matter injury with marked oligodendroglial loss, also called periventricular leukomalacia (PVL), resulting in motor, sensory, and cognitive impairment in the preterm infants. In addition, the abnormalities of hippocampus and cortical gray matter are also found in the premature brain (Brown *et al.*, 2009).

PND2–6 rodents, similar to 23–36 weeks of gestation in human, have shown similarities with the preterm infants in cellular development in the brain. For example, late oligodendrocyte progenitors ($O4^+/O1^-$) most sensitive to hypoxia-ischemia are predominant at PND2 and differentiate into immature oligodendrocytes at PND7, which is similar to the development of late oligodendrocyte progenitors in human fetus at 24–32 weeks. The optimized hypoxia-ischemia Rice-Vannucci rodent models can mimic many aspects of brain white matter injury in the preterm newborn. PND3 and PND6 rat HI models have been used to resemble PVL in the immature brain (Jantzie et al., 2015; Zhu et al., 2017). In a PND5 mouse HI model, hypoxia-ischemia induces a pattern of diffuse white and focal gray matter injury similar to that in preterm infants with PVL (Albertsson et al., 2014).

3. Neuro-immune communication in neonatal HI brain injury

The pathophysiology of HIE results from a reduction in blood flow and oxygen delivery to the brain. Neurons have been identified as a primary target in selectively vulnerable regions. Severe acute HI insult leads to rapid neuronal death via necrosis, whereas energy failure following less severe or prolonged HI insult causes slower neuronal death mainly through apoptosis (Nakajima *et al.*, 2000). Hypoxia-ischemia induced neuronal death have been

found in two phases (Vannucci and Hagberg, 2004). The severe HI insult causes primary energy failure leading to primary neuronal death, which is related to the significant reduction in energy (adenosine triphosphate (ATP)) and increased intracellular lactate production. The low levels of ATP cause failure of the energy-dependent cell membrane ion channels that maintain cell integrity, which results in an acute intracellular influx of calcium and sodium and cell membrane depolarization, as well as accumulation of extracellular glutamate. In addition, accumulated lactate directly increases reactive oxygen species (ROS) levels. These changes ultimately lead to rapid cell swelling and necrosis. The further injury in the secondary energy failure depends on the extent of primary energy failure. The severe HI insult reduces the latent period characterized by recovered blood flow and restored cerebral metabolism, promoting the onset of secondary energy failure. The secondary energy failure occurs at least six hours after the primary injury. This phase leads to delayed neuronal death with predominant apoptosis, which is related to several mechanisms, such as excitotoxicity, oxidative stress, and inflammation (Millar *et al.*, 2017). The delayed neuronal death contributes to a major proportion of final brain cell loss after HI insults (Figure 1).

The immune system is dispersed throughout the body to protect against disease-causing microbes and help in wound healing, as well as to maintain homeostasis. For a long period of time, the brain is considered to be free from normal immune surveillance. Growing evidence indicates that the privilege is only restricted to the brain parenchyma, but the immune responses in ventricles, choroid plexus and meninges are similar to those in other organs (Galea *et al.*, 2007; Louveau *et al.*, 2015; Shechter and Schwartz, 2013). In the healthy state, blood-brain barrier (BBB) prevents the infiltration of peripheral immune cells, and limits the entry of blood neurotoxic compounds into the central nervous system (CNS). Furthermore, the brain-immune interactions are limited by the immunosuppressive environment created by glia and neurons in the brain, as well as due to the quiescent phenotype of microglia under physiological conditions. However, CNS immune privilege is greatly undermined under the neuroinflammation condition.

Emerging evidence has demonstrated that hypoxia-ischemia induces the inflammatory responses in the brain parenchyma and the peripheral immune system, which play a critical role in mediating secondary neuronal death (Algra *et al.*, 2013; Wang *et al.*, 2010). In the neonatal brain, an immediate innate immune response occurs within minutes after the HI insult. It is thought that stressed or dead neurons directly result in the diffuse activation of neuroglial cells following injury in the immature brain. Activated neuroglial cells have direct influences on the neuronal apoptosis by releasing a large amount of pro-inflammatory cytokines and ROS. Furthermore, the release of matrix metalloproteinases (MMPs) and inflammatory mediators (chemokines and pro-inflammatory cytokines) from activated neuroglial cells helps in recruitment and activation of peripheral immune cells, including granulocytes and monocytes/macrophages. These activated peripheral inflammatory cells release neurotoxic agents, such as pro-inflammatory cytokines, nitric oxide (NO), ROS, and myeloperoxidase, to further enhance neuronal death (Figure 2).

On the other hand, there is increasingly persuasive evidence that the immune system in the context of HI brain injury also plays a beneficial role in supporting tissue healing (Bhalala *et al.*, 2014; Hagberg *et al.*, 2015). The reparative process is capable of promoting a release of

mediators that lead to the resolution of neuroinflammation. For example, phagocytosis of dead cells by microglia/macrophages promotes the production and release of anti-inflammatory cytokines, which help to suppress neuroinflammation and protect against death of remaining viable neurons. Some cells, such as microglia, which are initially associated with the inflammatory process, also produce the neuronal growth factors essential for axonal sprouting, neurogenesis, and angiogenesis. Thus, attempts to control the balance between the neurodestructive and neuroprotective effects of immune system may represent a promising therapeutic strategy for treating neonatal HI brain injury.

4. The role of innate immune cells in neonatal HI brain injury

4.1. Brain innate immune cells

4.1.1. Microglia—Microglia are derived from a distinct population of erythromyeloid progenitors that migrate into the brain starting at embryonic day 8.5 until the blood-brain barrier is formed (Matcovitch-Natan *et al.*, 2016). A large number of microglia are present in the developing periventricular white matter, comprising up to 20% of total glial population. Microglia are long-lived, and seldom need replacement from the bone marrow (BM) under physiological conditions (Perry *et al.*, 2010). Although both microglia and BM-derived monocytes/macrophages are positive for CD11b, microglia show intermediate expression of CD45 ($CD11b^{+}/CD45^{int}$) compared to high levels of CD45 in monocytes/macrophages ($CD11b^{+}/CD45^{hi}$). In addition, these cells express low levels of major histocompatibility complex class II (MHC-II), which accounts for a non-terminally differentiated state. Compared to adult microglia, neonatal microglia are slightly more active in normal condition, with higher levels of MHC-II and costimulatory molecules, such as CD86 and CD40. Neonatal microglia also have different proliferative responses to granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor, and are less dependent on astrocytes for the expansion (Santambrogio *et al.*, 2001).

Microglia are crucially important myeloid cells to act as the first and main form of immune defense against invading pathogens and environmental insults in CNS. In the healthy state, resting microglia show highly ramified and motile processes, forming a 3D non-overlapping network required for CNS immuno-surveillance of individual domain (Ransohoff and Perry, 2009). Microglia actively engage in synapse pruning and neurogenesis, and later, maintain homeostasis in the developing brain (Matcovitch-Natan *et al.*, 2016; Ransohoff and Perry, 2009). However, in the presence of an insult, microglia become activated with the rapid alterations in morphology, including increased cell body size and retraction of processes. Depending on the severity of injury, microglia can further be activated, becoming phagocytic, and travel to the injury sites and remove cellular debris (Kreutzberg, 1996). Besides their role as active phagocytes, microglia release pro-inflammatory cytokines and MMPs that initiate the neuroinflammation and result in the breakdown of the BBB (Iadecola and Anrather, 2011). As a result, the peripheral inflammatory cells infiltrate into the CNS and disrupt the immune privilege, which exacerbates neuroinflammation and subsequent CNS injury.

Hypoxia-ischemia results in notable microglial activation in the neonatal brain. A retrospective clinical study has shown that substantial microglial infiltration is detected in

the polymorphous layer of the dentate gyrus of infants who died from HIE, whereas the infants dying of trauma or sepsis have dramatically fewer microglia (Del Bigio and Becker, 1994). In a rat model of HI brain injury, microglial activation is detected as early as 2 hours following HI insult (Chen *et al.*, 2015; McRae *et al.*, 1995). Undoubtedly, microglia are activated by early neuronal death, while activated microglia play a central role in triggering neuroinflammation that leads to delayed cell death of other neurons and subsequent immature cerebral injury. Microglial contribution to secondary energy failure after hypoxia-ischemia is characterized by classic M1 activation that these cells exert toxic effects on neurons and glia in the surrounding zone through the release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-18), complement factors, proteases, and excitotoxic amino acids (Cowell *et al.*, 2002; Hagberg *et al.*, 1996; Hedtjarn *et al.*, 2002; Rocha-Ferreira and Hristova, 2015). In addition, the excessive production of ROS and NO by activated microglia can induce oxidative injury in the developing brain (Kaur *et al.*, 2013). One of the most potent mechanisms by which M1 activation is provoked is through the release of IL-1β and IL-18. Caspase–1, a cysteine protease, is essential for the proteolytic maturation and secretion of these two key pro-inflammatory cytokines. Caspase-1 is expressed primarily in microglia after HI insult, and caspase-1 deficiency significantly attenuates HI brain damage in neonatal mice (Liu *et al.*, 1999). Arvin et al. have demonstrated that an anti-inflammatory drug, minocycline, blocks the microglial responses and substantially reduces neonatal HI brain injury (Arvin *et al.*, 2002). These data support that early inflammatory responses of microglia aggravate neonatal brain HI injury. Thus, there is a growing interest in inhibiting the M1 activation of microglia for the development of novel therapeutic strategies in the neonatal HI brain injury.

Microglia have also been implicated as an equally crucial player in the resolution of inflammation and reparative processes after acute HI brain injury. Phagocytosis of debris by activated microglia is critical for the recovery of injured brain during the delayed phase after neonatal stroke (Faustino *et al.*, 2011). Selective ablation of microglia leads to elevated levels of pro-inflammatory cytokines and chemokines and exacerbation of brain injury in neonatal and adult stroke models (Faustino *et al.*, 2011; Lalancette-Hebert *et al.*, 2007). In the adult stroke model, the injection of microglia protects neurons against the ischemia-induced neuronal damage by preserving the levels of brain-derived neurotrophic factor (Hayashi *et al.*, 2006). A number of studies in adult stroke suggest that the initial M1 activation of microglia after ischemia may be followed by a switch to an anti-inflammatory M2 phenotype that results in the resolution of inflammation, clearance of debris and reactive species, and tissue repair (Hagberg *et al.*, 2015; Hu *et al.*, 2012; Patel *et al.*, 2013). Presently, the mechanisms of resolution of the pro-inflammatory phase in the injured brain after neonatal hypoxia-ischemia are not very clear.

4.1.2. Astrocytes—Astrocytes are the most prevalent in the CNS, which outnumber neurons by over five-fold. Astrocytes contiguously tile the CNS in a non-overlapping manner (Sofroniew and Vinters, 2010). In the healthy state, astrocytes are long-lived postmitotic cells whose turnover is low (Horner *et al.*, 2000; Sofroniew and Vinters, 2010). Astrocytes exert essential functions in the maintenance of the BBB integrity and neurons nearby. These cells are also responsible for glutamate uptake, suggesting that they may

prevent excitotoxic neuronal damage (Anderson and Swanson, 2000). In addition, they regulate the homeostasis of extracellular ions, including calcium and potassium, and are involved in the antioxidant defense of the CNS (Lian and Stringer, 2004; Ricci *et al.*, 2009). Astrocytes express a number of innate immunity-related functional receptors, mediating astrocyte responses to various kinds of CNS insults through a process commonly defined as reactive astrogliosis that is a pathological hallmark of CNS damage (Pekny and Pekna, 2014).

Pro-inflammatory cytokines and reactive species released by damaged neurons under HI conditions can trigger astrogliosis. Astrogliosis is prevalent within the white matter of up to 40% of infants with HI brain injury (Rezaie and Dean, 2002). Astrocytes in PND7 rat neonates are abundant within the penumbral zone 24 hours after hypoxia-ischemia (Derugin *et al.*, 2000). These findings indicate that astrogliosis may initiate detrimental effects on the developing brain after hypoxia-ischemia. One of mechanisms is that following HI injury impairment of glutamate transporters on astrocytes directly causes the accumulation of extracellular glutamate, which contributes to deleterious events in the developing brain (Dallas *et al.*, 2007). Of particular interest regarding detrimental functions of reactive astrocytes is growing evidence that astrogliosis mediates the innate immune response in the inflamed brain following hypoxia-ischemia. Despite not being viewed as traditional inflammatory cells, reactive astrocytes produce large amounts of pro-inflammatory cytokines, such as TNF-α, IL-1α and β, and IL-6, at the site of neuroinflammation in the ischemic brain in adult stroke (Tuttolomondo *et al.*, 2008; Van Wagoner *et al.*, 1999). These pro-inflammatory cytokines exacerbate toxicity of NO, promote both apoptosis and necrosis of neurons, and inhibit neurogenesis (Barreto *et al.*, 2011; Stoll *et al.*, 1998). Furthermore, reactive astrocytes secrete various inflammatory chemokines that attract migration of peripheral immune cells, such as neutrophils, into the injured site to further worsen the HI injury (Kim, 1996; Koh *et al.*, 2015; Miller *et al.*, 2005).

On the other hand, astrocytes release a number of cytokines, many of which in addition to pro-inflammatory role, have anti-inflammatory effects on the adult ischemic brain at later times (Dong and Benveniste, 2001). A line of evidence indicates that reactive astrocytes enhance production of anti-inflammatory cytokines, including IL-9, IL-10, and IL-11, and facilitate tissue repair by Toll-like receptor 3 (TLR3) activation (Bsibsi *et al.*, 2006). Thus, these data suggest that the astrocytes may also play an important role in the inflammation resolution and tissue repair in neonatal HI brain injury.

4.2. Peripheral innate immune cells

4.2.1. Neutrophils—Neutrophils are the most abundant peripheral immune cells, which comprise up to 75% of cells in the blood. Neutrophils as granular cells are considered as short-lived cells in circulation with approximately 5 days and 12 hours in humans and mice, respectively (Furze and Rankin, 2008). They are continuously generated in the bone marrow and are cleared by the bone marrow and liver macrophages after a short life. Neutrophils are a major component of the innate immunity and are the first peripheral immune cells to reach the site of inflammation after the injuries. Neutrophils are professional peripheral leukocytes that release large amounts of innate immune effector molecules, such as lytic enzymes,

cytokines, chemokines and free oxygen radicals, to defense against invading pathogens (Amulic *et al.*, 2012). In adult rodent stroke models, accumulation of neutrophils within the brain has been observed as early as 4–6 hours after ischemia, and remain there for 2–3 days (Jin *et al.*, 2010; Matsuo *et al.*, 1995). Neutrophils are capable of reducing microvascular flow and releasing large amounts of cytotoxic agents including proteases, cytokines, and free oxygen radicals into the vasculature and brain parenchyma, greatly contributing to ischemic brain injury (Gidday *et al.*, 2005; Matsuo *et al.*, 1995; Schmid-Schonbein, 1987). Several lines of evidence have demonstrated that neuroprotective effects can be achieved by administration of neutrophil inhibitory factors after cerebral ischemia in adult animals (Ikegame *et al.*, 2010; Jiang *et al.*, 1995; Jickling *et al.*, 2015).

Impaired infiltration of neutrophil has been observed in neonates, and neonatal neutrophils also show reduced extravasation from blood vessels (Anderson *et al.*, 1990). Impaired recruitment to sites of inflammation may be due not only to the reduced expression of adhesion molecules in neonatal neutrophils, but also to relatively high production of IL-6 by neonatal monocytes and antigen-presenting cells (APCs), which suppresses neutrophil migration to inflammatory sites, (Levy, 2007). Neutrophils have been observed in blood vessels of neonatal rats within a few hours after hypoxia-ischemia, but do not infiltrate into the brain during the first 42 hours of recovery post HI insult (Hudome *et al.*, 1997). By contrast, another study has revealed that neutrophil infiltration starts at 24 hours and peaks at 72–96 hours post HI insult in PND7 rats (Benjelloun *et al.*, 1999). Although the reports are variable, the data suggest that accumulation of neutrophils after hypoxia-ischemia in neonatal brain is in a much less extent than that in adults.

Interestingly, neutropenia induced prior to HI insult does reduce brain swelling as well as long-term brain injury (Palmer *et al.*, 2004). However, neutropenia is not protective when it is induced after hypoxia-ischemia (Palmer *et al.*, 2004). In term infants with HIE, the significantly elevated number of peripheral neutrophils in the first 96 hours of life is associated with the poor neurodevelopmental outcomes (Morkos *et al.*, 2007). A recent study suggests that the neuroprotective effects of granulocyte colony-stimulating factor (G-CSF) may be further enhanced if the peripheral neutrophils are depleted in a rat neonatal HI model (Doycheva *et al.*, 2014). Hence, neutrophils contribute to the exacerbation of neonatal brain damage, even though they do not rapidly accumulate in the developing brain after hypoxia-ischemia. A number of studies suggest that intravascular neutrophils may induce microvascular dysfunction and worsen brain damage by reducing red cell flow and oxygen delivery and subsequent energy metabolism during and after neonatal hypoxia-ischemia (Hudome *et al.*, 1997; Palmer *et al.*, 2004; Schmid-Schonbein and Lee, 1995).

4.2.2. Monocytes/Macrophages—Monocytes are important blood-borne cells that are involved in phagocytosis, bactericidal activities and antigen presentation to T lymphocytes. Blood monocytes are direct precursors of macrophages or dendritic cells (DCs) in tissues. Two functionally distinct subsets of blood monocytes have been identified by surface marker profiles and unique functions: Ly6C^{hi} and Ly6C^{lo} monocytes. Ly6C^{hi} monocytes, which express high levels of the chemokine receptor CCR2 and low levels of another chemokine receptor CX3CR1, are termed “inflammatory” monocytes. Ly6C^{lo} monocytes are initially termed “patrolling” monocytes that express high levels of CX3CR1 and are negative for

CCR2. Ly6C^{hi} monocytes are highly mobile and selectively migrate to inflammatory sites in response to the chemokine gradients and activation of endothelial cells. However, Ly6C^{lo} monocytes remain in the circulation with longer half-life and accumulate in peripheral tissues under homeostatic conditions.

During CNS inflammation, circulating monocytes are recruited to the brain and give rise to CNS macrophages (Ajami *et al.*, 2011; London *et al.*, 2013). Myeloid cell infiltration peaks at 24 hours following neonatal HI insult, and the infiltrating cells consist predominantly of monocytes (CD11b^{+}Gr1$^{lo/-}$Ly6C$^{int/hi}$) (Hagberg *et al.*, 2015). In an adult stroke model, the deficiency of CCR2 or its ligand CCL2 results in a smaller infarct size and impaired infiltration of monocytes and macrophages, as well as reduced production of pro-inflammatory cytokines (Dimitrijevic *et al.*, 2007; Hughes *et al.*, 2002). On the other hand, CCR2 deletion in bone marrow-derived monocytes/macrophages leads to delayed exacerbation and hemorrhagic conversion of the infarctions, suggesting that CCR2^{+} bone marrow-derived monocytes/macrophages play an essential role in maintaining integrity of the neurovascular unit after ischemia (Gliem *et al.*, 2012). In line with these results, CCR2 knock-out (KO) male mice exhibit markedly reduced numbers of activated macrophages in the damaged brain after neonatal hypoxia-ischemia, but experience long-term spatial learning deficits (Pimentel-Coelho *et al.*, 2015). CCR2/Ly6C^{hi} monocytes can differentiate into anti-inflammatory Ly6C^{lo}F4/80^{hi} macrophages. Therefore, depletion of infiltrating CCR2 monocytes may cause a reduction of beneficial macrophages in damaged brain at later phases, which compromises mechanisms of tissue repair. These findings point to the multi-functions of CCR2 monocytes/macrophages, which may contribute to tissue protection and regeneration, in addition to the detrimental effects on HI injury.

