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Review

# Inflammatory responses in hypoxic ischemic encephalopathy

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Inflammation plays a critical role in mediating brain injury induced by neonatal hypoxic ischemic encephalopathy (HIE). The mechanisms underlying inflammatory responses to ischemia may be shared by neonatal and adult brains; however, HIE exhibits a unique inflammation phenotype that results from the immaturity of the neonatal immune system. This review will discuss the current knowledge concerning systemic and local inflammatory responses in the acute and subacute stages of HIE. The key components of inflammation, including immune cells, adhesion molecules, cytokines, chemokines and oxidative stress, will be reviewed, and the differences between neonatal and adult inflammatory responses to cerebral ischemic injury will also be discussed.

**Keywords:** neonate; inflammatory response; hypoxic ischemic encephalopathy; microglia; leukocyte; cytokine; chemokine; adhesion molecules; oxidative stress

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## Introduction

Perinatal hypoxic-ischemic encephalopathy (HIE) is a major cause of neonatal death and long-term disability. Approximately 15% to 25% of affected newborns die in the postnatal period and 25% develop severe and permanent neuropsychological sequelae<sup>[1]</sup>, including cerebral palsy, seizures, visual impairment, mental retardation, learning impairment and epilepsy<sup>[2]</sup>. Two phases of HIE-induced neuronal death have been identified in both clinical and experimental studies<sup>[3–5]</sup>. The immediate phase, primary neuronal death, is related to cellular hypoxia with exhaustion of the cell's high-energy stores (primary energy failure). The second phase, delayed neuronal death<sup>[6]</sup>, occurs after a latent period of at least six hours, and is associated with encephalopathy and increased seizure activity. Delayed neuronal death accounts for a significant proportion of final cell loss even after very severe insults. The mechanisms involved in delayed neuronal death include excitotoxicity, apoptosis and microglial activation<sup>[7]</sup>. Microglia are the resident immune cells in the brain, and microglial activation is the initial step in inflammatory responses of the central nervous system (CNS) to various stimuli, including stroke<sup>[8]</sup>. This initial step is followed by the infiltration of circulating monocytes, neutrophils and T-cells<sup>[9]</sup>, which amplifies the inflammatory response in a stimulated brain.

Cerebral ischemia induces an inflammatory response in both the parenchyma and the systemic circulation. Within hours after an insult to the brain of an adult, cytokines are produced in large amounts, and leukocytes are activated and migrate into the injured brain<sup>[10–14]</sup>. In neonates, however, cerebral ischemia initiates an immediate innate immune response even minutes after the insult<sup>[15]</sup>. Age differences in the mechanisms of stroke, some of them very striking, stem from immaturity of the CNS, including differences in the cross-talk between excitotoxic, oxidative and inflammatory injury mechanisms, creating “windows of susceptibility” to hypoxic-ischemic injury during embryonic and early postnatal brain development<sup>[16]</sup>. Here, we review the data on specific aspects of neuroinflammation in the acute and subacute stages of HIE, and will also introduce known similarities and differences in adult and neonatal cerebral ischemic injury. Because the chronic inflammatory response to HIE may last for years and varies according to the developmental stage of the brain, this topic is beyond the scope of this review and will not be discussed.

## Immune cells

### Microglia/macrophages

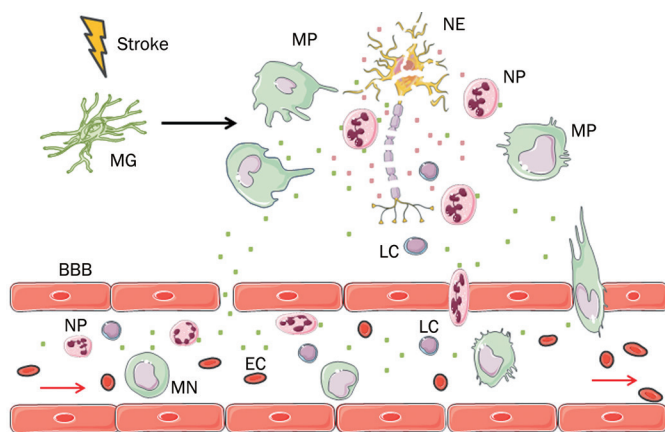
Microglia are a major glial component of the CNS and provide immuno-surveillance in the brain<sup>[17]</sup>. Resting microglia in a healthy brain, known as surveying microglia, are constantly extending and retracting their thin ramified processes to inspect the CNS microenvironment<sup>[18, 19]</sup>. When an ischemic event occurs, microglia are activated and develop

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macrophage-like abilities including phagocytosis, the production of inflammatory and anti-inflammatory cytokines, antigen presentation and the release of matrix metalloproteinases (MMPs), which lead to blood-brain barrier (BBB) breakdown<sup>[20]</sup>. As a result, peripheral leukocytes infiltrate the brain, and the normally immune-privileged brain environment is exposed to systemic responses that further exacerbate inflammation and brain damage (Figure 1). The innate immune response is characterized by the classical activation (M1) of microglia and the subsequent production of specific cytokines, chemokines and reactive intermediates, followed by resolution and alternative activation (M2) that leads to anti-inflammatory signaling (M2a), the clearance of reactive oxygen (ROS) and nitrogen (RNS) species (M2b), and wound healing (M2c)<sup>[21]</sup>. During disease progression, microglial activation phenotypes switch from M1 to M2 or vice versa depending on inflammatory signaling<sup>[22]</sup>. The M1 phenotype of microglia can lead to increased neuronal death compared to the alternatively activated M2 microglial phenotype<sup>[23]</sup>; therefore, there is a growing interest in controlling the classical activation phenotype of microglia.



**Figure 1.** Schematic diagram of inflammatory responses in ischemic stroke. When stroke occurs, microglia are activated and develop macrophage-like capabilities including phagocytosis, cytokine and chemokine production, antigen presentation and the release of MMPs that weaken the BBB. As a result, peripheral leukocytes infiltrate into the brain, leading to exacerbation of inflammation and neuronal injury. MG, microglia; MP, macrophage; NE, neuron; NP, neutrophil; LC, lymphocyte; MN, monocyte; EC, erythrocyte.

In addition to microglia, macrophages also inhabit various regions (choroid plexus, peri-vasculature and meninges) of the CNS<sup>[24]</sup>. The heterogeneous population of tissue macrophages can be continuously replenished by circulating monocytes, unlike microglia, which are thought to reside in the adult CNS from early development<sup>[25–27]</sup>. The theory that a second wave of microglia is established in the brain during the postembryonic period and is derived from peripheral monocytic precursors that last into adulthood is a subject of ongoing debate<sup>[25, 28]</sup>. However, one recent study suggested that a population of

dying microglia in the ischemic brain could be replenished by peripheral monocytes or macrophages infiltrating the injured region and then acquiring microglial phenotypes<sup>[29]</sup>.

Microglial activation and aggregation are pathological markers for HIE in human infants<sup>[30]</sup>. Retrospective clinical studies on the postmortem examinations of 178 brains from neonates found that patients who died from HIE had a dense infiltrate of microglia in the hippocampal dentate gyrus, whereas those neonates who died of other acute causes (trauma or sepsis) had significantly fewer microglia<sup>[30]</sup>. Emerging experimental data from disease models also outline the importance of microglial activation in hypoxia-induced neuroinflammation. HIE in preterm sheep resulted in profound activation and proliferation of microglia in the hippocampus and the periventricular and subcortical white matter, followed by a significant influx of neutrophils into the brain<sup>[31]</sup>. Ameboid microglia in the developing brain respond vigorously to hypoxia and accumulate in injured tissue<sup>[32–35]</sup>, producing excess amounts of inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , etc) along with glutamate, nitric oxide (NO) and ROS, which collectively cause oligodendrocyte death, axonal degeneration and disruption of the immature BBB<sup>[32, 33, 36]</sup>. Compared to adults, microglial activation in neonates is much more rapid following transient ischemia<sup>[37, 38]</sup> and excitotoxic injury<sup>[39]</sup> and continues for weeks<sup>[39–41]</sup>.

