

Causal Mechanisms Underlying Periventricular Leukomalacia and Cerebral Palsy

Hazim Kadhim^{1,2*}, Guillaume Sébire³, André Kahn¹, Philippe Evrard⁴ and Bernard Dan¹

¹Department of Pediatrics, Hôpital Universitaire des Enfants Reine Fabiola, Free University of Brussels (ULB), 15 Avenue JJ Crocq, 1020 Brussels, Belgium.

² Department of Pathology, Centre Hospitalier Universitaire Brugmann - Hôpital Universitaire des Enfants Reine Fabiola, Free University of Brussels (ULB), Brussels, Belgium.

³ Department of Pediatric Neurology, Centre Hospitalier Universitaire de Fleurimont, University of Sherbrooke, Canada.

⁴ Department of Pediatric Neurology, Hôpital Universitaire Robert Debré, Paris, France.

Abstract: Periventricular leukomalacia is a major neuropathological substrate underlying most of the neurologic morbidity in cerebral palsy. Etiopathogenesis of periventricular leukomalacia is believed to be multifactorial, involving hypoxic-ischemic insults and inflammatory processes. While emphasis was previously placed on hypoxia/ischemia, epidemiological, clinical, experimental and other studies conducted over the last decade have provided evidence for an important role of infective/inflammatory conditions and immune mediators in the pathogenesis of periventricular leukomalacia. Tumor necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β) and interleukin-2 (IL-2) are overexpressed in affected brains, and receptors for these cytokines are present on many inflammatory and neural cells in the white matter. These findings may be part of a wider network of cytokines, chemokines and other inflammatory factors. There is also evidence for interaction between infective/inflammatory conditions and ischemia/hypoxia as etiopathogenic factors in periventricular leukomalacia/cerebral palsy, as the former may enhance the effects of the latter. These developments in the understanding of the immune responses associated with perinatal brain damage, and the characterization of the implicated cellular and molecular mechanisms may have important implications for neuroprotection strategies aiming at prevention of periventricular leukomalacia and cerebral palsy.

André Kahn, Professor of Pediatrics at the Free University of Brussels (ULB) and Head of the Department of Pediatrics at the Hôpital Universitaire des Enfants Reine Fabiola in Brussels, Belgium, died unexpectedly on September 1, 2004, a few weeks after this work was submitted. We dedicate this article to him.

Keywords: Periventricular leukomalacia, cerebral palsy, spastic diplegia, inflammation, cytokines, TNF- α , IL-1, IL-2, IL-6.

INTRODUCTION

Cerebral palsy (CP) is a clinical construct commonly designating a group of conditions characterized by chronic motor impairment due to early occurrence of a nonprogressive lesion to the developing brain [1]. The definition of the concept is currently being reevaluated (Definition and Classification of Cerebral Palsy, NIH/NINDS, UCP & Castang Foundation). The CP concept covers a lot of heterogeneity in terms of etiology as well as types and severity of motor and associated disabilities. However, several relatively consistent associations have been described between etiology, pathology and clinical features, such as the sequence of neonatal hyperbilirubinemia, kernicterus and dyskinetic cerebral

palsy. An equally classic but more prevalent association is that of premature birth, periventricular leukomalacia (PVL) and spastic diplegia. The motor disability in this form of CP includes distinctive aspects of organization and control.

Perinatal deficit in oxygen supply is still regarded by many clinicians as a major causal factor in CP. It can be due to two major mechanisms, namely hypoxemia and ischemia. The major causes of neonatal hypoxemia include asphyxia with abnormal gas exchange across the placenta and respiratory failure at birth, postnatal respiratory insufficiency and right-to-left shunt secondary to cardiac disease or persistent fetal circulation [5]. The major causes of ischemia are antenatal or perinatal asphyxia itself leading to systemic and cerebral hemodynamic disturbances, and neonatal cardiac or circulatory insufficiency due to patent ductus arteriosus, congenital cardiac disease or vascular collapse. Although the hypoxic hypothesis went largely unchallenged for a long time, the possibility of other mechanisms has gradually been considered, including genetic and environmental factors acting in the antenatal or postnatal

*Address correspondence to this author at the Neuropathology (ANAPATH), CHU Brugmann, Place van Gehuchten 4, 1020 Brussels, Belgium; Tel: +32-2-477.2206; Fax: +32-2-477.2164; E-mail: Hazim.Kadhim@chu-brugmann.be

period. But even though emphasis was placed on various mechanisms at different times, the vast majority of cases were assigned to perinatal asphyxia until very recently. Moreover, most cases are now believed to be due to antenatal insult, including many of those with perinatal problems.

