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The role of inflammation in perinatal brain injury

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Abstract

Inflammation is increasingly recognized as being a critical contributor to both normal development and injury outcome in the immature brain. The focus of this Review is to highlight important differences in innate and adaptive immunity in immature versus adult brain, which support the notion that the consequences of inflammation will be entirely different depending on context and stage of CNS development. Perinatal brain injury can result from neonatal encephalopathy and perinatal arterial ischaemic stroke, usually at term, but also in preterm infants. Inflammation occurs before, during and after brain injury at term, and modulates vulnerability to and development of brain injury. Preterm birth, on the other hand, is often a result of exposure to inflammation at a very early developmental phase, which affects the brain not only during fetal life, but also over a protracted period of postnatal life in a neonatal intensive care setting, influencing critical phases of myelination and cortical plasticity. Neuroinflammation during the perinatal period can increase the risk of neurological and neuropsychiatric disease throughout childhood and adulthood, and is, therefore, of concern to the broader group of physicians who care for these individuals.

Introduction

The CNS is an immune-privileged site, which is appropriate for an organ with limited regenerative capacity.[1] However, its immune privilege is severely undermined once inflammation is established, and it is now clear that both peripheral and central immune

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signals can induce inflammatory responses within the CNS during the perinatal period. During this time, the immature brain passes through several essential stages of CNS development, and activation of the immune system during fetal and neonatal life affects critical phases of brain development, with long-lasting consequences for neurological and mental health.[2,3]

Brain injury in the perinatal period occurs in at least four clinical settings: neonatal encephalopathy in term infants, neonatal stroke, encephalopathy of prematurity (Box 1), and systemic infections (Figure 1). Neonatal encephalopathy often results from intrapartum asphyxia leading to hypoxia–ischaemia, and affects the brain globally. Perinatal arterial ischaemic stroke induces a focal brain lesion with a core and a penumbra similar to those in adult stroke.[4] Encephalopathy of prematurity is caused by exposure of the very immature brain to inflammatory triggers during fetal and postnatal life. In addition, CNS inflammation can occur as a result of systemic infections arising at any time during pregnancy or neonatal life; such inflammation can affect brain development directly or act in concert with the above-described insults.

Box 1

Clinical settings of perinatal brain injury

Neonatal encephalopathy at term (>36 weeks' gestational age), also known as hypoxic–ischaemic encephalopathy

- 3/1,000 live births
- Presentation: encephalopathy and changes in muscle tone
- Acute injury results in damage to basal ganglia; subacute injury can be restricted to watershed regions
- The insult causes primary energy depletion, and brain injury develops during the secondary and tertiary phases[112,121]
- Neurons are the primary target in selectively vulnerable regions
- Infections and inflammation can precede and predispose to neonatal encephalopathy (sensitization),[56] and brain injury itself initiates an immune response
- Outcomes depend on severity of injury
- Therapeutic hypothermia is now standard of care for babies if neonatal encephalopathy is recognized by 6 h of life

Perinatal arterial ischaemic stroke

- 1/2,300 live births
- Presentation: focal motor seizures in first day of life
- Focal injury detected on MRI in arterial (70%) or venous (30%) distribution

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- Perinatal stroke is caused by thrombus or embolus formation that can be associated with infection; inflammation contributes to progression of brain injury in the penumbral area[4,122]
- Affects babies *in utero* (20 weeks' gestation) and up to first 28 days of life
- 50% of affected infants have disability that includes epilepsy, behavioural deficits, learning disorders and cerebral palsy
- Currently no available therapies, except for anticoagulation therapy in sinovenous thrombosis

Encephalopathy of prematurity

- Preterm birth occurs in one in eight deliveries; about 5–10% have moderate to severe brain injury
- Damage is primarily to oligodendroglial precursors, but grey matter dysmaturation also occurs; the very immature brain is exposed to inflammation during fetal life
- Inflammation results in preterm birth, and the neonate might further be exposed to hypotension, hypoxia, inflammation, surgery, hypocarbia or hypercarbia during the intensive care period[123,124]
- Injury often goes undetected in the nursery
- Diffuse cortical white matter injury and cerebellar hypoplasia often observed
- Affects babies from 23–36 weeks' gestational age
- Results in cerebral palsy, cognitive disabilities, visual dysfunction, hearing impairments and epilepsy
- No specific therapies

In this Review, we will summarize how different perinatal insults activate the immune system and trigger peripheral and central responses that involve immune mediators (cytokines and chemokines), reactive oxygen species (ROS), reactive nitrosative species, excitotoxicity, mitochondrial impairment, and vascular integrity. We propose that the combined actions of these effectors can cause brain injury directly, modulate vulnerability or interfere with CNS development, thereby contributing to neurological or psychiatric disease (Figure 1).

PRRs in the innate immune response

Pattern recognition receptors (PRRs) of the innate immune system provide the organism with an intrinsic mechanism to distinguish self from non-self antigens and defend against invading microbes and viruses.[5,6] These receptors recognize different classes of pathogens, as well as endogenous molecules released from, or expressed by, damaged tissues. Thus, PRRs could be involved in both infection-induced injury and inflammatory responses that

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result from hypoxia–ischaemia or neonatal stroke. To date, the Toll-like receptor (TLR) family has been the most studied PRR type in the developing brain.

In the neonatal mouse, *TLR1-9* mRNAs are constitutively expressed in the forebrain,[7] and several *TLR* genes are expressed in the choroid plexus.[8] *TLR2* expression is relatively low before birth.[7,9] TLR3 and TLR8 have been suggested to regulate embryonic brain development, given that artificial upregulation of these molecules inhibits neurite outgrowth[10] and reduces cell proliferation *in vitro*.[9]

Glial TLR3 protein expression is increased in infants with periventricular white matter injury, suggesting that abnormal TLR3 expression influences developmental processes in the immature brain.[11] Moreover, in rodents, stimulation of TLRs during pregnancy or in the neonatal period results in robust inflammatory responses in the fetal and newborn brain, including marked microglial proliferation[12] and increased cytokine expression.[13,14] Prolonged periods of TLR stimulation result in notable blood–brain barrier (BBB) changes,[15] and infiltration of peripheral immune cells can further contribute to inflammation in the brain.

The choroid plexus—the site of the blood–cerebrospinal fluid (CSF) barrier in the brain—has been considered as a possible access route for peripheral immune signals and cells into the CNS. In neonatal mice, immune stimulation via administration of specific TLR ligands altered the expression of mRNAs encoding choroid plexus TLRs and the tight junction protein occludin.[8] In addition, the TLR2 ligand Pam_3CSK_4 induced the transcription of the tumour necrosis factor (*TNF*) gene, and also dramatically increased leukocyte diapedesis into the CSF.

Convincing data from several different species and experimental conditions show that activation of TLR3 or TLR4 in mid to late pregnancy, or during the early neonatal period, results in brain injury similar to that seen in human infants, including loss of myelin, astrogliosis and microgliosis, and disruption of thalamocortical function (Table 1). Other TLRs in the immature brain have been less well studied, but existing data suggest that TLR2 activation has detrimental effects on grey and white matter (Table 1).

Hypoxia–ischaemia and stroke at term

Experimental studies

Hypoxia–ischaemia triggers inflammatory processes that can continue for several weeks after the initial insult. The initial phase of inflammation (Figures 2 and 3) targets the region of injury, combats invading microbes and limits infectious processes to benefit the host, albeit at the cost of ‘bystander’ brain injury.[16] Several studies have shown that interventions that attenuate the early inflammatory phase confer neuroprotection (Table 2). After this early proinflammatory phase, the immune system shifts to favour an anti-inflammatory response, which is followed by a repair phase (Figure 2).

Hypoxia–ischaemia induces rapid activation of microglia and mast cells in the rodent brain.[17,18] During early reperfusion, neutrophils accumulate in post-capillary venules, and myeloid cells, T cells and natural killer cells infiltrate injured areas of the brain during the

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delayed recovery phase.[19–21] For example, in *Lys*-EGFP-*ki* mice,[22] a transgenic strain that enables the study of neuroinflammation, myeloid cell infiltration peaked at 24 h after hypoxia–ischaemia, and the infiltrating cells consisted predominantly of monocytes ($CD11b^{+}EGFP^{+}Gr1^{lo/-}$ $Ly6C^{int/hi}$); granulocytes accounted for about 10% of infiltrating cells. By contrast, in a neonatal stroke model, neutrophil infiltration was negligible.[23] Consistent with these findings, the BBB was found to undergo transient opening at 6–24 h after hypoxia–ischaemia,[24] whereas in the neonatal stroke model, the opening of the BBB was restricted.[23]

Both intrinsic and infiltrating cells produce proinflammatory cytokines and chemokines (Figures 2 and 3).[18–20] These cells also produce ROS, release excitatory amino acid agonists, and death receptor agonists including TNF, FasL, RANKL, TRAIL and TWEAK,[25] which could further contribute to cell death (Figure 3).