### Astrocytes

Both astrocytes and microglia are activated within minutes after injury by pro-inflammatory mediators, cytokines, and ROS that are secreted by injured neurons and glial cells<sup>[42]</sup>. The activation of astrocytes has both detrimental and beneficial roles in brain ischemia. Astrocyte support of neurons after a stroke can be achieved by several mechanisms, including the release of glutathione and superoxide dismutase (SOD)<sup>[43–45]</sup>, enhanced extra-synaptic glutamate uptake<sup>[46–48]</sup>, and the maintenance of ion gradients, such as that for potassium<sup>[49, 50]</sup>. However, activated astrocytes can also produce pro-inflammatory cytokines, including IL-6, TNF- $\alpha$ , IL-1 $\alpha$ , and  $\beta$  and interferon  $\gamma$ <sup>[42, 51, 52]</sup>. Rapid increases in the levels of these cytokines exacerbate an ischemic injury by directly inducing the apoptosis of neuronal cells<sup>[53]</sup>, increasing toxic NO levels and inhibiting neurogenesis<sup>[54]</sup>. Apart from cytokines, reactive astrocytes also secrete chemokines after ischemia, which results in the attraction of immune cells to the ischemic site and worsening of the brain injury<sup>[55, 56]</sup>.

In the brains of human neonates, astrocytes do not readily become reactive and responsive to injury signals until 20 to 23 weeks of gestation<sup>[57]</sup>. Experimental studies regarding astrocytic responses to HIE or systemic LPS stimulation performed in fetuses from various species, *eg*, lamb<sup>[58–60]</sup>, baboon<sup>[61]</sup>, and kitten<sup>[62]</sup>, found astrocyte hypertrophy and hyperplasia. These studies concluded that astrocytes generally are resistant to damage during the neonatal period and that the astrocytes adjacent to regions of necrosis are ready to proliferate. Similar to the findings in adult ischemic models, astrocytes in P7 rat neonates are rarely observed within the ischemic core but

are abundant in the penumbra area 24 h after HIE<sup>[37]</sup>. One unique role of neonatal astrocytes in HIE-induced inflammatory responses is that, in addition to the self-release of cytokines and chemokines, reactive astrocytes in neonatal brains have the ability to up-regulate the expression of inflammatory mediators in neuroblasts and angioblasts, which are chemotactic for bone marrow-derived immune cells<sup>[63]</sup>.

### Neutrophils

During ischemia, neutrophils can exacerbate brain injury through multiple mechanisms, including ROS production<sup>[35]</sup>, decreased microvascular flow resulting from capillary plugging by neutrophils<sup>[64]</sup>, the enhanced release of cytotoxic agents into the vasculature and brain parenchyma<sup>[65, 66]</sup>, and MMP-9 secretion<sup>[67]</sup>. The accumulation of neutrophils in ischemic brain tissue occurs as early as 4 h to 6 h after the onset of ischemia in adult animals<sup>[65, 68-70]</sup> and lasts to 48 h post insult, during the period while the brain injury is evolving<sup>[71-73]</sup>. In contrast to the exacerbated neutrophil infiltration observed in adults, neonates have a diminished ability to mount a neutrophil response to ischemia. Neonatal neutrophils show reduced extravasation from blood vessels<sup>[74, 75]</sup>. A previous study has shown that neutrophils did not transmigrate into the brains of P7 rats following HI injury within 42 h and were almost exclusively intravascular at all time periods examined<sup>[76]</sup>. Similarly, it has been reported that neutrophils were most often found within vessels and only transiently invaded brain tissue in the infarct region after induction by HI in P7 rats<sup>[35]</sup>. These studies indicated that neutrophils do not accumulate in ischemic brain parenchyma in neonatal rodents to the extent that they do in adults. Interestingly this concept translated well into the neuroprotection achieved with anti-neutrophil strategies; treatment with neutrophil inhibitory factor initiated after HI insult was neuroprotective in adult animals<sup>[77-79]</sup> but was less efficacious in neonatal rats. Beneficial effects were only observed when neutropenia was induced before the HI insult<sup>[80]</sup>, making this a less clinically relevant target for treating neonatal injury.

### Lymphocytes

Generally, lymphocytes are thought to play a negative role in acute ischemic brain pathogenesis. Yilmaz *et al*<sup>[81]</sup> reported that Rag1<sup>-/-</sup> mice, deficient in both T cells and B cells, had significantly smaller infarcts and neurologic damage compared to WT mice when subjected to middle cerebral artery occlusion (MCAO). In the same study, Rag1<sup>-/-</sup> mice reconstituted with splenocytes from WT mice were no longer protected from stroke, suggesting that the peripheral lymphocytic response plays an important role in mediating post-stroke injury. Infiltration of T cells and B cells into the ischemic brain can be observed as early as a few hours<sup>[82, 83]</sup>, and lasts days after injury in adult rodents<sup>[84, 85]</sup>. However, in neonates the infiltration of these cells following HIE and focal stroke may be less profound<sup>[35, 86]</sup> or only briefly present in the parenchyma<sup>[87]</sup>. The minimal involvement of lymphocytes in ischemia-induced inflammatory responses in the neonatal brain may reflect the immaturity of lymphoid progenitor cells. Recent clinical

studies showed that peripheral blood mononuclear cells of newborns are relatively undifferentiated and have a very low expression level of surface markers<sup>[88]</sup>. There are few studies investigating the role of lymphocytes in HIE. It is likely that a lymphocytic response is involved in the more chronic immunoinflammatory activation following HIE; the Hagberg group<sup>[35]</sup> found that CD4 lymphocytes invaded the infarct region quite late after injury (7 d after HIE) and persisted in damaged areas for 14 d to 35 d. Whether this lymphocytic response enhances damage or, conversely, enhances post-stroke repair is not yet clear. It is also unknown whether the presence of lymphocytes can lead to the development of later CNS autoimmunity, as has been observed in adult injury models<sup>[89]</sup>.

### Adhesion molecules

The recruitment of leukocytes in the cerebral vasculature and the subsequent migration to the ischemic brain tissue are initially mediated by three main groups of cell adhesion molecules: selectins, the immunoglobulin superfamily and integrins<sup>[90]</sup>. The recruitment process involves two stepwise stages, ie, an initial low affinity binding that is manifested as rolling and a later high affinity interaction that results in firm adhesion. Adhesion molecules may represent important therapeutic targets because inhibiting leukocyte adhesion with antibodies or inhibitors has improved histological and neurological outcomes in experimental stroke studies, whereas over-expression of adhesion molecules resulted in the exacerbation of infarcts<sup>[91]</sup>. Very few neonatal studies have reported the role of and changes in adhesion molecules in HIE; we have summarized the available data from studies in both HIE and other inflammatory diseases in Table 1.

### Selectins

Selectins play a key role in the early (rolling) stages of leukocyte/endothelial interactions in the ischemic cerebral microvasculature. Although all three selectins, L-, P-, and E-selectin, have been implicated in neutrophil rolling, P-selectin is the most important during the initial induction of neutrophil rolling after endothelial cell stimulation<sup>[92]</sup>. Compared to adults, decreased P-selectin expression in neonates has been found in activated platelets<sup>[93]</sup> and endothelial cells<sup>[94]</sup>. Similarly, L-selectin expression in term infant neutrophils is significantly lower than that in adult neutrophils either stimulated or unstimulated<sup>[95]</sup>. This may explain why the decreased adhesion of neutrophils to endothelial cells and delayed transendothelial cell migration of neutrophils have been consistently reported in neonatal animals and humans and may also contribute to susceptibility of neonates to infection<sup>[96, 97]</sup>. In immature animal brains during acute inflammation, E-selectin seems less important than other selectins because the blockade of E-selectin has no effect on neutrophil recruitment to the brain parenchyma, whereas the administration of P-selectin blocking monoclonal antibody inhibited neutrophil recruitment by 85% compared with controls<sup>[98]</sup>.