Ly6C^{lo}CX3CR1^{hi} monocytes are thought to accumulate at inflammatory sites less efficiently and to give rise to resident tissue cells. Ly6C^{lo} monocytes dominate in infarcted hearts on day 4 after myocardial infarction and attenuate inflammatory properties, as Ly6C^{lo} monocytes/macrophages are recruited *via* CX3CR1 to orchestrate tissue repair (Nahrendorf *et al.*, 2007). These cells not only are involved in the phagocytosis of debris, but also play a key role in regulating extracellular matrix production and angiogenesis. Moreover, deficiency of Nr4a1, a transcription factor for the development of Ly6C^{lo} monocytes, leads to the increasing atherosclerosis in adult mice (Hanna *et al.*, 2012). Therefore, Ly6C^{lo} monocytes may represent an anti-inflammatory subset. However, in adult transient ischemia models, CX3CR1 deficiency protects the brain against ischemic damage, which is correlated with a M2 pattern of macrophages in the ischemic region (Fumagalli *et al.*, 2013). These findings suggest that CX3CR1 favors a monocyte-mediated inflammatory milieu in the ischemic brain. Selective depletion of Ly6C^{lo} monocytes in Nr4a1 deficient bone marrow chimeras not only has no influence on the structural and functional outcomes, but also does not affect the activation or the number of macrophages after cerebral hypoxia-ischemia in adult mice (Michaud *et al.*, 2014). Gliem et al. have demonstrated that Ly6C^{lo} phagocytes within the lesion compartment are not recruited *via* CX3CR1 but are rather derived from CCR2^{+}Ly6C^{hi} monocytes, suggesting that Ly6C^{hi}/CCR2^{+} monocytes may give rise to Ly6C^{lo}/CX3CR1^{+} macrophages in the brain parenchyma in absence of Nr4a1 transcription factor (Gliem *et al.*, 2012). Thus far, there is very limited data available on the function of Ly6C^{lo} monocytes/macrophages in the neonatal HI model. These discrepancies in other

models highlight the necessity to understand the role of this subset in the initiation and resolution of inflammation in the neonatal injured brain.

4.2.3. Dendritic cells—Dendritic cells (DCs) are professional APCs that act as bridge between innate and adaptive immune systems. DCs specialize in antigen capture, processing, and presentation to T lymphocytes to induce either tolerance or adaptive immunity. The healthy brain parenchyma is recognized as being devoid of resident DCs. However, DCs have been seen in the meninges, choroid plexus and cerebrospinal fluid (CSF) of rats (McMenamin *et al.*, 2003). Vessel-associated $CD11c^+$ dendritic cells are considered as the APCs in a model of allergic encephalitis, which drive the activation of pathogenic $CD4^+$ T cells (Greter *et al.*, 2005). In a rat stroke model, the increased number of DCs expressing OX-62 and OX-6 (MHC-II) in ischemic brain has been observed as early as 1 hour, with a further increase at day 6, and the number of DCs in the brain is positively correlated with the infarct size (Kostulas *et al.*, 2002). Moreover, DCs not only produce the pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-12, in the ischemic brain, but also may initiate the T cell response in the inflammation secondary to brain ischemia (Felger *et al.*, 2010; Kostulas *et al.*, 2002).

The identity of the APCs in neonatal HI brain injury is still not clear. Pentraxin 3, a pattern recognition receptor prominently expressed on DCs, is induced by the transcription factor NF-kB in the early inflammatory phase (Doni *et al.*, 2003). A study globally looking at the inflammatory gene profile after neonatal hypoxia-ischemia has demonstrated the activation of Pentraxin 3 in the immature brain (Hedtjarn *et al.*, 2004), suggesting that DC may be involved in the inflammatory responses to neonatal HI insult. In addition, neonatal HI insult leads to activation of DCs with upregulation of costimulatory molecules, CD86 and MHC-II, in the injured brain and the periphery (Winerdal *et al.*, 2012). A significant increase in the percentage of DCs expressing CD86 occurs in the damaged brain one week after HI insult, which persists at three months (Winerdal *et al.*, 2012). Thus, these results provide evidence that active antigen presentation may exist in the inflamed brain and spleen during the delayed and tertiary phases after HI insult. Whether the presence of DCs contributes to the development of chronic inflammation after neonatal hypoxia-ischemia needs to be elucidated.

5. The role of adaptive immune cells in neonatal HI brain injury

5.1. T cells

The adaptive immune system creates the inflammatory response, anti-inflammatory response, or memory of immunological threats in CNS inflammation by recognizing specific antigens presented by APCs that result in the clonal expansion of effector cells. T cells, a major cell type of adaptive immunity, differ from other lymphocytes (B cells and natural killer cells) by the expression of T-cell receptor on the cell surface. T cells are functionally heterogeneous, and mainly consist of four subsets, including helper T cells, cytotoxic T cells, suppressor T cells and memory T cells. $CD4^+$ T cells are referred to as helper T cells, acting through cytokine production, while $CD8^+$ T cells are primarily cytotoxic. In adult stroke models, infiltration of T cells into the injured brain has been detected as early as a few

hours, and lasts days after ischemia (Brait *et al.*, 2010; Chu *et al.*, 2014). However, in neonatal HI models, the infiltration of $CD4^+$ and $CD8^+$ T cells to the immature brain occurs in the delayed and tertiary phases, usually starting after 24 hours and with a peak at 1 week and 2 weeks after HI insult, respectively (Benjelloun *et al.*, 1999; Winerdal *et al.*, 2012). A clinical study has shown that blood mononuclear cells in newborns are still in a relatively undifferentiated status, with low expression of surface markers (Wang and Lu, 2008). Thus, the delayed infiltration of T lymphocytes into the injured brain of neonates may be due to the immaturity of lymphoid cells during this developmental stage. Current support for the notion that T cells play a neurotoxic role in a short term in acute ischemic brain injury results from the findings that both blocking T cell migration and depletion of T cells reduce infarctions after ischemia in adult animals (Becker *et al.*, 2001; Hurn *et al.*, 2007; Liesz *et al.*, 2009). However, data of their function during the acute stage in neonatal HIE is still missing. Growing evidence suggests that T cells likely play a more important role in the chronic inflammation in neonatal models. In neonatal HI injury, $CD4^+$ T lymphocytes are detected in the damaged brain hemisphere with the first peak one week post HI insult, whereas recruitment of $CD8^+$ T cells peaks two weeks after HI insult (Winerdal *et al.*, 2012). Another more pronounced activation of $CD4^+$ and $CD8^+$ T cells with upregulated expression of activation markers, CD69 and CD25, is observed in the damaged brain as well as in the spleen even after three months (Winerdal *et al.*, 2012). Whether the long-term effects enhance toxic damage or, conversely, are important for resolution of inflammation is still entirely unknown. Thus, the persistent T cell activation after HI insult stresses the need to clarify the role of T lymphocytes in chronic neuroinflammation in neonatal HI brain injury.

5.2. B cells

B lymphocytes, unlike T cells, express B cell receptors that mediate the antibody responses by binding to a specific antigen. In addition, B cells present antigen, produce cytokines and regulate the activities of T cells in many inflammatory diseases. Neonatal B cells is naïve, lack antigenic exposure, have only a partially developed surface immunoglobulin repertoire, and is deficient in neonatal antibody production (Basha *et al.*, 2014), suggesting that B cells, similar to T cells, also exhibit immaturity in neonates. Winerdal et al. have recently demonstrated that the percentage of B lymphocytes expressing activation marker CD69 preferentially increases in the damaged hemisphere two days after neonatal hypoxia-ischemia, and returns to baseline three months after hypoxia-ischemia (Winerdal *et al.*, 2016). Notably, a decrease in B lymphocyte activation and frequency is associated with larger infarctions (Winerdal *et al.*, 2016). Thus, B cells may play a critical role in the resolution of inflammation and tissue repair in neonatal HI brain injury. In lines with these results, absence of B cells leads to larger infarct volumes in adult stroke and increases the numbers of microglia, monocytes, and activated T cells in the ischemic brain (Hurn *et al.*, 2007; Ren *et al.*, 2011). IL-10-secreting B cells have been found in rodent models of neonatal HIE and adult stroke (Offner and Hurn, 2012; Ren *et al.*, 2011; Winerdal *et al.*, 2016). Adoptive transfer of IL-10-secreting B cells into B-cell deficient mice reduces the infarct volumes following adult ischemic stroke (Bodhankar *et al.*, 2013a). Therefore, B cell protection against neonatal brain injury is most likely mediated through anti-inflammatory/immunomodulatory cytokine, IL-10.

6. Molecular mediators of inflammation in neonatal HI brain injury

6.1. Pro-inflammatory cytokines

As the HI cascade progresses, dead and dying neuronal cells release danger signals that trigger the innate immune responses in the brain. These danger associated molecular pattern molecules (DAMPs), including IL-33, high-mobility group protein B1 (HMGB1), and ATP, initiate a downstream pro-inflammatory cascade by activating pattern recognition receptors, such as TLRs and scavenger receptors, on the surface of microglia, astrocytes, brain endothelial cells and perivascular macrophages (Gadani *et al.*, 2015; Iadecola and Anrather, 2011). Early pro-inflammatory mediators in this cascade include pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 (Table 1), leading to the beginning of post-ischemic neuroinflammation.

TNF-α is a key inflammatory mediator implicated in the pathogenesis of HI brain injury. Although astrocytes and neurons are able to produce TNF-α, it is assumed that microglia are the major source of this cytokine during neuroinflammation (Hanisch, 2002; Welser-Alves and Milner, 2013). Human infants who suffer from HIE have higher levels of TNF-α in the peripheral blood and CSF, which is positively correlated with the severity of brain injury (Aly *et al.*, 2006; Liu and Feng, 2010; Silveira and Procianoy, 2003). In neonatal rats with HIE, the levels of TNF-α in serum or lesioned brain are significantly increased as early as 4 hours after hypoxia-ischemia (Li *et al.*, 2014; Szaflarski *et al.*, 1995). Hence, TNF-α may play a detrimental role in mediating the initial inflammatory response in neonatal HIE. TNF-α KO mice display the reduced infarct volumes in adult stroke model (Hallenbeck, 2002). TNF-α mainly acts through TNF-receptor-1 (TNF-R1) that is widely expressed on neurons and glia. The activation of TNF-R1 containing an intracellular death domain causes apoptotic cell death as well as necrotic cell death (Wajant *et al.*, 2003). Aberrant TNF-α/TNF-R1 signaling is associated with increased oligodendrocyte progenitor death after LPS-sensitized HI injury in the developing brain (Li *et al.*, 2008; Wang *et al.*, 2014b). TNF-α also induces neuronal apoptosis and enhances the expression of MHC-II and intercellular adhesion molecules 1 (ICAM-1) in astrocytes, leading to breakdown of BBB and infiltration of peripheral leukocytes (Hallenbeck, 2002). Indeed, the apoptotic rate of neurons is positively associated with the levels of TNF-α in neonatal rat brain with HI damage (Li *et al.*, 2014; Wang *et al.*, 2013). By contrast, in TNF-R KO adult mice, the infarct size is exacerbated 24 hours following ischemia (Bruce *et al.*, 1996). TNF-α signals are also mediated through TNF-R2, a receptor that is expressed on neurons and mediates survival signaling. NF-κB inhibition results in a switch to activation of the JNK/AP-1 signaling, which preserves TNF-α production and reduces brain damage after neonatal HI insult. It should be noted that when NF-κB is inhibited, TNF-R2 expression increases (Nijboer *et al.*, 2009). Thus, TNF-α may contribute to neuroprotection through TNF-R2 signaling in certain phases after neonatal hypoxia-ischemia.

Similar to TNF-α, mRNA expression of IL-1β in lesioned forebrain of neonatal rats with HIE is markedly increased at 3 hours after acute HI injury (Hagberg *et al.*, 1996). The elevated IL-1β levels in CSF or umbilical blood of human newborns are correlated with HIE severity more than other proinflammatory cytokines (Aly *et al.*, 2006; Liu and Feng, 2010).

Several lines of evidence indicate that the administration of IL-1 receptor antagonist and IL-1β provides the neuroprotective and neurotoxic effects on neonatal brain injury, respectively (Boutin *et al.*, 2001; Cai *et al.*, 2004; Rosenzweig *et al.*, 2014), suggesting that IL-1β is functionally detrimental in the pathogenesis of HIE. IL-1β can directly induce neuronal apoptosis and promote the expression of chemokines in microglia and astrocytes, as well as increase BBB physical permeability, leading to potentially enhanced immune cell infiltration into the CNS (Rothhammer and Quintana, 2015). Inflammatory actions of IL-1β are mediated by the type I IL-1 receptor (IL-1R). Recent evidence has shown that IL-1β decreases the proliferation and survival rates of neural precursor cells by activating IL-1β/IL-1R signaling and upregulating p53 and p53-mediated genes, which leads to cell cycle inhibition and Puma/Bax-mediated apoptosis in neural precursor cells (Guadagno *et al.*, 2015). Moreover, increased IL-1β production by microglia and astrocytes is linked to upregulated expression of IL-1R on oligodendrocytes and increased apoptosis of oligodendrocytes in periventricular white matter of neonatal brain (Deng *et al.*, 2014; Xie *et al.*, 2016). Thus, IL-1β is detrimental to immature brain with neonatal HIE through inhibiting the proliferation and survival of brain cells, such as neurons and oligodendrocytes.

Another representative pro-inflammatory cytokine, IL-6, is also induced by hypoxia-ischemia and follows a similar time-course of expression as IL-1β in neonatal rats with HIE (Hagberg *et al.*, 1996; Li *et al.*, 2014). In term infants who suffer from HIE, the levels of IL-6 are greatly elevated in blood and CSF after perinatal asphyxia, which is significantly associated with increased severity of HIE and neurological outcome (Aly *et al.*, 2006; Chiesa *et al.*, 2003; Savman *et al.*, 1998). Thus, these findings strongly suggest that IL-6 acts as a critical inflammatory mediator of neonatal brain damage. IL-6 has been implicated as a regulator of neurogenesis in neuroinflammation. IL-6 alone inhibits neurogenesis, which is primarily through inducing the nonspecific cell death and reducing the neuronal differentiation and accumulation (Monje *et al.*, 2003). IL-6 has been also suggested to promote hypoxia-ischemia-induced neuronal apoptosis in neonatal rats (Li *et al.*, 2014). In summary, these data suggest that rapid release of IL-6 following hypoxia-ischemia exacerbates neonatal brain injury by directly inducing the neuronal apoptosis and inhibiting neurogenesis.

6.2. Anti-inflammatory cytokines

Besides pro-inflammatory cytokines, activation of microglia/macrophages and astrocytes in response to neonatal hypoxia-ischemia also leads to the production of IL-10, a key immuno-regulatory cytokine antagonizing the pro-inflammatory response (Table 1). Immediately after birth, the concentration of IL-10 is higher in the serum of asphyxiated neonates than that in normal neonates (Okazaki *et al.*, 2006). Although the levels of IL-10 are induced at 6 hours in serum and brain of neonatal rats by hypoxia-ischemia, they peak at 48 hours and return back to normal by 7 days (Li *et al.*, 2014). In newborn mice subjected to intracerebral injection of ibotenate, an inducer of white matter lesions mimicking cerebral palsy, systemic treatment with IL-10 alone has no detectable effects, while IL-10 co-administered with IL-1β reduced the toxic effects of IL-1β (Mesples *et al.*, 2003), suggesting that exogenous IL-10 is neuroprotective in inflammatory contexts. IL-10 exerts its anti-inflammatory actions in leukocytes and glial cells by regulating multiple layers, such as inhibition of pro-

inflammatory cytokines, upregulation of pro-inflammatory cytokine antagonists, downregulation of pro-inflammatory cytokine receptors, and inactivation of NF-κB (Landskron *et al.*, 2014). Although some effects are mediated by hemeoxygenase-1, a heat-shock protein inhibiting pro-inflammatory cytokine synthesis and inducing anti-apoptotic processes (Lee and Chau, 2002), most of actions are attributable to the signal transducer and activator of transcription 3 (STAT3)/ SOCS3 signaling (Donnelly *et al.*, 1999; Ito *et al.*, 1999). Besides anti-inflammatory activity, IL-10 also protects hippocampal neurons against apoptotic cell death in ischemic brain injury. IL-10 has been shown to block the activity of pro-apoptotic protein caspase-3 (Bachis *et al.*, 2001), which has been implicated in participating in glutamate-induced neuronal apoptosis.

Growing evidence indicates that IL-6 plays ambivalent roles in acute ischemic brain injury, depending on the phases of ischemia (Table 1). In adult stroke models, ischemic brain damage is aggravated in IL-6-deficient mice or in anti-IL-6R antagonistic antibody-treated mice (Herrmann *et al.*, 2003; Yamashita *et al.*, 2005), suggesting that IL-6 plays a protective role in ischemic brain injury. IL-6 has the anti-inflammatory actions by inhibiting the synthesis of TNF-α and IL-1β, as well as by stimulating the production of their endogenous antagonists. Moreover, several studies have linked IL-6 to neuron protection against apoptosis by reducing N-methyl-D-aspartate toxicity (Ali *et al.*, 2000; Fang *et al.*, 2013; Liu *et al.*, 2011). In human neonates with perinatal asphyxia, the levels of IL-6 are significantly higher and show a biphasic pattern with early and delayed peaks (Jenkins *et al.*, 2012). IL-6 enhances anti-apoptotic activity of astrocytes through the IL-6/STAT3 signaling in neonatal rats with HI brain injury (Gu *et al.*, 2016). Thus, it is possible that IL-6 protects neurons and glia from apoptotic cell death at a delayed phase in the immature brain, but a significant anti-inflammatory role of IL-6 after neonatal HI insult has not been fully elucidated yet.

6.3. Chemokines

Chemokines are chemotactic cytokines that are involved in the recruitment of leukocyte in response to inflammation and play a fundamental role in mediating cellular communication. Chemokines are classified into four families (α (CXC), β (CC), γ (C), and neurotactin (CX_3C)) on the basis of the presence and position of the conserved cysteine residues. The α-chemokines are predominantly chemotactic to granulocytes whereas the β-chemokines are the agonists for mononuclear cells, including monocytes, dendritic cells, lymphocytes, and NK cells. In addition, the γ-chemokines are recognized as chemoattractants for lymphocytes and the neurotactin is mainly chemotactic to neutrophils. In a rat neonatal HI model, expression of α- and β-chemokines precedes infiltration of peripheral immune cells into the immature brain (Bona *et al.*, 1999), thus suggesting that these molecules may mainly mediate the recruitment of peripheral leukocytes following neonatal HI brain injury.

