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Inflammation and the Insulin-like Growth Factor System at Very Preterm Birth. Implications for Early Morbidity and Development.

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Inflammation and the Insulin-like Growth Factor System at Very Preterm Birth

Implications for Early Morbidity and Development

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Abstract
The intention of this thesis was to evaluate the effects of inflammation at very preterm birth on subsequent morbidity, as well as on the neuro-protective IGF-system. Prospective clinical studies of very preterm infants constituted a base for the evaluation. Temporal changes in levels of cytokines were chosen as markers of an induced inflammatory response and were evaluated together with components of the IGF-system at birth and during the first 3 postnatal days. The studies could describe associations between increased levels of pro-inflammatory cytokines and subsequent morbidity. Inflammation present in cord blood related to impaired developmental outcome at 2 years of age, as well as to changes in components of the IGF-system, which indicates that inflammation present already before delivery may injure the immature brain and interfere with neuro-protective mechanisms. A postnatal increase cytokines was on the other hand associated with early morbidity, such as arterial hypotension and cerebral hemorrhage. Concentrations of IGF-I displayed a temporal decrease from birth and onwards, suggesting a low endogenous production after very preterm birth. We could show that exogenous administration of IGF-I from adult donor plasma elevated low endogenous levels of IGF-I in extremely preterm infants without any side effects. In summary, these findings imply that the time point of an induced inflammatory reponse appears important for type of subsequent morbidity. This may be of relevance for determining an optimal time point of delivery and for intervention with anti-inflammatory or protective strategies, with the purpose to decrease brain injury after very preterm birth.

Key words
brain injury; cord blood; cytokines; cerebral palsy; child development; fresh frozen plasma, inflammation; insulin-like growth factor I; preterm birth

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Cover illustration: Hanna born after 23 gestational weeks as newborn and at the age of 2 years. Printed with permission from her parents.

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Lyckans minut

Är det sant att jag håller ett barn på min arm
och ser mig själv i dess blick,
att fjärdarna gnistra och jorden är varm
och himmelen utan en prick?
Vad är det för tid, vad är det för år,
vem är jag, vad bär jag för namn?
Du skrattande knyte med solblekt hår,
hur fick jag dig i min famn?

Jag lever. Jag lever! På jorden jag står.
Var har jag varit förut?
Jag väntade visst millioner år
på denna enda minut.

Erik Lindorm, 1920

Contents

Abstract

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List of papers

I. Hansen-Pupp I, Harling S, Berg A-C, Cilio C, Hellström-Westas L, Ley D. Circulating interferon-gamma and white matter brain damage in preterm infants.
Pediatric Research 2005;58:946-952

II. Hansen-Pupp I, Hallin A, Hellström-Westas L, Cilio C, Berg A-C, Stjernqvist K, Fellman V, Ley D. Inflammation at birth is associated with subnormal development in very preterm infants.
Pediatric Research, in press

III. Hansen-Pupp I, Hellström-Westas L, Cilio C, Andersson A, Fellman V, Ley D. Inflammation at birth and the insulin-like growth factor system in very preterm infants.
Acta Paediatrica 2007;96:830-836

IV. Hansen-Pupp I, Engström E, Niklasson A, Fellman V, Berg A-C, Löfqvist C, Hellström A, Ley D. Fresh-frozen plasma as a source of exogenous Insulin-like Growth Factor I in the very preterm infant.
Submitted for publication.

Abbreviations

ALS	acid labile subunit
BBB	blood brain barrier
BP	blood pressure
CA	chorioamnionitis
CBF	cerebral blood flow
Cl	clearance
CNS	central nervous system
CP	cerebral palsy
CSF	cerebrospinal fluid
DTI	diffusion tensor imaging
ELBW	extremely low birth weight
FFP	fresh frozen plasma
FIRS	fetal inflammatory response syndrome
GA	gestational age
GH	growth hormone
GW	gestational weeks
HI	hypoxic-ischemic
hp	high phosphorylated
IFN	interferon
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IGF-IR	insulin-like growth factor one receptor
iNOS	inducible nitric oxide synthase
IUGR	intrauterine growth restriction
IUI	intrauterine infection
IL	interleukin
lp	low phosphorylated
LPS	lipopolysaccharide
MABP	mean arterial blood pressure

MDI	mental developmental index
MRI	magnetic resonance imaging
NIRS	near infrared spectroscopy
NO	nitric oxide
NOS	neurological optimality score
OL	oligodendrocyte
PVL	periventricular leukomalacia
PDI	psychomotor developmental index
PROM	Premature rupture of membranes
rh	recombinant human
ROP	retinopathy of prematurity
ROS	reactive oxygen species
Th	T-helper
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TNFR	tumor necrosis factor receptor
Vd	volume of distribution
VLBW	very low birth weight
WMD	white matter damage

Background

Introduction

Survival of infants born after extremely preterm birth has improved significantly during the last two decades, due to improvements in perinatal and neonatal care. However, a considerable proportion of these infants survive with neurological and/or cognitive impairment. This has been attributed to a reduction in maturation and growth of the immature brain, but also to inflammatory insults present before or at preterm birth that interact with several fetal organ systems. Increased knowledge concerning mechanisms that are associated with perinatal brain injury may serve as a basis for future development of neuro-protective strategies for this vulnerable group of infants. This thesis is based on clinical studies of very preterm infants with the purpose to elucidate how inflammation and growth factors may interfere with brain development.

Perinatal brain damage in preterm infants

Incidence/epidemiology

Preterm deliveries are defined as deliveries occurring before 37 completed gestational weeks. The preterm delivery rate has increased during the last years and is in Europe 5-9% and in the United States 12-13% (1). Very preterm birth is defined as delivery before 32 gestational weeks (GW) and extremely preterm birth is defined as delivery before 28 GW. According to the Swedish birth registry, 0.9% of the infants were born before 32 GW and 0.3% before 28 GW in Sweden during 2005 (still birth before 28 GW not registered) (K. Källén PhD, personal communication, March 2008).

Improvements in antenatal and neonatal intensive care resulted during the 1990s in an increased survival of extremely preterm infants, which was accompanied by an increased *number* of infants surviving with neuro-developmental impairment, but also increased *rates* of survival without impairment (2). Recent studies now report declining rates in peri-

ventricular leukomalacia (PVL), cerebral palsy (CP) and cognitive impairment in preterm infants with very low birth weight (VLBW) or extremely low birth weight (ELBW) (3-5). In contrast, the incidence of intra-ventricular hemorrhage (IVH) grade III or parenchymal cerebral hemorrhage has not changed (3, 6). The decreased use of postnatal steroids during the last years has been suggested to be one reason of improved cognitive outcome (7). Despite these positive reports, long-term problems are frequent with 7-17% of ELBW infants having neuro-sensory impairments and 13-37% having delays in cognitive function (8). An even higher rate of disability has been described in the infants with the most extremely preterm birth (9, 10). These problems are also present in ELBW infants with normal cerebral ultrasound examinations (11).

Classification

Brain lesions in preterm infants can be classified into 3 principal categories: white matter damage (WMD), hemorrhages in non-parenchymal areas of the brain and lesions in other locations, such as cerebellum, basal ganglia and brain stem (12). WMD is the most important lesion and is today considered to comprise not only focal or diffuse WMD, but also parenchymal hemorrhage and ventricular enlargement (12). Hemorrhages in non-parenchymal areas of the brain can be graded according to the distribution of bleeding and can be either subependymal (grade I), intraventricular without ventricular dilatation (grade II) or intraventricular with ventricular dilatation (grade III) (13). Hemorrhagic lesions are often associated with WMD and it has been suggested that the two lesions share common etiologies (14). It has recently been proposed to abandon the grading scheme of hemorrhages in favour of a descriptive classification according to the localization of the hemorrhage (15).

Assessment of perinatal brain damage

Cerebral ultrasound

Cerebral ultrasound has been used as a standard non-invasive bedside technique, since more than 25 years. It is easy to perform and to repeat, since it causes little disturbance even in sick preterm infants. Cerebral ultrasound detects echodensities or echolucencies

as well as enlarged ventricles and signs of brain atrophy, defined as enlarged subarachnoid spaces or widened interhemispheric fissures. Cerebellar lesions or other lesions in the posterior fossa may be more difficult to diagnose with cranial ultrasound. Hemorrhages often present as echodensities and can be classified according to the definiton above. Parenchymal echodensities are often triangular shaped and located adjacent to the ventricular system. They are proposed to be secondary hemorrhages into an ischemic area. They usually evolve into a porencefalic cyst, often incorporated with the lateral ventricle. IVHs are frequently accompanied by ventricular dilatation, which is caused by impaired drainage of cerebrospinal fluid (CSF) from the ventricular system. Ventricular dilatation is further seen in association with white matter injury.