**Table 1.** Roles of adhesion molecules in pediatric inflammation.

Mediators	Investigated objects	Stimulation	Effects of stimulation	Compared to adults	References
L-selectin	New born infants	Acute bacterial infection	Down-regulation	N/A	[126]
	New born infants	LPS	Up-regulation	Lower	[127]
P-selectin	3–4 weeks rats	IL-1 $\beta$	Up-regulation	No difference	[98]
	P1 rats	Thioglycollate	Up-regulation	Lower	[94]
E-selectin	3–4 weeks rats	IL-1 $\beta$	Up-regulation	No difference	[98]
LFA-1	New born infants	IL-1	Up-regulation	Lower	[75]
MAC-1	New born infants	LPS	Up-regulation	Lower	[127]
VLA-4	P7 rats	Microglial activation	Up-regulation	N/A	[128]
ICAM-1	P7 rats	HIE	Up-regulation	N/A	[129]
	P2–3 mice	Pneumocystis carinii	Trend in increase	Lower	[130]
	P2–3 mice	TNF- $\alpha$	Up-regulation	N/A	[130]
ICAM-2	P4–10 mice	Antigen-specific	Up-regulation	N/A	[131]
VCAM-1	P2–3 mice	Pneumocystis carinii	Trend in increase	Lower	[130]
	P2–3 mice	TNF- $\alpha$	Up-regulation	N/A	[130]
PECAM-1	P1 piglets	HIE	Up-regulation	N/A	[132]

### Integrins and the immunoglobulin superfamily

The firm adhesion of leukocytes to the endothelium after rolling requires the activation and binding of leukocyte-expressed integrins to endothelial adhesion molecules<sup>[99]</sup>. Integrins are heterodimers consisting of a common  $\beta$  subunit and a variable  $\alpha$  subunit<sup>[100]</sup>. The major integrins expressed on neutrophils are the  $\beta$ 2 integrins LFA-1 ( $\alpha$ L $\beta$ 2, CD11a/CD18) and Mac-1 ( $\alpha$ M $\beta$ 2, CD11b/CD18). Monocytes adhere through the  $\beta$ 1 integrins VLA-4 ( $\alpha$ 4 $\beta$ 1, CD49d/CD29). To form a firm adhesion, integrins must bind to counter-receptors of the immunoglobulin superfamily expressed on inflamed endothelial cells, including ICAM-1, ICAM-2, VCAM-1, the mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1), platelet-endothelial cell adhesion molecule-1 (PECAM-1), and the receptor for advanced glycation end products (RAGE)<sup>[101–103]</sup>. Although no age-related differences in basal and stimulated LFA-1 surface expression were found in human neonatal and adult neutrophils<sup>[104–108]</sup>, Mac-1 expression remains low during the prenatal and postnatal periods and reaches adult levels by 11 months<sup>[108, 109]</sup>. The lower surface expression of Mac-1 on neonatal neutrophils has been directly linked to impaired transendothelial migration under chemotactic stimulation<sup>[75, 110]</sup> (Table 1).

Thus far, no data are available in neonates regarding the roles of integrins and the immunoglobulin superfamily in HIE. Experimental studies with adult stroke models have shown that blockade of LFA-1/Mac-1<sup>[111–115]</sup> and ICAM-1<sup>[116, 117]</sup> had beneficial effects on stroke outcomes. However, clinical trials of stroke patients given humanized antibodies against these adhesion molecules showed no effect<sup>[118, 119]</sup> or a worse outcome<sup>[120]</sup>. There are several reasons (see review<sup>[121]</sup>) for the failure of antibodies against these adhesion molecules to translate into a clinically relevant treatment strategy. For example, the study designs in the clinical trials did not mirror the laboratory models (such as late treatment or the absence of documented

recanalization to the occluded vessel). Another possibility is that changes in neutrophil integrins are different between humans and rodents. Indeed, recent work has highlighted the differences in the immune system between species<sup>[122]</sup>. These differences emphasize the importance of clinical biomarkers and early phase studies to confirm the targets in both adult stroke and neonatal HIE, particularly using accessible sources such as peripheral blood. Although intervention strategies targeting adhesion molecules appeared to be effective in preclinical studies, moving this work into humans remains a tremendous challenge. It is encouraging that natalizumab, a humanized monoclonal antibody against  $\alpha$ 4-integrin, has been used to treat multiple sclerosis for more than 5 years<sup>[123]</sup> and has been reported to decrease the risk of disability progression by 42% to 54% and to reduce the annualized rate of relapse by 68%<sup>[124]</sup>. Natalizumab treatment is associated with a risk of progressive multifocal leukoencephalopathy (PML), an opportunistic brain infection caused by the JC virus<sup>[125]</sup>. However, because its clinical benefits outweigh the risks involved, natalizumab remains on the market in the US under a special prescription program using risk stratification algorithms and PML management strategies<sup>[123]</sup>.

### Cytokines

Cytokines are important inflammatory mediators, and cerebral ischemic injury can trigger a cascade of cytokine induction that acts to orchestrate an *in situ* inflammatory reaction<sup>[133]</sup> and maintains brain tissue homeostasis<sup>[134]</sup>. In general, the roles of cytokines are pleiotropic, and whether the overall effects are pro- or anti-inflammatory in the context of ischemic insults remains controversial even in adult models, for which there are more data than for HIE. The most studied cytokines related to the inflammatory responses to stroke are IL-1, IL-6, IL-10, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and transforming growth factor- $\beta$  (TGF- $\beta$ )<sup>[135]</sup>.



IL-1 $\beta$  and TNF- $\alpha$  are among the best-characterized early response cytokines and are often expressed concurrently<sup>[136]</sup>. Several types of CNS cells secrete IL-1 $\beta$  and TNF- $\alpha$ , including microglia, astrocytes, and neurons, and these cytokines share potent pro-inflammatory actions. Human newborns with HIE have higher levels of IL-1 $\beta$  and TNF- $\alpha$  in peripheral blood samples at P1, P3, and P7 compared to controls, and the IL-1 $\beta$  levels correlate positively with HIE severity<sup>[137]</sup>. The neurotoxic consequences of IL-1 activation have been shown in experimental studies with HIE<sup>[138-140]</sup> and other inflammatory disease models<sup>[141-143]</sup>. The most convincing evidence that IL-1 is functionally detrimental in the pathogenesis of HIE is provided by the neuroprotective potential of IL-1 receptor antagonist administration in HIE models in rodents<sup>[144, 145]</sup> (Table 2).

### Chemokines

Chemokines, or chemoattractant cytokines, also play a pivotal role in cerebral damage in ischemic stroke, HIE and excitotoxic brain injury models<sup>[146]</sup>. Chemokines are classified based on the positions of key cysteine residues (C): C, CC, CXC, and CX3C, and act through specific and shared receptors belonging to the superfamily of G-protein-coupled receptors<sup>[147]</sup>. As their name indicates, chemokines play a central role in leukocyte physiology by controlling inflammatory cell trafficking. HIE modeled in P7 rats induces the up-regulation of alpha-chemokines [growth related gene and macrophage inflammatory protein-2 (MIP-2)] and beta-chemokines (MIP-1 $\alpha$ , MIP-1 $\beta$ , CCL-5) preceding the expression of markers for lymphocytes in the infarcted area<sup>[35]</sup>. In the neonatal brain, acute excitotoxic injury stimulates the expression of both monocyte chemoattractant protein-1 [MCP-1, also called chemokine ligand 2 (CCL2)] and its receptor CCR2, suggesting that MCP-1 regulates the

microglial/monocyte response to acute brain injury and contributes to the pathogenesis of acute neonatal brain injury<sup>[148, 149]</sup>. This has been confirmed by another study using the same model in which anti-MCP-1 antibody attenuated tissue injury in neonatal rats<sup>[150]</sup> (Table 2). Few data are available on the potential role of CXC chemokines in perinatal stroke. In experimental adult stroke models, stromal cell-derived factor 1 (SDF-1 or CXCL12) is expressed perivascularly in the injured region up to 30 d after the injury, suggesting that it could be a therapeutic target for tissue repair strategies<sup>[151]</sup>. However, in P7 mice, stroke induced up-regulation of CXCL12 was only observed up to 7 d after the injury but not at a later time point<sup>[63]</sup>, indicating a significantly smaller temporal window for CXCL12-mediated repair after a perinatal stroke.