Large pathological studies of CP have demonstrated varying combinations of lesions in the cerebral cortex, hemispheric white matter, basal ganglia and cerebellum. White matter lesions, particularly in the periventricular region (i.e. periventricular leukomalacia, PVL), are commonly found both in autopsy material and brain imaging. The type and site of lesions depend much on the 'stage' of brain maturation during which pathogenic events occurred. Prematurity is the single most important risk factor and infants with very low birth weight are at significant risk for PVL and/or CP. Early in the third trimester of gestation, the periventricular region is more readily affected, though this is by no means exclusive. PVL is the dominant pathological substrate in CP. As it is regarded as the cause of the motor impairment, it should be an essential focus for prevention. It is noteworthy that the full extent of white matter damage in PVL has not been well delineated. In particular, the 'diffuse component' of PVL has often been underestimated. PVL lesions are therefore not restricted to the deep white matter as originally suggested. Recent studies thus underlined the occurrence of a wider spectrum of white matter damage, especially in preterm neonates. It is likely that these lesions will be increasingly recognized with advances in neuroimaging and pathology.

Risk factors associated with PVL as diagnosed by neonatal cranial ultrasonography have been reviewed by Kuban and Leviton [6]. Factors related to pregnancy include placental vascular anastomosis, twin gestation and antepartum hemorrhage. During labor and delivery, inflammation of umbilical cord and membranes seems to be the principal risk factor for PVL. In the postnatal period, low gestational age is an important factor. As this factor is strongly associated with both PVL and CP, the role of other factors, such as acidosis, low Apgar scores, intracranial hemorrhage, hypotension, mechanical ventilation, patent ductus arteriosus and inflammatory factors deserves special attention in order to determine if they are primary, independent or epiphenomena.

A recent multi-center study of 4503 children with CP suggested that prenatal events occurring early in gestation contribute to rendering the brain susceptible to develop PVL [7]. Among these events, infections and coagulopathies are pointed out as risk factors.

In this review, we shall focus on recent advances contributing to the understanding of the etiopathogenesis of PVL lesions, which are the hallmark of leukomalacic spastic diplegia and are also found in other common forms of CP. According to current views, pathophysiology of PVL almost always involves multiple factors. These may include genetic, hemodynamic, metabolic, nutritional, endocrinological, toxic and infectious mechanisms. In most cases, the conjunction of these factors ultimately triggers neuronal death processes, while some factors may genetically or epigenetically interfere with other aspects of brain maturation. Recent

research in this context has focused on excitotoxicity, interleukin release and immune-mediated inflammatory processes. Whereas many authors have concentrated on ischemic/hypoxic factors, other studies have implicated the role of infection/inflammation. Recently, integrative hypotheses have been suggested, based on the inter-relationships between both concepts.

