Inflammation and Early Brain Injury in Term and Preterm Infants

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Early brain injury occurring during the prenatal, perinatal and postnatal periods is the most commonly recognized cause of severe, long-term neurological deficits in children [1,2]. There is no single cause for early brain injury, but a multiplicity of potential causes with complex underlying mechanisms [1,3]. The major proposed pathogenetic mechanisms are hypoxia-ischemia and inflammation [4-6]. Several lines of evidence suggest that neuronal injury related to inflammation can induce a cascade of immune responses that are involved in the pathogenesis of early brain injury [2,3,5,7]. However, until recently, the fetal-neonatal brain was viewed as an immune-privileged organ [2]. It is now clear that neuroinflammation is linked to the aggravation of early brain injury following hypoxia-ischemia and alone serves as a cause for brain injury [2]. Moreover, the unique anatomy and physiology of the fetal-neonatal brain underlies an exquisite sensitivity for inflammation [1,3,7]. In many neonates, both preterm and term, inflammation results in cell proliferation, cell differentiation and cell death, causing long-term brain injury leading to cerebral palsy, seizure disorders, sensory impairments and cognitive limitations [1,5,8,9]. However, neuroinflammatory responses are not necessarily harmful and provide beneficial results as well [10].

> **Neuronal injury related to inflammation can induce a cascade of immune responses, in both term and preterm infants, which is involved in the pathogenesis of early brain injury**

We assume that better understanding of the nature and mechanisms causing early brain injury will lead to the development of future pathways for improved prevention and treatment. In this review we focus on the relationships between inflammation and early brain injury, demonstrating the dominant role of inflammatory processes involved in early brain injury, which might lead to management strategies designed to limit early inflammatory responses and reduce the risk of early brain injury. Most studies and reviews on this subject focus solely on either term or preterm infants. We would like to introduce an unusual joint discussion of both term and preterm infants, since although different in many aspects of response and outcome they share basic mechanisms of early brain injury.

INFLAMMATION AND EARLY BRAIN INJURY

The common mechanism of early brain injury in both term and premature neonates is impaired cerebral blood flow [2,10]. At the cellular level, reduction of cerebral blood flow and oxygen delivery initiates a cascade of biochemical events leading to acidosis and cell death [10]. This is an evolving process, since it is usually followed by a phase of secondary energy failure [1,10]. The mechanisms involved in the secondary phase may involve mitochondrial dysfunction, calcium influx, excitatory neurotoxicity, oxygen free radicals, and nitric oxide formation [10]. During the secondary phase, neurons and oligodendroglia continue to die over hours, days, or possibly weeks and months [1,10]. The biochemical processes involved in cell death are numerous. Improved investigative methods enable detection of previously unrecognized factors that may have a role in causing brain injury among preterm and term neonates [2]. Animal models demonstrated several changes in hypoxia-inducible factors, among them pro-inflammatory cytokine expression in conjunction with neuronal damage [2,9]. In these models ischemia or hypoxia alone are not enough to cause cerebral damage, but additional inflammatory signals contribute to cell death [11]. We know that the brain is a major target for inflammatory mediator actions and that inflammation/cytokines could be neurotoxic with direct effects on the nerve cells; therefore, they have been associated with early brain damage [12]. Although the mechanisms involved in inflammation-induced early brain injury are not clearly understood, recent evidence has shown that inflammatory responses in the fetus and neonate can contribute towards inflammatory cerebral white matter damage [1,5,13]. During inflammation, systemic up-regulation of pro-inflammatory cytokines and diffuse activation of microglia in the neonatal brain occur, releasing inflammatory mediators, which enhance

brain injury [1,5,14]. Cytokine-activated cells release toxic substances (reactive oxygen species, proteolytic enzymes, myeloperoxidase) and activate cytotoxic T cells, natural killer cells, and lymphokine-activated killer cells, which enhance excessive cellular and tissue damage [8]. Significant 'key-players' in this process are:

MICROGLIA, PRO-INFLAMMATORY CYTOKINES AND INFLAMMATORY MEDIATORS

At the heart of this mechanism is a systemic up-regulation of pro-inflammatory cytokines and diffuse activation of microglia following an acute hypoxic insult [1,7,14]. When stimulated, the neonatal immune system can function in a fashion rather similar to the adult system and can lead to injury or death of neurons and premyelinating oligodendrocytes [1,5]. Microglia, the brain's resident immune cells, progressively populate the brain mainly during the second trimester [1,14]. These cells, present in large numbers in the developing periventricular white matter, are antigen-presenting cells in the brain [15]. They are known to be active macrophages that remove cellular debris during normal development as well as in pathological conditions [15]. Besides their role as active phagocytes, the presence of microglia has been associated with active myelination in the developing brain [15,16]. Microglial cells have been detected in periventricular lesions in children with signs of periventricular leukomalacia [17]. Microglia cells play an important role in the development of an inflammatory response in the developing brain [15-17]. It has been reported that microglia may be responsible for the early phase of an inflammatory response and enhance hypoxic injury by expressing inflammatory mediators such as tumor necrosis factor-alpha, interleukins 1 alpha and 1 beta (IL-1β), interleukins 2, 6, 8, 18, and lipopolysaccharide [1,5,8,14,15,17]. The inflammatory mediators enhance damage to the developing brain in different pathways, such as TNFα, which seems to play a key role in the immune cascade leading to periventricular white matter damage in the fetus/neonate [15,17]. The cytotoxic and inflammatory actions of TNFα are mediated through membrane receptors, among them TNF receptor-1 [15,18]. TNF-R1 has an intracellular death domain and its activation leads to cell apoptosis [15]. Aberrant TNFα/TNF-R1 signaling has a potentially major role in the early brain injury pathogenesis in which oligodendrocyte death and demyelination are primary pathological features [15]. The expression of TNF-R1 in oligodendrocytes increased significantly in the periventricular white matter in the developing brain [19]. This is coupled with increased cell death by apoptosis and necrosis in the periventricular white matter. Therefore, it seems that the pathway includes production of TNFα by microglial cells in

Hypoxia alone is not enough. There is an association between antenatal inflammation, white matter damage and long-term motor and cognitive deficits

hypoxic conditions which induce oligodendrocyte apoptosis via TNF-R1 [15,16,19]. An example of a different pathway is IL-1. Unlike TNFα, IL-1β is non-toxic to oligodendrocytes, but it can block oligodendrocyte proliferation [20]. A significant increase in IL-1β production by microglial cells along with expression of IL-1 receptor-1 on oligodendrocytes in periventricular white matter of the neonatal brain was observed [19]. It was suggested that activation and pro-inflammatory orientation of the IL-1 produced by microglial cells in hypoxic conditions delay the white matter development and recovery in hypoxic conditions [20,21].

Cytokine-activated cells may release toxic substances, such as reactive oxygen species and toxic granules including proteolytic enzymes and myeloperoxidase, resulting in cell injuries [8]. Moreover, these pro-inflammatory cytokines activate cytotoxic T cells, natural killer cells, lymphokine-activated killer cells and more phagocytes, which enhance excessive cellular and tissue damage in the inflammatory lesions [8]. The process induces responses such as cell proliferation, cell differentiation, and cell death, leading to the occurrence of early brain injury with severe damage especially to the vulnerable white matter, leading to long-term neurological damage [1,5,8,14].

TOLL-LIKE RECEPTOR

Innate immunity, the Toll-like receptors in particular, are vital players in the immune response in the brain [12]. It is not just that TLRs are important mediators of neuro-inflammation and tissue damage during infectious and non-infectious brain disease, but the dysregulation of this immunological response against brain-associated antigens could play a significant role in neonatal cerebral white matter damage [5]. Innate immunity in the brain depends, as mentioned above, primarily on the functions of glial cells such as astrocytes and microglia, which are important for the early control of pathogen replication, directing, recruiting and activating cells of the adaptive immune system [12]. Moreover, the activation of innate immunity via TLRs could play a role in pathology acquired after an infection and might initiate or amplify neuro-inflammation [12].