The initial inflammatory response is thought to depend on activation of innate immune receptors. TLRs are induced during recovery from neonatal hypoxia–ischaemia, and knocking out *Tlr2* in mice provides neuro protection.[7] In contrast to findings from TLR knockout mice, the majority of studies have found that deletion of two TLR adaptor proteins, MyD88 and TRIF, does not confer neuroprotection in neonatal hypoxia–ischaemia.[26,27] Indirect evidence from rodents suggests that NOD-like receptors that activate the inflammasome are important in neonatal injury. For example, hypoxia–ischaemia increases IL-1 production,[28] and administration of the IL-1 receptor antagonist IL-1ra ameliorates damage induced by hypoxia–ischaemia alone[28] or together with lipopolysaccharide (LPS) challenge.[29] Moreover, neonatal mice with a homozygous deletion of caspase-1[30] or IL-18[31] are resistant to hypoxia–ischaemia (Table 2).

The specific contributions of the different cell types to brain inflammation and cell death mechanisms in the immature brain continue to be the focus of many investigations. Below, we provide a summary of the findings to date.

Neutrophils—Depletion of neutrophils prior to hypoxia–ischaemia reduces injury, indicating that these cells contribute to injury progression, at least during (or just after) hypoxia–ischaemia. By contrast, neutrophil-targeted treatment after hypoxia–ischaemia is ineffective.[19] Neither genetic nor pharmacological inhibition of NADPH—which is required for phagocytosis by neutrophils— confers neuro protection in neonatal mice,[32] and modification of chemokine–neutrophil signalling, a pathway that protects against stroke in adult rats, exacerbates injury after neonatal focal stroke.[23] *Microglia* The pathophysiological role of microglia continues to be debated. Both hypoxia–ischaemia and neonatal stroke result in notable microglial activation in the neonatal brain.[17,33] The classic view—that these cells exert toxic effects, at least in the initial phase after hypoxia–ischaemia—is supported by several findings. First, IL-18 is produced preferentially by activated microglia, and genetic deletion of IL-18 confers protection.[31] Second, minocycline reduces the microglial response and reduces injury.[34] Last, caspase-1 is predominantly expressed in microglia after hypoxia–ischaemia, and genetic deletion of caspase-1 attenuates brain injury.[30] It should be noted, however, that none of the above-described interventions target microglia selectively. In the neonatal stroke model, microglial activation seems to

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predominate over extrinsic recruitment of monocytes. [33] Pharmacological depletion of microglia prior to neonatal stroke aggravates rather than improves outcome, and exacerbates the release of inflammatory cytokines, suggesting that at least a subpopulation of microglia have beneficial effects.[35]

Microglial phagocytosis of debris has been suggested to be critical for tissue recovery during the delayed phase after neonatal stroke;[35] this hypothesis is further supported by the finding that CD36 scavenger receptor deletion worsens injury in the neonatal mice.[36] The divergent results could relate to differing microglial phenotypes: depending on their phagocytic activity, some microglia might participate in acute early proinflammatory responses and aggravate injury, whereas others might be involved in the late anti-inflammatory responses and protect against injury (Figure 2). Microglia might assume distinct functional phenotypes during recovery from hypoxia–ischaemia, similar to the M1, M2a and M2b phenotypes suggested in other models.[37,38]

Mast cells—Despite a limited understanding of the contribution of mast cells to normal brain development, their involvement in several aspects of brain injury has now been established.[39,40] Data from experimental models of cerebral ischaemia and trauma have implicated mast cells as early contributors to BBB dysfunction, oedema and haemorrhage in the adult brain.[39] The immature brain features higher number of mast cells than does the adult brain, suggesting that mast cells might have an even more important role in the response to injury and/or inflammation in the neonatal brain.

Activated mast cells contribute to excitotoxic injury in neonatal mice by exacerbating transforming growth factor β1 (TGF-β1) toxicity, potentially via a histaminergic mechanism.[41] In neonatal rats, mast cells have been identified as the first responders to hypoxic–ischaemic brain damage by undergoing early degranulation and releasing TNF, and migration and/or proliferation of mast cells in the pia and infarct area remains enhanced for days to weeks.[18,42] Acute treatment with the mast cell stabilizer sodium cromoglycate prevents early mast cell activation and degranulation in this model, commensurate with markedly improved neuroprotection.[18]

Mast cell activation in response to neonatal stroke has been independently confirmed in the neonatal transient stroke model.[43] Mast cells seem to promote inflammation acutely after injury in the neonatal brain, but their contributions to the ongoing evolution of damage and reparative processes have not yet been studied.

Adaptive immune response—Neonatal hypoxia–ischaemia leads to central and peripheral activation of $CD11b^+$ and $CD11c^+$ antigen-presenting cells and the costimulatory molecules CD86 and MHC-II. This co-activation indicates active antigen presentation in the damaged hemisphere and in the spleen.[21] Infiltrating antigen-presenting cells and T lymphocytes have been observed in the damaged cerebral hemisphere up to 7 months after the initial insult (Figure 2).[21]

The site of the T-cell trafficking into the brain in hypoxia–ischaemia has not been established, but recent studies suggests that leukocyte entry via the choroid plexus is an

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important mechanism for resolution of CNS inflammation,[44] and that the infiltration augments recruitment of anti-inflammatory monocyte-derived macrophages.[45] Administration of the TLR2 ligand Pam_3CSK_4 to mice at postnatal day 8 (P8) results in a dramatic increase in leukocyte numbers in the CSF, presumably owing to entry the choroid plexus.[8] Furthermore, the choroid plexus has been recently recognized as an important immunological compartment, enriched with CNS-specific $CD4^+$ T cells.

Further support for the notion that T cells have an important role in ischaemic brain injury comes from the finding that adult lymphocyte-deficient mice are protected against ischaemic injury.[46] Stroke studies in chimaeric mice have shown that this protection is attributable to T cells rather than B cells.[47]

Early-life LPS exposure influences the generation of neuroprotective regulatory T cells[48] and modifies the inflammatory responses to autoimmune disease in mice.[49] Maternal polyinosinic:polycytidylic acid (Poly[I:C]) exposure alters adaptive immunity in offspring by priming their T cells towards a T helper 17 (T_H17) phenotype.[50] The inflammatory responses following activation of T_H1 cells are also toxic to premyelinating oligodendrocytes,[51] suggesting that αβ T cells can have a role in the pathogenesis of injury to the immature brain.

In summary, adaptive immune cells are detected in the immature brain after hypoxia–ischaemia in the delayed and tertiary phases, although their roles are not yet elucidated. Recent data, however, indicate that these cells can have toxic effects as well as being important for resolution of inflammation.

Synergy between TLRs and hypoxia–ischaemia—In addition to a direct effect on brain development, TLR activation makes the immature brain more susceptible to insults (Figure 1). For example, the LPS-induced increase in vulnerability to neonatal hypoxia–ischaemia[52] is dependent on TLR4, which functions as a sensor of LPS, and MyD88, an adapter protein used by most TLRs to activate nuclear factor-κB (NF-κB; Figure 4).[26,53]

Although *MyD88* knockout and wild-type mice show similar numbers of microglia after LPS insult, the cytokine–chemokine response to LPS is blunted in the transgenic mice, suggesting that the activation state of the microglia, rather than their absolute number, is the main factor affecting the inflammatory response to LPS.[26]

Anticytokine therapy can curtail TLR-dependent vulnerability to injury; for example, blockade of the TNF cluster[54] and treatment with IL-1ra[28] or anti-NF-κB peptides[55] all reduce brain injury (for mechanisms, see Figure 4). Similarly, we have demonstrated that Poly(I:C) increases the vulnerability of the immature brain to hypoxia–ischaemia in a TRIF-dependent manner.[27] Interestingly, this effect is associated not with a change in proinflammatory $CD86^+$ cells but, rather, a decrease in reparative $CD206^+$ immune cells (Figure 4).[27]

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Clinical studies

Neonatal encephalopathy and chorioamnionitis—Despite abundant preclinical evidence supporting a role for inflammation in neonatal encephalopathy and neonatal stroke at term, these findings await full validation in clinical studies.[56] Neonatal encephalopathy is accompanied by elevation of IL-6, IL-8 and IL-1β in the CSF,[57] and raised levels of these cytokines in both CSF and blood[58] are associated with adverse neurological outcome. Furthermore, increased levels of several cytokines in the neonatal blood at term correlate strongly with the likelihood of cerebral palsy.[59] It is unclear, however, whether inflammation is a result or a cause of the encephalopathy and neurodevelopmental sequelae. Distinct age-related susceptibility to injury of particular cell populations and mechanisms controlling local inflammation and immune cell infiltration might contribute to the development of the causal pathways. Indeed, nonclassical pathways of complement activation are not fully developed in term infants, and are likely to exert important age-dependent effects on the inflammatory response.[60]

Some human studies have shed light on the role of prior infection in subsequent brain damage, such as that seen in cerebral palsy. In a case–control study from the Kaiser Permanente Medical Care Program, a chart review of children with moderate to severe spastic or dyskinetic cerebral palsy evaluated the association between clinical chorioamnionitis and risk of cerebral palsy. The study found that chorioamnionitis or placental infection conferred a fourfold overall increased risk of cerebral palsy in term infants.[61] Among singleton births, the population attributable fraction of chorioamnionitis for cerebral palsy was 11%, and was even higher (27%) for spastic quadriplegia. Multiple logistic regression analyses identified chorioamnionitis, intrauterine growth restriction, maternal black ethnicity, and maternal age >25 years as independent risk factors for cerebral palsy.