The Ischemic/Hypoxic – Vascular/Hemodynamic Hypothesis

Insights into the ischemic/hypoxic processes that are implicated in CP have been derived from human studies as well as from a number of animal models including mice, rats and sheep. Cerebral ischemic insult to infants at risk for PVL may result from low systemic perfusion due to hypovolemia, venous infarction or intraparenchymal hemorrhage. Some investigators believe that the main pathogenic factor in PVL, particularly in the premature infant, is cerebral ischemia and perfusion failure, combined with a presumably cell-specific loss (due to particular vulnerability of oligodendrocyte precursors to ischemia). This concept has been presented in detail by Volpe [5]. According to this hypothesis, both the focal and the diffuse components of PVL relate in part to the development of the vascular supply to the cerebral white matter. This supply consists principally of the long (ventriculofugal) arteries, also called long medullary penetrating arteries, and short ventriculopetal (or short recurrent collaterals, also known as transventricular) arteries [8]. The focal component of PVL would occur in end-zones distribution regions of the long penetrators in the white matter. The distal fields of these vessels are thought to be not fully developed in the premature infant. Therefore, decrease in cerebral blood flow may result in severe ischemia in these areas leading to necrosis of all cell types. The diffuse component of the PVL would result from less severe ischemia in the border zones between long penetrators and in the end-zones of short penetrators, specifically affecting the 'sensitive' oligodendroglia. Impairment of cerebral blood flow was suggested to take place in the context of compromised cerebro-vascular autoregulation or pressure-passive cerebral circulation in a subset of ventilated premature infants. This vascular hypothesis has been questioned by others who disputed the presence of ventriculofugal arteries and consequently the validity of the watershed mechanism in this region [9].

Presumed oligodendroglial sensitivity is based on observed vulnerability of cultured oligodendroglial precursors to free radicals. Oxygen free-radicals have been shown to increase after cerebral hypoxemia. Elevated free-radical products have been found in the cerebrospinal fluid of very low birth weight infants with PVL [10]. The role of nitric oxide (NO) has been controversial, some data even suggesting a protective role to oligodendroglial precursors under conditions of oxidative stress. Vulnerability is also thought to be related to the absence of antioxidant maturation. Excitotoxic substances such as glutamate are increased in ischemic brains, and are thought to contribute to oligodendroglial death.

The special vulnerability of pre-oligodendrocytes as a basis for the development of PVL lesions has, however, been

disputed [6]. In addition, in most affected children, particularly those born at term and who develop CP later, the disorder cannot reasonably be ascribed to hypoxia/ischemia insult during delivery or to birth trauma [11-13]. Although oligodendroglial cell death seems to play an important role in the pathophysiology of CP/PVL, it may not occur as an independent mechanism. For example, lipopolysaccharides (LPS) have been shown to induce oligodendroglial cell death only in presence of microglia [14], supporting the hypothesis of involvement of other cells in the pathogenesis of white matter damage.

Role of Infection and Immune/Inflammatory Mechanisms

In the last decade, there has been mounting evidence from clinical, epidemiological, experimental, and pathological studies suggesting that maternal/neonatal infections and inflammatory conditions (whether infective or noninfective) are implicated in the pathogenesis of PVL lesions [15-18]. Furthermore, clinical and experimental studies showed that many biological mediators of immune-inflammatory processes, the so-called 'pro-inflammatory cytokines', were detected in association with CP or PVL [19-21]. These cytokines are produced by hematocytic and neural cells. They can induce expression of adhesion molecules such as ICAM-1 and VCAM-1 in brain parenchymal and vascular endothelial cells, and can promote microglial activation and demyelination [22]. Pro-inflammatory cytokines were detected in the amniotic fluid [20,21] and neonatal blood [19] of neonates with PVL, and high levels of cytokines, mainly TNF- α , IL-1 β , and IL-6, in neonatal blood of children developing spastic CP [15].

Whereas epidemiological studies showed that perinatal infections, chorioamnionitis and early onset sepsis are associated with an increased risk of PVL, experimental studies showed that injection of infectious agents caused fetal or neonatal brain white matter lesions [23,24].

Several issues have been addressed with regard to the role of the different molecular and cellular components involved in infectious/inflammatory mechanisms that are implicated in PVL. These include characterization of the different proinflammatory cytokines at play, their site of production and targets, interactions with other cytokines and non-cytokine agents, fetomaternal interactions and involvement of different age groups.