WMD may present as both echodensities and echolucencies. Usually a diffuse transient echodensity is the earliest sign on cerebral ultrasound, which may disappear, persist or evolve into cystic lesions. Periventriular echodenistes that persists for more than 7 days are usually considered to be a sign of WMD. PVL or cystic WMD in preterm infants can be divided into two entities, localized and extensive PVL. Extensive PVL is defined as the presence of multiple cysts, often located around the occipital horns of the lateral ventricles. They often develop within the first 2-3 weeks of life. Localized PVL is defined as the presence of smaller often singular cysts that usually are located fronto-parietally. These localized cysts are often transient. In a majority of infants, cysts develop beyond the first postnatal month and are often no longer visible at term age. Transient cysts are often followed by mild ventriculomegaly present at term (16, 17). In a study of very preterm infants with CP, major ultrasound abnormalities were detected after the first 4 postnatal weeks in almost one third of the infants (18). The detection of late or transient changes will therefore rely on sequential ultrasound examinations performed from birth and until term age (19).

Cerebral ultrasound can detect other changes than WMD which may be predictive of abnormal neurological outcome. Poor growth of corpus callosum during the first postnatal weeks, as well as signs of brain atrophy at term defined as enlarged

subarachnoidal spaces, widened inter-hemispheric fissure and a reduction in complex gyral folding have been associated with neuro-cognitive impairment and CP (20, 21).

Magnetic Resonance Imaging (MRI)

The MRI technique has developed rapidly during the last decade and is currently a valuable tool in clinical assessment of brain structure and outcome prediction in preterm infants. The most optimal time point for MRI scan of preterm infants is considered to be at term age, when myelination in the posterior limb of capsula interna starts to develop and can be compared to that of infants born at term (22). MRI has a better ability of detecting diffuse WMD, as compared to cranial ultrasound (23, 24). Diffuse WMD at term age has been defined by MRI as diffuse excessive high signal intensities (DEHSI) on T2 weighted images, reduction in white matter volume and ventricular dilatation (25-27). White matter changes are often accompanied by grey matter changes, defined as enlarged sub-arachnoidal space, immature gyral folding as well as reduced cortical surface (26, 28).

MRI has also been used to estimate cerebral volumes in gray and white matter. Preterm infants have a reduction in grey matter as well as in white matter volume, accompanied by an increase in CSF volume as compared to infants born at term (26, 29). Infants with white matter injury have in addition delayed maturation of white matter tracts, as defined by diffusion tension MRI (30-32). Many of the above described changes are persistent, where reduced volume in different cerebral regions, delayed maturation of white matter tracts as well as altered anatomical brain structure, can be demonstrated at school age and during adolescence and are accompanied by cognitive impairment (33-37).

Neuropathological features of cerebral white matter damage

WMD is the most important cerebral lesion, associated with developmental outcome. During the last years, it has become evident that WMD can be divided into two principal components, focal and diffuse WMD. Focal WMD or periventricular leukomalacia (PVL) is today infrequent, whereas a high proportion of very preterm infants have diffuse WMD (3, 26). Focal WMD is located deep in cerebral white matter, at the distribution areas of

the end-zones of the long penetrating arteries. It is characterized by necrosis of all cellular components and subsequent macroscopic cyst formation.

Diffuse WMD is characterized by diffuse injury to oligodendrocyte (OL) precursors, astrogliosis and infiltration of microglia and is located at the border zones between the individual long penetrating arteries and at the end-zones of the short penetrating arteries. The distal fields of the long and short penetrating arteries respectively are not completely developed in preterm infants rendering these watershed territories susceptible to ischemia (38).

Pathogenesis of cerebral white matter damage

The principal feature of WMD is a chronic disturbance of myelination in preferentially central (and deep) white matter. The hypomyelination is caused by damage to the myelin-producing cells in the white matter, the oligodendrocytes. There is no evidence of *primary* acute cortical damage to neurons, although cortical volume loss has been demonstrated with MRI in infants with WMD (39, 40). The reduction of cortical volume has been explained by a chronic disturbance of cortical neurons, secondary to injury to axons crossing the white matter and/or to damage of sub-plate neurons spreading throughout the white matter (39). The human OL lineage consists of 3 stages (1) the preOLs, (2) the immature OLs and (3) the mature OLs. WMD develops primarily between 24 to 32 GW and during the same period, the white matter is mostly populated by preOLs. Mature OLs are detected between 30 and 35 GW, with the first evidence of myelinogenesis in periventricular white matter, appearing around 30 GW (41). The preOLs have an increased vulnerability to NO-mediated and oxidative injury, whereas mature OLs are resistant to such injury (42, 43).

Either ischemia and feto-maternal infection or a *combination* of both of these insults lead to injury of developing OLs, which have been identified as the key cellular target in diffuse white matter injury. Ischemia is followed by excitotoxic cell damage, influx of calcium into the cell and subsequent production of reactive oxygen species (ROS), which is followed by later activation of microglia that may last several weeks. Feto-maternal

infection results in activation of the innate immune system and a subsequent inflammatory response which is mediated by activated microglia and astrocytes and includes release of cytokines, ROS and nitric oxide (NO) (44-46). This inflammatory response may last for weeks (Fig 1).

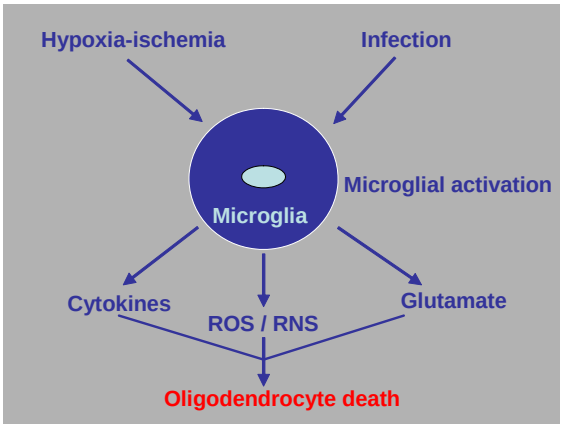

Figure 1. *Microglia plays a central role in the pathogenesis of white matter damage. ROS, reactive oxygen species RNS, reactive nitrogen oxygen species. Adapted from Khwaja et al 2008.*

Risk factors for brain injury and impaired developmental outcome

The very preterm infant encounters a multitude of different influences with a possible damaging effect to the immature brain. A schematic description of factors which may interfere with brain development is given in Fig 2.

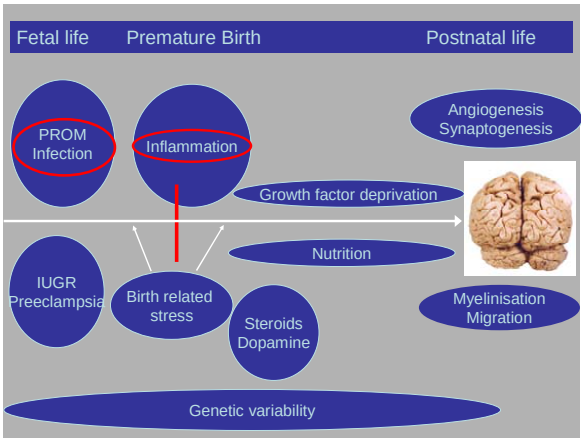

Figure 2. *Antenatal and neonatal risk factors associated with brain injury during the transition from fetal to neonatal life in preterm infants. PROM, premature rupture of membranes; IUGR intrauterine growth restriction.*

Gestational age and gender

The most important risk factors are decreased GA at birth and reduced BW, with low GA being considered to be most predictive of the two (47-50). The disadvantage of male sex at preterm birth has been described in many studies, but the biological mechanisms behind this gender-related difference are less well understood (9, 50-53).

Antenatal and neonatal infection

Clinical and histological signs of feto-maternal infection and premature rupture of membranes (PROM) are all significant risk factor for impaired neurological and pulmonary outcome in preterm infants. These risk factors are part of the main theme of this thesis and will therefore be expanded upon below. Additionally, not only antenatal but also postnatal infection has been associated with poor outcome (54, 55).

Intrauterine growth restriction (IUGR)

Preeclampsia is together with antenatal infection a common antecedent to preterm delivery and is often accompanied by intrauterine growth restriction (IUGR) and with abnormal fetal blood flow, indicative of fetal hypoxia. The risk of cerebral abnormalities as detected by ultrasound or MRI and of CP appears lower than in deliveries associated with maternal infection (26, 56, 57). However, impaired fetal blood flow and preeclampsia have been related to impaired cognitive outcome (58, 59). IUGR was not related to an increased risk for CP in one case-control study, whereas another study did show an increased risk (60, 61).

Postnatal growth, chronic lung disease and postnatal steroid treatment

Postnatal growth retardation due to insufficiency in meeting intrauterine nutritive requirements is common after extremely preterm birth and has been a predictive factor for poor neuro-developmental outcome and CP (62). In the same study, treatment with postnatal steroids was associated with impaired development and growth. The use of postnatal steroids as a treatment of chronic lung disease has decreased significantly during the last years, due to reports where postnatal steroids have been associated with both CP

and neuro-cognitive impairment (63). Further, chronic lung disease is by its own an independent risk factor for CP and impaired outcome (48, 50, 64-66).