In a recent study of term babies with neonatal encephalopathy who had signs of maternal infection (chorioamnionitis) or infant infection (sepsis), a clear dichotomy in outcomes depending on the source of infection was observed.[62] Neonates exposed to chorioamnionitis had a reduced risk of brain injury and adverse outcomes, whereas newborns with sepsis had an elevated risk of predominantly watershed injury, as detected with MRI.[62] The better outcomes among neonates exposed to chorioamnionitis might be explained by the timing of the prenatal infection, which could have preconditioned the developing brain against subsequent injury induced by hypoxia–ischaemia, as has been reported in animal studies.[63] However, the exact mechanisms underlying the protective effect remain unclear. In addition to the studies of placental infection, a recent study revealed that inflammation on the fetal side of the placenta was associated with elevated maternal IL-6 and IL-8 at delivery and fetal IL-1β, IL-6, IL-8, and TNF in the umbilical cord at birth, as well as worse neurological outcome at 6 months.[64]

Therapeutic hypothermia and inflammation—The advent of therapeutic hypothermia has brought improvements in clinical outcomes for babies with neo natal encephalopathy. Hypothermia affects several physiological parameters, one of which is the inflammatory response. In one small study from Japan, neonates treated with hypothermia had lower blood

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levels of high mobility group box 1 (HMGB1) than did untreated encephalopathic babies. HMGB1 is a DNA-binding protein that regulates transcription of genes encoding a number of inflammatory cytokines.[65]

In a study evaluating biomarkers of injury in encephalopathic newborns, some of whom underwent therapeutic hypothermia, elevated glial fibrillary acidic protein, IL-1, IL-6, IL-8, TNF, interferon and vascular endothelial growth factor (VEGF) levels at 6–24 h were associated with abnormal neurological outcomes, although the number of participants was limited in the outcome group owing to low sample size.[66] Serial measurements revealed that the levels of these molecules were not affected by hypothermia. Another study compared circulating leukocytes and serum chemokines between infants under therapeutic hypothermia and a normothermic group. In the hypothermia group, total white blood cells and certain subclasses of leukocytes were markedly depleted, and levels of chemokines, CCL2 and IL-8 were correlated negatively with leukocyte count.[67] These data indicate that hypothermia is immunosuppressive, which can be hazardous if neonatal sepsis occurs while the infant is under therapeutic hypothermia.

Stroke—With regard to perinatal arterial ischaemic stroke, the evidence is mixed. In a population-based, case–control study from Kaiser Permanente, 13 infants with stroke were identified, along with 86 randomly selected controls. [68] Several polymorphisms were tested, including variation in *IL6*, *LTA*, *TNF*, Leiden variant of coagulation factor V (*F5*), coagulation factor II *(F7)* and *MTRR*, and apolipoprotein E (*APOE*) alleles ε2 and ε4. Proinflammatory polymorphisms were not associated with stroke, whereas *APOE**ε4* was seen more often in infants with stroke than in controls. This study substantiated an earlier hospital-based cohort study of 49 newborns with stroke, in which 31 polymorphisms, including those representing pathways of inflammation, thrombosis, vascular tone and cellular adhesion, were evaluated.[69] In this study, no variant allele was found to be significantly more common in the stroke cohort than in the general population.

In a study evaluating risk factors for perinatal stroke in full-term infants, multivariate analysis revealed that risk factors independently associated with stroke included prolonged rupture of membranes (OR 3.8, 95% CI 1.1–12.8), chorioamnionitis (OR 3.4, 95% CI 1.1–10.5), and history of infertility and pre-eclampsia.[70] The risk of perinatal stroke increased dramatically when multiple risk factors were present. A more recent study from the Netherlands substantiated these findings in 52 infants: multivariate analysis indicated increased risk associated with maternal fever (OR 10.2, 95% CI 1.3–78.5) and early-onset sepsis and/or meningitis (OR 5.8, 95% CI 1.1–31.9).[71] Other risk factors were low Apgar score at 5 min, and hypoglycaemia, again suggesting multifactorial vulnerability.

Translational therapies—Other important aspects of the inflammatory response must be considered before therapies can be developed. Sex could be an important factor, as the response to therapy with 2-iminobiotin, an antioxidative and anti-inflammatory agent that acts as an inducible nitric oxide synthase inhibitor, protects female but not male neonates against hypoxia–ischaemia in a rat model, suggesting a sex-specific mechanism of protection.[72] In addition, intrinsic developmental differences in BBB basement membrane and extracellular matrix formation could contribute to the maintenance of barrier integrity

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after acute neonatal arterial stroke.[23] Taken together, these data suggest the need for a modified approach to any translational therapy, in which aspects such as sex and BBB permeability are taken into consideration. Clinical studies are needed to determine the best therapeutic approaches for term newborns with encephalopathy and stroke.

Inflammation and the preterm brain

Experimental models of preterm brain injury

Numerous studies have assessed the effects of microglial activation as a response to experimental infection or inflammation challenges, including infectious agents (*Escherichia coli*, *Ureaplasma parvum*, respiratory syncytial virus and cytomegalovirus), sterile inflammatory insults (TLR2, TLR3 or TLR4 agonists, IL-1β and IL-6), experimentally induced hypoxia–ischaemia, chronic hypoxia, or excitotoxic insults. These challenges have been tested across a large array of species (sheep, rabbit, rat, mouse, rat, piglet and guinea pig) to mimic exposures during various stages of pregnancy and neonatal life,[3,73] and the insults resulting from these challenges have been consistently shown to lead to white matter damage, often defined as myelin protein deficits. These deficits are accompanied by increased density of microglia and macrophages,[17,74–77] collectively pointing to a probable role for microglial activation in the pathophysiology of white matter damage (Figure 5 and Table 1).

Panels of M1–M2a–M2b markers in microglia have been validated in well-characterized *in vitro* conditions, [38,78] providing candidate molecules for involvement in progressive and temporal microglial activation that can be tested in future *in vivo* studies. Until recently, most experimental studies in the perinatal brain only evaluated the number and morphology of microglia at a small number of time points (acute, or acute and ‘long-term’), without assessing markers of differential activation and/or function over a protracted period of time. Timing is a key parameter that determines the predominant microglial phenotype and impact on lesion progression, as recently shown in adult mouse models of stroke,[79] brain trauma,[80] spinal cord injury and multiple sclerosis.[81] Microglial phenotype clearly depends on the type of injury, location (grey versus white matter), and temporal profile of toxic proinflammatory cytokines, and pro-repair and anti-inflammatory cytokines with neuroprotective properties.

Endogenous stem cells—Injuries that elicit neuroinflammation in the brain alter the composition of the pools of neural precursors in both the subventricular zone (SVZ) and the hippocampal subgranular zone (SGZ). In neonatal rats and mice, hypoxia–ischaemia stimulates an increase in the proliferation of SVZ neural precursors that is followed by an increase in the production of neurons and glia, which then also migrate towards regions of injury.[82,83] The mechanisms regulating this expansion of the neural precursor pool remain incompletely understood, although self-renewal, proliferation and fate specification of neural precursors are heavily influenced by the cytokines that are produced after injury (Table 3). For example, IL-6, which is markedly upregulated in developmental brain injury, enhances the growth, self-renewal and tripotentiality of neural precursors in the SVZ *in vitro*.[84,85]

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IL-6 induces expression of cyclooxygenase 2 (COX-2) and, thereby, the production of prostaglandins, such as prostaglandin E2. The effects of prostaglandin E2 on neural precursors were evaluated, and this compound was found to increase neural precursor expansion in the SVZ.[84] By contrast, indomethacin, which inhibits COX-2, decreased the initial reactive increase in neural precursors in SVZ after hypoxia–ischaemia. Indomethacin reduced the numbers of reactive microglia within and surrounding the SVZ, and reduced IL-6 production after hypoxia–ischaemia.[84] These data strongly implicate neuroinflammation— in particular, involvement of IL-6—in the increased expansion of primitive neural precursors in the SVZ after neonatal brain injury.