### Oxidative stress

Oxidative stress has recently been recognized as a common pathway in which different inflammatory cells mediate post-ischemic injury<sup>[159, 160]</sup>. After ischemic insults, the inflammatory cells in the brain are activated and then generate ROS via several enzyme systems to induce the expression of pro-inflammatory mediators including cytokines and adhesion molecules<sup>[160]</sup>. Superoxide is generated via cyclo-oxygenase (COX), xanthine dehydrogenase, xanthine oxidase, and NADPH oxidase, whereas myeloperoxidase (MPO) and monoamine oxidase (MAO) generate hypochlorous acid and H<sub>2</sub>O<sub>2</sub><sup>[121]</sup>. Compared to adult mice, P7 pups show the increased accumulation of H<sub>2</sub>O<sub>2</sub> in the brain after a HI injury, suggesting that the neonatal brain may be more damaged even after a milder degree of acute hypoxic-ischemic injury<sup>[161]</sup> (Table 3). Glutathione peroxidase (GPX) is a key enzyme responsible for the degradation of H<sub>2</sub>O<sub>2</sub><sup>[162]</sup>. The neonatal brain has limited

**Table 2.** Roles of cytokines and chemokines in HIE.

Mediators	Investigated objects	Stimulation	Expression after stimulation	Effects on HIE	References
IL-1 $\alpha$	P7 rats	HIE	Up-regulation	Detrimental	[144]
IL-1 $\beta$	P7 rats	HIE	Up-regulation	Detrimental	[144]
	P7 rats	HIE	Up-regulation	Detrimental	[152]
TNF- $\alpha$	P7 rats	HIE	Up-regulation	Detrimental	[152]
IL-18	P7 rats	HIE	Up-regulation	Detrimental	[153]
	P9 IL-18 <sup>-/-</sup> mice	HIE	N/A	Beneficial in KO	[154]
IL-2	Children 4.5 years (average age)	Perinatal stroke	Chronic up-regulation	N/A	[155]
IL-6	P7 rats	HIE	Up-regulation	Detrimental	[152]
IL-8	Children 4.5 years (average age)	Perinatal stroke	Chronic up-regulation	N/A	[155]
IL-9	P5 mice	Ibotenate+IL-9	N/A	Detrimental	[156]
IL-10	P5 mice	Ibotenate+IL-10	N/A	Beneficial	[157]
IL-4	P5 mice	Ibotenate+IL-4	N/A	Beneficial	[156]
IFN- $\gamma$	P1-3 rats	IFN- $\gamma$ treated	N/A	Detrimental	[158]
CCL3/MIP-1 $\alpha$	P7 rats	HIE	Up-regulation	Detrimental	[35]
CCL4/MIP-1 $\beta$	P7 rats	HIE	Up-regulation	Detrimental	[35]
CCL5/RANTES	P7 rats	HIE	Up-regulation	Detrimental	[35]
CCL2/MCP-1	P7 rats	HIE	Up-regulation	Detrimental	[150]
CXCL12/SDF1	P7 mice	HIE	Up-regulation	Detrimental	[63]

Data compared to the adults are not available.

**Table 3.** Roles of oxidative stress in HIE.

Mediators	Investigated objects	Stimulation	Expression after stimulation	Effects on HIE	Compared to adults	References
H <sub>2</sub> O <sub>2</sub>	P7 rats	HIE	Up-regulation	Detrimental	Higher	[161]
	hSOD1-Tg P7 mice	HIE	Up-regulation	Detrimental	N/A	[163]
COX-2	P7 rats	HIE+LPS	Up-regulation	Correlated	N/A	[167]
NO	P7 rats	HIE+LPS	Up-regulation	Correlated	N/A	[167]
	P7 rats	HIE	Up-regulation	Detrimental	N/A	[168]
	P7 rats	HIE	Up-regulation	Detrimental	N/A	[169]
Tyrosine nitration	P1–3 piglets	HIE+iNOS inhibitor	Down-regulation	Detrimental	N/A	[170]

GPX activity and is more susceptible to oxidative damage, as described in a study showing that H<sub>2</sub>O<sub>2</sub> rapidly accumulates in human-superoxide dismutase-1 (hSOD1) transgenic P7 mice, thus resulting in exacerbated HI brain injury, which is reversed in hGPX1-Tg mice<sup>[163]</sup>. However, the role of ROS in neonatal inflammatory responses following HIE is controversial. Inhibition of NADPH oxidase, the most important source of ROS<sup>[164]</sup>, increases HI injury and the level of IL-1 $\beta$  in P9 mice<sup>[165]</sup>. In contrast, it has been well established that NADPH oxidase can exacerbate inflammatory responses and stroke outcomes in adult animal models (see review<sup>[166]</sup>). Therefore, the results obtained in adult animals are not completely relevant to newborns and the role of oxidative stress in HIE remains to be fully investigated.

### Fetal inflammatory response syndrome (FIRS)

Originally defined in fetuses who experienced preterm labor and preterm premature rupture of the membranes (PROM), FIRS is a unique condition characterized by the systemic activation of the fetal innate immune system and by an elevation in fetal plasma IL-6 concentrations<sup>[171]</sup>. Currently, FIRS is characterized by a rapid increase in pro-inflammatory signaling (cytokines, chemokines, *etc*) and the mobilization of immune effector cells into the fetal circulation<sup>[172]</sup>. These pro-inflammatory mediators readily cross the BBB and induce the activation of microglia, which initiates a detrimental cerebral inflammatory response. The unique circumstances of the “patient” (fetus) and the environment (uterus) in FIRS make it distinguishable from other diseases; however, by definition, FIRS and inflammatory responses after HIE partly overlap in pathophysiology, and they share similar inflammatory mechanisms in the brain. There are multiple putative mechanisms by which the neonatal brain can sense FIRS signals in the systemic circulation, which will then lead to neuroinflammation. These mechanisms include the interface of macrophages in the circumventricular brain area, without a BBB, with circulating inflammatory molecules<sup>[173]</sup>, and the direct access of FIRS signals into the CNS through leakage of the BBB in the setting of peripheral inflammatory pain signaling through the vagal nerve<sup>[174]</sup>. The manner in which FIRS influences the response to HIE and whether HIE can induce FIRS and subsequent peripheral immune activation is an area of active study.

### Summary

HIE triggers a robust inflammatory response and accumulating data have linked post-ischemic inflammation to the exacerbation of brain damage. Many inflammatory mechanisms and pathways after cerebral ischemia have been assessed in various studies performed in adult subjects; however, caution should be exercised when attempting to extrapolate these findings to neonates. The mechanisms underlying cerebral ischemic injury and the following immune response are likely very different between the neonates and the adults.

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### References

- Lai MC, Yang SN. Perinatal hypoxic-ischemic encephalopathy. *J Biomed Biotechnol* 2011; 2011: 609813.
- Vannucci RC, Perlman JM. Interventions for perinatal hypoxic-ischemic encephalopathy. *Pediatrics* 1997; 100: 1004–14.
- Gluckman PD, Williams CE. When and why do brain cells die? *Dev Med Child Neurol* 1992; 34: 1010–4.
- Lorek A, Takei Y, Cady EB, Wyatt JS, Penrice J, Edwards AD, *et al*. Delayed (“secondary”) cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. *Pediatr Res* 1994; 36: 699–706.
- Penrice J, Cady EB, Lorek A, Wylezinska M, Amess PN, Aldridge RF, *et al*. Proton magnetic resonance spectroscopy of the brain in normal preterm and term infants, and early changes after perinatal hypoxia-ischemia. *Pediatr Res* 1996; 40: 6–14.
- Williams CE, Gunn A, Gluckman PD. Time course of intracellular edema and epileptiform activity following prenatal cerebral ischemia in sheep. *Stroke* 1991; 22: 516–21.
- Inder TE, Volpe JJ. Mechanisms of perinatal brain injury. *Semin Neonatol* 2000; 5: 3–16.
- Yenari MA, Kauppinen TM, Swanson RA. Microglial activation in stroke: therapeutic targets. *Neurotherapeutics* 2010; 7: 378–91.
- Zheng Z, Yenari MA. Post-ischemic inflammation: molecular mechanisms and therapeutic implications. *Neurol Res* 2004; 26: 884–92.
- Wang LW, Chang YC, Lin CY, Hong JS, Huang CC. Low-dose lipopoly-saccharide selectively sensitizes hypoxic ischemia-induced white matter injury in the immature brain. *Pediatr Res* 2010; 68: 41–7.
- Perego C, Fumagalli S, De Simoni MG. Temporal pattern of expres-