Cerebral abnormalites

Abnormalities in white and grey matter confirmed by ultrasound or MRI are strong predictors of CP, as well as of neuro-cognitive delay (50, 52, 65, 67-69). In infants with CP, ultrasound abnormalities were present in almost 80% and abnormalities identified by MRI were present in almost 90% of the performed examinations with white matter changes being the most common (18, 70). Extensive cystic PVL which today is declining in incidence is the lesion that shows the highest correlation with CP, where almost all infants (>90%) develop CP (3, 17, 18). The correlation between IVH grade III and CP is weaker, but still significant with about 40% developing CP (18). Additionally, IVH grade III irrespective of development of CP represents a risk of future cognitive impairment (18, 67).

Arterial hypotension and cerebral blood flow (CBF)

Systemic hypotension and low ventricular output are common during the first 24 h after very preterm birth (71, 72). Additionally, CBF is low in the immediate postnatal period, but is followed by a gradual increase, which has been suggested to be a result of increased cardiac output (34, 71, 72).

Previously hypotension was considered to be a risk factor of WMD/PVL, which may be theoretically expected, since white matter changes are located within watershed areas in the brain. More recent studies have however not observed an association between low blood pressure and brain injury, as defined by cerebral ultrasound (73, 74). This may be explained by a poor correlation between mean arterial blood pressure (MABP) and CBF as estimated by near infrared spectroscopy (NIRS). Infants with systemic hypotension may have a reduced CBF but may equally preserve an adequate CBF (75-77). Cerebral auto-regulation maintains an adequate CBF despite changes in systemic blood pressure. The opposite situation occurs, when fluctuations in blood pressure (BP) are accompanied by changes in CBF, which is defined as pressure passivity, probably due to immaturity of vasoregulatory mechanisms (78). Pressure passivity correlates with systemic hypotension,

but can also be transiently present during periods with normal BP in preterm infants (75, 78). Since blood flow to cerebral white matter is extremely low in preterm infants, pressure passivity during systemic hypotension may impair cerebral oxygen delivery preferentially in peri-ventricular white matter (79, 80).

Genetic risk factors

The importance of genetic factors influencing both the probability of preterm birth, as well as subsequent infant outcome has been emphasized in recent studies. Polymorphisms of genes associated with production of cytokines have been studied, where genotypes associated with increased production of IL-6 have been related to impaired cognitive outcome and those associated with increased production of IL-10 to a more favourable outcome in preterm infants (81, 82). Genotype-phenotype association studies require large study samples and carefully matched controls to reach sufficient power, which can be difficult to achieve in the preterm population. In addition, ethical aspects must be considered (83, 84).

The fetal and neonatal immune system

The immune system is divided into two different response systems, the *innate immune response* and the *adaptive immune response*. The innate immune response is an immediate non-specific reaction to foreign antigens. The primary effector cells of innate immunity are phagocytic cells (neutrophils, monocytes, macrophages) and natural killer cells. These cells promote migration and chemotaxis, with the purpose to ingest and destroy microbes. The recognition of microbial antigens by the innate immune system is effectuated by pattern recognition receptors, where the toll-like receptors (TLR) have a key role. The TLRs are trans-membrane proteins with one extra-cellular and one cytoplasmic domain, that recognize antigens either present on the cell membrane or within the cell. Coupling of a ligand to the TLR receptor, results in activation of an intra-cellular signalling cascade and release of the transcription factor NF-κβ, which induces activation of inflammatory cytokine genes. Different adaptor proteins are essential for the intracellular signalling cascade, where the major signalling pathway use the adaptor protein myelin differentiation

factor (MYD88) (85). The TLR system is dynamic and can be activated not only by exogenous microbial agents, but also by endogenous inflammatory stimuli (86).
The adaptive immune system mediates a slower and specific immune response. It consists of lymphocytes that are activated by antigen presenting cells from the innate immune system. The lymphocytes are capable of expressing two types of responses; a cellular response which is mediated by two subgroups of T-lymphocytes, either T-helper cells (CD4+ cells) or cytotoxic T-cells (CD8+cells) and a humoral response mediated by B-lymphocytes which upon activation produce antibodies (IgA, IgD, IgE, IgG, IgM) (87).

Both the innate and adaptive immune systems are developed and functional in the fetus but have a decreased capacity especially in the response to foreign antigens as compared to the adult immune response (88).

Cytokines

The interaction between and within the adaptive and innate immune systems is regulated by specific proteins collectively named cytokines, with a molecular mass of less than 30 kDa. These cytokines act as messengers of the immune system and regulate the intensity and duration of the immune response, by stimulating or inhibiting various immune cells and by regulating the secretion of antibodies or other cytokines. Cytokines act in an antigen non-specific manner, by binding to specific receptors on immune cells with a high affinity. The maintenance of antigen specificity is on the other hand regulated by the immune cell, which after interaction with an antigen expresses cytokine receptors on the cell surface.

Cytokines are primarily produced by macrophages and lymphocytes (Th cells). Production occurs in cascades, where release of some cytokines stimulates synthesis of other cytokines, which in turn controls production of previously released cytokines. In contrast to hormones that mediate endocrine effects, cytokines principally exert their effects at a short distance in an autocrine or paracrine fashion. The principally local actions of cytokines may be necessary due to their short half-life after secretion (89).

Polarization of T-helper cells (Th1/Th2)

T-helper cells are lymphocytes that can be divided in two subpopulations, Th1 and Th2 cells, which are designated to eliminate different type of pathogens and that produce different types of cytokines. The cytokine Interleukin (IL)-12 is the principal protein that induces Th1 differentiation, whereas IL-4 is critical for differentiation of Th2 cells. The Th cell subpopulations are however heterogenous and classification into Th1/Th2 subsets must be viewed as an approximative paradigm (90).

The Th1 cells produce interferon-gamma (IFN-γ), IL-1, IL-2 and tumor necrosis factor (TNF), promoting phagocyte dependent inflammation and cell mediated immunity. The Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13, which inhibit phagocyte functions and evoke antibody responses. In this perspective, the Th1 cytokines are considered to induce pro-inflammation, whereas the Th2 cytokines are considered to modulate or depress an inflammatory response, although Th2 cytokines in addition are capable of inducing phagocyte-independent inflammation. The balance between Th1 and Th2 polarization is dependent on genetical and environmental factors including properties of the microbial antigens (90, 91). This is exemplified by studies showing that gram-positive and gram-negative bacteria induce different cytokine profiles (92, 93).

In pregnancy, there is a reduced production of Th1 cytokines and increased production of Th2 cytokines. Shifting of balance towards Th2 polarization damps the fetus immune response and is an adaptive mechanism to preserve pregnancy. An imbalance in this polarization towards Th1 production of cytokines, may result in fetal loss or preterm birth (94). It has been proposed that intrauterine infections (IUI) may interfere with the Th1/Th2 balance during pregnancy which may result in preterm labour (85, 87). Increased concentrations of Th1 cytokines have been observed in placentas and in peripheral blood of women with preterm delivery (95, 96).

The Th2 predominance in preterm and newborn infants is followed by a reduced production of Th1 cytokines, thus impairing the infant's defense against microbial antigens, making them more susceptible to invasive infections. Mononuclear cells in cord blood have a reduced production of IFN-γ, probably due to a concomitant reduced

production of the IFN-γ inducing cytokines IL-12 and IL-18, as compared to adult cells (88). Neonatal macrophages have an impaired Th1 cytokine response to exposure of lipopolysaccharide (LPS), the endotoxin of gram-negative bacteria. This has been attributed to an unresponsiveness of the innate immune system and the TLR signalling. Although basal expression of TLRs are similar in newborns when compared to adults, the activation after stimulation seems to be different with an up-regulation of TLR2 but not of TLR4 (85). A reduced expression of TLR4 and decreased production of the adaptor protein MYD88 has been observed in monocytes from preterm infants, as compared to term infants and adults (97, 98).

The production of IL-6 is relatively higher than production of TNF-α after stimulation of the TLRs in neonatal monocytes in line with a polarization towards a Th2 inflammatory response (99). Adenosine is an ATP metabolite that is increased by ATP degradation in response to metabolic stress, presenting at significantly higher levels in plasma from newborn infants than in adults (100). Adenosine is produced by the placenta and crosses the placental-fetal barrier and has been suggested to have anti-inflammatory and Th2 polarizing properties. Stimulation of the adenosine receptor results in decreased production of TNF-α and NO and in increased production of IL-10 after LPS stimulation *in vitro* (101). The selective inhibition of TNF-α production described in neonatal cord blood monocytes seems to be mediated by adenosine through the TLR2 pathway (100).

