Review



# Microglial Polarization in Neonatal Hypoxic-Ischemic Encephalopathy: Roles and Therapeutic Potential

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# Microglial Polarization in Neonatal Hypoxic-Ischemic Encephalopathy: Roles and Therapeutic Potential

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**Abstract: Background:** Neonatal hypoxic-ischemic encephalopathy (HIE) is a major cause of neonatal mortality and long-term neurological impairments. Apart from therapeutic hypothermia, there are currently no effective pharmacological treatments. Microglia, as the resident immune cells of the nervous system, play a critical role in the pathogenesis and subsequent neural repair processes of HIE. However, the exact mechanisms underlying these roles remain unclear, and potential therapeutic targets for modulating microglial polarization still need to be explored. **Methods**: This review outlines the physiological functions of microglia in the developing brain and the role of microglial polarization in neonatal HIE. It discusses several strategies for targeting the modulation of microglial polarization and their underlying molecular mechanisms. **Results**: Microglial activation is one of the core pathophysiological mechanisms of neonatal HIE. During the acute phase of hypoxic-ischemic injury, microglia are typically activated to an M1 pro-inflammatory phenotype, mediating inflammatory responses and exacerbating neuronal damage. Over time, some microglia transition into the M2 phenotype, where they play a role in tissue repair and neuroprotection. The balance between pro-inflammatory and anti-inflammatory microglial phenotypes plays a crucial role in determining the prognosis of neonatal HIE. Several interventions targeting the modulation of microglial polarization, including IL-4, miRNA, and cyclic GMP-AMP synthase (cGAS), have shown promising results in animal models. However, the therapeutic mechanisms and safety of these approaches still require further investigation. **Conclusions**: Understanding how to effectively regulate microglial phenotype polarization to maximize neuroprotective effects and promote brain repair holds significant potential for the treatment of neonatal HIE. This could help open new therapeutic avenues and improve the prognosis of affected infants.

**Keywords:** microglia; polarization; inflammation; hypoxic-ischemic encephalopathy

## 1. Introduction

Neonatal hypoxic-ischemic encephalopathy (HIE) is a neonatal brain dysfunction syndrome caused by perinatal asphyxia, characterized by reduced or interrupted blood and/or oxygen supply to the brain before or during birth. This disease is also the leading cause of neonatal neurological developmental disorders and mortality[1–3]. HIE occurs in both preterm and full-term infants, with an incidence rate of approximately 1-4 per 1000 live births in developed countries, and a higher incidence rate of around 26 per 1000 in developing countries[4].Epidemiological data show that the incidence of neonatal HIE has been increasing in both developed and developing countries in recent years[5]. Although advancements in neonatal intensive care and perinatal nursing techniques over the past few decades have significantly improved the survival rates of neonates with HIE, long-term prognosis remains a major concern affecting the future quality of life of these infants. Depending on factors such as the severity of hypoxic-ischemic (HI) injury, brain maturity, and maternal/fetal health conditions, approximately 25% of infants with severe HIE die within the first two years of life.

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Furthermore, 35% of HIE survivors suffer from permanent neurological sequelae, including cerebral palsy, epilepsy, and developmental delays in cognition and behavior, significantly impairing quality of life and imposing a heavy burden on families and society[6,7].

Currently, therapeutic hypothermia (TH) is the only approved intervention for term neonates diagnosed with moderate to severe HIE. However, its efficacy and safety vary significantly by region. While infants in developed countries benefit from TH, many in low- and middle-income countries do not experience similar benefits and may even face adverse effects[8,9]. Additionally, due to the high cost of equipment and management, the narrow therapeutic window, and significant variability in outcomes, TH has not been widely adopted or implemented in clinical practice[10–12]. Therefore, understanding the cellular and molecular mechanisms at different stages of neonatal HIE pathophysiology is crucial for developing new interventions and treatment strategies.