- sion and colocalization of microglia/macrophage phenotype markers following brain ischemic injury in mice. *J Neuroinflammation* 2011; 8: 174.
- 12 Offner H, Subramanian S, Parker SM, Afentoulis ME, Vandenbark AA, Hum PD. Experimental stroke induces massive, rapid activation of the peripheral immune system. *J Cereb Blood Flow Metab* 2006; 26: 654–65.
- 13 Garcia JH, Liu KF, Yoshida Y, Chen S, Lian J. Brain microvessels: factors altering their patency after the occlusion of a middle cerebral artery (Wistar rat). *Am J Pathol* 1994; 145: 728–40.
- 14 Denker SP, Ji S, Dingman A, Lee SY, Derugin N, Wendland MF, *et al*. Macrophages are comprised of resident brain microglia not infiltrating peripheral monocytes acutely after neonatal stroke. *J Neurochem* 2007; 100: 893–904.
- 15 Algra SO, Groeneveld KM, Schadenberg AW, Haas F, Evens FC, Meerding J, *et al*. Cerebral ischemia initiates an immediate innate immune response in neonates during cardiac surgery. *J Neuroinflammation* 2013; 10: 24.
- 16 Ferriero DM. Neonatal brain injury. *N Engl J Med* 2004; 351: 1985–95.
- 17 Stoll G, Jander S. The role of microglia and macrophages in the pathophysiology of the CNS. *Prog Neurobiol* 1999; 58: 233–47.
- 18 Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, *et al*. ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat Neurosci* 2005; 8: 752–8.
- 19 Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 2005; 308: 1314–8.
- 20 Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med* 2011; 17: 796–808.
- 21 Varnum MM, Ikezu T. The classification of microglial activation phenotypes on neurodegeneration and regeneration in Alzheimer's disease brain. *Arch Immunol Ther Exp (Warsz)* 2012; 60: 251–66.
- 22 Jimenez S, Baglietto-Vargas D, Caballero C, Moreno-Gonzalez I, Torres M, Sanchez-Varo R, *et al*. Inflammatory response in the hippocampus of PS1M146L/APP751SL mouse model of Alzheimer's disease: age-dependent switch in the microglial phenotype from alternative to classic. *J Neurosci* 2008; 28: 11650–61.
- 23 Hu X, Li P, Guo Y, Wang H, Leak RK, Chen S, *et al*. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke* 2012; 43: 3063–70.
- 24 McMenamin PG. Distribution and phenotype of dendritic cells and resident tissue macrophages in the dura mater, leptomeninges, and choroid plexus of the rat brain as demonstrated in wholemount preparations. *J Comp Neurol* 1999; 405: 553–62.
- 25 Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch UK, Mack M, *et al*. Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci* 2007; 10: 1544–53.
- 26 Bechmann I, Priller J, Kovac A, Bontert M, Wehner T, Klett FF, *et al*. Immune surveillance of mouse brain perivascular spaces by blood-borne macrophages. *Eur J Neurosci* 2001; 14: 1651–8.
- 27 Lassmann H, Schmied M, Vass K, Hickey WF. Bone marrow derived elements and resident microglia in brain inflammation. *Glia* 1993; 7: 19–24.
- 28 Geissmann F, Auffray C, Palframan R, Wirrig C, Ciocca A, Campisi L, *et al*. Blood monocytes: distinct subsets, how they relate to dendritic cells, and their possible roles in the regulation of T-cell responses. *Immunol Cell Biol* 2008; 86: 398–408.
- 29 Varvel NH, Grathwohl SA, Baumann F, Liebig C, Bosch A, Brawek B, *et al*. Microglial repopulation model reveals a robust homeostatic process for replacing CNS myeloid cells. *Proc Natl Acad Sci U S A* 2012; 109: 18150–5.
- 30 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: 144–51.
- 31 Jellema RK, Passos VL, Zwanenburg A, Ophelders DR, De Munter S, Vanderlocht J, *et al*. Cerebral inflammation and mobilization of the peripheral immune system following global hypoxic-ischemia in preterm sheep. *J Neuroinflammation* 2013; 10: 13.
- 32 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.
- 33 McRae A, Gilland E, Bona E, Hagberg H. Microglia activation after neonatal hypoxic-ischemia. *Brain Res Dev Brain Res* 1995; 84: 245–52.
- 34 Ivacko JA, Sun R, Silverstein FS. Hypoxic-ischemic brain injury induces an acute microglial reaction in perinatal rats. *Pediatr Res* 1996; 39: 39–47.
- 35 Bona E, Andersson AL, Blomgren K, Gilland E, Puka-Sundvall M, Gustafson K, *et al*. Chemokine and inflammatory cell response to hypoxia-ischemia in immature rats. *Pediatr Res* 1999; 45: 500–9.
- 36 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.
- 37 Derugin N, Wendland M, Muramatsu K, Roberts TP, Gregory G, Ferriero DM, *et al*. Evolution of brain injury after transient middle cerebral artery occlusion in neonatal rats. *Stroke* 2000; 31: 1752–61.
- 38 Derugin N, Dingman A, Wendland MF, Fox C, Bollen A, Vexler ZS. Magnetic resonance imaging as a surrogate measure for histological sub-chronic endpoint in a neonatal rat stroke model. *Brain Res* 2005; 1066: 49–56.
- 39 Dommergues MA, Plaisant F, Verney C, Gressens P. Early microglial activation following neonatal excitotoxic brain damage in mice: a potential target for neuroprotection. *Neuroscience* 2003; 121: 619–28.
- 40 Renolleau S, Benjelloun N, Ben-Ari Y, Charriat-Marlangue C. Regulation of apoptosis-associated proteins in cell death following transient focal ischemia in rat pups. *Apoptosis* 1997; 2: 368–76.
- 41 Fox C, Dingman A, Derugin N, Wendland MF, Manabat C, Ji S, *et al*. Minocycline confers early but transient protection in the immature brain following focal cerebral ischemia-reperfusion. *J Cereb Blood Flow Metab* 2005; 25: 1138–49.
- 42 Tuttolomondo A, Di Raimondo D, di Sciacca R, Pinto A, Licata G. Inflammatory cytokines in acute ischemic stroke. *Curr Pharm Des* 2008; 14: 3574–89.
- 43 Swanson RA, Ying W, Kauppinen TM. Astrocyte influences on ischemic neuronal death. *Curr Mol Med* 2004; 4: 193–205.
- 44 Bambrick L, Kristian T, Fiskum G. Astrocyte mitochondrial mechanisms of ischemic brain injury and neuroprotection. *Neurochem Res* 2004; 29: 601–8.
- 45 Sims NR, Nilsson M, Muyderman H. Mitochondrial glutathione: a modulator of brain cell death. *J Bioenerg Biomembr* 2004; 36: 329–33.
- 46 Anderson CM, Swanson RA. Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* 2000; 32: 1–14.
- 47 Romera C, Hurtado O, Botella SH, Lizasoain I, Cardenas A, Fernandez-Tome P, *et al*. *In vitro* ischemic tolerance involves upregulation of glutamate transport partly mediated by the TACE/ADAM17-tumor necrosis factor-alpha pathway. *J Neurosci* 2004; 24: 1350–7.
- 48 Schousboe A, Waagepetersen HS. Glial modulation of GABAergic and glutamatergic neurotransmission. *Curr Top Med Chem* 2006; 6: 929–34.
- 49 Walz W. Role of astrocytes in the clearance of excess extracellular potassium. *Neurochem Int* 2000; 36: 291–300.
- 50 Stanimirovic DB, Ball R, Durkin JP. Glutamate uptake and Na,K-ATPase activity in rat astrocyte cultures exposed to ischemia. *Acta Neurochir Suppl* 1997; 70: 1–3.
- 51 Orzylowska O, Oderfeld-Nowak B, Zaremba M, Januszewski S, Mossakowski M. Prolonged and concomitant induction of astroglial immunoreactivity of interleukin-1beta and interleukin-6 in the rat hippocampus after transient global ischemia. *Neurosci Lett* 1999; 263: 72–6.
- 52 Lau LT, Yu AC. Astrocytes produce and release interleukin-1, inter-