CXCL8 (also known as IL-8) as a key α-chemokine is well known to neonatologists as a serum marker for neonatal sepsis (Berner *et al.*, 1998; Ng and Lam, 2006). Elevated levels of CXCL8 in the CSF of term newborns are associated with the severity of HIE and poor neurological outcome (Savman *et al.*, 1998). Microglia are considered as a potential production site of CXCL8 in the injured brain. In an adult rat stroke model, enhanced production of CXCL8 locally results in a rapid influx of blood polymorphonuclear

leukocytes, primarily neutrophils, to the sites of cerebral ischemia, thereby causing local inflammation (Domac and Misirli, 2008). CXCL8 also induces the accumulation of leukocytes in the microvessels of an injured area, which can obstruct these small vessels and decrease blood flow, leading to local brain ischemia. CXCL12 (also known as SDF-1, stromal cell-derived factor-1), is another interesting α-chemokine, whose expression is up-regulated in the ischemic penumbra, specifically in perivascular astrocytes after brain injury in PND7 mice (Miller *et al.*, 2005). CXCL12 plays a neuroprotective role in promoting angiogenesis and migration of neuronal and endothelial progenitor cells to the ischemic tissues (Fan *et al.*, 2010; Ohab *et al.*, 2006). However, CXCL12 receptor antagonists can attenuate the infiltration of peripheral immune cells into the ischemic hemisphere, which improves recovery of sensorimotor function after adult ischemia (Ruscher *et al.*, 2013). Thus, CXCL12 signaling most likely contributes to the deleterious homing of inflammatory cells in adult stroke. CXCL1 is also known to serve as a neutrophil chemoattractant in the ischemic brain. An intriguing study recently has revealed that hypoxia contributes to the activation of the HIF-1/TIM-3 axis in microglia and astrocytes, inducing the production of CXCL1 that causes increased infiltration of neutrophil and subsequent neuroinflammation in adult HI brain injury (Koh *et al.*, 2015). Whether hypoxia-ischemia induced CXCL1 can promote neonatal brain injury needs to be determined in future studies.

Similar to the α-chemokine, the β-chemokines also enhance post-ischemic inflammation. In the neonatal brain, one of the most important β-chemokines is CCL2 (also known as MCP-1, monocyte chemotactic protein-1), whose expression is significantly upregulated by acute HI insult in activated microglia (Faustino *et al.*, 2011; Galasso *et al.*, 2000). Hughes et al. have found that $CCL2^{-/-}$ mice develop a smaller infarct size in the adult stroke model (Hughes *et al.*, 2002). Consistently, myelin basic protein-JE transgenic mice overexpressing CCL2 have a larger brain infarct size after focal brain ischemia, accompanied by increased infiltration of macrophages and neutrophils into the ischemic region (Chen *et al.*, 2003). Thus, these studies offer the direct evidence for a detrimental role of CCL2 in ischemic brain injury. Another β-chemokine, CCL5 (also known as RANTES), can recruit a variety of immune cells into inflammatory sites. It has been implicated in HI pathophysiology as the significantly higher levels of CCL5 are measured in term neonates with HIE (Shalak *et al.*, 2002). Adult mice lacking CCL5 have smaller infarct volumes and decreased BBB permeability, accompanied by reduced recruitment of leukocytes and platelets, after focal brain ischemia and reperfusion (Appay and Rowland-Jones, 2001). Since the similar protective effects are noted in bone marrow chimeras with $CCL5^{-/-}$ hematopoietic cells (Terao *et al.*, 2008), it is suggested that blood cell-derived CCL5 mediates the inflammatory responses leading to tissue injury in the adult ischemic brain. The chemokines for lymphocyte infiltration remain largely elusive in neonatal HI brain injury. CCL12 and CCL20 play an essential role in exacerbating adult murine experimental autoimmune encephalomyelitis (EAE), a $CD4^{+}$ T cell-driving neurological injury (Reboldi *et al.*, 2009). Whether they also play a role in acute HI injury in the immature brain needs to be investigated. Further understanding of these insights in neonatal HIE could provide new therapeutic strategies for clinical interventions.

6.4. Adhesion molecules

Adhesion molecules, including integrins, selectins, and immunoglobulin superfamily, are essential for the recruitment of peripheral leukocytes into the injured brain. Pro-inflammatory cytokines and chemokines induce the expression of surface adhesion molecules in leukocytes and endothelial cells, promoting the adhesion and transendothelial migration of leukocytes. Adhesion molecules initially mediate a low affinity binding between leukocytes and endothelial cells, a process manifested as leukocyte rolling, which results in a later high affinity binding and firm adhesion. Hypoxia-ischemia upregulates expression of a number of genes involved in the adhesion process in the neonatal brain, such as CD44 antigen, ICAM-1, vascular cell adhesion molecule 1, decorin, thrombospondin 1, integrin β-2, and CD9 antigen (Hedtjarn *et al.*, 2004), suggest that adhesion molecules may play a critical role in the neonatal HI brain injury.

Upregulation of selectins on endothelial cells mediates a low affinity binding of leukocyte at the early stages in the ischemic cerebral microvasculature through interaction with glycoproteins on the surface of leukocytes, such as P-selectin glycoprotein ligand-1. All of selectins, including L-, P-, and E-selectin, participate in neutrophil rolling. However, in the immature brain, E-selectin appears to be less important as the blockade of E-selectin is not able to reduce the neutrophil recruitment to the brain inflammatory sites (Bernardes-Silva *et al.*, 2001). By contrast, the administration of P- (but not L-) selectin neutralizing antibody inhibits neutrophil recruitment up to 85% compared to controls (Ley *et al.*, 1995). P-selectin deficiency also leads to the smaller infarct size and significantly reduces infiltration of neutrophil in acute adult stroke (Connolly *et al.*, 1997). Thus, P-selectin may play the most important role in initiating the infiltration of peripheral inflammatory cells following endothelial cell stimulation in neonatal HI brain injury.

The activation of leukocyte-expressed integrins promotes a tight binding to the members of immunoglobulin gene superfamily on inflamed endothelial cells, which allow firm adhesion of leukocytes to the endothelium after rolling. Mac-1 (CD11b/CD18) and LFA-1 (CD11a/CD18) are the major β-2 integrins that are expressed on neutrophils and bind to two immunoglobulin superfamily members, ICAM-1 and ICAM-2. However, monocytes and lymphocytes bear β-1 integrins, such as VLA-4 (CD49d/CD29), binding to vascular cell adhesion molecule 1 on endothelial cells. Thus far, the role of integrins and the immunoglobulin superfamily in neonatal HI brain injury remains largely unknown. Preclinical studies in adult stroke models have indicated that blockade of LFA-1/Mac-1 offers neuroprotective effects on ischemic brain and ICAM-1 deficiency results in a smaller infarct size and dramatically less infiltration of neutrophil (Chen *et al.*, 1994; Connolly *et al.*, 1996; Garcia *et al.*, 1996). Thus, adhesion molecules-directed infiltration of peripheral leukocytes is a key determinant in adult ischemic brain injury. Additional work is needed to clearly define the role of adhesion molecules in mediating innate inflammatory response to neonatal HI insult and to explore the significance of the adhesion molecule as a therapeutic target in neonatal brain injury.

7. MiRNA modulation in neuroinflammation

MicroRNAs (miRNAs) are small noncoding RNAs (18 to 24 nucleotides) that negatively regulate the expression of target genes by degradation or translational inhibition of mRNA targets. As a single miRNA can target numerous mRNAs and several mRNAs are subject to regulation by more than one miRNA, these molecules are integral parts of gene expression networks that are capable of determining cell identity and function. Numerous studies have highlighted the vital role of miRNAs in normal development of the CNS (Bernstein *et al.*, 2003; Bhalala *et al.*, 2013; Davis *et al.*, 2008; De Pietri Tonelli *et al.*, 2008). In recent years, emerging evidence suggests that miRNAs act as key modulators during immune cell development/differentiation and immune response in the CNS. The dysfunction of miRNAs can lead to neuroinflammation that contributes to pathological conditions, such as autoimmune CNS injuries and adult stroke (Bhalala *et al.*, 2013; O'Connell *et al.*, 2010; Satoorian *et al.*, 2016; Soreq and Wolf, 2011). A clinical study has shown that neonatal HIE alters the expression profile of miRNAs in the umbilical cord blood (Looney et al., 2015). Furthermore, LPS *in vitro* stimulation rapidly alters the levels of a large number of miRNAs in the cord blood from infants, suggesting that miRNAs may play the key roles in modulating the innate inflammatory responses in the newborn (Chen *et al.*, 2014). Several miRNAs that are implicated in inflammatory gene regulation and modulation of TLR pathways might modulate the processes that enable the neuro-immune communication in the developing brain following HI injury (Figure 3).

A prominent example is miR-210, a signature of hypoxia. MiR-210 is significantly induced by transient focal ischemia and neonatal hypoxia-ischemia in the injured brain in adult and neonatal animal models (Jeyaseelan *et al.*, 2008; Ma *et al.*, 2016). It has been revealed that hypoxia synergizes with TCR and CD28 signaling to increase expression of miR-210 in activated T cells through HIF-1α, a key transcriptional factor of Th17 differentiation (Wang *et al.*, 2014a). Interestingly, miR-210 subsequently functions to inhibit HIF-1α in a negative feedback loop and thereby negatively modulates pathogenic T cell differentiation in hypoxia (Wang *et al.*, 2014a). In addition, TLR-induced NF-κB1 is known to bind the miR-210 promoter and induce its expression. In murine macrophages, miR-210 targets NF-κB1 and acts as an important negative feedback regulator for LPS-induced inflammatory response (Qi *et al.*, 2012). Thus, miR-210 seems to be a significant molecular brake on inflammatory reactions. In addition to miR-210, miR-146a and miR-124 have been shown to inhibit neuroinflammation. The upregulation of miR-146a after LPS stimulation has been observed in monocytes derived from newborn cord blood (Lederhuber *et al.*, 2011). Several lines of evidence support that miR-146a serves as a negative feedback regulator of the TLR-mediated NF-κB activity by targeting TRAF6 and IRAK1 in myeloid immune cells and microglia (Boldin *et al.*, 2011; Saba *et al.*, 2012; Taganov *et al.*, 2006). Notably, a recent study has shown that thymosin β4, a G-actin-sequestering molecule with anti-inflammatory features, inhibits microglia activation and neuroinflammation in neonatal rats following HI insult, which is associated with upregulated expression of miR-146a (Zhou *et al.*, 2015). Another CNS enriched miRNA, miR-124, is down-regulated in the brain of rats subjected to the transient focal ischemia (Zhu *et al.*, 2014). MiR-124 is highly expressed in microglia/CNS macrophages where it is directly involved in the maintenance of microglia/

macrophage quiescence by targeting both the master transcription factor C/EBP-α and its downstream gene, the myeloid cell differentiation-associated transcription factor PU.1 (Ponomarev *et al.*, 2011). MiR-124 also contributes to the alternative M2 activation of macrophages and microglia, since forced overexpression of miR-124 leads to an increase of M2 markers, such as IL-10, TGF-β, and arginase-1 (Ponomarev *et al.*, 2011). Thus, it is possible that miR-124 reduces neuronal apoptotic death and promotes tissue repair by controlling the balance of M1/M2 microglia/macrophages in the hypoxic-ischemic brain.

Conversely, miR-155 as a pro-inflammatory factor is directly linked to the classic M1 activation of microglia/macrophages. This miRNA enhances the expression of several key inflammatory mediators, including the inducible nitric oxide synthase, TNF-α, and IL-6, by targeting two negative regulators of AKT and IFN signaling pathway, Src homology 2-containing inositol phosphatase 1 (SHIP-1) and suppressor of cytokine signaling molecule 1 (SOCS1) (Cardoso *et al.*, 2012; O'Connell *et al.*, 2009). Furthermore, miR-155 inhibits the alternative M2 phenotype by downregulating SMAD2, a key component of the TGF-β signaling pathway, as well as C/EBP-β, a transcription factor responsible for the expression of arginase 1, IL-10 and CD206, further supporting that miR-155 plays a key in skewing microglia/macrophage activation to classic M1 phenotype (Louafi *et al.*, 2010; O'Connell *et al.*, 2008; Ruffell *et al.*, 2009). In the adult stroke model, the expression of miR-155 is significantly affected. Inhibition of miR-155 reduces the ischemic inflammatory responses, which contributes to the reduced brain tissue damage and promotes brain repair and functional recovery (Caballero-Garrido *et al.*, 2015; Pena-Philippides *et al.*, 2016).

MiRNA research in neonatal HI brain injury is still in its infancy thus far. In light of the pivotal roles of miRNAs in modulating neuroinflammation in adult CNS injuries, further studies need to define the exact roles of the miRNAs in neonatal HI brain injury in the next few years. A better understanding of miRNAs functioning in the neuroimmune interactions may have important therapeutic implications.

8. Long-term immunological consequences of neonatal HI brain injury

An intriguing study has provided evidence that the activation of APCs and T lymphocytes exists at three months after neonatal hypoxia-ischemia in the ischemic brain and periphery (Winerdal *et al.*, 2012), indicating that the long-lasting inflammation persists in neonates with HIE. T lymphocytes that infiltrate into the brain three months post HI insult are $CD4^{+}CD45RB^{lo}$ memory phenotype and auto-reactive (Winerdal *et al.*, 2012), suggesting that autoimmunity develops after neonatal HI brain injury. A detrimental role of autoimmune response in the development of ischemic brain injury emerges from the stroke studies. Autoreactive T cells and elevated levels of antibodies against brain antigens have been observed in adult stroke patients (Bornstein *et al.*, 2001; Dambinova *et al.*, 2003). Blocking of autoreactive T cell response with artificial T-cell ligands reduces lesion volume 96 hours after stroke (Subramanian *et al.*, 2009). Thus, it is possible that neonatal hypoxia-ischemia-induced autoimmunity may mediate neurodevelopmental impairments. Activation of peripheral T cells specific for brain antigens may increase the risk of brain injury later in life at a certain time point where the BBB allows the migration of autoreactive T cells into the

CNS. Therefore, there is a need for further investigations to address if perinatal HI brain injury causes the development of autoimmune CNS diseases in later life.

In preterm infants with cerebral palsy, at least in some patients with perinatal brain injury, a long-lasting inflammation is observed as increased levels of TNF-α in the plasma and the supernatants of peripheral blood cells after LPS stimulation are measured (Lin *et al.*, 2010). Indeed, another study has revealed that microglia remains activated many years after traumatic brain injury in human adult (Gentleman *et al.*, 2004). Although most studies only focus on the inflammatory responses during a short period after ischemic brain injury, the long-lasting immune responses may be of importance for future investigations as well. This long-term innate inflammatory responses could have detrimental effects on the development of the disease and/or on the clinical symptoms. Taken together, additional studies should further determine whether there is a long-term innate inflammatory process in the patients who suffer neonatal HIE and to define how it impacts on the development of immature brains. Understanding of such a pathophysiological event could shed lights on therapeutic interventions.

9. Bench to bedside: promising anti-inflammatory/immunomodulatory therapies for neonatal HI brain injury

Neonatal HI brain injury encounters an enormous therapeutic challenge. Currently, hypothermia is the only properly validated treatment for term HIE. However, it has a narrow therapeutic time window of less than 6 hours (before the secondary phase of energy failure), as well as an area of uncertainty regarding the optimal timing of initial cooling, and depth and duration of therapy (Dixon *et al.*, 2015). In addition, it is not always effective for neonates with severe HI injury. Hence, there is an urgent need to develop additional new therapies to treat neonates suffering from HIE. Growing evidence supports that inflammation plays a crucial role in the pathophysiology of neonatal HI brain injury. Post-HI inflammation not only promotes the exacerbation of neurological deficits, but is also involved in tissue repair in the recovery phase of neuronal injury. In order to create immune-related therapeutic strategies, it may be necessary to control the balance between pro-inflammatory and anti-inflammatory effects by targeting specific mediators and subsets of inflammation. Therefore, anti-inflammatory/immune-modulatory strategies represent the attractive therapies for the treatment of neonatal HI brain injury. These treatments not only bring great hope to extend the therapeutic window, but could also be used in combination for treating neonates with severe HIE.

9.1. Hypothermia

Although only 1 in 6 newborns with moderate to severe HIE could benefit from therapeutic hypothermia (TH), moderate hypothermia initiated before 6 hours of life and continued for 48–72 hours is the only therapeutic option that reduces mortality and improves neurological outcomes in the clinical setting (Davidson *et al.*, 2015; Shankaran *et al.*, 2005; Shankaran *et al.*, 2012). In addition to directly preventing neurons from oxidative injury and apoptosis, animal and clinical studies suggest that TH can regulate the inflammatory responses and has immunosuppressive effects on the neonatal HI brain injury. A randomized controlled trial of

systemic hypothermia in neonatal HIE has revealed that hypothermia therapy is associated with markedly lower counts of whole white blood cells, as well as with lower counts of neutrophils and lymphocytes (Jenkins *et al.*, 2013). Compared to HIE newborns with adverse outcome, the serum levels of pro-inflammatory cytokines, such as IL-1β, IL-2, and IL-6, have been significantly reduced in HIE newborns with favorable outcome at 24 hours of TH treatment (Orrock *et al.*, 2016). These evidence strongly suggest that TH regulates the peripheral immune responses in human neonates with HIE. In a neonatal animal study, hypothermia treatment for 72 hours significantly reduces the expression of pro-inflammatory cytokines and NO in rat microglia (Matsui and Kakeda, 2008). Another study also detects the inflammatory responses in the brains of PND7 rats treated with TH for 24 hours after hypoxia-ischemia. In the hypothermia treated group, the upregulated gene expression of GFAP is reversed, and the number of astrocytes is greatly reduced at day 3 and 7 (Xiong *et al.*, 2009). Importantly, the mRNA levels of inflammatory cytokines TNF-α and IL-6 in the brains are lower (Xiong *et al.*, 2009).

HMGB1 that initiates expression of inflammatory cytokine genes may mediate the immunosuppressive role of TH. A line of evidence supports this hypothesis that the levels of HMGB1 are significantly lower in the blood of HIE infants treated with TH compare to that of untreated HIE infants (Nakamura *et al.*, 2013). The activation of Janus kinase 2/STAT3 signaling pathway mediated by IL-6 results in expression of a series of genes involved in inflammation and apoptosis. Mild hypothermia attenuates phosphorylation and action of STAT3 (Choi *et al.*, 2011), which might be another possible mechanism of hypothermia-induced immunosuppression.

9.2. Anti-inflammatory agents

9.2.1. IL-1 receptor antagonist—IL-1β, a major cytokine exacerbating neonatal HI brain injury, is an attractive therapeutic target. The administration of recombinant human IL-1 receptor antagonist (rhIL-1ra) blocking the inflammatory action of IL-1 protects against ischemic brain injury in the adult stroke model (Loddick and Rothwell, 1996). A randomized phase II study of rhIL-1ra has shown that rhIL-1ra treatment greatly improves the clinical outcome of patients at 3 months after adult stroke (Emsley *et al.*, 2005). Importantly, it is safe and well tolerated in the patients. In the rat models of neonatal brain injury induced by prenatal LPS and/or hypoxia-ischemia, systemic administration of lowdose of IL-1ra attenuates neurological injury and associated functional deficits (Girard *et al.*, 2012; Girard *et al.*, 2010). Further elucidation of the therapeutic efficacy of postnatal systemic IL-1ra administration in clinical trials of term neonates with brain injury is needed.