Microglia are resident immune cells of the brain, playing a crucial role in the pathogenesis and potential recovery processes in HIE[13,14]. Increased blood-brain barrier (BBB) permeability and the release of microglia-regulated inflammatory mediators are key pathological mechanisms of HIE[15,16]. Moreover, microglia-mediated neuroinflammation is a critical factor in determining the prognosis of HIE. There is dense microglial infiltration in the dentate gyrus of the hippocampus in patients with HIE, and microglial activation and aggregation are pathological hallmarks of HIE[17]. When pathological changes occur in the brain due to hypoxia, ischemia, or inflammation, microglia become activated, manifested as an increase in quantity and functional activity. Following activation, microglia can exhibit different polarized phenotypes depending on the surrounding tissue microenvironment, performing various functions. The two most extensively studied phenotypes are pro-inflammatory microglia (M1) and anti-inflammatory microglia (M2)[18]. In the acute phase after HI injury, microglia are typically activated to the pro-inflammatory M1 phenotype. These M1 microglia release cytokines and reactive oxygen species (ROS), exacerbating neuronal damage and leading to secondary injury.Excessive M1 microglial polarization triggers an inflammatory cascade, which is a major contributor to the worsening of brain injury.However, over time, some microglia transition to the M2 phenotype, promoting tissue repair and neuroprotection[19]. Activated microglia can also functionally switch between the M1 and M2 phenotypes under specific environmental or inductive conditions[20,21].The microglial response typically lasts from several hours to days, so targeting microglia has a longer therapeutic window than other therapeutic measures. Understanding how to effectively regulate microglial phenotypic polarization to maximize neuroprotection and promote brain repair is of great importance in the treatment of neonatal HIE.This review will summarize the role of microglial polarization in neonatal HIE, aiming to open new therapeutic avenues for neonatal HIE and provide strategies for the development of related drugs.

## 2. Stages and Pathophysiology of Neonatal HIE

The pathogenesis of neonatal HIE is complex and progressive, involving two phases of energy failure, which can be divided into four stages[10,22,23]: the acute phase, latent phase, secondary phase, and tertiary phase. The acute phase is the primary energy failure period (0-6 hours after injury), during which reduced cerebral blood flow decreases the supply of nutrients (glucose) and oxygen, shifting cellular metabolism from aerobic to anaerobic and resulting in primary energy failure with a marked reduction in ATP production. The reduction in ATP disrupts the function of the sodium-potassium pump, impairing transmembrane transport and causing intracellular sodium ($Na^+$), calcium ($Ca^{2+}$), and water retention in neurons. This leads to membrane depolarization and excessive release of excitatory neurotransmitters like glutamate, causing excitotoxicity in neurons and glial cells. Anaerobic glycolysis during this phase results in lactate accumulation, leading to cellular dysfunction or cell apoptosis/necrosis[24–26]. Depending on the severity and duration of hypoxia-ischemia, a latent phase may occur. If effective resuscitation is provided during this phase, cerebral oxygenation and blood flow perfusion can be restored, mitigating the severity of energy failure. Neuronal metabolism may partially recover, reducing the extent of brain injury. Therefore, the latent phase is considered the optimal time to initiate neuroprotective therapies (such as TH or stem cell injections)

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to limit ongoing toxic processes. If treatment is ineffective or the acute phase is prolonged and severe, the latent phase is shortened, and the accumulation of lactate and pyruvate ions increases the production of ROS, causing damage to the BBB and changes in its permeability, leading to edema and leakage[27]. This is followed by a secondary phase (6-15 hours after injury), also called the secondary energy failure phase, which lasts approximately 24-72 hours. During this period, brain damage further deteriorates and cell death increases, characterized by excitotoxicity, mitochondrial failure, reperfusion-induced oxidative stress, increased microglial activation, acute inflammatory response, increased cytotoxicity and apoptosis, and increased seizures[28]. As the disease progresses, it may enter the tertiary phase (72 hours to several weeks post-injury), characterized by reduced neuronal plasticity, a decrease in neuronal numbers, brain tissue damage, and long-term neurological dysfunction. In severe cases, this can lead to disability or death in neonates with HIE[1,23,29](Figure 1).