A group from our laboratories therefore carried out a series of studies to look for possible *in situ* cytokine immunoreactivity in human brains with PVL. These studies were designed to (i) investigate and characterize the expression of various cytokines, adhesion molecules, and the associated inflammatory cells at the different stages during the evolution of PVL lesions; (ii) define the temporal expression of these inflammatory molecules; (iii) see if clinically proven infection modifies the expression of these cytokines in the brain; and (iv) compare the immunohistochemical and inflammatory characteristics of PVL brains with matched-controls without evidence of other neuropathologic findings. These studies showed inflammatory reaction in PVL brains starting at the very early stage of these lesions (coagulative necrosis stage) and

extending until the latest phase of cystic cavitation [25]. This reaction was characterized by prolonged (but declining) levels of cytokines, with high TNF- α and to a lesser extent IL-1 β expression. Cytokine immunoreactivity was detected in PVL cases both with and without infection. However, cytokine production was higher with infection. A different pattern of cytokine expression was observed in anoxic brains without PVL in which TNF- α immunoreactivity was significantly lower than the PVL group. Whereas previous animal and human studies showed elevation of IL-6 in amniotic fluid and blood, we did not detect any IL-6 *in situ* in PVL brains, suggesting that elevated systemic IL-6 does not cross into the brain. It was therefore inferred that an immune-mediated inflammatory process could play an important role in the pathogenesis of PVL.

In vitro studies in cell culture showed that TNF- α induces myelin degeneration and oligodendrocyte apoptosis [26-28]. TNF- α could also have deleterious effects on pre-oligodendrocyte maturation by inhibiting differentiation [29]. Moreover, overexpression of brain TNF- α may cause inflammatory demyelination with oligodendrocyte apoptosis and myelin vacuolation [30]. Furthermore, in a model of excitotoxic lesions in newborn rodents, pretreatment with TNF- α and IL-1 β , increased the white matter damage induced by intracerebral injection of a glutamatergic agonist [31]. This supports the idea that the high level of TNF- α we detected may have mediated damage to oligodendrocytes, which are the main cellular target in PVL. "Other animal studies also support the role of excitotoxicity and free radical-related damage [32-34]. These avenues pave the way for neuroprotective intervention".

We also found that IL-2 is expressed in the white matter lesions of human PVL brains [35], in parallel with high levels of pro-inflammatory cytokines particularly TNF- α . On the other hand, other studies showed that IL-2 could induce the production of potentially neuro-toxic pro-inflammatory cytokines namely TNF- α and IL-1 β [36], which are known to inflict deleterious effects on the maturation of oligodendrocytes precursors, to induce myelin degeneration, and to trigger oligodendrocyte apoptosis. These findings might therefore establish a link between the upregulated cerebral expression of IL-2 we documented in PVL brains, and between the elevated pro-inflammatory cytokine levels, namely TNF- α and IL-1 β , reported in the brain in this pathology. In addition, it was reported that IL-2 can, in certain circumstances, be directly myelinotoxic and might thus damage the white matter. IL-2 was shown to be toxic to oligodendrocytes *in vitro* [37] as well as *in vivo* in rodents. This is consistent with a possible role for IL-2 in the cascade of events implicated in the pathogenesis of PVL. "IL-2 is exclusively produced by activated T lymphocytes. It plays a crucial role in immune responses, mainly in clonal T cell proliferation after specific antigenic activation. However, very few CD3 positive lymphocytes were detected in the cerebral lesions we studied [35], suggesting that *in situ* detected IL-2 did not arise from local synthesis, but rather through diffusion from the blood compartment".

Duggan *et al.* [38] recently showed that higher concentrations of pro-inflammatory cytokines (especially TNF- α) and T lymphocyte marker CD45RO in umbilical

blood predicted cerebral lesions detected by magnetic resonance imaging (MRI) very soon after delivery. These findings further support the involvement of an immune-mediated mechanism in perinatal white matter damage. They also suggest that these immune mediators might originate as a reaction to a systemic immune stimulation, which later propagates to the brain. Heightened maternal C-reactive protein was also shown to be related to the abnormal brain MRI. These results suggest that the fetus responds *in utero* to an antigenic-induced T cell activation and produces pro-inflammatory mediators. This immune-inflammatory reaction therefore seems to start outside the brain (e.g. chorioamnionitis) and spread to involve the brain, in contrast to cerebral ischemia, where the inflammatory reaction is initiated inside the brain. Damman *et al.* [39] also favor this concept of a primary systemic immune response that subsequently propagates to the brain to cause white matter damage, proposing that the most harmful component of the systemic fetal inflammatory response is activated white blood cells that cross the blood-brain barrier and cause damage either directly or by activating local cells such as microglia and astrocytes. Cytokines within the brain tissue might then inflict direct deleterious effects, for instance on oligodendrocytes, as TNF- α and IL-2 can induce oligodendrocyte apoptosis and myelin degeneration.