Whereas SVZ neural precursors proliferate in response to injury, the opposite effect is often observed for the neural precursors in the SGZ: decreased neurogenesis in the hippocampus has been reported in murine LPS and Poly(I:C) models of prenatal inflammation. In a recently published study, LPS was administered to neonatal mice at P5, and its effects on microglio genesis, inflammation and neurogenesis in the developing hippo campus were examined.[12] LPS administration acutely, but transiently, increased the proliferation of resident microglia, leading to an increase in numbers of both M1 and M2 type micro glia. Neonatal LPS exposure did not lead to recruitment of peripheral monocyte-derived macrophages to the hippo campus. Microglial cell accumulation was followed by transient inhibition of hippocampal neuronal differentiation, due to specific effects on the type 3 neural precursors, that persisted for 2 weeks after LPS administration.

Oligodendroglial development—Postmortem studies of infants with white matter damage demonstrate that late oligodendrocyte progenitor cells (OPCs) are extremely vulnerable to injury.[86] Indeed, the age of highest incidence of white matter damage in the premature infant directly correlates with a predominance of late OPCs in the immature brain.

Hypomyelination can eventually be observed as the brain matures. Mechanisms implicated in hypomyelination include death of OPCs,[87] oligodendrocyte maturation blockade without notable cell death,[88] and depletion of the pool of proliferating OPCs.[87] In animals subjected to infectious, inflammatory, hypoxic, hypoxic–ischaemic or excitotoxic insults during the perinatal period, myelination deficits are seen in association with neuroinflammation,[75–77,89–91] suggesting a causal link similar to that seen in human preterm infants.

Neurons are classically regarded as the principal source of glutamate in the brain, but a number of studies have shown that microglia, when activated by proinflammatory stimuli, release substantial amounts of glutamate.[92] Several intrinsic properties of OPCs, such as high intracellular iron levels[93] and low superoxide dismutases levels,[94] render these cells vulnerable to injury mediated by excess glutamate. Multiple lines of *in vitro* and *in vivo* evidence support the hypothesis that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or kainate-type glutamate receptors are primarily responsible for glutamate-mediated death of OPCs.[95] Like immature oligodendrocytes, which are sensitive to high concentrations of glutamate, the late OPCs are extremely vulnerable to kainate-receptor-mediated and AMPA-receptor-mediated excitotoxic death.[96]

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The susceptibility of the brain to white matter lesions after intracerebral injections of AMPA into the pericallosal white matter seems to be age-dependent,[94] which is in line with the preferential expression of calcium-permeable AMPA receptors by late OPCs.[97] These data have led to the view that the late OPCs are intrinsically vulnerable to injury. In contrast to the classic *N*-methyl-d-aspartate-receptor-mediated excitotoxic death of neurons that occurs within a few hours, oligodendroglial death after hypoxia–ischaemia in the perinatal brain occurs over 24–48 h.[95,96] Glutamate has been demonstrated to activate AMPA and kainate receptors to increase Ca^{2+} influx in late OPCs, resulting in Bax trans location to the mitochondria, cytochrome *c* release, activation of caspase-9 and caspase-3, nuclear fragmentation, and cell death.[98,99]

TNF was one of the first cytokines to be shown to be elevated in premature infants with white matter injury,[100] and is consistently found to be strongly upregulated in the neonatal CNS after injury. As with glutamate, TNF seems to exert differential effects depending on the stage of development. TNF is not toxic for OPCs; in fact, it stimulates their proliferation by acting on TNF receptor 2 (TNFR2).[101] By contrast, as oligodendroglial cells mature, they become more sensitive to the toxic effects of TNF, which are mediated by TNFR1.[102] Like glutamate-mediated OPC cytotoxicity, TNF-mediated OPC death occurs in a dose-dependent manner that requires Bax translocation from the cytosol to the mitochondria.[103]

In an animal model of systemic inflammation induced by daily intraperitoneal injections of IL-1β during the first 5 days of life, the number of unmyelinated axons was increased and the number of large myelinated fibres was decreased.[77] The myelination impairment was associated with reduced fractional anisotropy on MRI and a number of behavioural deficits.

Cytokines of the IL-6 family have potent effects on OPCs. An *in vitro* study demonstrated that IL-6 could induce cell cycle withdrawal and maturation of OPCs.[104] Elevated levels of IL-6, therefore, could contribute to depletion of the pool of proliferating OPCs and premature maturation of the existing precursors into myelinating oligodendrocytes, thereby contributing to dysmyelination. Genetically engineered mice that over-expressed IL-6 developed severe neurological symptoms that include ataxia, tremor, seizures, and severe astrogliosis and microgliosis.[105] The white matter was not analysed in these mice.

Neuronal migration and survival—Until recently, migration of cortical neurons in the human brain was thought to be complete by 24–26 weeks of gestation. However, a 2011 study demonstrated that migration of interneurons to the neo-cortex is not complete until term,[106] suggesting that events coinciding with preterm delivery could affect this migration. Indeed, these migrating neurons might be influenced by factors released by neighbouring activated microglia, potentially leading to neuronal death or aberrant migration.[107]

Synaptogenesis—Chronic hypoxia during the neonatal period has been shown to reduce brain size in mice, largely due to decreased cortical volumes.[108] A transcriptome analysis of a mouse model of sublethal postnatal hypoxia demonstrated that hypoxia suppresses the expression of genes involved in synaptic maturation, postsynaptic function and neurotransmission.[109] Microglia have critical developmental roles in axonal growth and pruning, and in synaptic pruning and function; future studies must determine whether

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neuroinflammation and microglial activation have deleterious effects on such key events for brain connectivity and function.

Epigenetic changes—Epigenetic changes are crucial for every aspect of normal development and brain function. Epigenetic modifications include enzymatic regulation of transcription through modification of permissive tags (acetylation, methylation, ubiquitination, phosphorylation and sumoylation) on histones or DNA, or microRNA-mediated regulation of translation. In humans, acetylation regulates transcription of up to 5% of the genome, and a notable proportion of human genes can be regulated by at least one microRNA.[110] Epigenetic modifications are an essential mechanism by which injury and destructive prenatal environmental factors can lead to long-term disturbances of brain development, including cognitive, motor and behavioural impairments.[111]

Changes in epigenetic marks and microRNA expression could have key roles in the long-term consequences of perinatal brain insults, such as the failure of OPCs to adequately mature and differentiate. In addition, if these epigenetic changes occur in inflammatory cells such as microglia, they could represent an innate immune cell memory that would alter microglial function for months or years after the perinatal insult.[112] As such, epigenetic modifications are emerging as novel and promising targets for neuroprotection (Table 2).

Clinical evidence

Abnormal brain development and brain damage might result from infection *in utero* or perinatally, or from inflammation triggered by a variety of causes (including ischaemic insults). A large study from Switzerland reported that neonatal sepsis substantially contributed to neurodevelopmental impairment in extremely preterm infants, independently of other risk factors.[113]

Chorioamnionitis can lead to a fetal inflammatory response and white matter damage.[114] Systematic reviews[115,116] suggested a link between chorioamnionitis and cystic periventricular leukomalacia and cerebral palsy; although this association was later disputed, more-recent studies have substantiated the initial association. [113] The confusion resulted primarily from the inability to define chorioamnionitis and to document outcome with adequate quantitative MRI techniques coupled with standardized follow-up assessments.

In the absence of infection, intermittent or sustained systemic inflammation might be more detrimental to the brain than is inflammation of shorter duration (such as is seen in infectious diseases).[117] This hypothesis emphasizes the importance of ‘systemic’ inflammation affecting the brain—a process that has been documented in a mouse model of perinatal white matter damage,[77] in which systemic inflammation blocks oligodendrocyte maturation, resulting in persistent myelination defects. Similarly, in a rat model of neonatal stroke,[33] cytokine and chemokine levels are initially raised in plasma, and subsequently in the brain. This phenomenon might explain the secondary damage that accompanies stroke in the newborn.