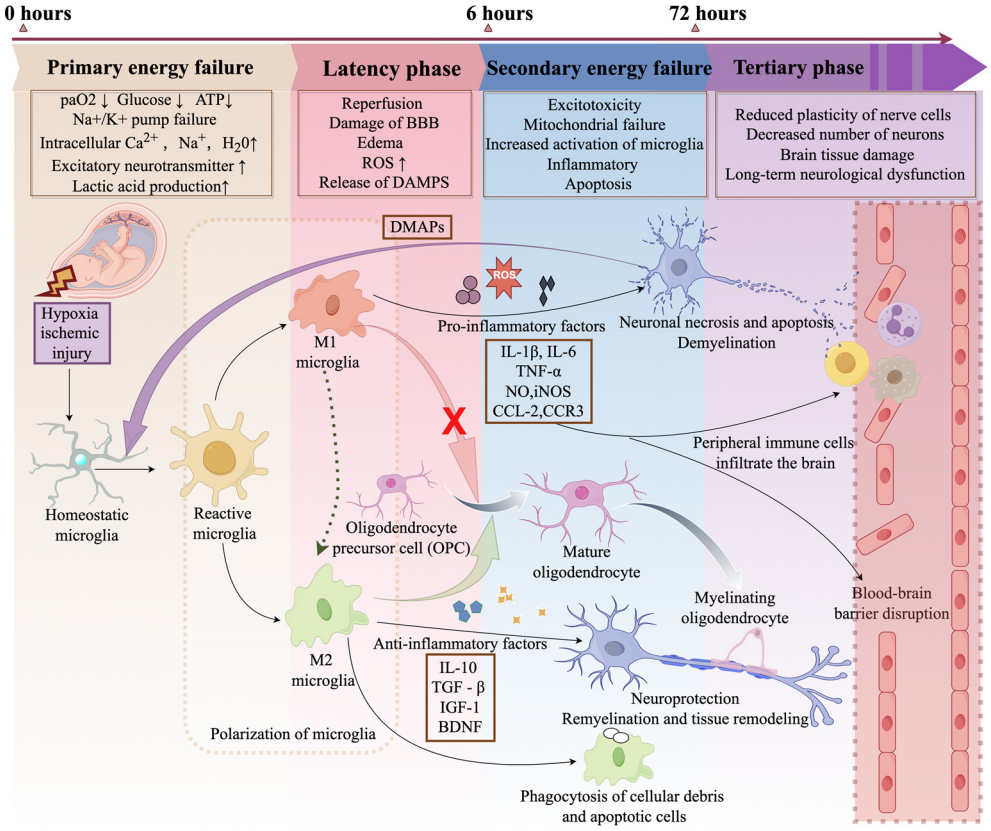


**Figure 1.** Stages of neonatal HIE and the role of microglia in the pathophysiology of HIE.

This schematic illustrates the staging of neonatal hypoxic-ischemic encephalopathy (HIE) and the typical injury features at each stage. It shows that, following hypoxic-ischemic signaling, microglia transition from a homeostatic to an activated state, then polarize into M1/M2 microglia, each of which performs distinct functional processes. M1 microglia mediate pro-inflammatory responses, secreting a large amount of pro-inflammatory factors that induce neuronal necrosis, apoptosis, and demyelination. They also disrupt the immature blood-brain barrier, allowing peripheral immune cells (neutrophils, monocytes, macrophages) to infiltrate the brain, thereby exacerbating neuroinflammation and neuronal injury. M2 microglia mediate anti-inflammatory responses, secreting anti-inflammatory, immunoregulatory, and neurotrophic molecules, thereby exerting neuroprotective effects. The potent phagocytic activity of M2 microglia enables them to engulf cellular debris and apoptotic cells, thereby inhibiting the spread of the inflammatory response. In terms of cellular crosstalk, M1 microglia inhibit the differentiation of oligodendrocyte precursor cells (OPCs) into myelinating oligodendrocytes (OLs), whereas M2 microglia promote the recruitment and differentiation of OPCs, thus facilitating myelin regeneration and tissue remodeling.

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Meanwhile, injured neurons trigger the release of damage-associated molecular patterns (DAMPs) within the brain, activating and proliferating microglia, which in turn initiate an inflammatory cascade.