- leukin-6, tumor necrosis factor alpha and interferon-gamma following traumatic and metabolic injury. *J Neurotrauma* 2001; 18: 351–9.
- 53 Stoll G, Jander S, Schroeter M. Inflammation and glial responses in ischemic brain lesions. *Prog Neurobiol* 1998; 56: 149–71.
- 54 Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. *Science* 2003; 302: 1760–5.
- 55 Sofroniew MV. Astrocyte failure as a cause of CNS dysfunction. *Mol Psychiatry* 2000; 5: 230–2.
- 56 Kim JS. Cytokines and adhesion molecules in stroke and related diseases. *J Neurol Sci* 1996; 137: 69–78.
- 57 Roessmann U, Gambetti P. Pathological reaction of astrocytes in perinatal brain injury. Immunohistochemical study. *Acta Neuropathol* 1986; 70: 302–7.
- 58 Ikeda T, Murata Y, Quilligan EJ, Choi BH, Parer JT, Doi S, *et al*. Physiologic and histologic changes in near-term fetal lambs exposed to asphyxia by partial umbilical cord occlusion. *Am J Obstet Gynecol* 1998; 178: 24–32.
- 59 Petersson KH, Pinar H, Stopa EG, Faris RA, Sadowska GB, Hanumara RC, *et al*. White matter injury after cerebral ischemia in ovine fetuses. *Pediatr Res* 2002; 51: 768–76.
- 60 Mallard C, Welin AK, Peebles D, Hagberg H, Kjellmer I. White matter injury following systemic endotoxemia or asphyxia in the fetal sheep. *Neurochem Res* 2003; 28: 215–23.
- 61 Inder T, Neil J, Kroenke C, Dieni S, Yoder B, Rees S. Investigation of cerebral development and injury in the prematurely born primate by magnetic resonance imaging and histopathology. *Dev Neurosci* 2005; 27: 100–11.
- 62 Gilles FH, Murphy SF. Perinatal telencephalic leucoencephalopathy. *J Neurol Neurosurg Psychiatry* 1969; 32: 404–13.
- 63 Miller JT, Bartley JH, Wimborne HJ, Walker AL, Hess DC, Hill WD, *et al*. The neuroblast and angioblast chemotactic factor SDF-1 (CXCL12) expression is briefly up regulated by reactive astrocytes in brain following neonatal hypoxic-ischemic injury. *BMC Neurosci* 2005; 6: 63.
- 64 Schmid-Schonbein GW. Capillary plugging by granulocytes and the no-reflow phenomenon in the microcirculation. *Fed Proc* 1987; 46: 2397–401.
- 65 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: 941–7.
- 66 Kochanek PM, Hallenbeck JM. Polymorphonuclear leukocytes and monocytes/macrophages in the pathogenesis of cerebral ischemia and stroke. *Stroke* 1992; 23: 1367–79.
- 67 Gidday JM, Gasche YG, Copin JC, Shah AR, Perez RS, Shapiro SD, *et al*. 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: H558–68.
- 68 Hallenbeck JM. Significance of the inflammatory response in brain ischemia. *Acta Neurochir Suppl* 1996; 66: 27–31.
- 69 Barone FC, Hillebrand LM, Price WJ, White RF, Lee EV, Feuerstein GZ, *et al*. Polymorphonuclear leukocyte infiltration into cerebral focal ischemic tissue: myeloperoxidase activity assay and histologic verification. *J Neurosci Res* 1991; 29: 336–45.
- 70 Garcia JH, Liu KF, Yoshida Y, Lian J, Chen S, del Zoppo GJ. Influx of leukocytes and platelets in an evolving brain infarct (Wistar rat). *Am J Pathol* 1994; 144: 188–99.
- 71 Matsuo Y, Onodera H, Shiga Y, Nakamura M, Ninomiya M, Kihara T, *et al*. Correlation between myeloperoxidase-quantified neutrophil accumulation and ischemic brain injury in the rat. Effects of neutrophil depletion. *Stroke* 1994; 25: 1469–75.
- 72 Zhang RL, Chopp M, Chen H, Garcia JH. Temporal profile of ischemic tissue damage, neutrophil response, and vascular plugging following permanent and transient (2H) middle cerebral artery occlusion in the rat. *J Neurol Sci* 1994; 125: 3–10.
- 73 Hallenbeck JM, Dutka AJ, Tanishima T, Kochanek PM, Kumaroo KK, Thompson CB, *et al*. Polymorphonuclear leukocyte accumulation in brain regions with low blood flow during the early postischemic period. *Stroke* 1986; 17: 246–53.
- 74 Anderson DC, Hughes BJ, Smith CW. Abnormal mobility of neonatal polymorphonuclear leukocytes. Relationship to impaired redistribution of surface adhesion sites by chemotactic factor or colchicine. *J Clin Invest* 1981; 68: 863–74.
- 75 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: 2613–21.
- 76 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: 607–16.
- 77 Jiang N, Chopp M, Chahwala S. Neutrophil inhibitory factor treatment of focal cerebral ischemia in the rat. *Brain Res* 1998; 788: 25–34.
- 78 Jiang N, Moyle M, Soule HR, Rote WE, Chopp M. Neutrophil inhibitory factor is neuroprotective after focal ischemia in rats. *Ann Neurol* 1995; 38: 935–42.
- 79 Zhang RL, Chopp M, Jiang N, Tang WX, Probst J, Manning AM, *et al*. Anti-intercellular adhesion molecule-1 antibody reduces ischemic cell damage after transient but not permanent middle cerebral artery occlusion in the Wistar rat. *Stroke* 1995; 26: 1438–42.
- 80 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: 549–56.
- 81 Yilmaz G, Arumugam TV, Stokes KY, Granger DN. Role of T lymphocytes and interferon-gamma in ischemic stroke. *Circulation* 2006; 113: 2105–12.
- 82 Brait VH, Jackman KA, Walduck AK, Selemidis S, Diep H, Mast AE, *et al*. Mechanisms contributing to cerebral infarct size after stroke: gender, reperfusion, T lymphocytes, and Nox2-derived superoxide. *J Cereb Blood Flow Metab* 2010; 30: 1306–17.
- 83 Jander S, Kraemer M, Schroeter M, Witte OW, Stoll G. Lymphocytic infiltration and expression of intercellular adhesion molecule-1 in photochemically induced ischemia of the rat cortex. *J Cereb Blood Flow Metab* 1995; 15: 42–51.
- 84 Stoll G, Jander S, Schroeter M. Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. *Adv Exp Med Biol* 2002; 513: 87–113.
- 85 Catania A, Lipton JM. Peptide modulation of fever and inflammation within the brain. *Ann N Y Acad Sci* 1998; 856: 62–8.
- 86 Northington FJ, Ferriero DM, Flock DL, Martin LJ. Delayed neurodegeneration in neonatal rat thalamus after hypoxia-ischemia is apoptosis. *J Neurosci* 2001; 21: 1931–8.
- 87 Benjelloun N, Renolleau S, Represa A, Ben-Ari Y, Charriat-Marlangue C. Inflammatory responses in the cerebral cortex after ischemia in the P7 neonatal Rat. *Stroke* 1999; 30: 1916–23.
- 88 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: 140–4.
- 89 Becker KJ. Activation of immune responses to brain antigens after stroke. *J Neurochem* 2012; 123: 148–55.
- 90 del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X, Feuerstein GZ. Inflammation and stroke: putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. *Brain Pathol* 2000; 10: 95–112.
- 91 Connolly ES Jr, Winfree CJ, Prestigiacomo CJ, Kim SC, Choudhri TF, Hoh BL, *et al*. 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: 304–10.
- 92 Ley K, Bullard DC, Arbones ML, Bosse R, Vestweber D, Tedder TF, *et al*. Sequential contribution of L- and P-selectin to leukocyte rolling *in vivo*. *J Exp Med* 1995; 181: 669–75.
- 93 Grosshaupt B, Muntean W, Sedlmayr P. Hyporeactivity of neonatal platelets is not caused by preactivation during birth. *Eur J Pediatr* 1997; 156: 944–8.
- 94 Lorant DE, Li W, Tabatabaei N, Garver MK, Albertine KH. P-selectin