9.2.2. Neutralizing antibodies—Blocking antibodies directly against the inflammatory mediators and/or immune cells represents a promising anti-inflammatory therapeutic strategy in neonate HI brain injury. TNF-α as a potent pro-inflammatory cytokine induces the neurotoxic effects after neonatal HI injury. Pretreatment with intravenous anti-TNF-α antibody significantly prevents the development of ischemic brain injury in the rat model of adult stroke (Lavine *et al.*, 1998), suggesting that anti-TNF-α may possess therapeutic value in treating neonatal HI brain injury. However, Nijboer et al. have demonstrated that NF-κB inhibition preserves hypoxia-ischemia-induced TNF-α expression by switching to JNK/

AP-1 activation and thereby provides neuroprotective effects through a TNFR2 dependent mechanism in neonatal HI models (Nijboer *et al.*, 2009). Thus, further studies are necessary to understand whether direct blocking of TNF-α after HI insult can protect against neonatal brain injury in preclinical experimental models.

Anti-neutrophil strategies after HI insult have shown neuroprotective effects in adult stroke models (Black *et al.*, 2010; Chalak *et al.*, 2012; Rocha-Ferreira and Hristova, 2016). In neonatal models, neuroprotection of neutrophil depletion has not been observed unless neutropenia is induced prior to HI insult (Palmer *et al.*, 2004), thus making it less relevant for clinical applications. G-CSF, a cytokine essential for BM cell proliferation and maturation, shows protective effects in adult and neonatal animal models of cerebral ischemia by inhibiting inflammation and neuronal apoptosis, as well as by stimulating neuronal differentiation (Folkerth, 2005; Gopagondanahalli *et al.*, 2016; Rice *et al.*, 1981; Rumajogee *et al.*, 2016). However, it promotes the development of neutrophilic granulocytes and leads to neutrophilia, which may ultimately exacerbate neuronal injury. Doycheva et al. have demonstrated that administration of anti-neutrophil antibody with GCSF after cerebral hypoxia-ischemia reduces the brain injury and significantly improves neurological function by decreasing blood neutrophil accumulation in a neonatal rat model (Doycheva *et al.*, 2014). Thus, anti-neutrophil strategies would be more effective in neonatal rats when co-administration of anti-neutrophil antibody with other anti-inflammatory and/or antiapoptotic candidates, such as G-CSF.

Programmed death-ligand 1 (PD-L1) is mainly induced in non-hematopoietic cells as well as APCs and placental cells within an inflammatory microenvironment. PD-L1 binds to PD-1, an inhibitory receptor expressed on T cells, which controls the activation and proliferation of T cells and assures T cell tolerance. Interestingly, PD-1 reduces stroke severity, but its ligand PD-L1 appears to play a pathogenic role in ischemic brain injury (Bodhankar *et al.*, 2013b). Injection of anti-PD-L1 monoclonal antibody causes a reduction in hemispheric infarct size and neurological deficit scores as well as the reduced infiltration of inflammatory cells with concurrent enhancement of $CD8^+CD122^+$ Treg cells in the lesioned brain in the stroke models (Bodhankar *et al.*, 2015). A human anti-PD-L1 mAb (MEDI4736) is currently being evaluated in 25 ongoing or planned clinical studies in multiple tumor types, with exciting results in >800 treated patients (Ibrahim *et al.*, 2015). This antibody has high affinity and specificity for PD-L1 and sustained exposure up to 1 year of dosing. Moreover, no immunogenicity impacting its bioactivity is reported thus far. Therefore, it is encouraging to test the efficacy of anti-PD-L1 antibody in the preclinical study of neonatal HI brain injury, as well as to make use of humanized anti-PD-L1 antibodies for immediate clinical trials for ischemic brain injury.

9.3. Immunomodulatory therapies

9.3.1. IL-10—IL-10 as a known anti-inflammatory cytokine has multiple immunomodulatory actions against inflammation after cerebral hypoxia-ischemia. In adult stroke models, IL-10 treatment after ischemia protects against ischemic brain injury (Dietrich *et al.*, 1999; Spera *et al.*, 1998). Moreover, several lines of evidence reveal that administration of IL-10 after maternal LPS exposure reduces white matter injury and rescues

sensorimotor development impairment in neonatal rodent models (Mittal *et al.*, 2010; Pang *et al.*, 2005; Wallace *et al.*, 2010). Thus, IL-10-based immunomodulatory approaches may be a promising strategy for the treatment of neonatal HI brain injury. Recombinant human IL-10 has been tested in phase I trials in healthy volunteers and patients with different diseases (Asadullah *et al.*, 2003). Pharmacokinetics of IL-10 at the doses ranging from 0.1 to 100 μg/kg in healthy volunteers indicates that maximum serum concentration and AUC are linearly correlated to dosage. In addition, IL-10 displays a short half-life in vivo and is eliminated through the kidney. Systemic administration of IL-10 in patients shows positive immunomodulatory effects without serious adverse effects at doses up to 25 μg/kg, but higher dose of IL-10 may result in the systemic side effects. Gene delivery to the injury sites provides the possibility of locally increasing the levels of therapeutic products, while minimizing exposure of healthy tissues to avoid deleterious side effects often related to systemic administration. Adenoviral-mediated gene transfer of IL-10 to the lateral ventricle post ischemia provides effective release of transgene IL-10 in the CSF, which markedly reduces the infarct volumes and attenuates the neuronal damages in the adult stroke model (Ooboshi *et al.*, 2005). These findings encourage us to further investigate the therapeutic potential of IL-10 therapy for neonatal HI brain injury.

9.3.2. Immunologic/hypoxic tolerance—Immunologic/hypoxic tolerance provides another example of protective immunomodulation. It is a phenomenon in which a weak and non-injurious insult to the organs prevents the organs from a subsequent injurious insult. Adoptive transfer of splenocytes from myelin basic protein-immunized animals protects naive adult animals against cerebral ischemia (Becker *et al.*, 2003). Similarly, in adult stroke model, ischemic postconditioning after reperfusion decreases infarct volumes and reduces the neurological dysfunction by blocking immune cell infiltration, as well as by attenuating peripheral lymphopenia associated with immunodepression (Joo *et al.*, 2013). Emerging evidence shows that in the neonatal rat brain, mild hypoxic pre- or post-conditioning after hypoxia-ischemia reduces brain tissue loss. Neuroprotection offered from hypoxic preconditioning might be mediated by modulating glial activity, inflammation, and apoptosis (Galle and Jones, 2013). Taken together, these animal studies justify additional investigations in this direction in neonates with HI brain injury.

9.3.3. T cell-based therapy—Over the past few years, the therapeutic potential of Treg cells has been highlighted in the field of ischemic brain injury. Treg cells act as a major immunomodulator after adult stroke by counteracting production of pro-inflammatory cytokines and by regulating infiltration and/or activation of microglia and lymphocytes in the ischemic brain *via* IL-10 signaling (Liesz *et al.*, 2009). Adoptive transfer of Treg cells, even up to 24 hours post ischemia, leads to a reduction in brain infarct size and a prolonged improvement of neurological functions lasting up to 4 weeks (Li *et al.*, 2013a). This therapy simultaneously dampens peripheral inflammation, especially the production of IL-6 and TNF-α in the blood, and corrects immunosuppression, as well as preserves blood-brain barrier integrity by inhibiting peripheral neutrophil-derived MMP9 (Li *et al.*, 2013a; Li *et al.*, 2013b). Boosting endogenous Treg cells can also be beneficial to the ischemic brain damage. Recently, a line of evidence has indicated that *in vivo* amplification of endogenous Treg cells by a CD28 superagonistic monoclonal antibody reduces brain injury and

attenuates the neuroinflammation after adult cerebral ischemia (Na *et al.*, 2015). Thus, these results suggest a novel Treg-based cell therapy for the treatment of hypoxic-ischemic brain injury. Since this strategy targets the delayed phase of the brain injury, leading to a wide therapeutic time window, it would be desirable for clinical applications. However, the role of T lymphocytes in neonatal HI brain injury is not well understood, and the implications of full translational potential of Treg-based cell therapy remain to be defined.

9.3.4. Umbilical cord blood cell/hematopoietic stem cell-based therapy—Stem cells hold great promise of new therapies for a wide range of diseases, including experimental cerebral ischemia. Systemic injection of lineage-negative bone marrow-derived hematopoietic stem cells (Lin^--HSCs) 24 hours after cerebral ischemia reduces infarctions and attenuate neuronal apoptosis (Schwarting *et al.*, 2008). Lin^--HSC therapy negatively regulates gene expression of pro-inflammatory cytokine and chemokine receptor in the spleen, and reduces T cell and macrophage infiltration into ischemic hemispheres (Schwarting *et al.*, 2008), suggesting that HSCs play an immunomodulatory role in mediating neuroprotection in ischemic brain injury.

Cord blood (CB), a rich source of HSCs, has been used to treat neurological diseases in animal models (Bliss *et al.*, 2007; Schira *et al.*, 2012). Autologous transplantation of CB has several advantages, such as easy access, no risk of incompatibility, minimal *ex vivo* manipulation, and storage properties. Growing evidence strongly supports that umbilical cord blood cell (UCBC) transplantation offers the neuroprotective benefits in animal models with neonatal HIE. Intraperitoneal injection of 10×10^6 UCBCs 24 hours after hypoxia-ischemia in PND7 rats improves motor performance (Meier *et al.*, 2006). In line with these results, 2×10^6 UCBCs injected i.p. in PND7 rats even 3 hours after hypoxia-ischemia also shows functional improvements of sensorimotor reflexes 4 days after insult (Pimentel-Coelho *et al.*, 2010). In another study, impaired motor asymmetry and motor coordination in PND7 rats with HIE can be significantly improved when 1.5×10^4 human UCBCs are injected i.v. even one week after hypoxia-ischemia. However, some groups have reported that intravenous administration of human UCBCs 24 or 48 hours after hypoxia-ischemia in rodent models leads to no significant improvement in either brain injuries or motor function (de Paula *et al.*, 2009; Ohshima *et al.*, 2016). Thus, a number of parameters, such as optimal dose, timing, administration route, duration of therapy, and immunosuppression, still need to be further investigated in order to provide better guidance for the full translation to clinical applications. Recently, a single i.p. injection of 2×10^6 rat UCBCs at 3 days after HI insult in PND7 rats attenuates neonatal HI brain injury (Nakanishi *et al.*, 2017), suggesting that an experimental system of allogeneic transplantation is essential for studying the therapeutic potential of UCBCs.

The mechanisms of UCBC beneficial actions after acute hypoxia-ischemia seem to include multiple aspects. Among them, modulation of immune responses emerges as a key feature of UCBC therapy for neonatal HI brain injury. UCBCs can reduce survival of microglia after hypoxia *in vitro* (Jiang *et al.*, 2010), and inhibit activation of microglia in the cerebral cortex after hypoxiaischemia in neonatal rats (Pimentel-Coelho *et al.*, 2010). Administration of human UCBCs modulates the balance of neuroinflammation, attenuates reactive gliosis, and down-regulates the expression of the major astrocytic gap junction protein connexin 43,

which in turn restores BBB function and substantially reduces the inflammatory cell influx into the brain (Wasielewski *et al.*, 2012). It has been also reported that human UCBC treatment preserves the number of $CD8^+$ T cells in the spleen and rescues the decreased spleen size in adult rats after stroke (Vendrame *et al.*, 2006). Moreover, human UCBCs increase the levels of IL-10 and reduce the production of TNF-α in the spleen of adult rats with ischemic brain injury (Vendrame *et al.*, 2006). Taken together, these findings suggest that in addition to exercising the anti-inflammatory effects on the brain, UCBCs act through immunomodulatory mechanism outside the brain.

A clinical phase I trial of autologous CB infusions in 23 term infants with HIE has thus far been accomplished at Duke University Medical Center (NCT00593242). In this study, up to 4 dose infusions of 10–50 million cells per dose are feasible and well tolerated, and no obvious infusion adverse reactions are observed, indicating the feasibility and safety of autologous CB therapy (Cotten *et al.*, 2014). This group is continuing to conduct a randomized phase II trial of autologous CB cells for neonatal HIE to provide further safety, feasibility, and efficacy information (NCT02612155). To date, a number of clinical trials have also been completed or listed for treatment of children with cerebral palsy using UCBCs. A pilot study has revealed that autologous CB infusion is well-tolerated in 15 of 20 children aged 2–10 years with cerebral palsy. Although neurological improvements are noted in 25% of participants, this study suggests the safety and potential of autologous CB therapy in children with cerebral palsy (Lee *et al.*, 2012). Autologous UCBCs is currently being used for treating cerebral palsy in two clinical trials conducted by different groups in USA. A highly encouraging trial at Duke University (NCT01147653) have been completed, but the results are not published yet. The other one at Georgia Regents University (NCT01072370) is recruiting additional patients for a large study.

10. Conclusions

Perinatal hypoxia-ischemia around the time of birth is a primary cause of neonatal deaths and long-term neurological deficits. Over the past two decades, the understanding of cellular processes and molecular mechanisms underlying neonatal HIE has been greatly advanced. The large body of accumulated evidence suggests that inflammation is of a key contributor to the pathogenic cascade of HIE. Inflammation plays an equally crucial role in participating in early or late brain injury after HI insult as well as in repair and recovery of brain tissues. Various immune cells and molecular mediators of inflammation, including cytokines, chemokines, and adhesion molecules, are involved in the brain-immune crosstalk in these processes that follow energy deprivation. If acute inflammation is not resolved properly, it will be shifted to chronic inflammation and adversely affect the developing brain, which increases the vulnerability of the brain to HI injury. It is possible that long-term consequences of hypoxia-ischemia in the perinatal period increase the future risk of a variety of neurological diseases, especially autoimmune CNS injury, in the adult/elderly.

The relationship between the brain and the immune system during the perinatal period is complex and still remains incompletely understood thus far. A large number of inflammatory mechanisms and pathways, including miRNA pathways, after cerebral ischemia have been assessed in the extensive studies in adult stroke or other CNS injuries.

Although many neurodegenerative disorders have similar pathogenic mechanisms, the mechanisms underlying the immune responses to cerebral ischemic injury are likely different between the adults and the neonates. Therefore, a hot frontier in future research of neonatal HIE involves efforts to further understand the detrimental and beneficial components of immune system after neonatal hypoxiaischemia, as well as their causative role and molecular mechanisms in secondary neuronal injury and long-term neurological impairments. In the longer perspective, these insights could lead to development of new immunotherapies that may significantly improve the neurological outcomes of HIE, and extend the therapeutic time window. To date, a number of antiinflammatory/immunomodulatory interventions have been proved to be neuroprotective in preclinical experimental models, and present the translational potential. Some of these strategies have even been pursued in clinical trials for adult stroke patients. More work needs to be done to apply the findings into infants with perinatal brain injury in the future.

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Abbreviations

APCs	antigen-presenting cells
ATP	adenosine triphosphate
BBB	blood brain barrier
BM	bone marrow
CB	cord blood
CNS	central nervous system
CSF	cerebrospinal fluid
DAMPs	danger associated molecular pattern molecules
DCs	dendritic cells
EAE	experimental autoimmune encephalomyelitis
G-CSF	granulocyte colony-stimulating factor
HI	hypoxic-ischemic
HIE	hypoxic-ischemic encephalopathy
ICAM-1	intercellular adhesion molecule 1
HMGB1	high-mobility group protein B1
KO	knock-out

Lin⁻-HSCs	lineage-negative bone marrow-derived hematopoietic stem cells
MHC-II	histocompatibility complex class II
miRNAs	microRNAs
MMPs	matrix metalloproteinases
NO	nitric oxide
PD-L1	programmed death-ligand 1
PND	Postnatal day
PVL	periventricular leukomalacia
rhIL-1ra	recombinant human IL-1 receptor antagonist
ROS	reactive oxygen species
SHIP-1	Src homology 2-containing inositol phosphatase 1
SOCS1	suppressor of cytokine signaling molecule 1
STAT3	signal transducer and activator of transcription 3
TH	therapeutic hypothermia
TNF-R1	TNF-receptor-1
TLR	Toll-like receptor
UCBC	umbilical cord blood cell

References

Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. Nat Neurosci. 2011; 14:1142–1149. [PubMed: 21804537]

Albertsson AM, Bi D, Duan L, Zhang X, Leavenworth JW, Qiao L, Zhu C, Cardell S, Cantor H, Hagberg H, Mallard C, Wang X. The immune response after hypoxia-ischemia in a mouse model of preterm brain injury. J Neuroinflammation. 2014; 11:153. [PubMed: 25187205]

Algra SO, Groeneveld KM, Schadenberg AW, Haas F, Evens FC, Meerding J, Koenderman L, Jansen NJ, Prakken BJ. Cerebral ischemia initiates an immediate innate immune response in neonates during cardiac surgery. J Neuroinflammation. 2013; 10:24. [PubMed: 23390999]

Ali C, Nicole O, Docagne F, Lesne S, MacKenzie ET, Nouvelot A, Buisson A, Vivien D. Ischemia-induced interleukin-6 as a potential endogenous neuroprotective cytokine against NMDA receptor-mediated excitotoxicity in the brain. J Cereb Blood Flow Metab. 2000; 20:956–966. [PubMed: 10894179]

Aly H, Khashaba MT, El-Ayouty M, El-Sayed O, Hasanein BM. IL-1beta, IL-6 and TNF-alpha and outcomes of neonatal hypoxic ischemic encephalopathy. Brain Dev. 2006; 28:178–182. [PubMed: 16181755]

Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. Annu Rev Immunol. 2012; 30:459–489. [PubMed: 22224774]

Anderson CM, Swanson RA. Astrocyte glutamate transport: review of properties, regulation, and physiological functions. Glia. 2000; 32:1–14. [PubMed: 10975906]

Anderson DC, Rothlein R, Marlin SD, Krater SS, Smith CW. Impaired transendothelial migration by neonatal neutrophils: abnormalities of Mac-1 (CD11b/CD18)-dependent adherence reactions. Blood. 1990; 76:2613–2621. [PubMed: 1979926]

Appay V, Rowland-Jones SL. RANTES: a versatile and controversial chemokine. Trends Immunol. 2001; 22:83–87. [PubMed: 11286708]

Arvin KL, Han BH, Du Y, Lin SZ, Paul SM, Holtzman DM. Minocycline markedly protects the neonatal brain against hypoxic-ischemic injury. Ann Neurol. 2002; 52:54–61. [PubMed: 12112047]

Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy--review of a new approach. Pharmacol Rev. 2003; 55:241–269. [PubMed: 12773629]

Bachis A, Colangelo AM, Vicini S, Doe PP, De Bernardi MA, Brooker G, Mocchetti I. Interleukin-10 prevents glutamate-mediated cerebellar granule cell death by blocking caspase-3-like activity. J Neurosci. 2001; 21:3104–3112. [PubMed: 11312295]