This has been documented in several models based on administration of *E. coli* or LPS in different species, including rabbits, kittens and sheep. Exposure to infection and inflammation contributes to the risk of CP/PVL [40]. On the other hand, white matter damage in this context can result from fetal inflammatory response to infection or other insults that either affect the fetus directly or occur outside the maternal compartment, including maternal systemic infections and chorioamnionitis. The majority of CP cases occur in infants born weighing more than 2500 g. The estimated proportion of cases that might be attributable to intrauterine infections (excluding the specific infections in the TORCH group, i.e. toxoplasmosis, rubella, cytomegalovirus, and herpes) was 12%, while the risk attributable to birth asphyxia was estimated to be 6% [41]. Infection in this age group may interact with other factors. For example, children born to women with intrauterine infection and potentially asphyxiating obstetrical complications were at much higher risk than those with only one of these risk factors, or neither [41]. In premature infants, infection appeared as an important risk factor for premature birth, especially before 30 weeks' gestational age. As low gestational age is associated with a high risk for CP and also with indicators of intrauterine infection, it was tempting to hypothesize that intrauterine infection causing preterm birth also causes CP in the prematurely born. However, the association of infection with CP was found to be different in very premature infants compared with term or near-term neonates. Thus, in contrast to the observed association of cytokine concentration with CP in term and near-term infants, cytokine levels in premature infants with CP have not differed from premature controls, either in amniotic fluid [20] or several days after birth, in peripheral blood [41]. This profile might reflect, differences in colonizing pathogens, in immune function in mothers and babies, in maturation of the immune/inflammatory system or

in vulnerabilities of constituents of the developing brain over the second half of gestation.

Interaction Between Inflammatory Cytokines and Ischemic Insult

Several possible interactions between infective/inflammatory conditions and ischemia/hypoxia as etiopathogenic factors in PVL/CP have been suggested. We [25] and others [43] found higher cerebral cytokine levels in infants with PVL and a history of infection than in infants with PVL only or infants having suffered anoxic conditions without PVL. Other studies also suggested that children born in a context of perinatal infections who also had potentially asphyxiating obstetrical complications had higher risk for CP than those with only one of these risk factors, or neither [41]. Experimental studies showed that pre-exposure to LPS enhances effects of subsequent hypoxia-ischemia in neonatal rat [44] but not sheep [45]. The precise mechanism of this interaction is not known. Preceding infection with cytokine production, amplifies the effect of insults such as hypoxia/ischemia. There have also been suggestions that the effect of infection may be mediated through ischemia/hypoxia [11]. However, while systemic LPS administration to the ovine fetus results in white matter damage, this injury does not occur as a result of cerebral ischemia. These latter results therefore suggest that endotoxin damages the brain by some mechanism other than cerebral ischemia. As cytokines levels increase within 6 hours of LPS exposure [46], LPS or systemic pro-inflammatory cytokines could act directly on the brain [47]. Hemodynamic consequences of LPS exposure may then be less important in the pathogenesis of PVL than the direct effect of LPS on the brain.

There could also be interactions between excitotoxicity molecules (triggered by ischemic conditions) and infection/inflammatory mediators such as cytokines. In this line, *in vitro* studies showed that TNF- α exacerbates glutamate-induced human fetal brain damage [48]. Further, TNF- α may itself be an inducer of ischemia, as demonstrated in piglets [49], and the pronounced vasoactive effects of this cytokine and of other mediators (e.g. NO) released as part of the inflammatory cascade [50] can increase risk for ischemic injury. In a mouse model, experimental data suggested that excitotoxic damage can produce neuropathological changes that are similar to those observed in CP [31].