*HIE, hypoxic-ischemic encephalopathy; BBB, blood brain barrier; DAMPS, damage-associated molecular patterns; ROS, Reactive Oxygen Species; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; NO, nitric oxide; iNOS, inducible NO synthase; CCL-2, C-C chemokine ligand 2; CCR3, C-C chemokine receptor 3; IL-10, interleukin-10; TGF-β, transforming growth factor-β; IGF-1, insulin-like growth factor 1; BDNF, brain-derived neurotrophic factors;*

## 3. Microglia and Their Role in the Developing Brain

Microglia are resident immune cells widely distributed throughout the central nervous system (CNS)[30]. They originate from the extraembryonic mesoderm of the yolk sac and colonize the neuroectoderm during embryonic development[31,32], a process that begins in the fourth week of human gestation[33]. After reaching the brain, microglia disperse throughout, but their distribution is regionally specific and heterogeneous. Particularly, microglia accumulate around the white matter in the early postnatal brain, forming a "microglial fountain[34]." These microglia exhibit an amoeboid morphology, which is distinct from the branched form found in the adult brain[35]. Microglia are the only cells in the brain with mononuclear phagocyte characteristics. Although they are relatively few in number, constituting about 5-20% of glial cells, they play essential roles in brain development and homeostatic regulation[36]. Under physiological conditions, the functions of microglia extend far beyond their role as immune cells. These functions include regulating embryonic brain angiogenesis and the development of the blood-brain barrier, regulating oligodendrocyte generation and neurogenesis, secreting neurotrophic factors, immune surveillance, and modulating synapse development and plasticity. The interaction between microglia and neurons is also essential for the construction of neural circuits during brain development. During development, neurons migrate to specific regions, extend axons to potential target cells, and establish circuits by forming synapses. Initially, neurons generate an excess of branches and synapses, which microglia can help remove inappropriate connections through axon pruning and/or synaptic elimination, playing a crucial role in shaping neural circuits. Microglia also contribute to the functional maturation and refinement of neural circuits by synaptic pruning and secreting brain-derived neurotrophic factors (BDNF), among others[37]. Microglial dysfunction can lead to neurodevelopmental disorders (NDDs) during childhood, such as autism spectrum disorder (ASD)[38]. Under pathological conditions, microglia can release inflammatory cytokines and phagocytose apoptotic cells and debris in the brain[39].

## 4. Polarization, Phenotype and Related Functions of Microglia

Under physiological conditions, microglia exist in a "homeostatic" state with small, spindle-shaped or branched cell bodies[40,41]. They monitor the brain tissue and the neurovascular unit (NVU) microenvironment, interacting with neurons and vascular components to maintain the dynamic balance of the CNS. Notably, microglia in the "homeostatic" state are not dormant; rather, they actively respond to changes in the CNS environment (adjacent tissue cells and the various factors they secrete) by continuously shrinking and extending their branches[42]. Under specific environmental stimuli, microglia transition from a "homeostatic" state to an activated state, presenting stable and functional phenotypes, a process known as microglial polarization. Microglia can become activated into two distinct phenotypic states: the "classically activated" M1 phenotype and the "alternatively activated" M2 phenotype[18]. M1 microglia exhibit cytotoxicity by promoting neuroinflammation through the release of pro-inflammatory cytokines and cytotoxic molecules, including interleukin-1β(IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-15 (IL-15), interleukin-18 (IL-18), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), prostaglandins, ROS, and nitric oxide (NO). While these inflammatory factors enhance the CNS's ability to defend against harmful stimuli, they also result in expanded neuroinflammation, increased neuronal death, and exacerbated neurotoxicity and tissue damage. In contrast, M2 microglia primarily exhibit protective functions by secreting anti-inflammatory, immunoregulatory, and neurotrophic molecules, aiding in

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inflammation control, BBB repair, neurovascular regeneration, and restoration of homeostasis[43–45]. M2 microglia can be further divided into several subtypes, each with distinct functions in the CNS[46]. M2a participates in repair (primarily through phagocytosis of cellular debris) and regeneration, M2b is involved in immunoregulation, and M2c contributes to neuroprotection and the production of anti-inflammatory cytokines, such as interleukin-10 (IL-10), transforming growth factor-β(TGF-β), and insulin-like growth factor 1(IGF-1), BDNF[47].