The concept of sustained damage also embraces the theory of tertiary brain damage, according to which children born prematurely have elevated levels of inflammatory

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mediators in their plasma long after birth.[118] The inflammation might persist through TLR-mediated signalling (as discussed above; Table 1), which is developmentally regulated. Epigenetic mechanisms might also contribute to this protracted event by shifting the balance between proinflammatory and anti-inflammatory gene regulation.[112]

Immunomodulatory therapies

A variety of drugs targeting neuroinflammation have been tested in animal models of perinatal brain injury (Table 2). The magnitude of neuroprotection observed in these studies has been quite variable, and sometimes the results are inconsistent between animal models and research cohorts (as seen, for example, in the case of minocycline). However, several compounds (for example, corticosteroids, melatonin, erythropoietin, COX inhibitors, cromolyn, histamine receptor blockers, *N*-acetylcysteine, etanercept, IL-1ra, simvastatin, and certain histone deacetylase inhibitors) that are already in clinical use for other indications have shown promising neuroprotective properties. In addition, innate defence regulatory peptides are currently being tested in clinical trials for other indications.

Two major issues must be taken into account during the investigation of potential immunomodulatory therapies for use in neonates. First, it is not known whether all the candidate drugs can cross the BBB, and additional testing in relevant *in vivo* models will be necessary to address this key point. Second, neonates at risk of developing brain damage are generally fragile, and their brains are undergoing major developmental changes that will determine the long-term cognitive and motor outcome. Consequently, the safety of compounds to be tested in neonates cannot be directly extrapolated from studies performed in adults or older children.

Most of the drugs tested, with the exception of TNF soluble receptor, IL-1ra and cromolyn, have multiple or even pleiotropic effects that go beyond pure anti-inflammatory effects, including antioxidant and antiapoptotic properties, neuronal activity modulation, mitochondrial protection, and induction of angiogenesis. Therefore, it is difficult to definitively link the neuroprotective properties of these drugs with their anti-inflammatory properties.

Most of the drugs that have been tested in the context of perinatal neuroinflammation have been assessed in a limited number of rodent models, without validation in gyrencephalic animals. The two notable exceptions are melatonin and erythropoietin, which are being tested in clinical trials in preterm infants, and in conjunction with hypothermia in term infants. A recent randomized trial based on a relatively small number of patients has shown that preterm infants exposed to erythropoietin to reduce transfusion needs had a better cognitive outcome than infants exposed to placebo.[119] In addition, in an analysis of secondary outcomes of a large randomized clinical trial, exposure of preterm infants to high dose of erythropoietin was associated with reduced brain damage on MRI.[120] While awaiting the complete results of these promising trials, present and future research will aim to define more-targeted approaches incorporating the multiple phenotypes of inflammatory cells, leading to modulatory therapies rather than indiscriminate anti-inflammatory strategies.

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Conclusions

Perinatal brain injury and developmental abnormalities can be caused by a number of conditions—for example, neonatal encephalopathy, perinatal arterial ischaemic stroke, premature birth, and systemic infections—that can affect the developing brain during fetal life, birth, and the postnatal period. Even though these clinical conditions are very different with respect to aetiology and clinical context, inflammation seems to be an important contributor to the pathogenetic cascade.

Inflammation can both have a priming effect (sensitization or preconditioning) and participate in early or late injury, as well as in repair and recovery after the insult. A number of immunomodulatory interventions that target inflammation have proved effective in experimental models, and might have translational potential.

A great deal of information has been gathered over the past decade about the innate immune response after exposure to TLR agonists and during the early phases of injury, in particular after hypoxia–ischaemia and stroke, but our understanding of inflammation during the perinatal period is still incomplete. More research is urgently needed to understand the role of adaptive immunity, especially during the late stages of disease, and its potential causative role in cognitive impairments acquired by preterm infants. Furthermore, the long-term consequences of inflammation during early fetal and postnatal life, including the possible involvement of epigenetic regulation and T cells, remain to be elucidated. Finally, more work needs to be done to confirm preclinical findings in humans.

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Key points

- Perinatal brain injuries result from a spectrum of conditions, including neonatal encephalopathy, arterial ischaemic stroke, prematurity, and systemic infections
- Depending on the timing and context, inflammation can prime the brain for injury or exert protective actions
- Pattern recognition receptors, such as Toll-like receptors on innate immune cells (microglia, mast cells and macrophages), are important participants in the early phases of injury, and can increase CNS vulnerability (sensitization)
- Inflammation during preterm labour and intensive care of premature infants affects the very immature brain during critical phases of brain development, with serious consequences for myelination and cortical development
- Understanding the involvement of inflammation in perinatal brain injury can aid identification of new strategies for prevention and treatment that could reduce neurological and neuropsychiatric morbidities in maturing infants

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Review criteria

We searched PubMed for articles published in English from January 1950 to August 2014, with the terms “inflammation”, “mitochondria and brain”, “neonatal brain injury”, “preconditioning”, “sensitization”, “immune and neonatal brain”, “adaptive immunity and immature brain”, “innate immunity and immature brain”, “neonatal hypoxia–ischaemia”, “neonatal encephalopathy”, “neonatal neuroprotection”, “neonatal neuroinflammation”, “neonatal brain oxidative stress”, and “neonatal stroke”. We selected articles reporting clinical, experimental and preclinical findings relevant to understanding of the role of inflammation in brain development and brain injury.

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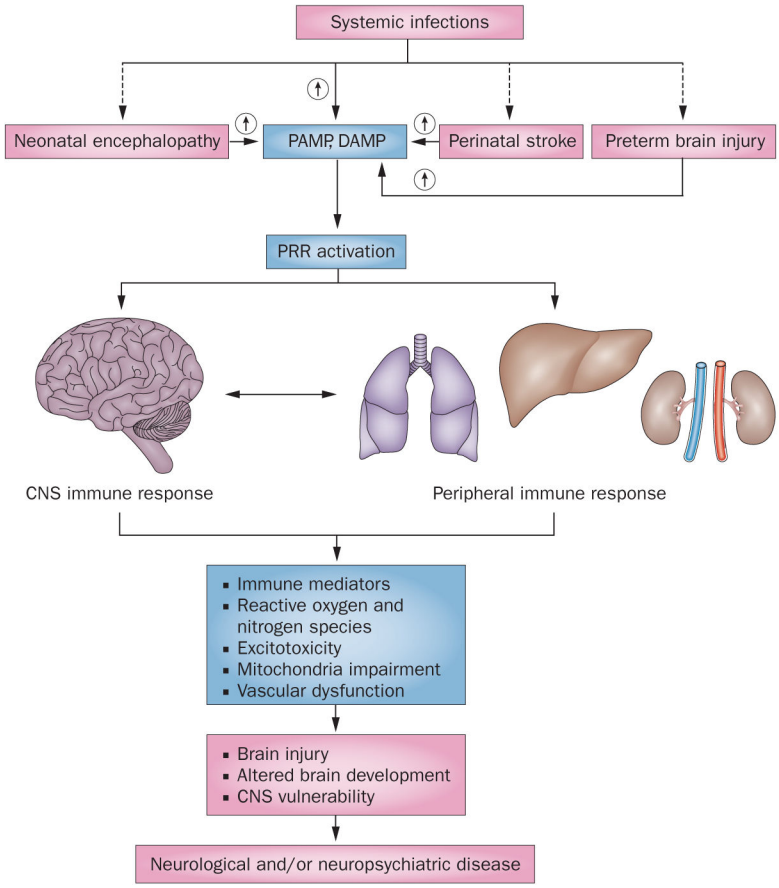

Figure 1.

Inflammation in the developing brain. Neonatal encephalopathy, perinatal stroke, preterm brain injury and systemic infections trigger release of PAMP and DAMP, which activate PRRs. Under some conditions, systemic infection can also be an antecedent of the other insults (dashed arrows). PRRs trigger inflammation in the periphery and in the brain. Inflammation can act in concert with hypoxia–ischaemia to induce activation of immune mediators, reactive oxygen and nitrogen species, excitotoxicity, mitochondrial impairment and vascular disruption. These effectors can cause brain injury directly, interfere with brain development and modulate CNS vulnerability, all of which may contribute to neurological and neuropsychiatric disease. Abbreviations: DAMP, damage-associated molecular pattern; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor.