Finally, it should be noted that while pro-inflammatory cytokines are liberated in response to infection, several other conditions including ischemia, trauma, inflammation and autoimmunity are also known to cause cytokine production. These cytokines, could therefore form a "final common pathway" in the cascade of molecular interactions leading to brain damage in PVL whether triggered by infection or ischemia.

CONCLUSION

Data presented in this review underline the importance of inflammatory processes and in particular of cytokines in the causal pathways of PVL/CP. Their role along this pathway remains to be clarified. Whereas they might cause PVL, their presence in sites of cerebral damage might alternatively be

secondary to injury. Infections may trigger a chain of molecular mediators that is detrimental to the developing brain. It must be borne in mind that infections, including chorioamnionitis, intrauterine or urinary tract infection, can be subclinical, lacking the classic manifestations of fever, leukocytosis or tenderness. In addition to the processes directly associated with maternal or neonatal infection, increased cytokine production amplifies the effect of hypoxia/ischemia in a 'double-impact' mechanism to cause brain damage. Therefore, the context of perinatal, intrapartum or intrauterine environmental injury should be expanded beyond pure hypoxic insult in these forms of neonatal encephalopathy to include intrauterine exposure to infection or inflammation and the ensuing fetal and neonatal immune-inflammatory response. These neurobiological insights into the understanding of the immune responses implicated in perinatal brain damage, and the characterization of the various involved cellular and molecular mechanisms might have major implications in developing novel approaches in neuroprotection in PVL/CP as well as therapeutic strategies targeting cytokines or their receptors [25,35,51]. These might include anti-inflammatory cytokines, cytokine binding proteins used as blockers, cytokine receptor antagonists, CAM antibodies and caspase inhibitors.