Microglia are highly plastic, and activated microglia can functionally switch between the M1 and M2 phenotypes under specific environmental or inductive conditions. Theoretically, reducing M1 polarization while increasing M2 polarization can enhance neuroprotection during CNS injury[48]. Li et al. found that using L-3-n-butylphthalide (NBP) as a drug to induce M1 microglia to shift toward the M2 phenotype reduced infarct size in a transient cerebral ischemia mouse model and promoted neurological recovery[49]. Additionally, several studies have confirmed that the conversion of microglia from a pro-inflammatory to an anti-inflammatory phenotype alleviates neuroinflammation and improves the prognosis of neurological diseases such as Alzheimer's and Parkinson's[50,51]. Microglial polarization is not limited to the traditional M1/M2 phenotypes. With the advancement of new technologies and research methodologies, a growing number of microglial phenotypes have been identified. Newly discovered microglial phenotypes often exhibit disease, region, and function specificity, such as CD11c+ microglia, dark microglia(DM), disease-associated microglia (DAM), and white matter-associated microglia (WAMs). These cells participate in various stages of CNS development, stress responses, and disease progression[52–54]. A recent study identified a novel microglial subset: Arginase-1 (Arg-1$^+$)-expressing microglia, which are primarily located in the basal forebrain and ventral striatum of the developing brain. These cells contribute to the establishment of the cholinergic neuronal system and are involved in several critical functions during early brain development. Genomic sequencing analysis revealed that Arg-1$^+$ microglia exhibit a distinct gene expression profile compared to non-Arg-1-expressing microglia. Specific knockdown of the Arg-1 gene in microglia leads to deficits in cholinergic innervation, and in the hippocampus, where cholinergic neurons project, dendritic spine maturation is impaired, ultimately resulting in cognitive and behavioral deficits in mice[55].

## 5. The Role of Microglia in Neonatal HIE: Activation, Polarization, and Crosstalk with Other Cells

Microglial activation is a central pathogenic mechanism in HIE, acting as a "double-edged sword" in the pathology of HIE. Studies have shown that microglia begin to activate rapidly within 2 hours of HI, peaking on the second or third day and persisting for several weeks thereafter[56]. These temporal changes are region-dependent, with hippocampal microglia showing earlier activation compared to microglia in the cortex, striatum, and white matter[57]. M1/M2 microglia alternate in exerting dominant roles during different stages of HIE: in the early inflammatory stage, M1 microglia play a pro-inflammatory role, while in the later stages, M1 microglia transition to M2 and exert anti-inflammatory effects. One study found that 3 hours after HI, M1 microglia dominated the ipsilateral hemisphere, while a rapid increase in M2 microglia was observed at 24 hours[58]. The two polarized states of microglia often coexist during the development and recovery phases of HIE. Under specific disease stages and intervention measures, microglia can switch between these states; however, the underlying mechanisms of this transition remain poorly understood and require extensive research. A study found that in an ischemic brain environment, extracellular vesicles secreted by microglia were rich in TGF-β1, which stimulated microglial polarization toward an anti-inflammatory phenotype, thus aiding in anti-inflammatory responses[59].

In the early stages of HIE, brain tissue hypoxia, metabolic disruption, and oxidative stress trigger damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) within the brain. These processes lead to the release of inflammatory cytokines and chemokines, which in turn stimulate intracranial inflammation and immune responses[60]. Microglia are highly sensitive to these stimuli. Their surface pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), and retinoic