Although several studies indicate that preterm and also term newborn infants have an immature immune system, others suggest that they are capable of mounting a significant inflammatory response. Higher levels of pro-inflammatory cytokines in cord blood as well as enhanced synthesis of IL-6 and IL-8 after LPS stimulation as compared to adults have been observed in preterm infants (102, 103). The counter regulatory anti-inflammatory response that follows an initial inflammatory response seems however, to be decreased in newborn and preterm infants (104).

Fetal inflammation

Definition

The fetal inflammatory response syndrome (FIRS) was originally defined as an elevation of fetal plasma interleukin (IL)-6 concentration in fetuses with preterm labour or PROM (105). FIRS is the fetal counterpart of the systemic inflammatory response syndrome described in adults (106). There are several target organs for FIRS including the brain, the lung, the hematopoetic system, the heart, the adrenal and the skin (107-109). Histologically, FIRS is characterized by fetal vasculitis which is described as presence of polymorphonuclear leukocytes in the blood vessel walls of the chorion (chorionic vasculitis) and in the umbilical cord (funisitis) (110). Funisitis is associated with endothelial activation and up-regulation of adhesion molecules, resulting in transmigration of leukocytes and tissue damage (111).

The maternal inflammatory response to an intrauterine infection is chorioamnionitis (CA) and the leukocytes that invade preterm fetal membranes have been described to be of maternal origin (112). On the other hand, polymorphonuclear leukocytes in amniotic fluid have been identified as fetal in women with preterm labour (113). Histological CA has a higher predictive value for abnormal neurological outcome than clinical CA (114). Further, it has been proposed that fetal rather than maternal inflammation is associated with fetal organ injury and later neurological impairment (65, 110, 115-117).

Etiology

Intrauterine infection (IUI) is considered to be a major cause of preterm labour. The frequency of histologic CA increase with decreasing GA (118). One of the most common routes for microorganisms to gain access to the intrauterine cavity and to the fetus is by an ascending infection from the vagina or cervix. Other routes are hematogenous dissemination of microorganisms through the placenta, retrograde seeding through the peritoneal cavity or accidental introduction of microbes during invasive procedures (109, 118) (Fig 3).

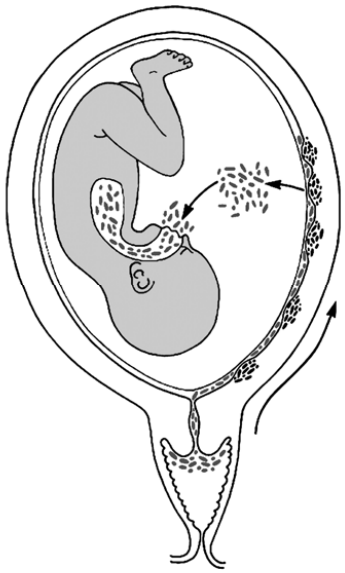

Figure 3. *The ascending route of intrauterine infection. Adapted from Romero et al 2006.*

Microorganisms that are frequently associated with IUI are *ureaplasma urealyticum*, *fusobacterium* species and *mycoplasma hominis* (118). There is now increasing evidence that intra-amniotic microbes may be present long before delivery without any overt clinical symptoms. The maternal and fetal genotypes and the innate and adaptive immune system may determine if an invasive IUI occurs or not (118). PROM early in pregnancy is closely associated with IUI and with a high prevalence of intra-amniotic inflammation. Presence of intra-amniotic inflammation independent of presence of microbes has been associated with early spontaneous delivery and adverse neonatal outcome (119, 120).

The fetal inflammatory response can initiate preterm labour, which is thought to be a survival mechanism to prevent the fetus from remaining in an inflamed intrauterine environment with the risk of progressive organ damage (121). Cytokines play a central role in initiation of preterm labour, an active contribution has especially been ascribed to IL-1β and TNF-α (107, 109). Increased levels of IL-6, IL-8 and IL-18 in cervical and amniotic fluid women with preterm labour was a strong predictor of histological chorioamnionits, and an increase in maternal serum levels of IL-6 before delivery in women with preterm PROM was predicitive of funisitis (122, 123). The inflammatory

response produced in gestational tissue after preterm labour is counter regulated by a negative feed back loop where IL-10 plays a central role (94, 124). Available studies on placental transfer of cytokines between the maternal and fetal circulation are contradictory (125, 126), and LPS-stimulation seems to cause different cytokine responses in preterm and term placentas (127). Preterm labour can be initiated without IUI. Placental vascular diseases have been associated with production of pro-inflammatory cytokines as well as with a reduction of the modulatory cytokine IL-10, which may increase the risk of preterm labour (128-130).

Fetal inflammation and perinatal brain damage

Experimental studies

The immature brain is vulnerable to hypoxic and inflammatory insults, where the immature oligodendrocytes are particularly sensitive. Extensive research has been performed using LPS to evaluate the effects of induced inflammation. LPS is a component of the bacterial wall of gram-negative bacteria and consists of a polysaccharide chain and a lipid component (lipid A). LPS is an endotoxin where lipid A exerts the toxic effect and can induce a strong response from the innate immune system. The effects of LPS are mediated through interaction with the TLR4 receptor located on the immune cell. TLR-signalling activates intracellular events such as induction of the transcription factor NF-$\varkappa\beta$, which induces production of cytokines. Microglia are macrophages located within the central nervous system (CNS) and are the only glial cells that express TLR-4 receptors (131). Presence of TLR4 receptors and therefore of microglia is essential for the damaging effect of LPS to immature OLs (131).

Routes of induced inflammation

Different routes of LPS administration have been used in animal models to evaluate the hypothetically damaging effects to the immature brain. LPS has been administrated either directly to the fetus (intravenously, intracerebrally) or to the mother (intravenously, intraperitoneally, intrauterine, peripherally) (132). Fetal LPS administration is generally associated with more extensive structural brain lesions than maternal administration. It is

not clear how the inflammatory response induced by LPS is mediated to the brain. Peripheral LPS can bind to receptors of endothelial cells in the brain and does not seem to cross the blood brain barrier (BBB) (133). Instead, LPS seems rather to modulate the passage over BBB (134). Intraperitoneal LPS administration in rats and opossums was associated with increased BBB permeability preferentially located within white matter and restricted to periods during early fetal development (135).

Fetal intracerebral administration of LPS directly in to the subcortical white matter of rat fetuses, induced white matter injury with increased expression of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 and of inducible nitric oxide synthase (iNOS) as well as loss of oligodendrocyte precursors. No lesions in the grey matter were observed. (131, 136, 137).

Fetal intravenous LPS administration to preterm lamb fetuses results in similar changes as intracerebral administration, with both cystic and diffuse white matter lesions as well as an inflammatory response with activation of NF-κβ (138-142). Interestingly, in two of the studies where *low* dose LPS was administrated data indicated that the brain injury in fact was induced by inflammation and not by cerebral ischemia (140, 142). On the other hand intravenous administration of *high* dose of LPS has been observed to impair compensatory mechanisms to hypoxemia with a reduction in both placental and cerebral blood flow (143).

Maternal intravenous or intraperitoneal LPS administration studied in rats and mice is considered to induce a milder injury than fetal intravenous or intracerebral administration with only higher LPS doses appearing to affect the fetal brain. LPS has not been proven to pass the placenta and probably exerts indirect effects on the fetal brain by other mediators of inflammation (144, 145). Increased cytokine expression in the fetal rat brain can however be detected as early as 1 h following maternal LPS injection (146). Besides white matter injury one study observed additional injury in deep gray matter (147).

Maternal intrauterine LPS administration can be either intracervical or intraamniotic and is considered to mimic chorioamnionitis better than intraperitoneal administration. This

route of LPS exposure has been applied to both rat and sheep models. Similar to maternal intraperitoneal or intravenous administration the resulting lesions are milder than with fetally administrated LPS and consist of subcortical white matter changes accompanied by hypomyelination as well as grey matter lesions. No cystic lesions have been detected (148, 149). An inflammatory response in the fetal brain has been shown within hours after intrauterine LPS administration with activation of TNF-α and IFN-γ as well as up-regulation of several Th1/Th2 pathway genes for cytokine production (150, 151).

An association between detected white matter changes and subsequent neuro-cognitive function following LPS administration has not yet been proven (152). However, LPS administrated to pregnant rats has been associated with behavioural changes in the adult offspring (153).

Tolerance/sensitization

Sensitization occurs when an insult *e.g.* antenatal inflammation makes the tissue vulnerable to another later insult *e.g.* hypoxia-ischemia (HI). Antenatal administration of LPS induces a sensitizating effect that can last for up to 2 weeks in immature animals. Sensitization may be involved in the clinical observation where antenatal infection was associated with an increased risk of CP in term infants as compared to asphyxia alone (154).