REFERENCES

- [1] Dan B, Cheron G. Reconstructing cerebral palsy. *J Pediatr Neurol* 2004; 2: 57-64.
- [2] Dan B, Bouillot E, Bengoetxea A, Boyd SG, Cheron G. Distinct multi-joint control strategies in spastic diplegia associated with prematurity or Angelman syndrome. *Clin Neurophysiol* 2001; 112: 1618-1625.
- [3] Mewasingh LD, Demil A, Christiaens F, Missa AM, Cheron G, Dan B. Motor strategies in standing up in leukomalacic spastic diplegia. *Brain Dev* 2002; 24: 291-295.
- [4] Dan B, Bouillot E, Bengoetxea A, Devalck C, Christophe C, Cheron G. Gait control in spinal palsy. *Brain Dev* 2004; 26:463-468.
- [5] Volpe JJ. *Neurology of the Newborn*. 4th edn. Philadelphia, W.B. Saunders, 2001.
- [6] Kuban KCK, Leviton A. Cerebral palsy. *N Engl J Med* 1994; 330: 188-195.
- [7] Jarvis S, Glinianaia SV, Torrioli MG et al. Cerebral palsy and intrauterine growth in single birth: European collaborative study. *Lancet* 2003; 362:1106-1111.
- [8] Kuban KCK, Gilles FH. Human telencephalic angiogenesis. *Ann Neurol* 1985; 17: 539-548.
- [9] Graham DI, Lantos PL. *Greenfield's neuropathology*. London, Arnold, 1997.
- [10] Inder T, Mocatta T, Darlow B et al. Elevated free radical products in the cerebrospinal fluid of VLBW infants with cerebral white matter injury. *Pediatr Res* 2002; 52: 213-218.
- [11] Illingworth RS. A paediatrician asks – Why is it called birth injury? *Br J Obstet Gynaecol* 1985; 92: 122-130.
- [12] Freeman JM, Nelson KB. Intrapartum asphyxia and cerebral palsy. *Pediatrics* 1988; 82: 240-249.
- [13] Stanley F, Blair E, Alberman E. *Cerebral Palsies: Epidemiology & Causal Pathways*. London, Mac Keith Press, 2000.
- [14] Lehnardt S, Lachance C, Patrizi S et al. The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. *J Neurosci* 2002; 22: 2478-2486.
- [15] Nelson KB, Dambrosia JM, Grether JK, Phillips TM. Neonatal cytokines and coagulation factors in children with cerebral palsy. *Ann Neurol* 1998; 44: 665-675.
- [16] Zupan V, Gonzalez P, Lacaze-Masmonteil T, et al. Periventricular leukomalacia: risk factors revisited. *Dev Med Child Neurol* 1996; 38: 1961-1967.
- [17] Baud O, Foix-L'Heliass L, Kaminski M, et al. Antenatal glucocorticoid treatment and cystic periventricular leukomalacia in very premature infants. *N Engl J Med* 1999; 341: 1190-1196.
- [18] Leviton A, Paneth N. White matter damage in preterm newborns – an epidemiologic perspective. *Early Hum Dev* 1990; 24: 1-22.
- [19] Yoon BH, Romero R, Yang SH, et al. Interleukin-6 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with periventricular leukomalacia. *Am J Obstet Gynecol* 1996; 174: 1433-1440.
- [20] Yoon BH, Jun JK, Romero R, et al. Amniotic fluid inflammatory cytokines (interleukine-6, interleukine-1, and tumor necrosis factor-), neonatal brain white matter lesions, and cerebral palsy. *Am J Obstet Gynecol* 1997; 177: 19-26.
- [21] Baud O, Emilie D, Pelletier E, et al. Amniotic fluid concentrations of interleukin-1beta, interleukin-6 and TNF-alpha in chorioamnionitis before 32 weeks of gestation: histological associations and neonatal outcome. *Br J Obstet Gynaecol* 1999; 106: 72-77.
- [22] Aloisi F, Ria F, Adorini L. Regulation of T-cell responses by CNS antigen-presenting cells: Different roles for microglia and astrocytes. *Immunol Today* 2000; 21: 141-147.
- [23] Cai Z, Pan Z-L, Pang Y, Evans OB, Rhodes PG. Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. *Pediatr Res* 2000; 47: 64-72.
- [24] Yoon BH, Romero R, Kim CJ, et al. Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits. *Am J Obstet Gynecol* 1997; 177: 797-802.
- [25] Kadhim H, Tabarki B, Verellen G, De Prez C, Rona AM, Sébire G. Inflammatory cytokines in the pathogenesis of periventricular leukomalacia. *Neurology* 2001; 56: 1278-1284.
- [26] Selmaj KW, Raine CS. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. *Ann Neurol* 1988; 23: 339-346.
- [27] Selmaj K, Raine CS, Farooq M, Norton WT, Brosnan CF. Cytokine cytotoxicity against oligodendrocytes: apoptosis induced by lymphotoxin. *J Immunol* 1991; 147: 1522-1529.
- [28] Louis JC, Magal E, Takayama S, Varon S. CNTF protection of oligodendrocytes against natural and tumor necrosis factor-induced death. *Science* 1993; 259: 689-692.
- [29] Cammer W. Effects of TNF alpha on immature and mature oligodendrocytes and their progenitors in vitro. *Brain Res* 2000; 864: 213-219.
- [30] Probert L, Akassoglou K, Pasparakis M, Kontogeorgos G, Kollias G. Spontaneous inflammatory demyelinating disease in transgenic mice showing central nervous system-specific expression of tumor necrosis factor alpha. *Proc Natl Acad Sci USA* 1995; 92: 11294-11298.
- [31] Dommergues M-A, Patkai J, Renauld J-C, Evrard P, Gressens P. Proinflammatory cytokines and interleukin-9 exacerbate excitotoxic lesions of the newborn murine neopallium. *Ann Neurol* 2000; 47: 54-63.
- [32] Laudenbach V, Medja F, Zoli M et al. Selective activation of central subtypes of the nicotinic acetylcholine receptor has opposite effects on neonatal excitotoxic brain injuries. *FASEB J* 2002; 16: 423-425.
- [33] Husson I, Mesples B, Bac P, Vamecq J, Evrard P, Gressens P. Melatoninergic neuroprotection of the murine periventricular white matter against neonatal excitotoxic challenge. *Ann Neurol* 2002; 51: 82-92.
- [34] Baud O, Daire JL, Dalmaz Y et al. Gestational hypoxia induces white matter damage in neonatal rats: a new model of periventricular leukomalacia. *Brain Pathol* 2004; 14: 1-10.
- [35] Kadhim H, Tabarki B, De Prez C, Rona A-M, Sébire G. Interleukin-2 in the pathogenesis of perinatal white matter damage. *Neurology* 2002; 58: 1125-1128.
- [36] Ellison D, Merchant RE. Appearance of cytokine-associated central nervous system myelin damage coincides temporally with serum tumor necrosis factor induction after recombinant interleukin-2 infusion in rats. *J Neuroimmunol* 1991; 33: 245-251.
- [37] Curatolo L, Valsasin B, Caccia C, Raimondi GL, Orsini G, Bianchetti A. Recombinant human IL-2 is cytotoxic to oligodendrocytes after *in vitro* self aggregation. *Cytokine* 1997; 9: 734-739.
- [38] Duggan PJ, Maalouf EF, Watts TL, et al. Intrauterine T-cell activation and increased proinflammatory cytokine concentrations