Barreto G, White RE, Ouyang Y, Xu L, Giffard RG. Astrocytes: targets for neuroprotection in stroke. Cent Nerv Syst Agents Med Chem. 2011; 11:164–173. [PubMed: 21521168]

Basha S, Surendran N, Pichichero M. Immune responses in neonates. Expert Rev Clin Immunol. 2014; 10:1171–1184. [PubMed: 25088080]

Becker K, Kindrick D, McCarron R, Hallenbeck J, Winn R. Adoptive transfer of myelin basic protein-tolerized splenocytes to naive animals reduces infarct size: a role for lymphocytes in ischemic brain injury? Stroke. 2003; 34:1809–1815. [PubMed: 12791945]

Becker K, Kindrick D, Relton J, Harlan J, Winn R. Antibody to the alpha4 integrin decreases infarct size in transient focal cerebral ischemia in rats. Stroke. 2001; 32:206–211. [PubMed: 11136938]

Benjelloun N, Renolleau S, Represa A, Ben-Ari Y, Charriaut-Marlangue C. Inflammatory responses in the cerebral cortex after ischemia in the P7 neonatal Rat. Stroke. 1999; 30:1916–1923. discussion 1923-1914. [PubMed: 10471445]

Bernardes-Silva M, Anthony DC, Issekutz AC, Perry VH. Recruitment of neutrophils across the blood-brain barrier: the role of E- and P-selectins. J Cereb Blood Flow Metab. 2001; 21:1115–1124. [PubMed: 11524616]

Berner R, Niemeyer CM, Leititis JU, Funke A, Schwab C, Rau U, Richter K, Tawfeek MS, Clad A, Brandis M. Plasma levels and gene expression of granulocyte colony-stimulating factor, tumor necrosis factor-alpha, interleukin (IL)-1beta, IL-6, IL-8, and soluble intercellular adhesion molecule-1 in neonatal early onset sepsis. Pediatr Res. 1998; 44:469–477. [PubMed: 9773833]

Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ. Dicer is essential for mouse development. Nat Genet. 2003; 35:215–217. [PubMed: 14528307]

Bhalala OG, Srikanth M, Kessler JA. The emerging roles of microRNAs in CNS injuries. Nat Rev Neurol. 2013; 9:328–339. [PubMed: 23588363]

Bhalala US, Koehler RC, Kannan S. Neuroinflammation and neuroimmune dysregulation after acute hypoxic-ischemic injury of developing brain. Front Pediatr. 2014; 2:144. [PubMed: 25642419]

Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, Jha P, Campbell H, Walker CF, Cibulskis R, Eisele T, Liu L, Mathers C. Child Health Epidemiology Reference Group of, W.H.O, Unicef. Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet. 2010; 375:1969–1987. [PubMed: 20466419]

Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. Stroke. 2007; 38:817–826. [PubMed: 17261746]

Bodhankar S, Chen Y, Lapato A, Dotson AL, Wang J, Vandenbark AA, Saugstad JA, Offner H. PD-L1 Monoclonal Antibody Treats Ischemic Stroke by Controlling Central Nervous System Inflammation. Stroke. 2015; 46:2926–2934. [PubMed: 26306753]

Bodhankar S, Chen Y, Vandenbark AA, Murphy SJ, Offner H. IL-10-producing B-cells limit CNS inflammation and infarct volume in experimental stroke. Metab Brain Dis. 2013a; 28:375–386. [PubMed: 23640015]

Bodhankar S, Chen Y, Vandenbark AA, Murphy SJ, Offner H. PD-L1 enhances CNS inflammation and infarct volume following experimental stroke in mice in opposition to PD-1. J Neuroinflammation. 2013b; 10:111. [PubMed: 24015822]

Boldin MP, Taganov KD, Rao DS, Yang L, Zhao JL, Kalwani M, Garcia-Flores Y, Luong M, Devrekanli A, Xu J, Sun G, Tay J, Linsley PS, Baltimore D. miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. J Exp Med. 2011; 208:1189–1201. [PubMed: 21555486]

Bona E, Andersson AL, Blomgren K, Gilland E, Puka-Sundvall M, Gustafson K, Hagberg H. Chemokine and inflammatory cell response to hypoxia-ischemia in immature rats. Pediatr Res. 1999; 45:500–509. [PubMed: 10203141]

Bornstein NM, Aronovich B, Korczyn AD, Shavit S, Michaelson DM, Chapman J. Antibodies to brain antigens following stroke. Neurology. 2001; 56:529–530. [PubMed: 11222800]

Boutin H, LeFeuvre RA, Horai R, Asano M, Iwakura Y, Rothwell NJ. Role of IL-1alpha and IL-1beta in ischemic brain damage. J Neurosci. 2001; 21:5528–5534. [PubMed: 11466424]

Brait VH, Jackman KA, Walduck AK, Selemidis S, Diep H, Mast AE, Guida E, Broughton BR, Drummond GR, Sobey CG. Mechanisms contributing to cerebral infarct size after stroke: gender, reperfusion, T lymphocytes, and Nox2-derived superoxide. J Cereb Blood Flow Metab. 2010; 30:1306–1317. [PubMed: 20145655]

Brown NC, Inder TE, Bear MJ, Hunt RW, Anderson PJ, Doyle LW. Neurobehavior at term and white and gray matter abnormalities in very preterm infants. J Pediatr. 2009; 155:32–38. 38 e31. [PubMed: 19394041]

Bruce AJ, Boling W, Kindy MS, Peschon J, Kraemer PJ, Carpenter MK, Holtsberg FW, Mattson MP. Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. Nat Med. 1996; 2:788–794. [PubMed: 8673925]

Bsibsi M, Persoon-Deen C, Verwer RW, Meeuwsen S, Ravid R, Van Noort JM. Toll-like receptor 3 on adult human astrocytes triggers production of neuroprotective mediators. Glia. 2006; 53:688–695. [PubMed: 16482523]

Burnsed JC, Chavez-Valdez R, Hossain MS, Kesavan K, Martin LJ, Zhang J, Northington FJ. Hypoxia-ischemia and therapeutic hypothermia in the neonatal mouse brain--a longitudinal study. PLoS One. 2015; 10:e0118889. [PubMed: 25774892]

Caballero-Garrido E, Pena-Philippides JC, Lordkipanidze T, Bragin D, Yang Y, Erhardt EB, Roitbak T. In Vivo Inhibition of miR-155 Promotes Recovery after Experimental Mouse Stroke. J Neurosci. 2015; 35:12446–12464. [PubMed: 26354913]

Cai Z, Lin S, Pang Y, Rhodes PG. Brain injury induced by intracerebral injection of interleukin-1beta and tumor necrosis factor-alpha in the neonatal rat. Pediatr Res. 2004; 56:377–384. [PubMed: 15201401]

Cardoso AL, Guedes JR, Pereira de Almeida L, Pedroso de Lima MC. miR-155 modulates microglia-mediated immune response by down-regulating SOCS-1 and promoting cytokine and nitric oxide production. Immunology. 2012; 135:73–88. [PubMed: 22043967]

Chalak LF, Rollins N, Morriss MC, Brion LP, Heyne R, Sanchez PJ. Perinatal acidosis and hypoxic-ischemic encephalopathy in preterm infants of 33 to 35 weeks' gestation. J Pediatr. 2012; 160:388–394. [PubMed: 22033298]

Chen CY, Sun WZ, Kang KH, Chou HC, Tsao PN, Hsieh WS, Fu WM. Hypoxic Preconditioning Suppresses Glial Activation and Neuroinflammation in Neonatal Brain Insults. Mediators Inflamm. 2015; 2015:632592. [PubMed: 26273140]

Chen H, Chopp M, Zhang RL, Bodzin G, Chen Q, Rusche JR, Todd RF 3rd. Anti-CD11b monoclonal antibody reduces ischemic cell damage after transient focal cerebral ischemia in rat. Ann Neurol. 1994; 35:458–463. [PubMed: 8154873]

Chen J, Liu Z, Yang Y. In vitro screening of LPS-induced miRNAs in leukocytes derived from cord blood and their possible roles in regulating TLR signals. Pediatr Res. 2014; 75:595–602. [PubMed: 24513687]

Chen Y, Hallenbeck JM, Ruetzler C, Bol D, Thomas K, Berman NE, Vogel SN. Overexpression of monocyte chemoattractant protein 1 in the brain exacerbates ischemic brain injury and is associated with recruitment of inflammatory cells. J Cereb Blood Flow Metab. 2003; 23:748–755. [PubMed: 12796723]

Chiesa C, Pellegrini G, Panero A, De Luca T, Assumma M, Signore F, Pacifico L. Umbilical cord interleukin-6 levels are elevated in term neonates with perinatal asphyxia. Eur J Clin Invest. 2003; 33:352–358. [PubMed: 12662167]

Choi JS, Park J, Suk K, Moon C, Park YK, Han HS. Mild Hypothermia Attenuates Intercellular Adhesion Molecule-1 Induction via Activation of Extracellular Signal-Regulated Kinase-1/2 in a Focal Cerebral Ischemia Model. Stroke research and treatment. 2011; 2011:846716. [PubMed: 21716663]

Chu HX, Kim HA, Lee S, Moore JP, Chan CT, Vinh A, Gelderblom M, Arumugam TV, Broughton BR, Drummond GR, Sobey CG. Immune cell infiltration in malignant middle cerebral artery infarction: comparison with transient cerebral ischemia. J Cereb Blood Flow Metab. 2014; 34:450–459. [PubMed: 24326388]

Connolly ES Jr, Winfree CJ, Prestigiacomo CJ, Kim SC, Choudhri TF, Hoh BL, Naka Y, Solomon RA, Pinsky DJ. Exacerbation of cerebral injury in mice that express the P-selectin gene: identification of P-selectin blockade as a new target for the treatment of stroke. Circ Res. 1997; 81:304–310. [PubMed: 9285631]

Connolly ES Jr, Winfree CJ, Springer TA, Naka Y, Liao H, Yan SD, Stern DM, Solomon RA, Gutierrez-Ramos JC, Pinsky DJ. Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion. Role of neutrophil adhesion in the pathogenesis of stroke. J Clin Invest. 1996; 97:209–216. [PubMed: 8550836]

Cotten CM, Murtha AP, Goldberg RN, Grotegut CA, Smith PB, Goldstein RF, Fisher KA, Gustafson KE, Waters-Pick B, Swamy GK, Rattray B, Tan S, Kurtzberg J. Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. J Pediatr. 2014; 164:973–979 e971. [PubMed: 24388332]

Cowell RM, Xu H, Galasso JM, Silverstein FS. Hypoxic-ischemic injury induces macrophage inflammatory protein-1alpha expression in immature rat brain. Stroke. 2002; 33:795–801. [PubMed: 11872906]

Dallas M, Boycott HE, Atkinson L, Miller A, Boyle JP, Pearson HA, Peers C. Hypoxia suppresses glutamate transport in astrocytes. J Neurosci. 2007; 27:3946–3955. [PubMed: 17428968]

Dambinova SA, Khounteev GA, Izykenova GA, Zavolokov IG, Ilyukhina AY, Skoromets AA. Blood test detecting autoantibodies to N-methyl-D-aspartate neuroreceptors for evaluation of patients with transient ischemic attack and stroke. Clin Chem. 2003; 49:1752–1762. [PubMed: 14500616]

Davidson JO, Wassink G, van den Heuij LG, Bennet L, Gunn AJ. Therapeutic Hypothermia for Neonatal Hypoxic-Ischemic Encephalopathy - Where to from Here? Front Neurol. 2015; 6:198. [PubMed: 26441818]

Davis TH, Cuellar TL, Koch SM, Barker AJ, Harfe BD, McManus MT, Ullian EM. Conditional loss of Dicer disrupts cellular and tissue morphogenesis in the cortex and hippocampus. J Neurosci. 2008; 28:4322–4330. [PubMed: 18434510]

de Paula S, Vitola AS, Greggio S, de Paula D, Mello PB, Lubianca JM, Xavier LL, Fiori HH, Dacosta JC. Hemispheric brain injury and behavioral deficits induced by severe neonatal hypoxia-ischemia in rats are not attenuated by intravenous administration of human umbilical cord blood cells. Pediatr Res. 2009; 65:631–635. [PubMed: 19430381]

De Pietri Tonelli D, Pulvers JN, Haffner C, Murchison EP, Hannon GJ, Huttner WB. miRNAs are essential for survival and differentiation of newborn neurons but not for expansion of neural progenitors during early neurogenesis in the mouse embryonic neocortex. Development. 2008; 135:3911–3921. [PubMed: 18997113]

Del Bigio MR, Becker LE. Microglial aggregation in the dentate gyrus: a marker of mild hypoxic-ischaemic brain insult in human infants. Neuropathol Appl Neurobiol. 1994; 20:144–151. [PubMed: 8072645]

Deng Y, Xie D, Fang M, Zhu G, Chen C, Zeng H, Lu J, Charanjit K. Astrocyte-derived proinflammatory cytokines induce hypomyelination in the periventricular white matter in the hypoxic neonatal brain. PLoS One. 2014; 9:e87420. [PubMed: 24498101]

Derugin N, Wendland M, Muramatsu K, Roberts TP, Gregory G, Ferriero DM, Vexler ZS. Evolution of brain injury after transient middle cerebral artery occlusion in neonatal rats. Stroke. 2000; 31:1752–1761. [PubMed: 10884483]

Dietrich WD, Busto R, Bethea JR. Postischemic hypothermia and IL-10 treatment provide long-lasting neuroprotection of CA1 hippocampus following transient global ischemia in rats. Exp Neurol. 1999; 158:444–450. [PubMed: 10415151]

Dimitrijevic OB, Stamatovic SM, Keep RF, Andjelkovic AV. Absence of the chemokine receptor CCR2 protects against cerebral ischemia/reperfusion injury in mice. Stroke. 2007; 38:1345–1353. [PubMed: 17332467]

Dixon BJ, Reis C, Ho WM, Tang J, Zhang JH. Neuroprotective Strategies after Neonatal Hypoxic Ischemic Encephalopathy. Int J Mol Sci. 2015; 16:22368–22401. [PubMed: 26389893]

Domac FM, Misirli H. The role of neutrophils and interleukin-8 in acute ischemic stroke. Neurosciences (Riyadh). 2008; 13:136–141. [PubMed: 21063307]

Dong Y, Benveniste EN. Immune function of astrocytes. Glia. 2001; 36:180–190. [PubMed: 11596126]

Doni A, Peri G, Chieppa M, Allavena P, Pasqualini F, Vago L, Romani L, Garlanda C, Mantovani A. Production of the soluble pattern recognition receptor PTX3 by myeloid, but not plasmacytoid, dendritic cells. Eur J Immunol. 2003; 33:2886–2893. [PubMed: 14515272]

Donnelly RP, Dickensheets H, Finbloom DS. The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes. J Interferon Cytokine Res. 1999; 19:563–573. [PubMed: 10433356]

Douglas-Escobar M, Weiss MD. Hypoxic-ischemic encephalopathy: a review for the clinician. JAMA Pediatr. 2015; 169:397–403. [PubMed: 25685948]

Doycheva DM, Hadley T, Li L, Applegate RL 2nd, Zhang JH, Tang J. Anti-neutrophil antibody enhances the neuroprotective effects of G-CSF by decreasing number of neutrophils in hypoxic ischemic neonatal rat model. Neurobiol Dis. 2014; 69:192–199. [PubMed: 24874543]

Emsley HC, Smith CJ, Georgiou RF, Vail A, Hopkins SJ, Rothwell NJ, Tyrrell PJ, Acute Stroke I. A randomised phase II study of interleukin-1 receptor antagonist in acute stroke patients. J Neurol Neurosurg Psychiatry. 2005; 76:1366–1372. [PubMed: 16170078]

Fan Y, Shen F, Frenzel T, Zhu W, Ye J, Liu J, Chen Y, Su H, Young WL, Yang GY. Endothelial progenitor cell transplantation improves long-term stroke outcome in mice. Ann Neurol. 2010; 67:488–497. [PubMed: 20437584]

Fang XX, Jiang XL, Han XH, Peng YP, Qiu YH. Neuroprotection of interleukin-6 against NMDA-induced neurotoxicity is mediated by JAK/STAT3, MAPK/ERK, and PI3K/AKT signaling pathways. Cell Mol Neurobiol. 2013; 33:241–251. [PubMed: 23161148]

Faustino JV, Wang X, Johnson CE, Klibanov A, Derugin N, Wendland MF, Vexler ZS. Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke. J Neurosci. 2011; 31:12992–13001. [PubMed: 21900578]

Felger JC, Abe T, Kaunzner UW, Gottfried-Blackmore A, Gal-Toth J, McEwen BS, Iadecola C, Bulloch K. Brain dendritic cells in ischemic stroke: time course, activation state, and origin. Brain Behav Immun. 2010; 24:724–737. [PubMed: 19914372]

Folkerth RD. Neuropathologic substrate of cerebral palsy. J Child Neurol. 2005; 20:940–949. [PubMed: 16417840]

Fumagalli S, Perego C, Ortolano F, De Simoni MG. CX3CR1 deficiency induces an early protective inflammatory environment in ischemic mice. Glia. 2013; 61:827–842. [PubMed: 23440897]

Furze RC, Rankin SM. Neutrophil mobilization and clearance in the bone marrow. Immunology. 2008; 125:281–288. [PubMed: 19128361]

Gadani SP, Walsh JT, Lukens JR, Kipnis J. Dealing with Danger in the CNS: The Response of the Immune System to Injury. Neuron. 2015; 87:47–62. [PubMed: 26139369]

Galasso JM, Miller MJ, Cowell RM, Harrison JK, Warren JS, Silverstein FS. Acute excitotoxic injury induces expression of monocyte chemoattractant protein-1 and its receptor, CCR2, in neonatal rat brain. Exp Neurol. 2000; 165:295–305. [PubMed: 10993690]

Galea I, Bechmann I, Perry VH. What is immune privilege (not)? Trends Immunol. 2007; 28:12–18. [PubMed: 17129764]

Galle AA, Jones NM. The neuroprotective actions of hypoxic preconditioning and postconditioning in a neonatal rat model of hypoxic-ischemic brain injury. Brain Res. 2013; 1498:1–8. [PubMed: 23274537]

Garcia JH, Liu KF, Bree MP. Effects of CD11b/18 monoclonal antibody on rats with permanent middle cerebral artery occlusion. Am J Pathol. 1996; 148:241–248. [PubMed: 8546211]

Gentleman SM, Leclercq PD, Moyes L, Graham DI, Smith C, Griffin WS, Nicoll JA. Long-term intracerebral inflammatory response after traumatic brain injury. Forensic Sci Int. 2004; 146:97–104. [PubMed: 15542269]

Gidday JM, Gasche YG, Copin JC, Shah AR, Perez RS, Shapiro SD, Chan PH, Park TS. Leukocyte-derived matrix metalloproteinase-9 mediates blood-brain barrier breakdown and is proinflammatory after transient focal cerebral ischemia. Am J Physiol Heart Circ Physiol. 2005; 289:H558–568. [PubMed: 15764676]