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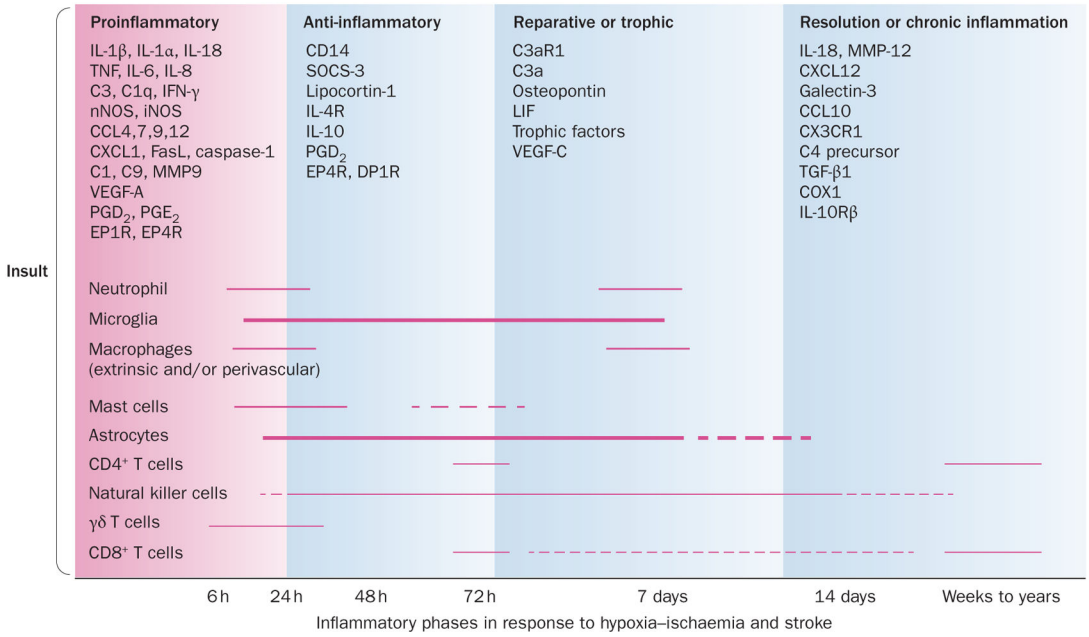

Figure 2.

Stages of inflammation in the immature brain after hypoxia–ischaemia and stroke. The hypoxic or ischaemic insult triggers a proinflammatory response followed by anti-inflammatory and reparative phases. These events result either in resolution of inflammation or in chronic inflammation. The critical phases of inflammation are regulated by multiple cytokines, chemokines, prostaglandins and other immune mediators, leading to activation and participation of inflammatory cells that are part of both innate and adaptive immune responses. The figure is a summary based on multiple experimental and clinical studies.[4,17,19,21,28,31–33,57–59,122,125] Abbreviations: C, complement; CD, cluster of differentiation; COX, cyclooxygenase; DPR, prostaglandin D receptor; EPR, prostaglandin E receptor; FasL, Fas ligand; iNOS, inducible NOS; LIF, leukemia inhibitory factor; MMP, matrix metalloproteinase; nNOS, neuronal NOS; NOS, nitric oxide synthase; PG, prostaglandin; SOCS, suppressor of cytokine signalling; TGF, transforming growth factor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor. Chemokines are abbreviated according to the new classification.

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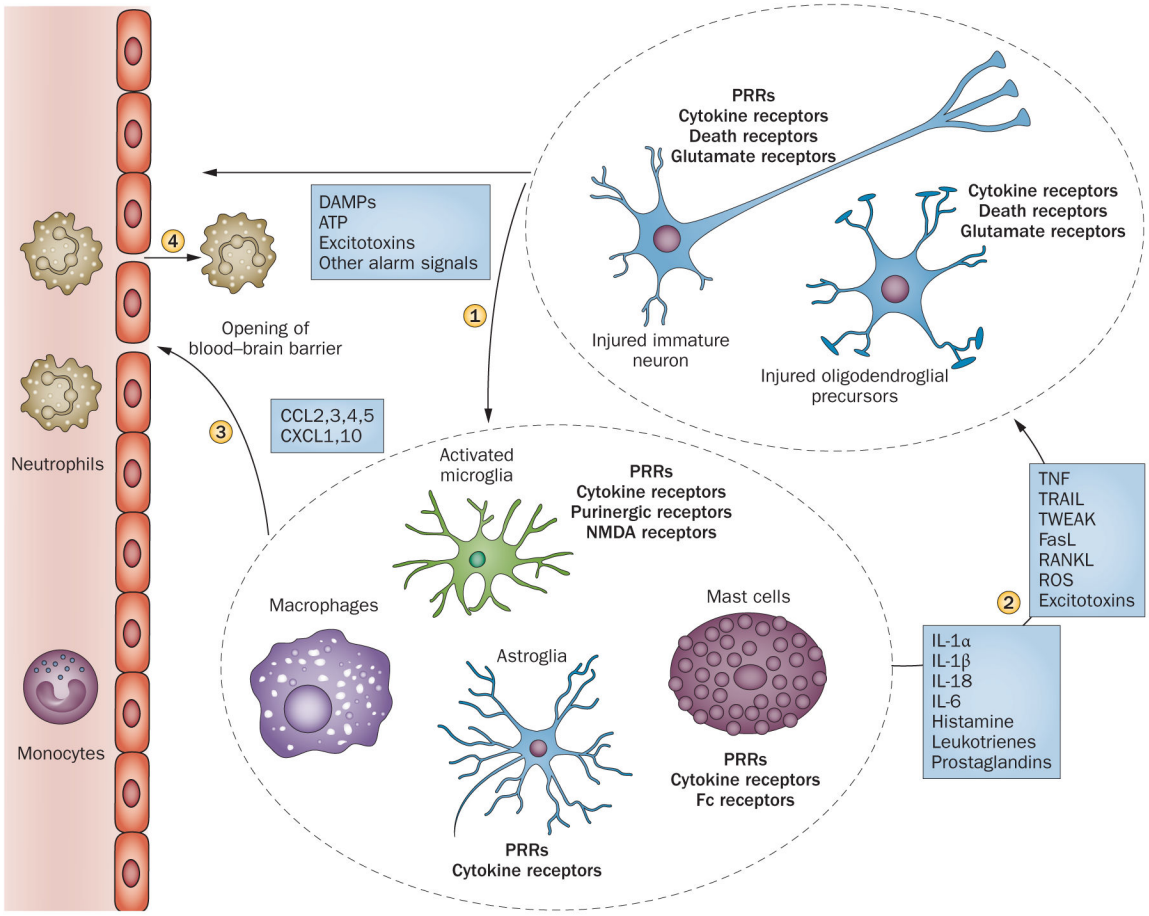

Figure 3.

Early innate response to hypoxia–ischaemia. Immune effector cells (microglia, macrophages, astroglia, mast cells) sense alarm signals from injured parenchymal cells via PRRs and cytokine receptors (1). The triggered innate immune response has proinflammatory and toxic influences on the neurons, oligodendroglial precursors (2) and vascular bed (3); increased blood–brain barrier permeability contributes to the recruitment of immune cells from the periphery (4). Abbreviations: DAMP, damage-associated molecular pattern; NMDA, *N*-methyl-D-aspartate; PRR, pattern recognition receptor; RANKL, receptor activator of nuclear factor-κB ligand; ROS, reactive oxygen species; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TWEAK, TNF-like weak inducer of apoptosis. Chemokines are abbreviated according to the new classification.

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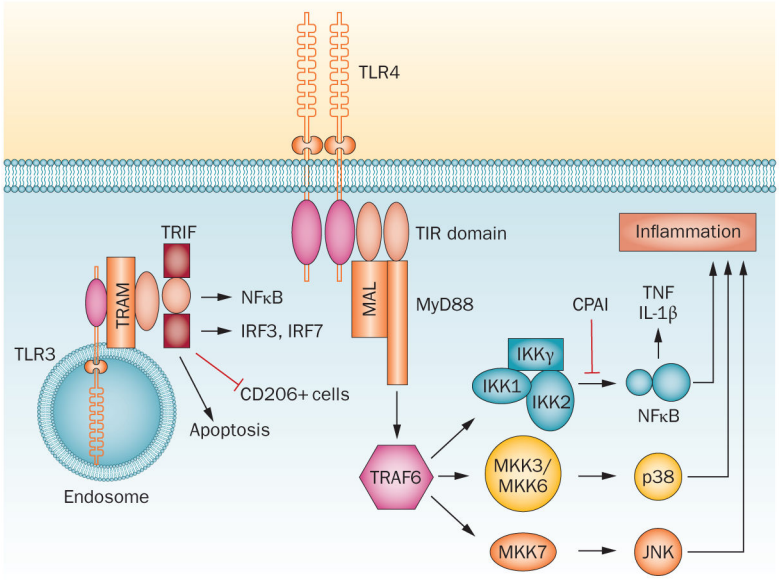

Figure 4.

Mechanisms of TLR4 and TLR3 sensitization. TLR4 increases vulnerability of the immature CNS through activation of the MyD88-dependent pathway, leading to NF-κB-dependent production of IL-1β and TNF and activation of JNK. Endosomal TLR3 induces sensitization through TRIF-dependent activation of NF-κB, IRF and apoptosis, and inhibition of potentially cytoprotective $CD206^{+}$ cells.[27] LPS and hypoxia–ischaemia induce proteolytic activity of tPA, but this can be blocked by CPAI, which reduces NF-κB signalling, microglial activation, and production of proinflammatory cytokines in the brain.[126] JNK inhibition also significantly reduces neuroinflammation, blood–brain barrier leakage and oligodendrocyte progenitor apoptosis[127] after LPS sensitization. Abbreviations: CPAI, plasminogen activator protein-1; IRF, interferon regulatory factor; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; Myd88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB; TLR, Toll-like receptor; TNF, tumour necrosis factor; tPA, tissue plasminogen activator; TRIF, TIR-domain-containing adapter-inducing IFN-β.