- expression by endothelial cells is decreased in neonatal rats and human premature infants. *Blood* 1999; 94: 600–9.
- 95 Rebeck N, Gibson A, Finn A. Neutrophil adhesion molecules in term and premature infants: normal or enhanced leucocyte integrins but defective L-selectin expression and shedding. *Clin Exp Immunol* 1995; 101: 183–9.
- 96 Nussbaum C, Sperandio M. Innate immune cell recruitment in the fetus and neonate. *J Reprod Immunol* 2011; 90: 74–81.
- 97 Mariscalco MM, Vergara W, Mei J, Smith EO, Smith CW. Mechanisms of decreased leukocyte localization in the developing host. *Am J Physiol Heart Circ Physiol* 2002; 282: H636–44.
- 98 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: 1115–24.
- 99 Zarbock A, Ley K. Mechanisms and consequences of neutrophil interaction with the endothelium. *Am J Pathol* 2008; 172: 1–7.
- 100 Albelda SM. Endothelial and epithelial cell adhesion molecules. *Am J Respir Cell Mol Biol* 1991; 4: 195–203.
- 101 Danton GH, Dietrich WD. Inflammatory mechanisms after ischemia and stroke. *J Neuropathol Exp Neurol* 2003; 62: 127–36.
- 102 Frommhold D, Kamphues A, Hepper I, Pruenster M, Lukic IK, Socher I, *et al*. RAGE and ICAM-1 cooperate in mediating leukocyte recruitment during acute inflammation *in vivo*. *Blood* 2010; 116: 841–9.
- 103 Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol* 2007; 7: 678–89.
- 104 Moriguchi N, Yamamoto S, Isokawa S, Andou A, Miyata H. Granulocyte functions and changes in ability with age in newborns; Report No 2: activation of granulocyte functions by cytokines. *Pediatr Int* 2006; 48: 22–8.
- 105 Strunk T, Temming P, Gembruch U, Reiss I, Bucsky P, Schultz C. Differential maturation of the innate immune response in human fetuses. *Pediatr Res* 2004; 56: 219–26.
- 106 Kim SK, Keeney SE, Alpard SK, Schmalstieg FC. Comparison of L-selectin and CD11b on neutrophils of adults and neonates during the first month of life. *Pediatr Res* 2003; 53: 132–6.
- 107 Bikoue A, D'Ercole C, George F, Dameche L, Mutin M, Sampol J. Quantitative analysis of leukocyte membrane antigen expression on human fetal and cord blood: normal values and changes during development. *Clin Immunol Immunopathol* 1997; 84: 56–64.
- 108 McEvoy LT, Zakem-Cloud H, Tosi MF. Total cell content of CR3 (CD11b/CD18) and LFA-1 (CD11a/CD18) in neonatal neutrophils: relationship to gestational age. *Blood* 1996; 87: 3929–33.
- 109 Storm SW, Mariscalco MM, Tosi MF. Postnatal maturation of total cell content and up-regulated surface expression of Mac-1 (CD11b/CD18) in polymorphonuclear leukocytes of human infants. *J Leukoc Biol* 2008; 84: 477–9.
- 110 Anderson DC, Abbassi O, Kishimoto TK, Koenig JM, McIntire LV, Smith CW. Diminished lectin-, epidermal growth factor-, complement binding domain-cell adhesion molecule-1 on neonatal neutrophils underlies their impaired CD18-independent adhesion to endothelial cells *in vitro*. *J Immunol* 1991; 146: 3372–9.
- 111 Bows MP, Rothlein R, Fagan SC, Zivin JA. Monoclonal antibodies preventing leukocyte activation reduce experimental neurologic injury and enhance efficacy of thrombolytic therapy. *Neurology* 1995; 45: 815–9.
- 112 Chen H, Chopp M, Zhang RL, Bodzin G, Chen Q, Rusche JR, *et al*. Anti-CD11b monoclonal antibody reduces ischemic cell damage after transient focal cerebral ischemia in rat. *Ann Neurol* 1994; 35: 458–63.
- 113 Clark WM, Madden KP, Rothlein R, Zivin JA. Reduction of central nervous system ischemic injury in rabbits using leukocyte adhesion antibody treatment. *Stroke* 1991; 22: 877–83.
- 114 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: 241–8.
- 115 Bednar MM, Wright SD, Raymond-Russell SJ, Kohut JJ, Gross CE. IB4, a monoclonal antibody against the CD18 leukocyte adhesion protein, reduces intracranial pressure following thromboembolic stroke in the rabbit. *Neurol Res* 1996; 18: 171–5.
- 116 Kitagawa K, Matsumoto M, Mabuchi T, Yagita Y, Ohtsuki T, Hori M, *et al*. Deficiency of intercellular adhesion molecule 1 attenuates microcirculatory disturbance and infarction size in focal cerebral ischemia. *J Cereb Blood Flow Metab* 1998; 18: 1336–45.
- 117 Connolly ES Jr, Winfree CJ, Springer TA, Naka Y, Liao H, Yan SD, *et al*. 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: 209–16.
- 118 Becker KJ. Anti-leukocyte antibodies: LeukArrest (Hu23F2G) and Enlimomab (R6.5) in acute stroke. *Curr Med Res Opin* 2002; 18: s18–22.
- 119 Krams M, Lees KR, Hacke W, Grieve AP, Orgogozo JM, Ford GA. Acute stroke therapy by inhibition of neutrophils (ASTIN): an adaptive dose-response study of UK-279,276 in acute ischemic stroke. *Stroke* 2003; 34: 2543–8.
- 120 Enlimomab A. Use of anti-ICAM-1 therapy in ischemic stroke: results of the Enlimomab Acute Stroke Trial. *Neurology* 2001; 57: 1428–34.
- 121 Vexler ZS, Tang XN, Yenari MA. Inflammation in adult and neonatal stroke. *Clin Neurosci Res* 2006; 6: 293–313.
- 122 Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, *et al*. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A* 2013; 110: 3507–12.
- 123 Rudick R, Polman C, Clifford D, Miller D, Steinman L. Natalizumab: bench to bedside and beyond. *JAMA Neurol* 2013; 70: 172–82.
- 124 Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, *et al*. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2006; 354: 899–910.
- 125 Bloomgren G, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, *et al*. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med* 2012; 366: 1870–80.
- 126 Buhner C, Graulich J, Stibenz D, Dudenhausen JW, Obladen M. L-selectin is down-regulated in umbilical cord blood granulocytes and monocytes of newborn infants with acute bacterial infection. *Pediatr Res* 1994; 36: 799–804.
- 127 Torok C, Lundahl J, Hed J, Lagercrantz H. Diversity in regulation of adhesion molecules (Mac-1 and L-selectin) in monocytes and neutrophils from neonates and adults. *Arch Dis Child* 1993; 68: 561–5.
- 128 Hailer NP, Jarhult JD, Nitsch R. Resting microglial cells *in vitro*: analysis of morphology and adhesion molecule expression in organotypic hippocampal slice cultures. *Glia* 1996; 18: 319–31.
- 129 Miao H, Jiang L, Huang L. Effects of simvastatin on the expression of intercellular adhesion molecule-1 mRNA in neonatal brain with hypoxic-ischemic damage. *J Nanosci Nanotechnol* 2005; 5: 1261–5.
- 130 Qureshi MH, Cook-Mills J, Doherty DE, Garvy BA. TNF-alpha-dependent ICAM-1- and VCAM-1-mediated inflammatory responses are delayed in neonatal mice infected with *Pneumocystis carinii*. *J Immunol* 2003; 171: 4700–7.
- 131 Reiss Y, Hoch G, Deutsch U, Engelhardt B. T cell interaction with ICAM-1-deficient endothelium *in vitro*: essential role for ICAM-1 and ICAM-2 in transendothelial migration of T cells. *Eur J Immunol* 1998; 28: 3086–99.
- 132 Nadeau S, Baribeau J, Janvier A, Perreault T. Changes in expression of vascular endothelial growth factor and its receptors in neonatal hypoxia-induced pulmonary hypertension. *Pediatr Res* 2005; 58: 199–205.
- 133 Saliba E, Henrot A. Inflammatory mediators and neonatal brain damage. *Biol Neonate* 2001; 79: 224–7.
- 134 Hopkins SJ. The pathophysiological role of cytokines. *Leg Med (Tokyo)* 2003; 5: S45–57.
- 135 Han HS, Yenari MA. Cellular targets of brain inflammation in stroke. *Curr Opin Investig Drugs* 2003; 4: 522–9.
- 136 Silverstein FS, Barks JD, Hagan P, Liu XH, Ivacko J, Szafarski J. Cytokines and perinatal brain injury. *Neurochem Int* 1997; 30: 375–83.