Preconditioning or tolerance is defined as an event where a prior insult (inflammation or hypoxia) makes the tissue less sensitive to a severe insult that is initiated after a time delay. Activation of NF-κβ is involved in LPS preconditioning but the role in the immature brain is unclear. The protective effect of hypoxic preconditioning may last for several weeks in the immature brain and includes a stimulation of cerebral vascularisation (155, 156). Hypoxic preconditioning can induce a vascular response with an increase in vascular density (157).

The time interval between the first and second insult defines whether sensitization or tolerance will occur. The same dose of LPS increased the immature brain vulnerability to HI when administrated at 6 h or 3 days before HI but conversely had protective effects

when administrated 24 h before HI (156, 158). Similarly a low dose of LPS 24 h before a subsequent high dose LPS had a protective effect in pregnant mice (159).

Role of cytokines

Pro-inflammatory cytokines are directly involved in mechanisms inducing cerebral injury. Cytokines are produced within the brain by activated microglia and astrocytes. Increased expression of pro-inflammatory cytokines (IL-2, TNF-α, IL-1β and IFN-γ) have been observed in human neonatal brains affected by PVL (160-164). Pro-inflammatory cytokines are involved in cytotoxic injury to oligodendrocyte precursors and neurons through several mechanisms such as induction of excitotoxicity (glutamate), increase in apoptosis (caspase activity) and increased production of NO.

TNF-α

TNF-α belongs to a family of both ligands and receptors, which are expressed in the cell membrane indicating local sites of action under normal conditions (165). TNF-α plays a role in embryogenesis and is involved in modulation of synaptic connectivity (166, 167). The biological effect of TNF-α is mediated primarily by interaction with the tumor necrosis factor receptor (TNFR)-1 and TNFR-2. TNFR-1 is expressed by all cells whereas TNFR-2 is expressed mainly by immune cells and endothelial cells. Generally TNFR-1 is considered to mediate apoptosis whereas TNFR2 mediate anti-inflammatory and anti apoptotic functions, however the two receptors seem to cooperate. In addition TNFR1 mediates activation of the transcriptional factor NF-κβ (165, 168).

TNF-α is considered to be a key cytokine in inflammatory and ischemic brain damage. After intravenous LPS injection to healthy adults, TNF-α is the first cytokine that appears in plasma within 1 h after LPS administration and the protein expression is accompanied by an parallel increase in gene expression (169). Several studies have shown deleterious effects of TNF-α on both the immature and mature brain. TNF-α inhibits proliferation in neural precursor cells (170), induces apoptosis in immature oligodendrocytes and in neurons (171, 172) and facilitates glutamate dependent excitotoxicity (166). Over-expression of TNF-α in transgenic mice evokes astrogliosis, hypomyelination and

activation of microglia (173). Further, TNF-α seems to modulate capillary BBB permeability (174).

TNF-α can also participate in repairing processes, such as oligodendrocyte regeneration (175). It has been proposed that TNF-α exerts a toxic effect during acute injury, but promotes repair mechanisms during the chronic post-injury period (168, 176, 177).This dual role by TNF-α is considered to be mediated by activation of different TNF receptors (132, 168, 178). In experimental settings TNF-α administrated at the time of ischemia exacerberates brain injury, whereas preconditioning with LPS or TNF-α suppress the deleterious effects of TNF-α at the time of ischemia (179, 180).

IFN-γ

Expression of the Th1 cytokine IFN-γ is induced by IL-12 and IL-18 (90). After LPS stimulation, production of IFN-γ is detected after 24 h *i.e.* significantly later than other pro-inflammatory cytokines (181). In the brain IFN-γ may be primarily produced by astrocytes (45). IFN-γ participates in similar neurotoxic pathways as TNF-α and the cytotoxic effects of IFN-γ seems to be potentiated by TNF-α (160, 170, 172, 182). The OLs appear to be especially vulnerable to IFN-γ mediated toxicity, where the immature OLs seem to have the highest susceptibility (183, 184). Combination of LPS and IFN-γ had an additive effect on oligodendrocyte cytotoxicity and was accompanied by increased NO production especially in the differentiated cells. Immature human OLs express IFN-γ receptors and an increased expression of IFN-γ has been observed in neonatal brains with PVL (160). After intrauterine LPS IFN-γ was detected only within the fetal brain but not in placenta or in other fetal tissue (150).

IL-1β

IL-1β is synthesized as a precursor protein which is cleaved to its active form by the enzyme caspase IL1-1β and is together with TNF-α, IFN-γ and IL-18 expressed in the brain after ischemic or inflammatory brain injury. IL-1β activates NF-κβ signalling after HI injury and induces production of IL-6, TNF-α and NO (176, 185, 186). Presence of IL-1β after traumatic injury has been associated with up-regulation of nerve growth factors and promotes remyelination, also suggesting a role of IL-1β in repairing processes

(176). Both TNF-α and IL-1β seem therefore important in the inflammatory process beneficial for recovery.

IL-18

IL-18 belongs to the IL-1 family of cytokines and its precursor protein is triggered by the same pathway as IL-1β. It activates the transcription factor NF-κβ by interaction with the IL-18 receptor and is a strong inducer of Th1 mediated immune response. IL-18 is expressed primarily in the developing brain and is down-regulated thereafter (187). After exposure to HI injury, IL-18 is expressed in astrocytes and microglia and has been associated with development of white and grey matter injury *in vivo* (188, 189).

IL-10

IL-10 is an anti-inflammatory or modulatory cytokine that down-regulates pro-inflammatory cytokines. The balance between pro- and anti-inflammatory cytokines has been suggested to be important in the context of brain injury. Although counter-regulatory mechanisms seem to be present in preterm infants with systemic infections, a decreased anti-inflammatory capacity has been observed in term and preterm infants (104, 190). IL-10 has neuroprotective effects after inflammatory insults *in vivo* (191, 192). IL-10 reduces white matter injury by suppressing microglia activation and by inhibiting endogenous NO-production in oligodendrocytes *in vitro* (184, 193).

IL-6

IL-6 is often expressed together with TNF-α and IL-1β in brain injury models, but the individual contribution of IL-6 to brain injury has been questioned (194). It is released after TNF-α and the release seems partly to be triggered by TNF-α (195). Although frequently detected during inflammatory conditions, IL-6 seems rather to play an anti-inflammatory role by controlling the release of pro-inflammatory cytokines (196).

IL-8

IL-8 is a chemokine that is predominantly produced by monocytes and macrophages and participates in the innate immune response by mediating recruitment of neutrophil granulocytes. It has been considered to be a sensitive marker of both maternal and

neonatal infection and shows a more prolonged increase after LPS-stimulation than TNF-α (181, 197). IL-8 induced chemotaxis increases after birth stress which has been suggested to enhance capacity of the newborn immune response (198).

Clinical studies

Maternal fever, PROM, chorioamnionitis and funisitis are all indicative of an ongoing antenatal inflammation and have in a large number of studies been related to IVH, PVL/WMD, CP as well as later neurocognitive impairment in preterm infants (26, 57, 116, 117, 199-204).

Increased levels of pro-inflammatory cytokines or other markers of inflammation in cord blood at delivery indicate the presence of a fetal inflammatory response. In preterm infants, an elevation of IL-1β, IL-6, IL-8 and IL-18 as well as activated T-lymfocytes and neutrophils in cord blood have been associated with PVL/WMD and with CP (205-210). Indeed, presence of an inflammatory response in cord blood has been associated with cerebral lesions detected by MRI shortly after delivery (205). These studies together strongly suggest that the inflammatory response associated with brain damage is initiated *in utero* and that fetuses responding to an inflammatory stimuli have a higher risk of cerebral injury (205).

Early postnatal cytokines have not been so extensively studied as cord blood cytokines. In one study an increase in IL-6 concentrations up to >100 pg/ml within the first 12 postnatal hours was predictive of IVH grade III or parenchymal hemorrhage (211).
Some studies have failed to show associations between IUI, cytokines in cord blood or neonatal blood and later neurological morbidity (56, 212, 213). These studies will be discussed below.

Antenatal inflammation has been associated with hemodynamic impairment in the fetus and the preterm infant. Fetuses to mothers with preterm PROM have echocardiographic signs indicating diastolic dysfunction (108). Histological signs of feto-maternal infection (chorioamnionitis, fetal vasculitis) and increased cord blood concentrations of IL-6 have

been associated with decreased blood pressure and cardiac hemodynamic changes corresponding to increased cardiac output during the first postnatal hours in very preterm infants (214). Increased cardiac output suggests lower systemic vascular resistance. Cytokines up-regulate the endothelial production of prostaglandins and NO, substances that decrease vascular tone (106, 214). Oxidative stress has also been suggested to play a role in vascular dysfunction induced by inflammation. Administration of LPS to adults induces vasodilatation that is reversed by antioxidants (215). Catecholamines (epinephrine, norepinephrine and dopamine) are substances that exert hemodynamic effects by interfering with cardiac output as well as vascular tone. A stress response with a concomitant increase in catecholamines can modulate cytokine profiles towards an anti-inflammatory response with inhibition of production of TNF- α and IFN-γ and with enhanced production of IL-6 and IL-10 (216, 217).