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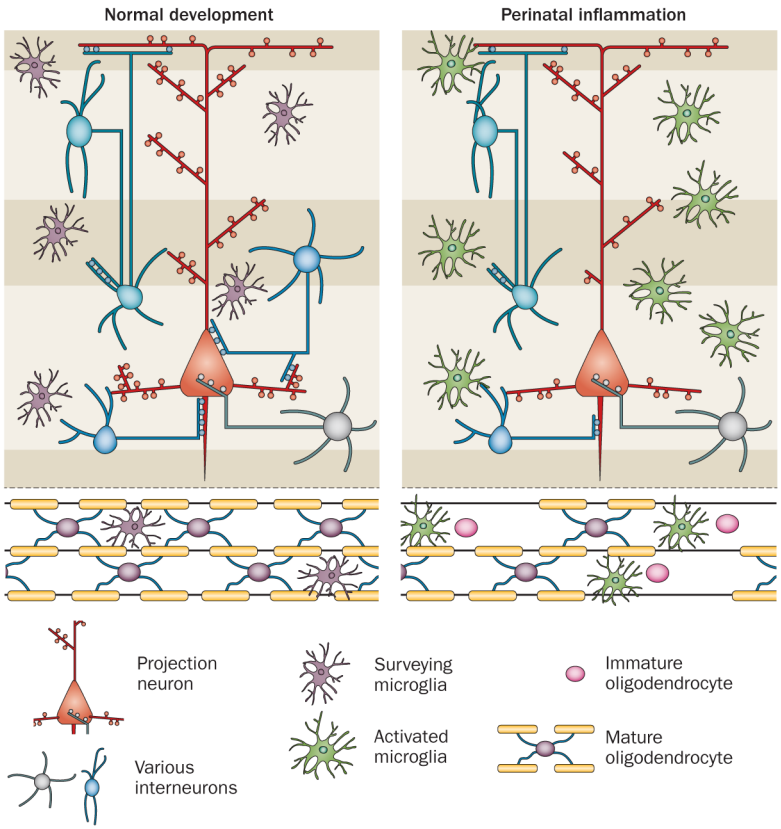

Figure 5.

Effects of perinatal inflammation on brain development. Infants born at extremely low gestational age have a markedly increased risk of brain dysfunction, which is attributable to damage and developmental impairment in both white and grey matter. In such situations, microglia become activated. The resulting CNS inflammation impairs oligodendrocyte precursor maturation, which leads to a myelination defect. Furthermore, the cerebral cortex and deep grey matter will be affected by impairments in interneuron survival, axonal integrity, neurite branching, spine density, and synaptogenesis, leading to abnormal connectivity and brain microstructure.

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Table 1

Effects of TLR2, TLR3 and TLR4 activation on the developing brain

Subjects	Target	Treatment	Effects
Mice, P3–11	TLR2	Pam_3CSK_4 5 mg/kg, i.p, daily injections	Elevated IL-1β, IL-6, CXCL1 and CCL2 in brain; transient decrease in grey and white matter volume Brain deficits resolved by P50[128]
Cultured cells (E9 chick, E14 mouse), P4 mice	TLR3	Poly(I:C) 20–100 μg/ml, (*in vitro*); Poly(I:C) 3 mg/kg intrathecally to mice	Growth cone collapse and irreversible inhibition of neurite extension in culture experiments; righting reflex and negative geotaxis impaired at P9 No neuropathological analysis of the forebrain[129]
Pregnant mice, E9 or E17	TLR3	Poly(I:C) 5 mg/kg, i.v.	Poly(I:C) at E9 impaired sensorimotor gating and reduced dopamine D1 receptors in adulthood Poly(I:C) at E17 impaired working memory, potentiated locomotor reaction, and reduced hippocampal *N*-methyl-D-aspartate receptor type 1 expression[130]
Pregnant spiny mice, E20	TLR3	Poly(I:C) 0.5 mg/kg, s.c.	Impairments in non-spatial memory and learning tasks and motor activity in off-spring at P100 Decreased reelin expression, increased GFAP expression and increased numbers of activated microglia, specifically in the hippocampus[131]
Pregnant SD rats, E10.5	TLR4	LPS 1 mg/kg, i.p.	TNF increased in mesencephalon in P21 offspring Substantia nigra volume and number of tyrosine hydroxylase-positive cells reduced[132]
Fetal sheep, 70% of gestation	TLR4	LPS 1 μg/kg, i.v. several doses over 5 days	Infrequent neural injury, but injury more common in cerebral white matter Corticospinal tract cross-sectional area reduced by 30% Very high LPS dose for fetal sheep[133]
Fetal sheep, 70% of gestation	TLR4	LPS 100 ng/kg, i.v., single bolus	Focal inflammation and cystic lesions in periventricular white matter in two of five animals, but with no neuron-specific injury Loss of astrocytes and oligodendrocytes in white matter[75]
Pregnant Wistar rats, E19 and E20	TLR4	LPS 0.3 mg/kg, i.p.	Increased IL-1β in brain at P1 Increased cell death, astrogliosis and hypomyelination at P7 Delays in neonatal behaviour; myelination and most motor deficits normalized by adulthood[76]
Pregnant Sprague–Dawley rats, E15	TLR4	LPS 0.1 mg/kg, i.p.	Reduction in complexity and spine numbers of cortical and hippocampal pyramidal neurons up to P60[134]
Mice, P3–P11	TLR4	LPS 0.3 mg/kg, i.p., daily	Grey matter volume, myelin basic protein, CNPase staining area and number of $Olig2^+$ cells decreased in white matter at P12[135]
Fetal sheep, 70% of gestation	TLR4	LPS 200 ng/kg, i.v., single bolus	Reduced white matter and cortical volumes shown by MRI and histology Maturation deficits in fetal EEG activity[89]
Pregnant rabbits, E28	TLR4	LPS 20 mg/kg, i.u.	PET and immunohistochemical findings indicate newborn rabbits from LPS-treated mothers to have reduced cortical 5-HT and disruption of 5-HT-regulated thalamocortical development[136]
Pregnant rats, E15	TLR4	LPS 0.25 mg/kg, i.p.	Reduced social preference and exploration behaviours in offspring; affected genes that regulate migration of GABAergic interneurons[137]
Pregnant mice, E17	TLR4	LPS 50 μg for each dam, i.u.	At P90–P100 disturbances in the circadian rhythm in the offspring, with longer time spent in non-rapid eye movement sleep states during the dark cycle compared with controls (measured by EEG and electromyography)[138]

Abbreviations: 5-HT, 5-hydroxytryptamine; CNPase, 2′,3′-cyclic nucleotide 3′ phosphodiesterase; E, embryonic day; GABA, γ-aminobutyric acid; GFAP, glial fibrillary acidic protein; i.p., intraperitoneal; i.u., intrauterine; i.v., intravenous; LPS, lipopolysaccharide; Olig2, oligodendrocyte lineage transcription factor 2; P, postnatal day; Poly(I:C), polyinosinic:polycytidylic acid; TLR, Toll-like receptor; TNF, tumour necrosis factor. Chemokines are abbreviated according to the international classification.