- in preterm infants with cerebral lesions. *Lancet* 2001; 358: 1699-1700.
- [39] Damman O, Durum S, Leviton A. Do white cells matter in white matter damage? *Trends Neurosci* 2001; 24 : 320-324.
- [40] Nelson KB, Willoughby RE. Infection, inflammation and the risk of cerebral palsy. *Curr Opin Neurol* 2000; 13: 133-139.
- [41] Nelson KB, Grether JK. Potentially asphyxiating conditions and spastic cerebral palsy in infants of normal birth weight. *Am J Obstet Gynecol* 1998; 179: 507-513.
- [42] Nelson KB, Grether JK, Dambrosia JM et al. Cytokine concentrations in neonatal blood of preterm children with cerebral palsy. (Abstract) *Am J Obstet Gynecol* 2000; 182: S47.
- [43] du Plessis AJ, Volpe JJ. Perinatal brain injury in the preterm and term newborn. *Curr Opin Neurol* 2002; 15: 151-157.
- [44] Eklind S, Mallard C, Leverin AL et al. Bacterial endotoxin sensitizes the immature brain to hypoxic-ischemic injury. *Eur J Neurosci* 2001; 13: 1101-1106.
- [45] Peebles DM, Miller S, Newman JP et al. The effect of systemic administration of lipopolysaccharide on cerebral haemodynamics and oxygenation in the 0.65 gestation ovine fetus in utero. *BJOG* 2003; 110: 735-743.
- [46] Duncan JR, Cock ML, Scheerlinck JP et al. White matter injury after repeated endotoxin exposure in the preterm ovine fetus. *Pediatr Res* 2002; 52: 941-949.
- [47] Lehnardt S, Massillon L, Follett P et al. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. *Proc Nat Acad Sci (USA)* 2003; 100: 8514-8519.
- [48] Chao CC, Hu S. Tumor necrosis factor-alpha potentiates glutamate neurotoxicity in human fetal brain cell cultures. *Dev Neurosci* 1994; 16: 172-179.
- [49] Megyeri P, Abraham CS, Temesvari P et al. Recombinant human tumor necrosis factor -alpha constricts pial arterioles and increases blood-brain barrier permeability in newborn piglets. *Neurosci Lett* 1992; 148: 137-140.
- [50] Brian JE, Faraci FM. Tumor necrosis factor-alpha induced dilatation of cerebral arterioles. *Stroke* 1998; 29: 509-515.
- [51] Kadhim HJ, De prez C, Rona A-M, Sébire G. Molecular pathways involved in cell injury in periventricular leukomalacia. *Ann Neurol* 2002; 52 (Suppl. 1): S121.