ENDOPLASMIC RETICULUM

ER stress promotes cell survival in fully myelinated mature oligodendrocytes; on the other hand however, ER stress leads actively myelinating oligodendrocytes to cell death [22]. Non-inflammatory stimuli such as hypoxia can initiate inflammatory phenomena via ER stress, leading to the unfolded protein response, which might then lead to brain damage [23]. The

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TNFα = tumor necrosis factor-alpha
TNF-R1 = TNF receptor-1

IL = interleukin
TLR = Toll-like receptors
ER = endoplasmic reticulum

neonatal brain might be especially vulnerable to ER stress because of the abundant protein synthesis by pre-oligodendrocytes and myelinating oligodendrocytes [23]. Moreover, TNFα can induce the unfolded protein response, which in turn, is able to attenuate interleukin-1 and interferon-gamma signaling – all causing or enhancing early brain injury [19,20,23,24]. So it is suggested that non-inflammatory stimuli (hypoxia) can lead to ER stress, promoting systemic inflammation that contributes to early brain injury [23]. There are specific and sometimes different aspects related to neonatal age.

CNS INFLAMMATION IN TERM INFANTS

Several major events can induce inflammation in the term infant brain.

ISCHEMIA

Ischemia induces microglial activation, initiating an inflammatory response, increases regional cerebral blood flow, induces perivascular inflammatory reactions with chemotactic activity, and alters both neuronal and glial function, causing brain injury [10]. Endothelial cells further contribute to the inflammatory response by producing pro-inflammatory mediators, which alter vascular permeability and regional blood flow and promote leukocyte adhesion [7]. Cytokines are injurious to the white matter by inhibiting differentiation of developing oligodendrocyte precursors, inducing glial apoptosis, and causing myelin degeneration [1,25].

Term newborn infants who suffer from hypoxic-ischemic encephalopathy were found to have elevated cerebrospinal fluid IL-6 and TNFα levels [14,25]. Shalak et al. [26] reported a significant association between abnormalities in the neurological examination and cytokine concentrations, with the highest cytokine concentrations (IL-6, IL-8) in term infants who developed clinical encephalopathy with seizures.

INFECTION

Ascending infections causing inflammation occur most commonly in the presence of rupture of membranes, but they are also possible with intact membranes [27]. The immune suppression inherent in pregnancy, which prevents rejection of the fetus by the mother, might explain how vaginal and lower genital tract organisms overcome natural barriers and gain access to the uterine cavity, and why the mother tolerates the presence of these organisms over long periods without showing any clinical manifestations [28]. Other routes of infection include hematogenous or transplacental infection, retrograde infection from the pelvis, and even transuterine infection caused by medical procedures [29]. Intraamniotic infection is usually polymicrobial and in most cases is caused

by a combination of anaerobic and aerobic organisms [28,29]. The most frequently isolated pathogens are found in the vaginal flora [28,29]. The presence of bacteria induces the release of pro-inflammatory cytokines (IL-1 and TNF), causing an inflammatory process [27,28]. Prolonged fetal exposure to these microorganisms might contribute to prolonged inflammation and to neonatal brain injury and subsequent cerebral palsy [28]. Chorioamnionitis is strongly associated with brain injury leading to CP among term infants, and increases the risk by two to twelvefold through multiple pathways [28]. Involved mechanisms include elevated fetal cytokine levels, which cause direct injury to the fetal brain, and inflammation of the placental membranes, which results in hypoxic-ischemic brain injury in the fetus [26,29]. This might result in compromised placental circulation or the exacerbation of existing hypoxic brain injury [28].