Whether circulatory cytokines pass the BBB is unclear, however experimental studies have demonstrated that cytokines and chemokines can modulate BBB permeability (177, 218-220). In preterm infants, cytokine concentrations in cerebrospinal fluid (CSF) and plasma did not correlate with each other, which however, may be related to the varying interval between the CSF and blood sampling, up to 24-48 h in some infants (221, 222).

Concentrations of cytokines in CSF in preterm infants have been related to feto-maternal infection as well as to neurological injury. Infants exposed to histologic chorioamnionitis or fetal vasculitis had higher concentrations of IL-6 and IL-8 in CSF within the first 24 hours after birth (223). Cerebral ultrasound abnormalities and white matter damage as defined by MRI have been associated with increased CSF concentrations of TNF-α, IL-6 and IL-10 in preterm infants (221, 222). Post-hemorrhagic hydrocephalus in preterm infants has been related to a pro-inflammatory response in the CSF with higher CSF concentrations of IFN-γ in a subgroup developing cystic WMD (224, 225).

The Insulin-like growth factor (IGF) system

Classification and biological actions

The insulin-like growth factors (IGF-I and IGF-II) are peptides with a molecular weight of 7.5 kDa that were described for the first time 50 years ago (226). The most important function of the IGFs is stimulation of cell proliferation but they possess anti-apoptotic properties as well. Both IGF-I and IGF-II share structures homologous with pro-insulin. While insulin is stored intracellularly in the β cells and released by stimulation, the IGFs exists in an extra-cellular pool that is controlled by specific insulin-like growth factor binding proteins (IGFBPs) (227). Both IGF-I and IGF-II mediate cellular actions by binding to the IGF-I receptor (IGF-IR) located on the surface of different cell types in all tissues thereby activating intracellular signalling pathways. The IGF-IR shares similarities with the insulin receptor and IGF-I is capable of binding to the insulin receptor although with an affinity approximately 100-fold lower than that for insulin (226). IGF-I mediates independent glucose lowering effects and increases insulin sensitivity (228, 229). The IGF-II receptor is structurally different to the IGF-IR and does not appear to be a signalling receptor for IGF-I and IGF-II. It has been suggested that the IGF-II receptor primarily acts as a clearance receptor for IGF-II (226, 227).

Circulatory IGF-I is primarily produced by the hepatocytes in the liver and the production is regulated by pituitary growth hormone (GH) via specific GH-receptors (227). In addition, nutritional factors can regulate production of IGF-I both directly and indirectly via insulin and by changes in expression of GH receptors (230). Whereas IGF-I is essential for promoting longitudinal postnatal growth the physiological postnatal role of IGF-II is not well defined. Production of IGF-II seems neither to be regulated by GH nor nutrition (231).

Almost the entire pool of extracellular IGFs (99 %) are bound to six different IGFBPs (IGFBP-1 to IGFBP-6) with a higher binding affinity than to the IGF-receptor. The IGFBPs regulate the biological activitiy of the IGFs through several complex mechanisms. They can either inibit or potentiate cellular IGF responses, influence distribution and elimination of the IGFs and can also exert exclusive intracellular actions

that are either independent or modulated by the IGFs. Further, it has been hypothesized that the IGFBPs may be involved in gene transcription and that they may regulate protease activity (227).

The main part (75 %) of the IGFs are carried in a ternary complex with a molecular weight of 150 kDa that consists of IGF-I or IGF-II together with IGFBP-3 (50 kDa) and an acid labile subunit (ALS) (88 kDa) (226). Production of IGFBP-3 and ALS is GH-dependent and occurs in hepatocytes (ALS) and in Kupffer cells (IGFBP-3) in the liver (232). The ternary complex retains the IGFs within the circulation and prolongs their half lifes which is between 12 to 15 h as compared to half-life of free IGF-I which is less than 10 min (233). Other IGFBPs only form binary complexes with the IGFs where the lower molecular weight of the binary complexes facilitates movement from the circulation to the extra-cellular compartments. The ternary complex can be modified by cleavage of ALS resulting in a binary complex which is capable of leaving the circulatory reservoir. Another described mechanism is cleavage of IGFB-3 by specific proteases which decreases the affinity between IGFBP-3 and the IGFs although both proteins remain within the ternary complex (227, 234). IGFBP-3 proteolysis is more extensive in the extra-cellular compartment and occurs during catabolic conditions, during pregnancy and under circumstances with insulin resistance. The proteolysis is considered to be a physiologic reaction with the purpose to increase availability of free IGFs for tissue-uptake (227, 234, 235).

IGFBP-1 is produced in the liver by hepatocytes (232). The production is not GH–dependent but is negatively regulated by insulin which inhibits IGFBP-1 transcription. Concentrations of IGFBP-1 show a diurnal variation and follow the variation in levels of insulin and food intake. Concentrations of IGFBP-1 decrease when insulin concentrations increase and IGFBP-1 is therefore regarded to be a marker of insulin resistance (236). Further, IGF-I and IGF-II seem to inhibit expression of IGFBP-1 although insulin is the major regulator of IGFBP-1 production (237). IGFBP-1 is generally considered to inhibit IGF-actions. IGFBP-1 consist of two phospho-isoforms, where the high phosphorylated form (hpIGFBP-1) has several fold higher binding affinity to IGF-I and is more resistant to proteolysis than the non- or low-phosphorylated form

(lpIGFB-I) (234, 238). Degree of phosphorylation thus influences the likelihood of IGF-I of binding to its receptors, where hpIGF-I inhibits IGF-action and lpIGFBP-1 stimulates it. Degree of IGFBP-1 phosphorylation does not influence the binding affinity between IGF-II and IGFBP-1.

The remaining binding proteins IGFBP-2, IGFBP-4 and IGFBP-5, IGFBP-6 inhibit whereas IGFBP-5 potentiates IGF-actions. IGFBP-2, IGFBP-5 and IGFBP-6 preferentially bind to IGF-II (239).

The IGF-system and fetal growth

The hormonal pathways that mediate fetal growth differ from those that influence postnatal growth. Postnatal longitudinal growth is mediated by GH that stimulates hepatic production of IGF-I. Levels of GH decline postnatally concomitant with a rise in IGF-I. Fetal insulin is the major fetal growth hormone and promotes fetal growth by direct action on tissue uptake of nutrients, as well as by stimulation of IGF-I production. GH does not seem to be a major regulator of fetal growth and the expression of GH-receptors in the fetus is low. Since IGF-I exerts a negative feed back control of pituitary GH-production, high fetal levels of GH may rather be a result of low IGF-I levels secondary to reduced amount or dysfunctional GH receptors (240).

Both IGF-I and IGF-II are expressed early during gestation. IGFs are synthesized by all fetal tissues indicating that autocrine or paracrine mechanisms are important especially during early gestation. Plasma concentrations of both IGF-I and IGF-II increase during gestation with several fold higher concentrations of IGF-II (241). IGF-II is the principal regulator of embryonic growth and of early placental development whereas IGF-I promotes growth and placental function later in gestation and especially after birth (231, 242, 243). Both proteins mediate tissue exert specific effects that are essential for development of different organ systems. IGF-I exclusively promotes development of the central nervous system whereas IGF-II is required for development of placenta and fetal adrenal cortex. The growth promoting effects are mediated by the IGF-IR and for IGF-II probably also by the insulin receptor (244).

Fetal plasma levels of IGF-I are closely related to maternal nutritional status and fetal intravenous infusion of IGF-I increases fetal uptake of nutrients from the placenta (245). Fetal IGF-levels are regulated by an interaction between placenta and the fetus with both placental uptake and release of IGF-I depending on levels of fetal IGF-I. This feto-placental interaction thus regulates the substrate distribution between the maternal and fetal compartments (246). In the fetal compartment glucose concentrations rather than amino acids seems to be the primary regulator of IGF-I levels (247).

The primary IGFBPs during pregnancy are IGFBP-1 and IGFBP-2, both with inhibitory effects on IGF action. IGFBP-3 begins to increase during the last trimester to become the dominating IGFBP postnatally. Changes in phosphorylation status of IGFBP-1 occurs during pregnancy with increased concentration of lpIGFBP-1, considered to be a physiological mechanism to increase tissue availability of IGF-I and thus promote fetal growth (248, 249). Sampling of fetal blood during the second and third trimester has shown that concentrations IGF-I, IGF-II, IGBP-2 and IGFBP-3 increase, whereas concentrations of IGFBP-1 decrease with advancing GA (250). This is in agreement with the findings of a positive correlation between IGF-I or IGFBP-3 and BW and a negative correlation between IGFBP-1 and BW in cord blood samples at birth (251, 252).