- 137 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: 178–82.
- 138 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: 1331–9.
- 139 Girard S, Kadhim H, Larouche A, Roy M, Gobeil F, Sebire G. Pro-inflammatory disequilibrium of the IL-1 beta/IL-1ra ratio in an experimental model of perinatal brain damages induced by lipopolysaccharide and hypoxia-ischemia. *Cytokine* 2008; 43: 54–62.
- 140 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: 377–84.
- 141 Green HF, Treacy E, Keohane AK, Sullivan AM, O'Keefe GW, Nolan YM. A role for interleukin-1beta in determining the lineage fate of embryonic rat hippocampal neural precursor cells. *Mol Cell Neurosci* 2012; 49: 311–21.
- 142 Favrais G, van de Looij Y, Fleiss B, Ramanantsoa N, Bonnin P, Stoltenburg-Didingier G, *et al.* Systemic inflammation disrupts the developmental program of white matter. *Ann Neurol* 2011; 70: 550–65.
- 143 Crampton SJ, Collins LM, Toulouse A, Nolan YM, O'Keefe GW. Exposure of foetal neural progenitor cells to IL-1beta impairs their proliferation and alters their differentiation – a role for maternal inflammation? *J Neurochem* 2011; 120: 964–73.
- 144 Hagberg H, Gilland E, Bona E, Hanson LA, Hahin-Zoric M, Blennow M, *et al.* Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxia-ischemia in neonatal rats. *Pediatr Res* 1996; 40: 603–9.
- 145 Martin D, Chinookoswong N, Miller G. The interleukin-1 receptor antagonist (rhIL-1ra) protects against cerebral infarction in a rat model of hypoxia-ischemia. *Exp Neurol* 1994; 130: 362–7.
- 146 Mirabelli-Badenier M, Braunersreuther V, Viviani GL, Dallegrì F, Quercioli A, Veneselli E, *et al.* CC and CXC chemokines are pivotal mediators of cerebral injury in ischaemic stroke. *Thromb Haemost* 2011; 105: 409–20.
- 147 Baggiolini M. Chemokines in pathology and medicine. *J Intern Med* 2001; 250: 91–104.
- 148 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.
- 149 Szaflarski J, Ivacko J, Liu XH, Warren JS, Silverstein FS. Excitotoxic injury induces monocyte chemoattractant protein-1 expression in neonatal rat brain. *Brain Res Mol Brain Res* 1998; 55: 306–14.
- 150 Galasso JM, Liu Y, Szaflarski J, Warren JS, Silverstein FS. Monocyte chemoattractant protein-1 is a mediator of acute excitotoxic injury in neonatal rat brain. *Neuroscience* 2000; 101: 737–44.
- 151 Hill WD, Hess DC, Martin-Studdard A, Carothers JJ, Zheng J, Hale D, *et al.* SDF-1 (CXCL12) is upregulated in the ischemic penumbra following stroke: association with bone marrow cell homing to injury. *J Neuropathol Exp Neurol* 2004; 63: 84–96.
- 152 Szaflarski J, Burtrum D, Silverstein FS. Cerebral hypoxia-ischemia stimulates cytokine gene expression in perinatal rats. *Stroke* 1995; 26: 1093–100.
- 153 Hedtjarn M, Leverin AL, Eriksson K, Blomgren K, Mallard C, Hagberg H. Interleukin-18 involvement in hypoxic-ischemic brain injury. *J Neurosci* 2002; 22: 5910–9.
- 154 Hedtjarn M, Mallard C, Iwakura Y, Hagberg H. Combined deficiency of IL-1beta18, but not IL-1alpha18, reduces susceptibility to hypoxia-ischemia in the immature brain. *Dev Neurosci* 2005; 27: 143–8.
- 155 Yurur D, Teber S, Deda G, Egin Y, Akar N. The relation between cytokines, soluble endothelial protein C receptor, and factor VIII levels in Turkish pediatric stroke patients. *Clin Appl Thromb Hemost* 2009; 15: 545–51.
- 156 Dommergues MA, Patkai J, Renaud JC, Evrard P, Gressens P. Proinflammatory cytokines and interleukin-9 exacerbate excitotoxic lesions of the newborn murine neopallium. *Ann Neurol* 2000; 47: 54–63.
- 157 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.
- 158 Mann SA, Versmold B, Marx R, Stahlhofen S, Dietzel ID, Heumann R, Berger R. Corticosteroids reverse cytokine-induced block of survival and differentiation of oligodendrocyte progenitor cells from rats. *J Neuroinflammation* 2008; 5:39.
- 159 Pun PB, Lu J, Mochhala S. Involvement of ROS in BBB dysfunction. *Free Radic Res* 2009; 43: 348–64.
- 160 Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. *J Leukoc Biol* 2010; 87: 779–89.
- 161 Lafemina MJ, Sheldon RA, Ferriero DM. Acute hypoxia-ischemia results in hydrogen peroxide accumulation in neonatal but not adult mouse brain. *Pediatr Res* 2006; 59: 680–3.
- 162 Armogida M, Nistico R, Mercuri NB. Therapeutic potential of targeting hydrogen peroxide metabolism in the treatment of brain ischaemia. *Br J Pharmacol* 2012; 166: 1211–24.
- 163 Sheldon RA, Jiang X, Francisco C, Christen S, Vexler ZS, Tauber MG, *et al.* Manipulation of antioxidant pathways in neonatal murine brain. *Pediatr Res* 2004; 56: 656–62.
- 164 Brandes RP, Kreuzer J. Vascular NADPH oxidases: molecular mechanisms of activation. *Cardiovasc Res* 2005; 65: 16–27.
- 165 Doverhag C, Keller M, Karlsson A, Hedtjarn M, Nilsson U, Kapeller E, *et al.* Pharmacological and genetic inhibition of NADPH oxidase does not reduce brain damage in different models of perinatal brain injury in newborn mice. *Neurobiol Dis* 2008; 31: 133–44.
- 166 Tang XN, Cairns B, Kim JY, Yenari MA. NADPH oxidase in stroke and cerebrovascular disease. *Neurol Res* 2012; 34: 338–45.
- 167 Yang L, Sameshima H, Yamaguchi M, Ikenoue T. Expression of inducible nitric oxide synthase and cyclooxygenase-2 mRNA in brain damage induced by lipopolysaccharide and intermittent hypoxia-ischemia in neonatal rats. *J Obstet Gynaecol Res* 2005; 31: 185–91.
- 168 Higuchi Y, Hattori H, Kume T, Tsuji M, Akaike A, Furusho K. Increase in nitric oxide in the hypoxic-ischemic neonatal rat brain and suppression by 7-nitroindazole and aminoguanidine. *Eur J Pharmacol* 1998; 342: 47–9.
- 169 Tsuji M, Higuchi Y, Shiraishi K, Kume T, Akaike A, Hattori H. Protective effect of aminoguanidine on hypoxic-ischemic brain damage and temporal profile of brain nitric oxide in neonatal rat. *Pediatr Res* 2000; 47: 79–83.
- 170 Peeters-Scholte C, Koster J, Veldhuis W, van den Tweel E, Zhu C, Kops N, *et al.* Neuroprotection by selective nitric oxide synthase inhibition at 24 hours after perinatal hypoxia-ischemia. *Stroke* 2002; 33: 2304–10.
- 171 Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. *Am J Obstet Gynecol* 1998; 179: 194–202.
- 172 Kuypers E, Ophelders D, Jellema RK, Kunzmann S, Gavilanes AW, Kramer BW. White matter injury following fetal inflammatory response syndrome induced by chorioamnionitis and fetal sepsis: lessons from experimental ovine models. *Early Hum Dev* 2012; 88: 931–6.
- 173 Malaeb S, Dammann O. Fetal inflammatory response and brain injury in the preterm newborn. *J Child Neurol* 2009; 24: 1119–26.
- 174 Willis CL, Brooks TA, Davis TP. Chronic inflammatory pain and the neurovascular unit: a central role for glia in maintaining BBB integrity? *Curr Pharm Des* 2008; 14: 1625–43.



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