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acid-inducible gene-I-like receptors (RIG-1), efficiently recognize these stimuli and activate the "classical pathway" of M1 microglial activation. This results in microglial proliferation and the release of large amounts of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, the production of superoxides, and upregulation of chemokines such as C-C chemokine ligand 2 (CCL-2) and C-C chemokine receptor 3 (CCR3). This cascade triggers demyelination and neurotoxicity, damaging normal CNS cells, neurons, and disrupting synaptic structure and function. Pro-inflammatory factors can also disrupt the immature BBB, allowing peripheral immune cells (such as neutrophils, monocytes, and macrophages) to infiltrate the brain and synergize with activated microglia, further exacerbating the immune-inflammatory response[61–63]. Although excessive activation of these "pro-inflammatory responses" may result in neurotoxicity, the pro-inflammatory response should not inherently be viewed as harmful. In essence, it represents a physiological immune response, which is necessary under physiological conditions. When brain homeostasis is challenged and disrupted, microglia-driven acute inflammatory processes initiate subsequent "defense and repair" mechanisms[64]. As HIE progresses, microglia polarize to the M2 phenotype through the "alternative pathway," promoting neurological recovery by releasing anti-inflammatory cytokines, neurotrophic factors, phagocytosing cellular debris, and maintaining neurovascular integrity.

One of the main pathological features of neonatal HIE is extensive neuronal apoptosis. Microglia, as the brain's primary phagocytic cells, clear apoptotic neurons through a process called efferocytosis. Efferocytosis is the physiological process by which phagocytes efficiently clear apoptotic cells from tissues, preventing secondary necrosis and inflammatory responses[65]. When the efferocytosis function of microglia is impaired, the accumulation of apoptotic cells and their subsequent necrosis lead to excessive inflammatory responses and immune dysregulation, worsening brain injury[66]. Studies have shown that both M1 and M2 microglia exhibit phagocytic activity, with M2 microglia being more efficient at clearing apoptotic neurons and providing neuroprotective effects, while M1-mediated phagocytosis may damage healthy neurons[67,68]. Appropriately inducing or inhibiting microglial efferocytosis at different stages of the disease may improve the prognosis of neonatal HIE. In the early stages of neonatal HIE, inhibiting microglial efferocytosis can protect damaged neurons that might recover later from being phagocytosed. In the later stages, inducing microglial efferocytosis can help control inflammatory responses, thereby improving prognosis[69,70].

Throughout the entire pathophysiological process of HIE, the dynamic interactions between microglia and various types of cells in the CNS are crucial[71]. This crosstalk between cells has a significant impact on the prognosis of HIE. Among neural cells, neurons are most susceptible to HI injury due to their high energy demands for maintaining neurotransmission and transmembrane ion gradients. Specific neuronal populations and brain regions in the developing brain, such as the cortex, thalamus, and striatum, have been shown to be particularly vulnerable, a phenomenon known as selective vulnerability[72,73]. When neurons deplete their energy reserves, lactate accumulates, leading to acidosis. This acidic environment promotes oxidative stress and the production of large amounts of ROS, causing neuronal dysfunction and death. Damaged neurons secrete lipocalin-2 and IL-4, which can induce microglia to transition to an anti-inflammatory phenotype. At the same time, IGF-1 secreted by microglia helps maintain neuronal survival. The IGF-1/IGF-1 receptor axis promotes neurogenesis and angiogenesis in ischemic areas, reducing brain damage caused by HI[74,75]. Astrocytes are not only the most abundant cell type in the central nervous system but also an integral part of the brain's innate immune system[76]. Activated astrocytes exhibit two polarized states: the neurotoxic or pro-inflammatory phenotype (A1) and the neuroprotective or anti-inflammatory phenotype (A2). Microglia can trigger the activation of astrocytes. It has been shown that activated microglia can induce A1 astrocyte activation by releasing cytokines such as IL-1αand TNF-α. A1 astrocytes lose their phagocytic function and are capable of inducing neuronal and oligodendrocyte death[77]. Microglia can also induce A2 astrocyte activation by downregulating astrocytic P2Y1 purinergic receptors, forming astrocytic scars to exert neuroprotective and reparative functions[78]. During the course of HIE, astrocytes can modulate microglial inflammatory responses by increasing the production of heme oxygenase-1 (HO-1) and downregulating ROS levels in microglia, thus preventing excessive inflammation in the brain[79]. The primary function of

 doi:10.20944/preprints202411.2228.v1



oligodendrocytes is to insulate axons in the CNS by forming myelin sheaths, facilitating the efficient saltatory conduction of electrical signals, and maintaining and protecting neuronal function. Inflammatory factors such as ROS and IL-1β released by M1 microglia induce oligodendrocyte damage and death, leading to demyelination. M2 microglia promote the recruitment and differentiation of oligodendrocyte progenitor cells (OPCs), aiding their maturation into oligodendrocytes and promoting myelin regeneration[80]. (Figure 1).