Girard S, Sebire H, Brochu ME, Briota S, Sarret P, Sebire G. Postnatal administration of IL-1Ra exerts neuroprotective effects following perinatal inflammation and/or hypoxic-ischemic injuries. Brain Behav Immun. 2012; 26:1331–1339. [PubMed: 22982341]

Girard S, Tremblay L, Lepage M, Sebire G. IL-1 receptor antagonist protects against placental and neurodevelopmental defects induced by maternal inflammation. J Immunol. 2010; 184:3997–4005. [PubMed: 20181892]

Gliem M, Mausberg AK, Lee JI, Simiantonakis I, van Rooijen N, Hartung HP, Jander S. Macrophages prevent hemorrhagic infarct transformation in murine stroke models. Ann Neurol. 2012; 71:743–752. [PubMed: 22718543]

Gopagondanahalli KR, Li J, Fahey MC, Hunt RW, Jenkin G, Miller SL, Malhotra A. Preterm Hypoxic-Ischemic Encephalopathy. Front Pediatr. 2016; 4:114. [PubMed: 27812521]

Goren B, Cakir A, Ocalan B, Serter Kocoglu S, Alkan T, Cansev M, Kahveci N. Long-term cognitive effects of uridine treatment in a neonatal rat model of hypoxicischemic encephalopathy. Brain Res. 2017; 1659:81–87. [PubMed: 28126402]

Greter M, Heppner FL, Lemos MP, Odermatt BM, Goebels N, Laufer T, Noelle RJ, Becher B. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. Nat Med. 2005; 11:328–334. [PubMed: 15735653]

Gu Y, He M, Zhou X, Liu J, Hou N, Bin T, Zhang Y, Li T, Chen J. Endogenous IL-6 of mesenchymal stem cell improves behavioral outcome of hypoxic-ischemic brain damage neonatal rats by supressing apoptosis in astrocyte. Sci Rep. 2016; 6:18587. [PubMed: 26766745]

Guadagno J, Swan P, Shaikh R, Cregan SP. Microglia-derived IL-1beta triggers p53-mediated cell cycle arrest and apoptosis in neural precursor cells. Cell Death Dis. 2015; 6:e1779. [PubMed: 26043079]

Hagberg H, Gilland E, Bona E, Hanson LA, Hahin-Zoric M, Blennow M, Holst M, McRae A, Soder O. Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxia-ischemia in neonatal rats. Pediatr Res. 1996; 40:603–609. [PubMed: 8888290]

Hagberg H, Gressens P, Mallard C. Inflammation during fetal and neonatal life: implications for neurologic and neuropsychiatric disease in children and adults. Ann Neurol. 2012; 71:444–457. [PubMed: 22334391]

Hagberg H, Mallard C, Ferriero DM, Vannucci SJ, Levison SW, Vexler ZS, Gressens P. The role of inflammation in perinatal brain injury. Nat Rev Neurol. 2015; 11:192–208. [PubMed: 25686754]

Hallenbeck JM. The many faces of tumor necrosis factor in stroke. Nat Med. 2002; 8:1363–1368. [PubMed: 12457181]

Hanisch UK. Microglia as a source and target of cytokines. Glia. 2002; 40:140–155. [PubMed: 12379902]

Hanna RN, Shaked I, Hubbeling HG, Punt JA, Wu R, Herrley E, Zaugg C, Pei H, Geissmann F, Ley K, Hedrick CC. NR4A1 (Nur77) deletion polarizes macrophages toward an inflammatory phenotype and increases atherosclerosis. Circ Res. 2012; 110:416–427. [PubMed: 22194622]

Hayashi Y, Tomimatsu Y, Suzuki H, Yamada J, Wu Z, Yao H, Kagamiishi Y, Tateishi N, Sawada M, Nakanishi H. The intra-arterial injection of microglia protects hippocampal CA1 neurons against global ischemia-induced functional deficits in rats. Neuroscience. 2006; 142:87–96. [PubMed: 16844302]

Hedtjarn M, Leverin AL, Eriksson K, Blomgren K, Mallard C, Hagberg H. Interleukin-18 involvement in hypoxic-ischemic brain injury. J Neurosci. 2002; 22:5910–5919. [PubMed: 12122053]

Hedtjarn M, Mallard C, Hagberg H. Inflammatory gene profiling in the developing mouse brain after hypoxia-ischemia. J Cereb Blood Flow Metab. 2004; 24:1333–1351. [PubMed: 15625408]

Herrmann O, Tarabin V, Suzuki S, Attigah N, Coserea I, Schneider A, Vogel J, Prinz S, Schwab S, Monyer H, Brombacher F, Schwaninger M. Regulation of body temperature and neuroprotection by endogenous interleukin-6 in cerebral ischemia. J Cereb Blood Flow Metab. 2003; 23:406–415. [PubMed: 12679717]

Higgins RD, Raju T, Edwards AD, Azzopardi DV, Bose CL, Clark RH, Ferriero DM, Guillet R, Gunn AJ, Hagberg H, Hirtz D, Inder TE, Jacobs SE, Jenkins D, Juul S, Laptook AR, Lucey JF, Maze M, Palmer C, Papile L, Pfister RH, Robertson NJ, Rutherford M, Shankaran S, Silverstein FS, Soll RF, Thoresen M, Walsh WF. Eunice Kennedy Shriver National Institute of Child, H., Human Development Hypothermia Workshop, S., Moderators. Hypothermia and other treatment options for neonatal encephalopathy: an executive summary of the Eunice Kennedy Shriver NICHD workshop. J Pediatr. 2011; 159:851–858 e851. [PubMed: 21875719]

Horner PJ, Power AE, Kempermann G, Kuhn HG, Palmer TD, Winkler J, Thal LJ, Gage FH. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. J Neurosci. 2000; 20:2218–2228. [PubMed: 10704497]

Hu X, Li P, Guo Y, Wang H, Leak RK, Chen S, Gao Y, Chen J. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. Stroke. 2012; 43:3063–3070. [PubMed: 22933588]

Huang BY, Castillo M. Hypoxic-ischemic brain injury: imaging findings from birth to adulthood. Radiographics : a review publication of the Radiological Society of North America, Inc. 2008; 28:417–439. quiz 617.

Hudome S, Palmer C, Roberts RL, Mauger D, Housman C, Towfighi J. The role of neutrophils in the production of hypoxic-ischemic brain injury in the neonatal rat. Pediatr Res. 1997; 41:607–616. [PubMed: 9128280]

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. 2002; 22:308–317. [PubMed: 11891436]

Hurn PD, Subramanian S, Parker SM, Afentoulis ME, Kaler LJ, Vandenbark AA, Offner H. T- and B-cell-deficient mice with experimental stroke have reduced lesion size and inflammation. J Cereb Blood Flow Metab. 2007; 27:1798–1805. [PubMed: 17392692]

Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nat Med. 2011; 17:796–808. [PubMed: 21738161]

Ibrahim R, Stewart R, Shalabi A. PD-L1 blockade for cancer treatment: MEDI4736. Semin Oncol. 2015; 42:474–483. [PubMed: 25965366]

Ikegame Y, Yamashita K, Hayashi S, Yoshimura S, Nakashima S, Iwama T. Neutrophil elastase inhibitor prevents ischemic brain damage via reduction of vasogenic edema. Hypertens Res. 2010; 33:703–707. [PubMed: 20485441]

Ito S, Ansari P, Sakatsume M, Dickensheets H, Vazquez N, Donnelly RP, Larner AC, Finbloom DS. Interleukin-10 inhibits expression of both interferon alpha- and interferon gamma- induced genes by suppressing tyrosine phosphorylation of STAT1. Blood. 1999; 93:1456–1463. [PubMed: 10029571]

Jantzie LL, Hu MY, Park HK, Jackson MC, Yu J, Maxwell JR, Jensen FE. Chloride cotransporter NKCC1 inhibitor bumetanide protects against white matter injury in a rodent model of periventricular leukomalacia. Pediatr Res. 2015; 77:554–562. [PubMed: 25585037]

Jenkins DD, Lee T, Chiuzan C, Perkel JK, Rollins LG, Wagner CL, Katikaneni LP, Bass WT, Kaufman DA, Horgan MJ, Laungani S, Givelichian LM, Sankaran K, Yager JY, Martin R. Altered circulating leukocytes and their chemokines in a clinical trial of therapeutic hypothermia for neonatal hypoxic ischemic encephalopathy*. Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies. 2013; 14:786–795.

Jenkins DD, Rollins LG, Perkel JK, Wagner CL, Katikaneni LP, Bass WT, Kaufman DA, Horgan MJ, Languani S, Givelichian L, Sankaran K, Yager JY, Martin RH. Serum cytokines in a clinical trial of hypothermia for neonatal hypoxic-ischemic encephalopathy. J Cereb Blood Flow Metab. 2012; 32:1888–1896. [PubMed: 22805873]

Jeyaseelan K, Lim KY, Armugam A. MicroRNA expression in the blood and brain of rats subjected to transient focal ischemia by middle cerebral artery occlusion. Stroke. 2008; 39:959–966. [PubMed: 18258830]

Jiang L, Saporta S, Chen N, Sanberg CD, Sanberg P, Willing A. The effect of human umbilical cord blood cells on survival and cytokine production by post-ischemic astrocytes in vitro. Stem Cell Rev. 2010; 6:523–531. [PubMed: 20680520]

Jiang N, Moyle M, Soule HR, Rote WE, Chopp M. Neutrophil inhibitory factor is neuroprotective after focal ischemia in rats. Ann Neurol. 1995; 38:935–942. [PubMed: 8526467]

Jickling GC, Liu D, Ander BP, Stamova B, Zhan X, Sharp FR. Targeting neutrophils in ischemic stroke: translational insights from experimental studies. J Cereb Blood Flow Metab. 2015; 35:888–901. [PubMed: 25806703]

Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. J Leukoc Biol. 2010; 87:779–789. [PubMed: 20130219]

Joo SP, Xie W, Xiong X, Xu B, Zhao H. Ischemic postconditioning protects against focal cerebral ischemia by inhibiting brain inflammation while attenuating peripheral lymphopenia in mice. Neuroscience. 2013; 243:149–157. [PubMed: 23590905]

Kaur C, Rathnasamy G, Ling EA. Roles of activated microglia in hypoxia induced neuroinflammation in the developing brain and the retina. J Neuroimmune Pharmacol. 2013; 8:66–78. [PubMed: 22367679]

Kim JS. Cytokines and adhesion molecules in stroke and related diseases. J Neurol Sci. 1996; 137:69–78. [PubMed: 8782158]

Koh HS, Chang CY, Jeon SB, Yoon HJ, Ahn YH, Kim HS, Kim IH, Jeon SH, Johnson RS, Park EJ. The HIF-1/glial TIM-3 axis controls inflammation-associated brain damage under hypoxia. Nat Commun. 2015; 6:6340. [PubMed: 25790768]

Kostulas N, Li HL, Xiao BG, Huang YM, Kostulas V, Link H. Dendritic cells are present in ischemic brain after permanent middle cerebral artery occlusion in the rat. Stroke. 2002; 33:1129–1134. [PubMed: 11935071]

Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 1996; 19:312–318. [PubMed: 8843599]

Lalancette-Hebert M, Gowing G, Simard A, Weng YC, Kriz J. Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. J Neurosci. 2007; 27:2596–2605. [PubMed: 17344397]

Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA. Chronic inflammation and cytokines in the tumor microenvironment. Journal of immunology research. 2014; 2014:149185. [PubMed: 24901008]

Lavine SD, Hofman FM, Zlokovic BV. Circulating antibody against tumor necrosis factor-alpha protects rat brain from reperfusion injury. J Cereb Blood Flow Metab. 1998; 18:52–58. [PubMed: 9428305]

Lederhuber H, Baer K, Altiok I, Sadeghi K, Herkner KR, Kasper DC. MicroRNA-146: tiny player in neonatal innate immunity? Neonatology. 2011; 99:51–56. [PubMed: 20616571]

Lee TS, Chau LY. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. Nat Med. 2002; 8:240–246. [PubMed: 11875494]

Lee YH, Choi KV, Moon JH, Jun HJ, Kang HR, Oh SI, Kim HS, Um JS, Kim MJ, Choi YY, Lee YJ, Kim HJ, Lee JH, Son SM, Choi SJ, Oh W, Yang YS. Safety and feasibility of countering neurological impairment by intravenous administration of autologous cord blood in cerebral palsy. Journal of translational medicine. 2012; 10:58. [PubMed: 22443810]

Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. Nat Rev Immunol. 2007; 7:379–390. [PubMed: 17457344]

Ley K, Bullard DC, Arbones ML, Bosse R, Vestweber D, Tedder TF, Beaudet AL. Sequential contribution of L- and P-selectin to leukocyte rolling in vivo. J Exp Med. 1995; 181:669–675. [PubMed: 7530761]

Li J, Ramenaden ER, Peng J, Koito H, Volpe JJ, Rosenberg PA. Tumor necrosis factor alpha mediates lipopolysaccharide-induced microglial toxicity to developing oligodendrocytes when astrocytes are present. J Neurosci. 2008; 28:5321–5330. [PubMed: 18480288]

Li P, Gan Y, Sun BL, Zhang F, Lu B, Gao Y, Liang W, Thomson AW, Chen J, Hu X. Adoptive regulatory T-cell therapy protects against cerebral ischemia. Ann Neurol. 2013a; 74:458–471. [PubMed: 23674483]

Li P, Mao L, Zhou G, Leak RK, Sun BL, Chen J, Hu X. Adoptive regulatory T-cell therapy preserves systemic immune homeostasis after cerebral ischemia. Stroke. 2013b; 44:3509–3515. [PubMed: 24092548]

Li SJ, Liu W, Wang JL, Zhang Y, Zhao DJ, Wang TJ, Li YY. The role of TNF-alpha, IL-6, IL-10, and GDNF in neuronal apoptosis in neonatal rat with hypoxic-ischemic encephalopathy. Eur Rev Med Pharmacol Sci. 2014; 18:905–909. [PubMed: 24706318]

Lian XY, Stringer JL. Astrocytes contribute to regulation of extracellular calcium and potassium in the rat cerebral cortex during spreading depression. Brain Res. 2004; 1012:177–184. [PubMed: 15158175]

Liesz A, Suri-Payer E, Veltkamp C, Doerr H, Sommer C, Rivest S, Giese T, Veltkamp R. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. Nat Med. 2009; 15:192–199. [PubMed: 19169263]

Lin CY, Chang YC, Wang ST, Lee TY, Lin CF, Huang CC. Altered inflammatory responses in preterm children with cerebral palsy. Ann Neurol. 2010; 68:204–212. [PubMed: 20695013]

Liu J, Feng ZC. Increased umbilical cord plasma interleukin-1 beta levels was correlated with adverse outcomes of neonatal hypoxic-ischemic encephalopathy. J Trop Pediatr. 2010; 56:178–182. [PubMed: 19822562]

Liu XH, Kwon D, Schielke GP, Yang GY, Silverstein FS, Barks JD. Mice deficient in interleukin-1 converting enzyme are resistant to neonatal hypoxic-ischemic brain damage. J Cereb Blood Flow Metab. 1999; 19:1099–1108. [PubMed: 10532634]

Liu Z, Qiu YH, Li B, Ma SH, Peng YP. Neuroprotection of interleukin-6 against NMDA-induced apoptosis and its signal-transduction mechanisms. Neurotox Res. 2011; 19:484–495. [PubMed: 20717763]

Loddick SA, Rothwell NJ. Neuroprotective effects of human recombinant interleukin-1 receptor antagonist in focal cerebral ischaemia in the rat. J Cereb Blood Flow Metab. 1996; 16:932–940. [PubMed: 8784237]

London A, Cohen M, Schwartz M. Microglia and monocyte-derived macrophages: functionally distinct populations that act in concert in CNS plasticity and repair. Front Cell Neurosci. 2013; 7:34. [PubMed: 23596391]

Looney AM, Walsh BH, Moloney G, Grenham S, Fagan A, O'Keeffe GW, Clarke G, Cryan JF, Dinan TG, Boylan GB, Murray DM. Downregulation of Umbilical Cord Blood Levels of miR-374a in Neonatal Hypoxic Ischemic Encephalopathy. J Pediatr. 2015; 167:269–273 e262. [PubMed: 26001314]

Louafi F, Martinez-Nunez RT, Sanchez-Elsner T. MicroRNA-155 targets SMAD2 and modulates the response of macrophages to transforming growth factor-{beta}. J Biol Chem. 2010; 285:41328–41336. [PubMed: 21036908]

Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J. Structural and functional features of central nervous system lymphatic vessels. Nature. 2015; 523:337–341. [PubMed: 26030524]

Ma Q, Dasgupta C, Li Y, Bajwa NM, Xiong F, Harding B, Hartman R, Zhang L. Inhibition of microRNA-210 provides neuroprotection in hypoxic-ischemic brain injury in neonatal rats. Neurobiol Dis. 2016; 89:202–212. [PubMed: 26875527]

Matcovitch-Natan O, Winter DR, Giladi A, Vargas Aguilar S, Spinrad A, Sarrazin S, Ben-Yehuda H, David E, Zelada Gonzalez F, Perrin P, Keren-Shaul H, Gury M, Lara-Astaiso D, Thaiss CA, Cohen M, Bahar Halpern K, Baruch K, Deczkowska A, Lorenzo-Vivas E, Itzkovitz S, Elinav E, Sieweke MH, Schwartz M, Amit I. Microglia development follows a stepwise program to regulate brain homeostasis. Science. 2016; 353:aad8670. [PubMed: 27338705]

Matsui T, Kakeda T. IL-10 production is reduced by hypothermia but augmented by hyperthermia in rat microglia. Journal of neurotrauma. 2008; 25:709–715. [PubMed: 18533891]

Matsuo Y, Kihara T, Ikeda M, Ninomiya M, Onodera H, Kogure K. Role of neutrophils in radical production during ischemia and reperfusion of the rat brain: effect of neutrophil depletion on

extracellular ascorbyl radical formation. J Cereb Blood Flow Metab. 1995; 15:941–947. [PubMed: 7593354]

McMenamin PG, Wealthall RJ, Deverall M, Cooper SJ, Griffin B. Macrophages and dendritic cells in the rat meninges and choroid plexus: three-dimensional localisation by environmental scanning electron microscopy and confocal microscopy. Cell Tissue Res. 2003; 313:259–269. [PubMed: 12920643]

McRae A, Gilland E, Bona E, Hagberg H. Microglia activation after neonatal hypoxic-ischemia. Brain Res Dev Brain Res. 1995; 84:245–252. [PubMed: 7743644]

Meier C, Middelanis J, Wasielewski B, Neuhoff S, Roth-Haerer A, Gantert M, Dinse HR, Dermietzel R, Jensen A. Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. Pediatr Res. 2006; 59:244–249. [PubMed: 16439586]

Mesples B, Plaisant F, Gressens P. Effects of interleukin-10 on neonatal excitotoxic brain lesions in mice. Brain Res Dev Brain Res. 2003; 141:25–32. [PubMed: 12644245]

Michaud JP, Pimentel-Coelho PM, Tremblay Y, Rivest S. The impact of Ly6Clow monocytes after cerebral hypoxia-ischemia in adult mice. J Cereb Blood Flow Metab. 2014; 34:e1–9.