IUGR and maternal preeclampsia have been associated with decreased cord blood levels of IGF-I and IGFBP-3 with a corresponding increase for IGFBP-1 in both preterm and term infants. These changes of the IGF-system are suggested to be protective mechanisms to restrict IGF-I mediated fetal growth when placental nutrient supply is limited (252-255). IUGR has further been associated with a concomitant reduction of IGF-I receptors in the placenta (256). During acute or chronic hypoxia circulatory levels of IGF-I decrease, with a corresponding increase in IGFBP-1 (252, 257-261). Up regulation of IGFBP-1 levels *in vitro* has been related to changes in gene transcription in the hepatocytes mediated by hypoxia inducible factors (262).

Cortisol may affect fetal growth by interaction with IGF-system. Cortisol appears to affect fetal IGF gene expression, either directly or indirectly by changes in expression of

GH-receptors and has been suggested to be involved in the switch from paracrine production to endocrine production of IGF-I during the transition from fetal to postnatal life (242). The growth inhibiting effect of glucocorticoids may be caused by an increase in IGFBP-1 expression (237, 263).

IGF-I, IGF binding proteins and brain development

IGF-I influences all parts of normal brain development except migration, which means that IGF-I is involved in differentiation, proliferation and maturation of progenitors of neural stem cells and of precursors of neuronal cell lineages as well as in apoptotic mechanisms (244, 264). Overexpression of IGF-I increases brain size and number of neural cell lineages (265). IGF-I plays an important role in differentiation and proliferation of oligodendrocyte progenitor cells and in promoting remyelination both *in vivo* and *in vitro* (266-269).

IGF-I receptors in the brain are expressed early and are abundant during fetal development (266). Due to the blood brain barrier (BBB) many of the IGF actions in the brain are local *i.e.* autocrine or paracrine but probably endocrine IGF-I produced by the liver is also essential for normal brain function (244, 264, 270). IGF-I seems to cross the BBB using special transport mechanisms, which may involve IGFBPs (271, 272). The choroid plexus has been suggested to be one major site for transport of IGF-I into the brain (244).

IGFBP-2, IGFBP-4 and IGBBP-5 are expressed in the rat brain during development whereas in the human fetal brain exclusively IGFBP-3 has been visualized in cerebral cortex (244, 273). Although IGFBP-1 has not been described to be present in the brain during development, over-expression of IGFBP-1 in transgenic mice has been associated with impaired brain development that can be due to direct effects of IGFBP-I on the brain or to indirect effects by inhibition of IGF activity (274, 275).

Nutrition is the most important environmental factor that influences brain development. Maternal and fetal under-nutrition during pregnancy has been associated with impaired

fetal brain growth, with corresponding changes in the IGF-system *i.e.* a decrease in levels of IGF-I and an increase in IGFBP-1 (244).

Neuro-protective mechanisms of IGF-I

IGF-I appears important for endogenous protection following brain injury. Local production of IGF-I is increased in the brain after HI injury. Induced perinatal asfyxia is followed by two phases of brain energy failure with subsequent neuronal death. The first stage of cell death occurs in close proximity to the insult and involves cytotoxic mechanisms whereas the second protracted stage may last for weeks and is characterized by apoptotic neuronal cell loss. The neuro-protective effects of IGF-I appear to be confined to the second stage of brain injury by reducing apoptotic cell death in both gray and white matter. This opens opportunities for post-insult administration (276). The anti-apoptotic effects have been related to transport of nutrients essential for cell survival over the cell membrane (244). Binding of IGF to the IGF-IR, results in activation of intracellular pathways that mediate expression of glucose transport proteins in the cell membrane (277). Other anti-apoptotic mechanisms are protection of IGF-I against glutamate mediated toxicity (278). Following HI injury, intracerebrally administrated IGF-I protects oligodendrocyte precursor cells and improves neurological function (279-282). IGF-I protects oligodendrocytes and preserves myelination after an inflammatory insult induced by TNF-α *in vitro* and *in vivo* (283, 284). The protection is mediated by inhibition of intracellular apoptotic pathways (285).

A modified IGF-I protein has been identified in the fetal brain which is the result of cleavage of a tripeptide (glycin-prolin-glutamate; GPE) from the original IGF-I protein. This IGF-I variant demonstrates increased affinity to the IGF-IR concomitant with reduced affinity to the IGFBPs. Moreover, the cleavage product *i.e.* the tripeptid GPE has been shown to possess unique neuro-protective properties not mediated via the IGF-IR. After an HI insult, peripheral and central administration of a GPE analogue to juvenile rats reduces brain injury by reducing inflammation and promoting neovascularisation and astrogliosis (286, 287).

Interaction between IGF-I and immune system

The IGF-system interacts closely with the immune system (288). IGF-I has been shown to promote maturation and induce cytokine production in neonatal mononuclear cells (289). Overexpression of TNF-α in transgenic mice reduces brain growth with corresponding changes of IGF-system components, where expression of IGF-I is reduced and IGFBP-3 is increased (290). Acute and chronic inflammation is associated with alterations in the IGF-system. Childhood diseases characterized by chronic inflammation are associated with retarded growth, and in juvenil idiopatic arthritis IL-6 levels correlate inversely with levels of IGF-I (291).

Pro-inflammatory cytokines may decrease the capacity of the IGF-system through several mechanisms as observed in transgenic mice models over-expressing circulatory IL-6. These animals had a marked reduction in growth rate and decreased levels of IGF-I in spite of a normal GH-production (291). The hepatocyte production of IGF-I and ALS was not primarily affected and the decrease in IGF-I was attributed to a decreased IGFBP-3 production, impaired formation of the ternary complex and thus an increased clearance of IGF-I. IGFBP-3 is produced by a different hepatic cell type (Kupffer cells) than IGF-I and ALS and these cells constitutively express the IL-6 receptor. An increased IGFBP-3 proteolysis and a decrease in hepatic GH receptors was additionally observed (292, 293).

In another *in vivo* model TNF-α and IL-1β induced a decreased hepatic production of both IGF-I and ALS but with preserved IGFBP-3 levels which suggests that other cytokines may interfere with the IGF-system through other pathways (294, 295). Pro-inflammatory cytokines may induce a GH insensitivity and interfere with the IGF-IR *in vitro* and *in vivo* (288, 292, 293, 296-299). Finally, TNF-α, IL-1β and IL-6 up-regulate levels of IGFBP-1 and induce insulin resistance *in vivo* and *in vitro* (300-304).

The IGF-system after preterm birth

After birth an immediate decrease in circulatory IGF-concentrations occurs due to interruption of maternal/placental supply. In term infants, a restitution appears within the

first few days whereas preterm infants continue to have lower postnatal IGF concentrations than corresponding fetal levels during several weeks (250, 305, 306). Postnatal levels of IGF-I and IGFBP-3 have been associated with short term growth velocity in preterm infants (307-309). Persisting low levels of IGF-I and IGFBP-3 have been observed up to school age in children with very preterm birth, which has been ascribed to a partial GH-resistance (310).

Concentrations of IGF-I and IGFBP-3 are nutritionally regulated and correlate with protein and energy intake (307, 311) in preterm infants. At preterm birth the nutrient intakes of protein and energy do not reach required intrauterine needs (312). Thus a catabolic state due to under-nutrition occurs. Protein malnutrition has been proposed to be a major determinant of low IGF-concentrations after preterm birth where low protein intake during the first postnatal weeks has been associated with a delayed increase in circulatory IGF-concentrations (313). Early nutritional deficits and poor postnatal growth may impair brain development and later neuro-cognitive functions (62, 314). Changes in expression of IGF-I or IGF-IR in different regions of the brain as well as a protective effect of IGF-I on myelination have been observed after nutritional deprivation in murine models (315-317). In preterm infants postnatal under-nutrition has been associated with delayed maturation of EEG background (318).

IGF-I is important not only for neurogenesis but also for angiogenesis in the brain. IGF-I and IGFBP-3 have been observed to promote retinal and brain vessel growth in mice (319, 320). Persisting low postnatal levels of IGF-I and retarded head growth in very preterm infants have been observed to be predictive of development of retinopathy of prematurity (ROP) (321, 322).

Administration of IGF-I

Exogenous administration of recombinant (rh) IGF-I increases insulin sensitivity and has been used to improve glycemic control in patients with diabetes (323). RhIGF-I is also used to treat children with primary IGF-I deficiency including GH-insensitivity syndromes (324, 325). A complex with an equi-molar combination of rhIGF-I and

IGFBP-3 has been introduced during the last years, having the advantage of less side effects and a slower clearance (326). Administration of enteral IGF-I to sheep fetuses improved fetal growth and gut maturation (327). No studies have so far been carried out evaluating systemic postnatal adminstration of rhIGF-I to very preterm infants.