CNS INFLAMMATION IN PRETERM INFANTS

Inflammation has an important role in inducing both preterm labor and brain injury in the premature infant, especially at very low gestation [28]. Prematurity is a major cause of neonatal morbidity and mortality, and cerebral white matter damage is the predominant pattern of brain injury and a major clinical issue [1,30]. The risk of CP is 70 times greater at delivery < 28 weeks compared to delivery at term [28,29]. WMD is also associated with an increased risk for cognitive limitations, behavioral problems, and visuospatial difficulties [1,30]. Epidemiological studies show that perinatal infections, chorioamnionitis and early-onset sepsis are associated with an increased risk of PVL [17]. Early models of WMD etiology were based on the assumption that much of WMD occurrence among preterm infants is attributable to brain vulnerability associated with prematurity itself [7]. Dammann and Leviton [7] suggested that a certain "factor" might lead to both prematurity and WMD, thus increasing the possibility of damage to the premature brain. Prenatal inflammatory response might be a candidate for this "factor," leading to both preterm birth and brain damage [9]. The presence of pro-inflammatory cytokines in the CNS inhibits proliferation of neuronal precursor cells, activates astrogliosis and stimulates oligodendrocyte cell death, all of which increase the risk of WMD [28]. The oligodendrocyte appears to be particularly vulnerable [15,28,29]. Activation of cytokine receptors on the surface of oligodendrocytes may result in early cell death. Overproduction of pro-inflammatory cytokines TNFa, IL-1β, IL-6 and IL-2, along with adhesion molecules such as intercellu-

> Better understanding of the nature and mechanisms leading to inflammatory early brain injury will help focus and develop future pathways for improved prevention and treatment

CP = cerebral palsy
WMD = white matter damage
PVL = periventricular leukomalacia

lar adhesion molecule-1 and vascular cell adhesion molecule-1 can decrease the number of oligodendrocyte progenitors by causing their apoptosis and has been implicated in the pathogenesis of PVL [6,7,9,13,15].

Intraamniotic bacterial endotoxins trigger a release of cytokines in maternal and fetal tissue that leads to a release of additional cytokines, leukocyte migration, and prostaglandin [31]. This prostaglandin release leading to rupture of the fetal membranes and to the initiation of uterine contractions can be one of the direct mechanisms causing preterm labor [30,31]. Together with the evidence that inflammation can damage the developing white matter, it means that prenatal inflammation is involved in both directly causing brain WMD in preterm infants and in causing preterm labor, which is associated with WMD, as suggested by Leviton and Damman [7].

INTRAUTERINE INFECTION

Intrauterine infections increase the risk for cystic PVL and CP [23]. The rate of these complications increases dramatically with decreasing gestational age at delivery. The mechanism whereby infection appears to lead to neurological injury in the fetus and neonate is similar to the mechanism believed to cause premature rupture of membranes and preterm labor [28]. The presence of bacteria induces the release of pro-inflammatory cytokines (IL-1 and TNF) by macrophages, amnion, decidua and myometrium. IL-1 and TNFα as well as endotoxins released by the bacteria induce an increased production of prostaglandins, endothelin and corticotropin-releasing hormone in decidual, chorionic and amniotic cells [27]. Furthermore, IL-1 and TNFα induce the release of IL-6 from decidual and chorionic cells, which increases the placental secretion of prostaglandins and endothelin and mediates the release of IL-8 from decidual, chorionic, amniotic and cervical cells [28]. Elevated concentrations of bacterial endotoxins and pro-inflammatory cytokines as well as prostaglandins and endothelin in the amniotic fluid are detectable in patients with chorioamnionitis [27,28]. This leads to the activation and recruitment of granulocytes that release elastase in high concentrations and contribute to the reduction of the extracellular matrix, causing preterm labor [27]. As mentioned above, this also facilitates the process that leads to WMD.