Millar LJ, Shi L, Hoerder-Suabedissen A, Molnar Z. Neonatal Hypoxia Ischaemia: Mechanisms, Models, and Therapeutic Challenges. Front Cell Neurosci. 2017; 11:78. [PubMed: 28533743]

Miller JT, Bartley JH, Wimborne HJ, Walker AL, Hess DC, Hill WD, Carroll JE. The neuroblast and angioblast chemotaxic factor SDF-1 (CXCL12) expression is briefly up regulated by reactive astrocytes in brain following neonatal hypoxic-ischemic injury. BMC Neurosci. 2005; 6:63. [PubMed: 16259636]

Mittal R, Gonzalez-Gomez I, Panigrahy A, Goth K, Bonnet R, Prasadarao NV. IL-10 administration reduces PGE-2 levels and promotes CR3-mediated clearance of Escherichia coli K1 by phagocytes in meningitis. J Exp Med. 2010; 207:1307–1319. [PubMed: 20498022]

Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. Science. 2003; 302:1760–1765. [PubMed: 14615545]

Morkos AA, Hopper AO, Deming DD, Yellon SM, Wycliffe N, Ashwal S, Sowers LC, Peverini RL, Angeles DM. Elevated total peripheral leukocyte count may identify risk for neurological disability in asphyxiated term neonates. J Perinatol. 2007; 27:365–370. [PubMed: 17443199]

Na SY, Mracsko E, Liesz A, Hunig T, Veltkamp R. Amplification of regulatory T cells using a CD28 superagonist reduces brain damage after ischemic stroke in mice. Stroke. 2015; 46:212–220. [PubMed: 25378432]

Nahrendorf M, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo JL, Libby P, Weissleder R, Pittet MJ. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. J Exp Med. 2007; 204:3037–3047. [PubMed: 18025128]

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. 2000; 20:7994–8004. [PubMed: 11050120]

Nakamura T, Yamada S, Yoshioka T. Brain hypothermic therapy dramatically decreases elevated blood concentrations of high mobility group box 1 in neonates with hypoxic-ischemic encephalopathy. Disease markers. 2013; 35:327–330. [PubMed: 24191125]

Nakanishi K, Sato Y, Mizutani Y, Ito M, Hirakawa A, Higashi Y. Rat umbilical cord blood cells attenuate hypoxic-ischemic brain injury in neonatal rats. Sci Rep. 2017; 7:44111. [PubMed: 28281676]

Ng PC, Lam HS. Diagnostic markers for neonatal sepsis. Curr Opin Pediatr. 2006; 18:125–131. [PubMed: 16601490]

Nijboer CH, Heijnen CJ, Groenendaal F, van Bel F, Kavelaars A. Alternate pathways preserve tumor necrosis factor-alpha production after nuclear factor-kappaB inhibition in neonatal cerebral hypoxia-ischemia. Stroke. 2009; 40:3362–3368. [PubMed: 19628795]

Northington FJ. Brief update on animal models of hypoxic-ischemic encephalopathy and neonatal stroke. ILAR journal. 2006; 47:32–38. [PubMed: 16391429]

O'Connell RM, Chaudhuri AA, Rao DS, Baltimore D. Inositol phosphatase SHIP1 is a primary target of miR-155. Proc Natl Acad Sci U S A. 2009; 106:7113–7118. [PubMed: 19359473]

O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. Nat Rev Immunol. 2010; 10:111–122. [PubMed: 20098459]

O'Connell RM, Rao DS, Chaudhuri AA, Boldin MP, Taganov KD, Nicoll J, Paquette RL, Baltimore D. Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med. 2008; 205:585–594. [PubMed: 18299402]

Offner H, Hurn PD. A novel hypothesis: regulatory B lymphocytes shape outcome from experimental stroke. Transl Stroke Res. 2012; 3:324–330. [PubMed: 23175646]

Ohab JJ, Fleming S, Blesch A, Carmichael ST. A neurovascular niche for neurogenesis after stroke. J Neurosci. 2006; 26:13007–13016. [PubMed: 17167090]

Ohshima M, Taguchi A, Sato Y, Ogawa Y, Saito S, Yamahara K, Ihara M, Harada-Shiba M, Ikeda T, Matsuyama T, Tsuji M. Evaluations of Intravenous Administration of CD34+ Human Umbilical Cord Blood Cells in a Mouse Model of Neonatal Hypoxic-Ischemic Encephalopathy. Developmental neuroscience. 2016; 38:331–341. [PubMed: 28103599]

Okazaki K, Nishida A, Kato M, Kozawa K, Uga N, Kimura H. Elevation of cytokine concentrations in asphyxiated neonates. Biol Neonate. 2006; 89:183–189. [PubMed: 16244469]

Ooboshi H, Ibayashi S, Shichita T, Kumai Y, Takada J, Ago T, Arakawa S, Sugimori H, Kamouchi M, Kitazono T, Iida M. Postischemic gene transfer of interleukin-10 protects against both focal and global brain ischemia. Circulation. 2005; 111:913–919. [PubMed: 15710762]

Orrock JE, Panchapakesan K, Vezina G, Chang T, Harris K, Wang Y, Knoblach S, Massaro AN. Association of brain injury and neonatal cytokine response during therapeutic hypothermia in newborns with hypoxic-ischemic encephalopathy. Pediatr Res. 2016; 79:742–747. [PubMed: 26717001]

Palmer C, Roberts RL, Young PI. Timing of neutrophil depletion influences long-term neuroprotection in neonatal rat hypoxic-ischemic brain injury. Pediatr Res. 2004; 55:549–556. [PubMed: 14739365]

Pang Y, Rodts-Palenik S, Cai Z, Bennett WA, Rhodes PG. Suppression of glial activation is involved in the protection of IL-10 on maternal E. coli induced neonatal white matter injury. Brain Res Dev Brain Res. 2005; 157:141–149. [PubMed: 15878785]

Patel AR, Ritzel R, McCullough LD, Liu F. Microglia and ischemic stroke: a double-edged sword. Int J Physiol Pathophysiol Pharmacol. 2013; 5:73–90. [PubMed: 23750306]

Patel SD, Pierce L, Ciardiello A, Hutton A, Paskewitz S, Aronowitz E, Voss HU, Moore H, Vannucci SJ. Therapeutic hypothermia and hypoxia-ischemia in the term-equivalent neonatal rat: characterization of a translational preclinical model. Pediatr Res. 2015; 78:264–271. [PubMed: 25996893]

Pekny M, Pekna M. Astrocyte reactivity and reactive astrogliosis: costs and benefits. Physiol Rev. 2014; 94:1077–1098. [PubMed: 25287860]

Pena-Philippides JC, Caballero-Garrido E, Lordkipanidze T, Roitbak T. In vivo inhibition of miR-155 significantly alters post-stroke inflammatory response. J Neuroinflammation. 2016; 13:287. [PubMed: 27829437]

Perry VH, Nicoll JA, Holmes C. Microglia in neurodegenerative disease. Nat Rev Neurol. 2010; 6:193–201. [PubMed: 20234358]

Pimentel-Coelho PM, Magalhaes ES, Lopes LM, deAzevedo LC, Santiago MF, Mendez-Otero R. Human cord blood transplantation in a neonatal rat model of hypoxic-ischemic brain damage: functional outcome related to neuroprotection in the striatum. Stem Cells Dev. 2010; 19:351–358. [PubMed: 19296724]

Pimentel-Coelho PM, Michaud JP, Rivest S. C-C chemokine receptor type 2 (CCR2) signaling protects neonatal male mice with hypoxic-ischemic hippocampal damage from developing spatial learning deficits. Behav Brain Res. 2015; 286:146–151. [PubMed: 25746456]

Ponomarev ED, Veremeyko T, Barteneva N, Krichevsky AM, Weiner HL. MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-alpha-PU. 1 pathway. Nat Med. 2011; 17:64–70. [PubMed: 21131957]

Qi J, Qiao Y, Wang P, Li S, Zhao W, Gao C. microRNA-210 negatively regulates LPS-induced production of proinflammatory cytokines by targeting NF-kappaB1 in murine macrophages. FEBS Lett. 2012; 586:1201–1207. [PubMed: 22575656]

Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol. 2009; 27:119–145. [PubMed: 19302036]

Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, Uccelli A, Lanzavecchia A, Engelhardt B, Sallusto F. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat Immunol. 2009; 10:514–523. [PubMed: 19305396]

Reinboth BS, Koster C, Abberger H, Prager S, Bendix I, Felderhoff-Muser U, Herz J. Endogenous hypothermic response to hypoxia reduces brain injury: Implications for modeling hypoxic-ischemic encephalopathy and therapeutic hypothermia in neonatal mice. Exp Neurol. 2016; 283:264–275. [PubMed: 27349408]

Ren X, Akiyoshi K, Dziennis S, Vandenbark AA, Herson PS, Hurn PD, Offner H. Regulatory B cells limit CNS inflammation and neurologic deficits in murine experimental stroke. J Neurosci. 2011; 31:8556–8563. [PubMed: 21653859]

Rezaie P, Dean A. Periventricular leukomalacia, inflammation and white matter lesions within the developing nervous system. Neuropathology. 2002; 22:106–132. [PubMed: 12416551]

Ricci G, Volpi L, Pasquali L, Petrozzi L, Siciliano G. Astrocyte-neuron interactions in neurological disorders. J Biol Phys. 2009; 35:317–336. [PubMed: 19669420]

Rice JE 3rd, Vannucci RC, Brierley JB. The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann Neurol. 1981; 9:131–141. [PubMed: 7235629]

Rocha-Ferreira E, Hristova M. Antimicrobial peptides and complement in neonatal hypoxia-ischemia induced brain damage. Front Immunol. 2015; 6:56. [PubMed: 25729383]

Rocha-Ferreira E, Hristova M. Plasticity in the Neonatal Brain following Hypoxic-Ischaemic Injury. Neural plasticity. 2016; 2016:4901014. [PubMed: 27047695]

Rosenzweig JM, Lei J, Burd I. Interleukin-1 receptor blockade in perinatal brain injury. Front Pediatr. 2014; 2:108. [PubMed: 25340046]

Rothhammer V, Quintana FJ. Control of autoimmune CNS inflammation by astrocytes. Seminars in immunopathology. 2015; 37:625–638. [PubMed: 26223505]

Ruffell D, Mourkioti F, Gambardella A, Kirstetter P, Lopez RG, Rosenthal N, Nerlov C. A CREB-C/EBPbeta cascade induces M2 macrophage-specific gene expression and promotes muscle injury repair. Proc Natl Acad Sci U S A. 2009; 106:17475–17480. [PubMed: 19805133]

Rumajogee P, Bregman T, Miller SP, Yager JY, Fehlings MG. Rodent Hypoxia-Ischemia Models for Cerebral Palsy Research: A Systematic Review. Front Neurol. 2016; 7:57. [PubMed: 27199883]

Ruscher K, Kuric E, Liu Y, Walter HL, Issazadeh-Navikas S, Englund E, Wieloch T. Inhibition of CXCL12 signaling attenuates the postischemic immune response and improves functional recovery after stroke. J Cereb Blood Flow Metab. 2013; 33:1225–1234. [PubMed: 23632969]

Saba R, Gushue S, Huzarewich RL, Manguiat K, Medina S, Robertson C, Booth SA. MicroRNA 146a (miR-146a) is over-expressed during prion disease and modulates the innate immune response and the microglial activation state. PLoS One. 2012; 7:e30832. [PubMed: 22363497]

Santambrogio L, Belyanskaya SL, Fischer FR, Cipriani B, Brosnan CF, Ricciardi-Castagnoli P, Stern LJ, Strominger JL, Riese R. Developmental plasticity of CNS microglia. Proc Natl Acad Sci U S A. 2001; 98:6295–6300. [PubMed: 11371643]

Satoorian T, Li B, Tang X, Xiao J, Xing W, Shi W, Lau KH, Baylink DJ, Qin X. MicroRNA223 promotes pathogenic T-cell development and autoimmune inflammation in central nervous system in mice. Immunology. 2016; 148:326–338. [PubMed: 27083389]

Savman K, Blennow M, Gustafson K, Tarkowski E, Hagberg H. Cytokine response in cerebrospinal fluid after birth asphyxia. Pediatr Res. 1998; 43:746–751. [PubMed: 9621983]

Schira J, Gasis M, Estrada V, Hendricks M, Schmitz C, Trapp T, Kruse F, Kogler G, Wernet P, Hartung HP, Muller HW. Significant clinical, neuropathological and behavioural recovery from acute spinal cord trauma by transplantation of a well-defined somatic stem cell from human umbilical cord blood. Brain. 2012; 135:431–446. [PubMed: 21903726]

Schmid-Schonbein GW. Capillary plugging by granulocytes and the no-reflow phenomenon in the microcirculation. Fed Proc. 1987; 46:2397–2401. [PubMed: 3552737]

Schmid-Schonbein GW, Lee J. Leukocytes in capillary flow. Int J Microcirc Clin Exp. 1995; 15:255–264. [PubMed: 8852624]

Schwarting S, Litwak S, Hao W, Bahr M, Weise J, Neumann H. Hematopoietic stem cells reduce postischemic inflammation and ameliorate ischemic brain injury. Stroke. 2008; 39:2867–2875. [PubMed: 18658037]

Shalak LF, Laptook AR, Jafri HS, Ramilo O, Perlman JM. Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. Pediatrics. 2002; 110:673–680. [PubMed: 12359779]

Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, Fanaroff AA, Poole WK, Wright LL, Higgins RD, Finer NN, Carlo WA, Duara S, Oh W, Cotten CM, Stevenson DK, Stoll BJ, Lemons JA, Guillet R, Jobe AH. National Institute of Child, H., Human Development Neonatal Research, N. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. N Engl J Med. 2005; 353:1574–1584. [PubMed: 16221780]

Shankaran S, Pappas A, McDonald SA, Vohr BR, Hintz SR, Yolton K, Gustafson KE, Leach TM, Green C, Bara R, Petrie Huitema CM, Ehrenkranz RA, Tyson JE, Das A, Hammond J, Peralta-Carcelen M, Evans PW, Heyne RJ, Wilson-Costello DE, Vaucher YE, Bauer CR, Dusick AM, Adams-Chapman I, Goldstein RF, Guillet R, Papile LA, Higgins RD. Eunice Kennedy Shriver, N.N.R.N. Childhood outcomes after hypothermia for neonatal encephalopathy. N Engl J Med. 2012; 366:2085–2092. [PubMed: 22646631]

Shechter R, Schwartz M. CNS sterile injury: just another wound healing? Trends Mol Med. 2013; 19:135–143. [PubMed: 23279948]

Sheldon RA, Sedik C, Ferriero DM. Strain-related brain injury in neonatal mice subjected to hypoxia-ischemia. Brain Res. 1998; 810:114–122. [PubMed: 9813271]

Silveira RC, Procianoy RS. Interleukin-6 and tumor necrosis factor-alpha levels in plasma and cerebrospinal fluid of term newborn infants with hypoxic-ischemic encephalopathy. J Pediatr. 2003; 143:625–629. [PubMed: 14615734]

Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. Acta Neuropathol. 2010; 119:7–35. [PubMed: 20012068]

Soreq H, Wolf Y. NeurimmiRs: microRNAs in the neuroimmune interface. Trends Mol Med. 2011; 17:548–555. [PubMed: 21813326]

Spera PA, Ellison JA, Feuerstein GZ, Barone FC. IL-10 reduces rat brain injury following focal stroke. Neurosci Lett. 1998; 251:189–192. [PubMed: 9726375]

Stoll G, Jander S, Schroeter M. Inflammation and glial responses in ischemic brain lesions. Prog Neurobiol. 1998; 56:149–171. [PubMed: 9760699]

Subramanian S, Zhang B, Kosaka Y, Burrows GG, Grafe MR, Vandenbark AA, Hurn PD, Offner H. Recombinant T cell receptor ligand treats experimental stroke. Stroke. 2009; 40:2539–2545. [PubMed: 19443805]

Szaflarski J, Burtrum D, Silverstein FS. Cerebral hypoxia-ischemia stimulates cytokine gene expression in perinatal rats. Stroke. 1995; 26:1093–1100. [PubMed: 7762028]

Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci U S A. 2006; 103:12481–12486. [PubMed: 16885212]

Ten VS, Wu EX, Tang H, Bradley-Moore M, Fedarau MV, Ratner VI, Stark RI, Gingrich JA, Pinsky DJ. Late measures of brain injury after neonatal hypoxia-ischemia in mice. Stroke. 2004; 35:2183–2188. [PubMed: 15272130]

Terao S, Yilmaz G, Stokes KY, Russell J, Ishikawa M, Kawase T, Granger DN. Blood cell-derived RANTES mediates cerebral microvascular dysfunction, inflammation, and tissue injury after focal ischemia-reperfusion. Stroke. 2008; 39:2560–2570. [PubMed: 18635850]

Tuttolomondo A, Di Raimondo D, di Sciacca R, Pinto A, Licata G. Inflammatory cytokines in acute ischemic stroke. Curr Pharm Des. 2008; 14:3574–3589. [PubMed: 19075734]

Van Wagoner NJ, Oh JW, Repovic P, Benveniste EN. Interleukin-6 (IL-6) production by astrocytes: autocrine regulation by IL-6 and the soluble IL-6 receptor. J Neurosci. 1999; 19:5236–5244. [PubMed: 10377335]

Vannucci SJ, Hagberg H. Hypoxia-ischemia in the immature brain. The Journal of experimental biology. 2004; 207:3149–3154. [PubMed: 15299036]

Vendrame M, Gemma C, Pennypacker KR, Bickford PC, Davis Sanberg C, Sanberg PR, Willing AE. Cord blood rescues stroke-induced changes in splenocyte phenotype and function. Exp Neurol. 2006; 199:191–200. [PubMed: 16713598]

Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. Cell Death Differ. 2003; 10:45–65. [PubMed: 12655295]

Wallace KL, Lopez J, Shaffery JP, Wells A, Paul IA, Bennett WA. Interleukin-10/Ceftriaxone prevents E. coli-induced delays in sensorimotor task learning and spatial memory in neonatal and adult Sprague-Dawley rats. Brain Res Bull. 2010; 81:141–148. [PubMed: 19883741]

Wang H, Flach H, Onizawa M, Wei L, McManus MT, Weiss A. Negative regulation of Hif1a expression and TH17 differentiation by the hypoxia-regulated microRNA miR-210. Nat Immunol. 2014a; 15:393–401. [PubMed: 24608041]

Wang J, Lu Q. Expression of T subsets and mIL-2R in peripheral blood of newborns with hypoxic ischemic encephalopathy. World J Pediatr. 2008; 4:140–144. [PubMed: 18661772]

Wang LW, Chang YC, Chen SJ, Tseng CH, Tu YF, Liao NS, Huang CC, Ho CJ. TNFR1-JNK signaling is the shared pathway of neuroinflammation and neurovascular damage after LPS-sensitized hypoxic-ischemic injury in the immature brain. J Neuroinflammation. 2014b; 11:215. [PubMed: 25540015]

Wang LW, Chang YC, Lin CY, Hong JS, Huang CC. Low-dose lipopolysaccharide selectively sensitizes hypoxic ischemia-induced white matter injury in the immature brain. Pediatr Res. 2010; 68:41–47. [PubMed: 20351655]