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Table 2

Immunomodulatory therapeutic possibilities for perinatal brain injury

Experimental insult and animal model	Intervention	Outcome
Corticosteroids (DEX)		
HI, P7 rats	DEX given between P1 and P3	Exacerbated brain damage, decreased glutamate reuptake; role of microglia not specifically addressed[90]
HI, P7 rats	DEX given 24 h and 4 h before the insult	Neuroprotection, increased brain VEGF production; role of microglia not specifically addressed[139]
HI + LPS, P7 rats	DEX given 24 h and 4 h before the insult	Neuroprotection and decreased CXCR4 receptor density[140,141]
Minocycline		
HI, P7 rats	Minocycline given either immediately before or after the insult	Marked neuroprotection; role of microglia not specifically addressed[34]
HI, P8 mice	Minocycline given either immediately before or 12 h before the insult	Exacerbated brain damage; role of microglia not specifically addressed[142]
Ibo, P5 mice	Repeated minocycline injections either before or after Ibo	Protection of GM and WM; reduced microglial density[143]
LPS (intracerebral), P5 rats	Minocycline given for 3 days, starting 12 h before insults	Protection, reduced microglial activation[144]
Hyperoxia, P6 rats	Minocycline given during exposure to hyperoxia	Protection, long-lasting reduction in microglial activation[145]
Melatonin		
Ibo, P5 mice	Melatonin administered immediately after Ibo	Reduced microglial density, beneficial effect on WM[146]
Experimentally induced stroke, P7 rats	Melatonin given as either a single dose before ischaemia or a double-dose regimen, combining one before ischaemia and one 24 h after reperfusion	Improved myelination, no effect on infarct size, reduced microglial density[147]
UAL, E18 rats (growth restriction model)	Melatonin given from P0 to P3	Improved myelination, reduced microglial density[148]
UCO, E92 sheep	Melatonin infused for 6 h after UCO	Reduced cell death, oxidative stress and microglial density[149]
UCO, E130 sheep	Melatonin infused to the ewe for 2 h, before and after UCO	Protection, reduced microglial density[150]
HI (βNTP at 40% baseline for 12.5 min), P0 piglets	Combined melatonin–hypothermia treatment	Neuroprotection, did not affect microglial density[151]
EPO		
HI, P3 rats	EPO given once a day during the first week after HI (P3 to P10) and then 3 times/week until P25	Improved WM microstructure, no effect on cortex; role of inflammation not specifically addressed[152]
HI, P7 rats	EPO was studied as an add-on to hypothermia	No overall protection by EPO or hypothermia, alone or in combination; role of microglia not specifically addressed[153]
Stroke, P7 rats	EPO given at reperfusion, 24 h, and 7 days after stroke	Lasting decreased brain damage and improved function, neurogenesis enhanced; role of inflammation not specifically addressed[154]
Ibo, P5 mice	Single dose of EPO 1 h after Ibo	Protection in GM and WM; role of inflammation not specifically addressed[155]

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Experimental insult and animal model	Intervention	Outcome
UCO E165–172, macaques	EPO given on days 1, 2, 3, and 7 after UCO + hypothermia	Improved anisotropy and cognitive functions; role of inflammation not specifically addressed[156]
Cyclooxygenase inhibitors		
IL-1β P1–P5 and Ibo P5, mice	Nimesulide (COX-2) or indomethacin (COX-1+2) given in combination with IL-1β	Blockade of IL-1β-induced sensitization of brain injury and inflammatory response in the brain[91]
IL-1β P1–P5 and Ibo P5, mice	Tianeptine given for 5 days before Ibo	Blockade of IL-1β-induced sensitization in GM and WM, no effect in absence of IL-1β; effect on microglia not specifically addressed[157]
Pifithrin-μ		
HI, P7 rats	Pifithrin-μ given after HI	Protection of GM and WM, reduced microglial density[158]
Cromolyn		
IL-9 P1-P5 and Ibo P5, mice	Cromoglycate given 1 h before Ibo	Blockade of IL-9-induced sensitization, protection in WM and GM, but no effect in the absence of IL-9; reduced MC density[41]
HI, P7 rats	Cromoglycate given before and/or following HI	Neuroprotection, inhibition of microglial activation, and MC migration[18,42]
Innate defence regulatory peptide		
LPS and HI, P8–9 mice	Innate defence regulatory peptide 1018 given 3 h after HI	Reduced injury in WM and GM, microarray analysis demonstrated decrease in proinflammatory and cell-death-related pathways[159]
NAC		
Maternal LPS, E19, rat NAC	in drinking water from E17 to birth	Prevented oxidative stress and restored long-term potentiation in the hippocampus and spatial recognition performance (effects found only in males)[160]
Newborn piglets, hypoxia	NAC given as bolus + 24 h infusion, started 5 min after reoxygenation	Attenuated caspase-3 and lipid hydroperoxide in the cortex, short (48 h) recovery period[161]
HI, P7 rats	NAC, daily until sacrifice, hypothermia for 2 h post HI	Reduced brain infarct volume and improved behavioural outcome, assessed up to 4 weeks after HI[162]
Intrauterine LPS, E28 rabbit	NAC administered in dendrimers as a single dose within 6 h after birth	D-NAC taken up by astroglia and microglia; reductions in motor deficits, oxidative injury, expression of proinflammatory genes, microglial activation, and loss of WM and GM[163]
TNF receptor blockade (etanercept)		
HI, P7 rats	Etanercept given immediately after HI	Etanercept detectable in the brain after intraperitoneal administration, reduced the neuroprotective effect of NF-κB inhibition[164]
IL-1b + Ibo, P1–P5, mice	Etanercept given before or after Ibo	Reduced brain damage by 50%; protective only when given after combined insult[165]
IL-1ra		
HI, P7 rats	IL-1ra, intracerebroventricular, before or after HI	Improved brain wet weight, but neuropathology not assessed[28]

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Experimental insult and animal model	Intervention	Outcome
LPS, P5 rats	Co-administration of IL-1ra with LPS	Improved myelination, reduction of lateral ventricle enlargement; neuroinflammation not investigated[166]
HI, P7 rats	Intracerebroventricular injection of IL-1ra 2 h after HI	Reduced cell death and caspase 3 activity in the hippocampus and cortex; reduced NF-κB activity, iNOS and COX-2[167]
LPS (E20–E22) + HI (P1), rats	IL-1ra treatment every 12 h from P1 to P9	Normalized motor function, exploratory behaviour, and density of immature neurons and astrocytes[29]
TAT-NBD		
HI, P7 rats	TAT-NBD given up to 12 h after HI	Neuroprotection, including improved long-term motor and cognitive outcome when given within 6 h after HI; effect independent of cytokines[168]
LPS + HI, P7 rats	Intranasal delivery of TAT-NBD 10 min after HI	Prevents brain injury after LPS + HI, blocks NF-κB signalling; not neuroprotective in HI alone[169]
Simvastatin		
HI, P7 rats	Pre-HI treatment	Neuroprotective, improved behaviour; effect on microglia not addressed; only male rats investigated[170]
HI, P7, rats	Pre-HI treatment	Improved GM and WM injury, reduced microglial activation[171]
PTB mice	Pravastatin or simvastatin given 24 h before and 2 h after LPS intravaginal administration	Protected cortical neurons in the fetus, protection mediated by Akt/PKB signalling pathways; effect on microglia not addressed[172]
Histone deacetylase inhibitors (TSA, valproate)		
Hippocampal Ibo, P7 rats	Injected daily from day after surgery until adulthood	Improved some behavioural characteristics, but not anxiety; did not protect against hippocampal lesions; neuroinflammation not specifically addressed[173]
LPS + HI, P8–9 mice	TSA or valproate given at the same time as LPS	Valproate increased mortality; TSA reduced GM and WM injury and improved learning in the fear conditioning test in females, but did not affect number of microglia after injury[174]
Unilateral carotid artery ligation, P12 rats	Treatment with valproate, TSA or vehicle for 2 weeks after insult	Both TSA and valproate increased neurogenesis, but valproate also increased mortality and impaired weight gain; neuroinflammation not specifically addressed[175]

Abbreviations: βNTP, β-nucleotide triphosphate; Akt/PKB, protein kinase B; COX, cyclooxygenase; DEX, dexamethasone; d-NAC, dendrimer NAC; E, embryonic day; EPO, erythropoietin; GM, grey matter; HI, hypoxia–ischaemia; Ibo, ibotenate; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MC, mast cells; NAC, N-acetylcysteine; NF-κB, nuclear factor-κB; P, postnatal day; PTB, preterm birth; TAT-NBD, Tat-NEMO-binding domain; TSA, trichostatin A; UAL, uterine artery ligation; UCO, umbilical cord occlusion; VEGF, vascular endothelial growth factor; WM, white matter. Chemokines and their receptors are named and abbreviated according to the international classification.

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Table 3

Effects of microglial cytokines on neural precursors of subventricular and subgranular zones

Cytokine	Effect on subventricular zone	Effect on subgranular zone
IL-1α	Not known	Stimulates proliferation[176]
IL-1β	Maintains stemness[177]	Inhibits proliferation[178]
TNF	TNF receptor 1 inhibits proliferation[179]	TNF receptor 1 binding inhibits neural precursor proliferation,[180] whereas TNF receptor 2 binding promotes neural precursor proliferation[180]
IL-6	Increases neural precursor proliferation and self-renewal[84]	Decreases neural precursor proliferation[176] and suppresses differentiation of neurons[181]
Vascular endothelial growth factor C (VEGF-C)	Promotes neural stem cell self-renewal and proliferation[182] and enhances oligodendroglial precursor proliferation[183]	Not known
IFN-γ	Decreases neural precursor proliferation and self-renewal[184]	Not known
Nitric oxide	Decreases neural precursor proliferation and self-renewal[185]	Increases neural precursor proliferation[186]
Transforming growth factor β (TGF-β)	Increases neural precursor proliferation[187]	Increases neural precursor proliferation[188]

Abbreviation: TNF, tumour necrosis factor.