INTRAUTERINE INFLAMMATION

As in the term infant, pro-inflammatory cytokines in the premature brain inhibit proliferation of neuronal precursor cells, activate astrogliosis, and stimulate oligodendrocyte cell death [3]. It has been suggested that neurological damage in the preterm infant results from the innate and adaptive immune systems reinforcing each other [5]. Innate immune mechanisms include the inflammatory reactions of neutrophils and monocytes triggered by microbial infectious products, such as endotoxin and nucleic acids. Adaptive immunity refers to

the responses of lymphocytes that recognize specific microbial antigens. Leviton and Damman [5] suggest that the interaction between these immune systems provides a more intense and prolonged inflammatory response. Much of this mutual reinforcement occurs peripherally and does not require the presence of lymphocytes or a foreign inflammatory stimulus in the infant's brain [5,9].

Infants born at 23–29 weeks gestation and who developed WMD in the first days after birth were found to have an increased level of memory T cells [32]. The presence of these cells in cord blood is presumptive evidence for antigen exposure in utero. Another support is the presence of IL-2, produced exclusively by activated T lymphocytes, which is toxic to oligodendrocytes and myelin in areas of cerebral WMD in newborns, even when lymphocytes were not identified [5]. These findings suggest that IL-2 originated outside the brain. Activated microglia are found in high concentrations in the immature brain and produce various inflammatory mediators, including IL-1β, TNFα, and IL-6 [14,27]. This is particularly true in PVL-affected areas. In addition, these cells produce free radicals, which potentially amplify white matter injury by upregulating the production of cytokines [14]. Oligodendrocytes appear to be particularly vulnerable to this injury, causing PVL in both in vitro and in vivo studies [28].

POSTNATAL INFECTION AND INFLAMMATION

The majority of premature (especially very low birth weight) infants develop at least one neonatal infection during their hospital stay [33]. An overwhelming systemic inflammatory response may be generated in response to early or late-onset sepsis, meningitis or necrotizing enterocolitis, resulting in brain injury or death. In a large cohort study from the NICHD Neonatal Research Network, Stoll et al. [33] reported that premature infants with neonatal infections were more likely to have cerebral palsy, lower cognitive scores, lower psychomotor developmental index scores, visual impairment, and impaired growth compared with those who were not infected [27].

DOES INFLAMMATION ALWAYS MEAN 'BAD NEWS'?

Intuitively, if the neonatal immune system is capable of producing an inflammatory response, the blockade of inflammatory chemokines may contribute to the prevention of early brain damage. However, the same cytokines causing the unfavorable inflammation in the neonatal brain appear to have a beneficial neurotropic effect [10]. Cytokines play a vital role in elimination of cellular debris, and in growth and repair, thus contributing to tissue recovery [10,15]. Some researchers could not find an association between histological inflammation of placenta and lesions and regional volumes of the brain in very preterm infants, or a direct association between the risk

of white matter injury and the severity of fetal and maternal inflammatory responses [34]. Others have demonstrated that the activation of glial cells triggers release of factors, such as colony-stimulating factor-1 and IL-6, resulting in marked neuroprotection, and is necessary for neuron survival [10]. Compensatory antioxidants and IL-8 elevation could be protective of perinatal asphyxic brain injury [35]. This dual effect complicates the task of developing targeted interventions to reduce the inflammatory response [10].

WHAT CAN BE DONE?

There is no clear answer to this question but several aspects of treatment and prevention have been suggested in the literature: namely, prevention of perinatal infection and inflammation and anti-inflammatory treatment such as corticosteroids, indomethacin and recombinant human erythropoietin. There has even been a suggestion regarding endogenous protectors and genetic regulation of inflammatory processes, but this is beyond the scope of this review [36-40].

CONCLUSIONS

Early brain injury is a continuous process, initiated most probably by in utero preconditioning in many neonates, born premature or at term. The injury is initiated during the primary insult and extends through the recovery phase. Hypoxia alone is not enough. There is an association between antenatal inflammation, WMD and long-term motor and cognitive deficits. Clearer understanding of the mechanisms and roles of key cellular and vascular components is crucial for the development of more effective prophylactic and therapeutic strategies. Better markers are needed for a reliable, earlier and more certain diagnosis of the newborn at risk. The hope is that there are interventions that might help to minimize the intensity and extent of damage, thereby decreasing morbidity and mortality in term and preterm infants alike.

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