## 6. Strategies for Targeting Microglial Polarization

M1 microglia primarily secrete pro-inflammatory factors and mediate inflammatory functions, causing apoptosis and secondary injury. Excessively activated M1 microglia exhibit significant neurotoxic effects. In contrast, M2 microglia secrete anti-inflammatory cytokines and play a crucial role in promoting inflammation resolution and repair. This suggests that during different stages of neonatal HIE treatment, appropriate interventions—such as early inhibition of excessive M1 microglial activation and later induction of M1-to-M2 microglial conversion—may help control inflammatory responses and improve the prognosis of HIE patients. This section summarizes several strategies for targeting microglial polarization and describes the related mechanisms.

### *6.1. IL-4*

IL-4 is primarily produced by activated T cells and modulates various immune cells in the body. Damaged neurons can secrete IL-4, which exerts neuroprotective effects by regulating microglial activation pathways[81]. IL-4 stimulates macrophages and microglia to transition to an anti-inflammatory phenotype, inhibiting the progression of inflammation, promoting tissue repair, and exerting neuroprotective effects[82]. IL-4 induces the transition of microglia to the M2 phenotype primarily through the JAK1-STAT6 pathway[83]. Upon binding to its receptor, IL-4 promotes JAK1 phosphorylation (pJAK1), which subsequently phosphorylates STAT6 (pSTAT6), leading to the upregulation of M2 phenotype-related gene expression in microglia. He et al. demonstrated that IL-4 induces M1-to-M2 microglial polarization via the JAK1-STAT6 pathway, reducing neuroinflammation in hemorrhagic stroke[84]. Radpour et al. found that in a rat model of traumatic brain injury (TBI), IL-4 induced anti-inflammatory microglial polarization, reducing brain injury volume, decreasing the number of damaged neurons, and suppressing seizures in rats[85].

### *6.2. cGAS Inhibitors*

The cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) signaling pathway is present in various cells and plays a critical role in bacterial infections, viral infections, immune disorders, and inflammatory diseases[86]. This pathway leads to the phosphorylation of nuclear factor κB (NF-κB) inhibitor κB (IκB) and its degradation via the ubiquitin-proteasome pathway. NF-κB then translocates to the nucleus, inducing an inflammatory cascade[87]. The NOD-like receptor protein 3 (NLRP3) inflammasome is an innate immune protein complex that mediates the release of pro-inflammatory mediators, including IL-1βand IL-18, and plays a role in regulating inflammatory responses[88]. A study found that the cGAS/STING axis is significantly elevated in a rat model of HIE, which is associated with increased neuroinflammation in the brain. Using the specific inhibitor RU.521 to block cGAS inhibited STING levels and NLRP3 inflammasome activation, significantly reducing brain inflammation. Furthermore, RU.521 promoted the transition of microglia to the M2 phenotype[89], suggesting that cGAS is a promising therapeutic target for treating neuroinflammation in HIE.

### *6.3. MicroRNAs (miRNAs)*

MicroRNAs are small, non-coding RNAs that bind to the 3′ untranslated regions (UTRs) of target genes, inducing mRNA degradation and/or translation inhibition. miR-210 is considered the "master miRNA" in response to hypoxia and can be strongly induced by hypoxia in various cells and tissues[90,91]. Studies have shown that in neonatal HIE, miR-210 expression is significantly