Wang Y, Cao M, Liu A, Di W, Zhao F, Tian Y, Jia J. Changes of inflammatory cytokines and neurotrophins emphasized their roles in hypoxic-ischemic brain damage. Int J Neurosci. 2013; 123:191–195. [PubMed: 23110519]

Wasielewski B, Jensen A, Roth-Harer A, Dermietzel R, Meier C. Neuroglial activation and Cx43 expression are reduced upon transplantation of human umbilical cord blood cells after perinatal hypoxic-ischemic injury. Brain Res. 2012; 1487:39–53. [PubMed: 22796290]

Welser-Alves JV, Milner R. Microglia are the major source of TNF-alpha and TGF-beta1 in postnatal glial cultures; regulation by cytokines, lipopolysaccharide, and vitronectin. Neurochem Int. 2013; 63:47–53. [PubMed: 23619393]

Winerdal M, Winerdal ME, Kinn J, Urmaliya V, Winqvist O, Aden U. Long lasting local and systemic inflammation after cerebral hypoxic ischemia in newborn mice. PLoS One. 2012; 7:e36422. [PubMed: 22567156]

Winerdal M, Winerdal ME, Wang YQ, Fredholm BB, Winqvist O, Aden U. Adenosine A1 receptors contribute to immune regulation after neonatal hypoxic ischemic brain injury. Purinergic Signal. 2016; 12:89–101. [PubMed: 26608888]

Wood T, Osredkar D, Puchades M, Maes E, Falck M, Flatebo T, Walloe L, Sabir H, Thoresen M. Treatment temperature and insult severity influence the neuroprotective effects of therapeutic hypothermia. Sci Rep. 2016; 6:23430. [PubMed: 26997257]

Xie D, Shen F, He S, Chen M, Han Q, Fang M, Zeng H, Chen C, Deng Y. IL-1beta induces hypomyelination in the periventricular white matter through inhibition of oligodendrocyte progenitor cell maturation via FYN/MEK/ERK signaling pathway in septic neonatal rats. Glia. 2016; 64:583–602. [PubMed: 26678483]

Xiong M, Yang Y, Chen GQ, Zhou WH. Post-ischemic hypothermia for 24h in P7 rats rescues hippocampal neuron: association with decreased astrocyte activation and inflammatory cytokine expression. Brain Res Bull. 2009; 79:351–357. [PubMed: 19406216]

Yamashita T, Sawamoto K, Suzuki S, Suzuki N, Adachi K, Kawase T, Mihara M, Ohsugi Y, Abe K, Okano H. Blockade of interleukin-6 signaling aggravates ischemic cerebral damage in mice: possible involvement of Stat3 activation in the protection of neurons. J Neurochem. 2005; 94:459–468. [PubMed: 15998296]

Zhou T, Huang YX, Song JW, Ma QM. Thymosin beta4 inhibits microglia activation through microRNA 146a in neonatal rats following hypoxia injury. Neuroreport. 2015; 26:1032–1038. [PubMed: 26457369]

Zhu F, Liu JL, Li JP, Xiao F, Zhang ZX, Zhang L. MicroRNA-124 (miR-124) regulates Ku70 expression and is correlated with neuronal death induced by ischemia/reperfusion. J Mol Neurosci. 2014; 52:148–155. [PubMed: 24166354]

Zhu L, Huang L, Wen Q, Wang T, Qiao L, Jiang L. Recombinant human erythropoietin offers neuroprotection through inducing endogenous erythropoietin receptor and neuroglobin in a neonatal rat model of periventricular white matter damage. Neurosci Lett. 2017; 650:12–17. [PubMed: 28359933]

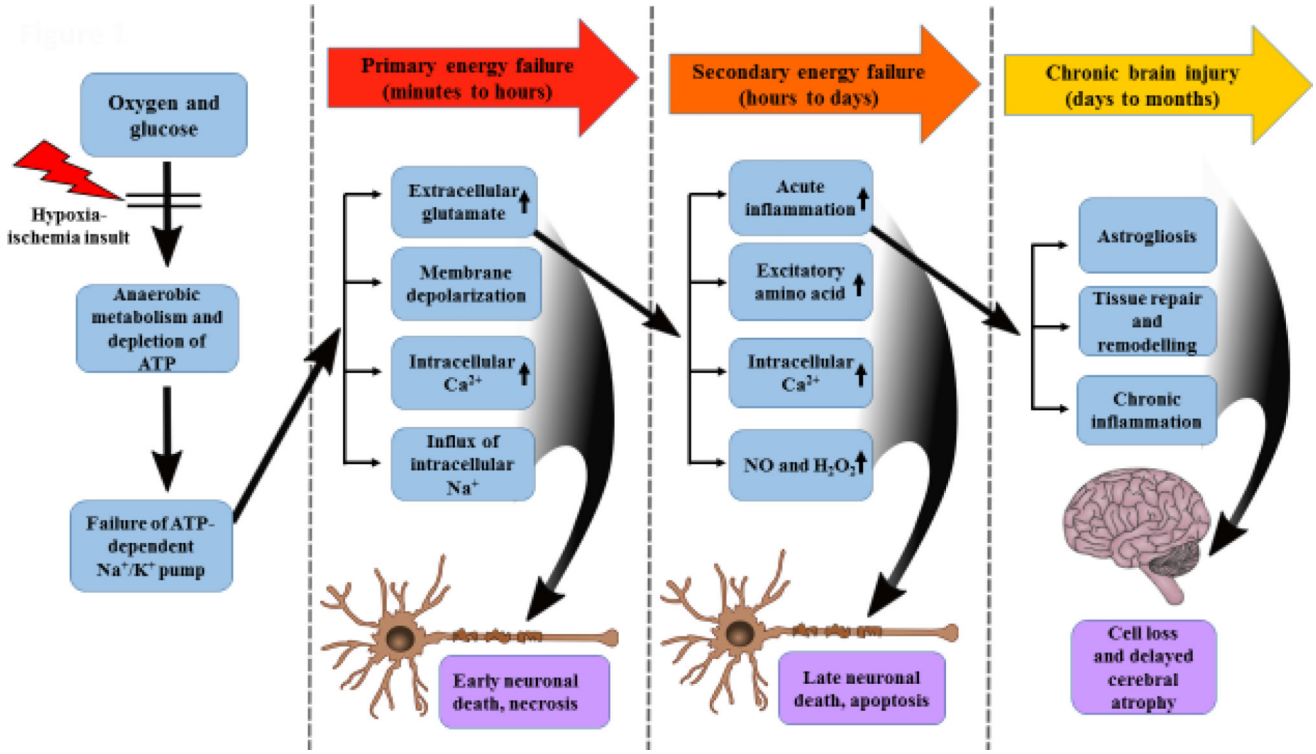

Figure 1. The pathogenesis of hypoxic-ischemic encephalopathy

Neonatal hypoxia-ischemia results in deprivation of energy substrates, oxygen and glucose, to the brain tissue, and transforms the cells to anaerobic metabolism. The reduction in ATP contributes to the failure of the energy-dependent cell membrane ion channels, which causes an acute intracellular influx of calcium and sodium and cell membrane depolarization, as well as accumulation of extracellular glutamate. This cascade consequently leads to cell swelling and necrotic cell death. If the initial insult is prolonged or severe, it may result in a secondary delayed energy failure within hours that most cell death occurs due to the apoptosis. During this phase, excitotoxicity, inflammation, and continual uptake of intracellular calcium as well as release of reactive oxygen species are observed. After the secondary phase of injury, another chronic phase of injury occurs within days and continues for months, maybe up to years. This phase includes astrogliosis, chronic inflammation, and tissue repair and remodeling, which further contribute to loss of brain cells and cerebral atrophy.

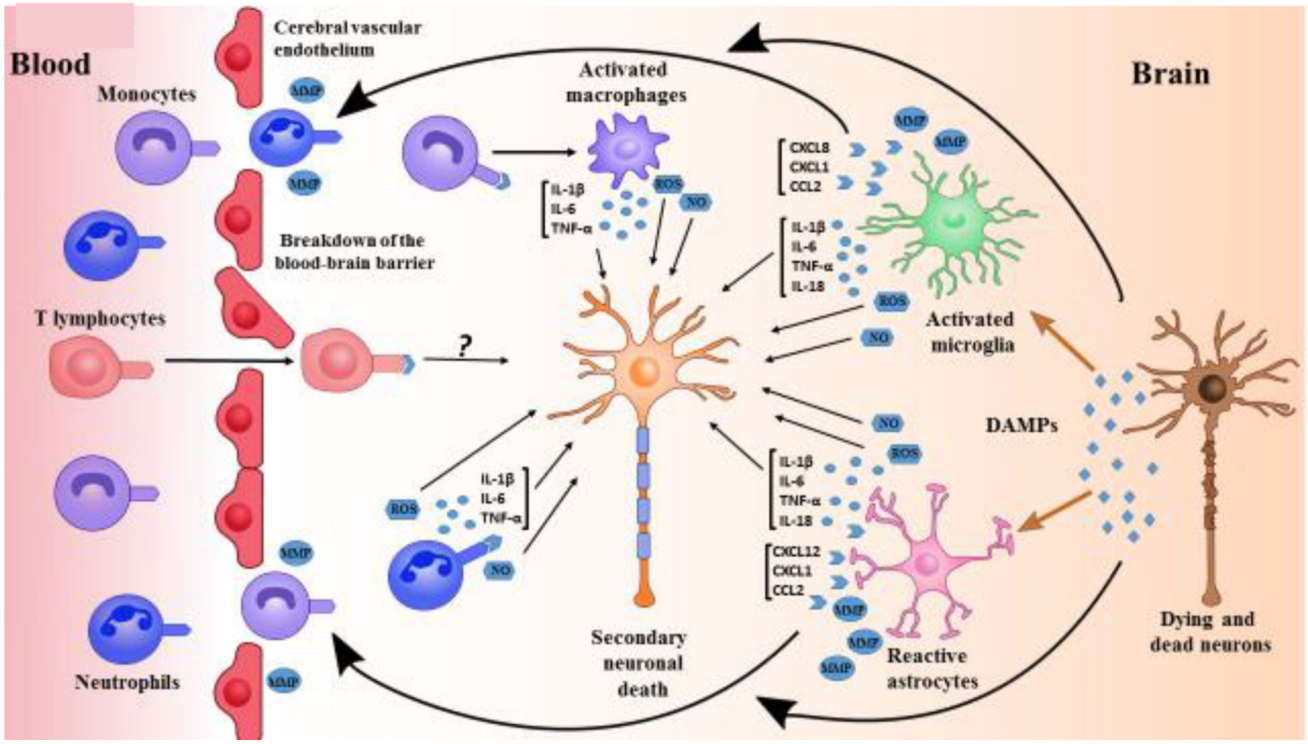

Figure 2. Early inflammatory response to neonatal hypoxia-ischemia in the developing brain

Neonatal hypoxia-ischemia leads to necrotic neuronal death that releases danger associated molecular pattern molecules (DAMPs). Resident immune effector cells (microglia and astrocytes) first sense these danger signals through pattern recognition receptors, such as TLRs and cytokine receptors, which results in inflammatory activation of microglia and astrocytes. Activated glia cells have a direct neurotoxic role in promoting the neuronal apoptosis by releasing a large amount of pro-inflammatory cytokines, nitric oxide (NO) and reactive oxygen species (ROS). In addition, synthesis and release of chemokines and matrix metalloproteinases (MMPs) by activated glia, together with DAMPs, increase blood-brain barrier (BBB) permeability, which contributes to the recruitment of peripheral inflammatory cells to injured brain, leading to further exacerbation of neuroinflammation and subsequent neuronal death.

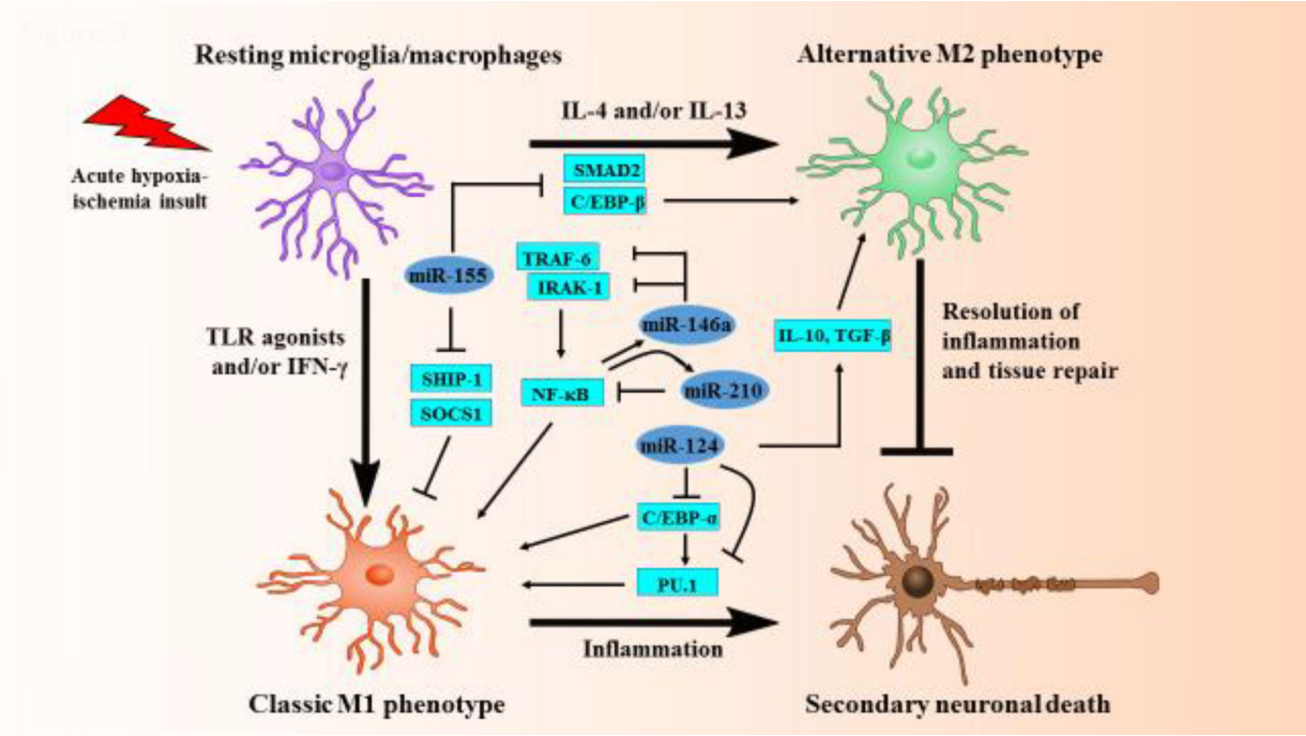

Figure 3. Proposed miRNA pathways in modulating activation phenotypes of microglia/macrophages in the developing brain with HI injury

Following rapid activation of TLRs, NF-κB induces miR-210 expression in microglia/macrophages; however, miR-210 serves as a negative feedback regulator for the M1 activation by targeting NF-κB. Similarly, TLR-driven NF-kB activation upregulates the expression of miR-146a, which in turn down-regulates NF-kB activity by targeting two NF-kB upstream signaling transducers, TRAF6 and IRAK1. CNS enriched miRNA, miR-124, is down-regulated upon the LPS stimulation in microglia/macrophages. MiR-124 promotes the quiescent state of microglia/macrophages by direct repression of C/EBP-α and its downstream PU.1, two myeloid cell differentiation-associated transcription factors. Furthermore, miR-124 also contributes to the M2 phenotype of microglia/macrophages by increasing the production of IL-10 and TGF-β. In contrast, miR-155 expression is induced by TLR stimulation. This miRNA targets two negative regulators of M1 activation, SHIP-1 and SOCS1, thereby enhancing the M1 inflammatory responses. In addition, miR-155 is able to inhibit the M2 phenotype by targeting SMAD2, a protein involved in the TGF-β pathway, and C/EBP-β, a transcription factor important for the expression of M2 markers. Thus, dysregulation of these miRNA pathways may skew microglia/macrophage activation to classic M1 phenotype that induces secondary neuronal death. Accordingly, decreasing the levels of miR-155 or increasing the levels of miR-146a or miR-124 might switch the activation to alternative M2 phenotype, thereby protecting the neurons against apoptosis and promoting the resolution of inflammation and tissue repair in the developing brain with HI injury.

Table 1

Cytokines act as the mediators of inflammation in neonatal hypoxic-ischemic brain injury

Cytokine	Human	Animal	Animal
TNF-a	Upregulated in blood and CSF at 24h, newborns (Aly *et al.*, 2006; Liu and Feng, 2010; Silveira and Procianoy, 2003)	Upregulated in serum and lesioned brain as early as 4h after HI, P7 rats (Li *et al.*, 2014; Szaflarski *et al.*, 1995)	1) Increases oligodendrocyte progenitor death and neuronal apoptosis by TNF-receptor-1 binding (Hallenbeck, 2002; Li *et al.*, 2008; Wang *et al.*, 2014b); 2) enhances peripheral leukocyte infiltration by upregulating the expression of MHC-II and ICAM-1 in astrocytes (Hallenbeck, 2002); 3) promotes neuronal cell survival by TNF-receptor-2 binding (Nijboer *et al.*, 2009).
IL-1β	Upregulated in serum and CSF at 24h, newborns (Aly *et al.*, 2006; Liu and Feng, 2010)	Upregulated in lesioned brain at 3h after HI, P7 rats (Hagberg *et al.*, 1996)	1) Induces apoptosis of oligodendrocytes and neural precursor cells by IL-1 receptor binding and p53 activation (Deng *et al.*, 2014; Guadagno *et al.*, 2015; Xie *et al.*, 2016) 2) enhances peripheral leukocyte infiltration by upregulating the expression of chemokines (Rothhammer and Quintana, 2015).
IL-6	Upregulated in serum and CSF within 24h, newborns (Aly *et al.*, 2006; Chiesa *et al.*, 2003; Jenkins *et al.*, 2012; Savman *et al.*, 1998)	Upregulated in lesioned brain at 3h after HI, P7 rats (Hagberg *et al.*, 1996; Li *et al.*, 2014)	1) Induces nonspecific cell death and reduces the neuronal differentiation and accumulation (Monje *et al.*, 2003); 2) promote HI-induced neuronal apoptosis (Li *et al.*, 2014); 3) protects neurons against apoptosis by reducing N-methyl-D-aspartate toxicity (Ali *et al.*, 2000; Fang *et al.*, 2013; Liu *et al.*, 2011); 4) enhances astrocyte survival through IL-6/STAT3 signaling (Gu *et al.*, 2016).
IL-10	Upregulated in serum within 24h, newborns (Okazaki *et al.*, 2006)	Upregulated in serum and lesioned brain from 6h after HI and peaked at 48h, P7 rats (Li *et al.*, 2014)	1) Inhibits pro-inflammatory cytokines (Lee and Chau, 2002; Landskron *et al.*, 2014); 2) downregulates pro-inflammatory cytokine receptors (Landskron *et al.*, 2014); 3) inactivates NF-κB (Landskron *et al.*, 2014); 4) protects neurons against apoptosis (Bachis *et al.*, 2001; Lee and Chau, 2002).