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upregulated within 3 hours. Inhibition of miR-210 significantly reduced HI-induced brain injury in neonatal rats and improved long-term neurobehavioral recovery[92,93]. Li et al. confirmed that miR-210 expression was significantly upregulated in activated microglia following neonatal HIE. miR-210 targets the SIRT1 gene to reduce deacetylation of the NF-κB subunit p65, thereby enhancing NF-κB signaling activity, promoting M1 microglial activation in primary neonatal rat microglia, and increasing the expression of innate pro-inflammatory cytokines[94]. In vivo administration of miR-210 inhibitors effectively suppressed M1 microglial activation, reduced microglia-mediated neuroinflammation, and improved the prognosis of HIE. Given the broad efficacy of miRNAs, their ease of administration, and their small molecular size, allowing them to cross the BBB, these molecules have become promising therapeutic targets for improving neonatal brain injury and developmental disorders after HIE[95].

### *6.4. Atorvastatin*

Atorvastatin is a statin drug that inhibits HMG-CoA reductase and is commonly used to lower cholesterol and reduce cardiovascular risk. The Wnt/β-catenin signaling pathway is a key pathway involved in maintaining neuronal homeostasis and plays an important role in regulating microglial function following brain injury[96]. Sclerostin (SOST) recombinant protein can antagonize the binding of Wnt to its receptor, thereby inhibiting the Wnt/β-catenin signaling pathway[97]. Yu et al. found that atorvastatin inhibits SOST expression, activating the Wnt/β-catenin signaling pathway and promoting the conversion of M1 microglia to the M2 phenotype, reducing inflammation, decreasing infarct volume in hypoxic-ischemic brain damage (HIBD) model rats, and improving their learning and memory abilities[98].

### *6.5. Scoparone (SCO)*

Many studies have shown that the TLR4/MyD88/NF-κB signaling pathway plays a critical role in HIE. Some natural compounds can protect against HIE by inhibiting different nodes of this pathway[99–101]. SCO, a coumarin derivative derived from the Chinese herb Artemisia capillaris, is commonly used to treat liver diseases. SCO has multiple beneficial pharmacological effects, including antioxidant, anti-inflammatory, and anti-apoptotic properties[102,103]. TLR4 is one of the most extensively studied TLRs and can recognize pathogen-associated and damage-associated molecular patterns. In the brain, TLR4 is highly expressed in neurons, astrocytes, and microglia. Upon stimulation by hypoxia-ischemia signals, TLR4 activates its main downstream adaptor, Myeloid differentiation primary response 88 (MyD88), initiating signal transduction pathways and ultimately leading to the upregulation of pro-inflammatory markers via activation of the key inflammatory transcription factor, NF-κB. One study found that SCO promotes the conversion of M1 microglia to the M2 phenotype by inhibiting the TLR4/MyD88/TRAF-6/TAK-1/NF-κB axis and NLRP3 inflammasome activation, thereby reducing neuroinflammation and neurodegeneration in an Alzheimer's disease model[104]. Wu et al. used SCO to intervene in oxygen-glucose deprivation/reperfusion (OGD/R)-induced hippocampal neuron in mice. They found that OGD/R-induced hippocampal neuronal apoptosis was significantly reduced, with decreased expression of the pro-apoptotic gene bax and increased expression of the anti-apoptotic gene bcl-2[105]. The above research suggests that the research and development of SCO has great prospects in the treatment of HIE.

## 7. Conclusions

Neonatal HIE is a complex cascade of neuronal injury, and there is still a lack of effective drug or surgical treatment options for this disease. Microglial polarization plays a "double-edged sword" role in the pathogenesis of neonatal HIE. Targeting the induction of microglial polarization from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype can protect neurons and promote the repair of brain tissue damage. Effectively regulating the polarization states of M1 and M2 microglia may become a new therapeutic strategy for neonatal HIE. How to consider the balance

 doi:10.20944/preprints202411.2228.v1



between pro-inflammatory and anti-inflammatory responses and select the appropriate time point for treatment also need to be explored urgently. This review provides a overview of the physiological roles of microglia and their involvement in neonatal HIE, including microglial activation, polarization, efferocytosis, and their crosstalk with other neural cells. Several strategies for targeting microglial polarization are summarized, looking forward to providing reference and support for in-depth exploration of related research.

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