Fresh frozen plasma (FFP) is frequently used during neonatal intensive care as volume expansion or as a source of coagulation factors. Concentrations of IGF-I and IGFBP-3 are about 10 times higher in FFP from adult donors as compared to postnatal concentrations in very preterm infants and are stabile proteins that remain intact after freeze-thaw treatment of plasma samples (328). FFP may therefore theoretically be an exogenous source of IGF-I and IGFBP-3.

Aims of the thesis

Inflammation affecting the immature fetus may have a damaging effect on the developing brain. The insulin-like growth factor system is essential for fetal growth and has described neuro-protective properties. These rationales constituted the basis for the following specific study aims:

- (I) to evaluate the temporal profile of pro-inflammatory and modulatory cytokines in fetal and neonatal blood in very preterm infants and determine their relationship to hemodynamic impairment and to morphological brain damage

- (II) to determine the predictive value of pro-inflammatory and modulatory cytokines in fetal and neonatal blood for neurological and developmental outcome at 2 years of corrected age in very preterm infants

- (III) to assess the effect of pro-inflammatory cytokines on components of the insulin-like growth factor system at very preterm birth

- (IV) to evaluate the effect of administration of fresh-frozen plasma on concentrations of IGF-I and IGFBP-3 in extremely preterm infants

Subjects and study design

Temporal profiles of cytokines in very preterm infants (I)

To this prospective cohort study, very preterm infants were recruited, who were born at the University Hospital in Lund between February 2001 and February 2003 and admitted to the Neonatal Intensive Care Unit (NICU). All pregnancies were dated by ultrasound at 17-18 GW. Pregnant women with a risk of delivery before 32 GW were identified. Both parents were informed, using both verbal and written information. Inclusion criteria were antenatal informed consent, GA < 32 weeks at birth and absence of major anomalies.

Seventy-four infants were included in the study. Antenatal characteristics of the study population are presented in Table 1. During the period of recruitment to the study a further 95 infants with a GA < 32 weeks were born and admitted to the NICU in Lund. Neonatal characteristics of the study population and of non-participating infants are presented in Table 2 for comparative purposes.

Table 1. *Antenatal characteristics of the study population in paper I and III (N=74).*

Clinical chorioamnionitis	5 (7)
Maternal antibiotic treatment	53 (72)
Suspected maternal infection	22 (30)
Premature rupture of membranes	30 (40)
Preeclampsia	18 (24)
Preterm labor	29 (39)
Antenatal steroids	74 (100)
Caesarean section	52 (70)

All values expressed as number (percentage)

Table 2. *Neonatal characteristics of the study population (paper I and III) and of non-participating infants born at Lund University Hospital before 32 gestational weeks during the same time period (February 2001 to February 2003).*

	Study population N=74	Non-participating infants N=95	*p*-value
Gestational age (weeks)	27.1 (2.0)	28.0 (2.4)	0.015
Birth weight (g)	1007 (280)	1179 (457)	0.003
Twins or triplets	34 (46)	32 (34)	NS
Small for gestational age	20 (27)	16 (17)	NS
Apgar Score < 7 at 5 min	24 (32)	16 (17)	0.018
Male gender	39 (53)	54 (57)	NS
White matter damage, n (%)	8 (11)	2 (2)	0.021
Cerebral hemorrhage (I/II/III/PH)	7/3/5/1	4/2/2/2	___
Any cerebral hemorrhage	17 (24)	10 (10)	0.023
Severe cerebral hemorrhage	6	4 (4)	NS
Mortality, n (%)	3 (4)	9 (10)	NS

Values expressed as mean (SD), or number (percentage). Severe cerebral hemorrhage; intraventricular hemorrhage grade III or parenchymal hemorrhage (PH)

An umbilical or peripheral arterial catheter was inserted within one hour after birth for blood sampling and for continuous monitoring of BP during the first 72 postnatal hours. Sampling for analysis of pro-inflammatory (TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-12) and modulatory (IL-4 and IL-10) cytokines was performed from umbilical cord blood and from arterial blood through the indwelling arterial line at 6 h, 24 h and 72 h of postnatal age. Cerebral ultrasound was performed after 1, 3 and 7 postnatal days, at 6 weeks and at term. Antenatal and neonatal clinical data was prospectively registered from the maternal and infants records until home discharge.

Temporal profiles of cytokines and developmental outcome in very preterm infants (II)

The study population consisted of all surviving infants from the cohort in paper I (N=71). Parents of all surviving infants were asked for participation in the follow-up study and a separate informed consent was obtained. The parents of three infants declined participation and one infant was lost to follow-up. None of these four infants had cerebral hemorrhage or WMD as detected by neonatal ultrasound. Sixty-seven children (94%) were assessed at a corrected age of mean (SD) 24.0 (0.53) months.

At follow-up, neurological assessment was performed by one examiner (I H-P) using a standardized neurological examination Neurological Optimality Score (NOS) and developmental outcome was assessed by one examiner (AH) using Bayley Scales of Infant Development with the two different index scales, Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI). The developmental and neurological assessments were performed on the same day and the examiners were blinded to each others results. Maternal and paternal educational levels were registered in three categories: completed compulsory school, upper secondary education or university degree.

Blood sampling for analysis of cytokines and registration of antenatal and clinical data was performed as described in paper I. Clinical risk variables relevant for neurological and developmental outcome were evaluated in relation to outcome variables (NOS, MDI, PDI and CP) .The following clinical risk variables were evaluated: PROM, clinical maternal infection, clinical chorioamnionitis, preeclampsia, mode of delivery, multiple pregnancy, gender, GA in days, SGA, Apgar score**,** treatment with dopamine during the first 72 h, medical treatment or surgical ligation of persistent ductus arteriosus, postnatal septicaemia, ventilator treatment (days), supplemental oxygen at 36 GW, retinopathy of prematurity and accumulated dose (mg/kg) of hydrocortisone and/or betamethasone administered from birth until discharge.

Cytokines and the IGF-system at birth in very preterm infants (III)

The study population was identical to that of paper I. Blood sampling for analysis of IGF-I, IGFBP-3, lp/hpIGFBP-1 was performed from cord blood and from arterial blood at 72 h postnatal age. Blood sampling for analysis of cytokines, cerebral ultrasound examinations and prospective registration of clinical neonatal and maternal data were performed as described in study I. Treatment with hydrocortisone, betamethasone and insuline during the first 72 postnatal hours was registered. Enteral, parenteral and total nutritional intake of protein (g/kg) and energy (kcal/kg) was calculated prospectively from birth until time point of sampling of IGF-I, IGFBP-3 and lp/hp IGFBP-1 at 72 h.

Effects of fresh-frozen plasma on levels of IGF-I and IGFBP-3 in extremely preterm infants (IV)

The study was performed in a cohort of very preterm infants born at the University Hospital in Lund and at Drottning Silvias Hospital, Göteborg between December 2005 and July 2006. All pregnancies were dated by ultrasound at 17-18 gestational weeks.
Inclusion criteria were requirement of FFP for clinical purposes (arterial hypotension, decreased urinary production, and signs of dehydration or bleeding tendency), GA < 28 weeks at birth, postnatal age less than 7 days, and informed parental consent from both parents. Exclusion criteria were ongoing treatment with insulin and/or major congenital anomalies. Twenty infants were enrolled in the study. The mean (SD) GA was 25.3 (1.3) weeks and BW 724 (177) g. Thirteen infants (65%) were boys and ten infants (50%) were SGA.

Transfusion of FFP (10 ml/kg) was performed as a continuous infusion through a separate peripheral or central intravenous line. Sampling for analysis of IGF-I and IGFBP-3 was performed from the batch of FFP immediately before initiation of transfusion. Continued sampling was performed from arterial blood through an indwelling arterial catheter immediately before initiation of transfusion, directly after transfusion and at 6, 12, 24 and 48 h after completed transfusion. Plasma glucose concentrations in neonatal blood were analyzed before FFP transfusion, immediately after and at 6 h after completed FFP transfusion.

Antenatal and neonatal clinical data were prospectively recorded with registration of packed red blood cells (ml/kg) or FFP (ml/kg) administered on clinical indication during 24 h before study entry and during the study period, accumulated dose of hydrocortisone (mg/kg) or insulin (U/kg) administered during the last 24 h before study entry and intravenous glucose intakes (mg/kg/min) during 6 h before, during FFP infusion and the following 6 h.

Methods

Quantitative analyses of cytokines

Blood samples were collected in a Vacutainer tube containing the anticoagulant EDTA (Becton Dickinson (BD) biosciences, San Jose, CA, U.S.A.), put on ice and delivered within 20 minutes to the local chemical laboratory where the plasma separated and stored in a freezer (-70°C) until analyzed in one batch. Levels of 9 different cytokines (TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-12, IL-4, IL-10) cytokines in plasma were determined by cytometric bead array (CBA) (BD biosciences) and flow cytometry according to the manufacturer's recommendations. This assay is based on a mixture of six microbead populations with distinct fluorescent intensities (FL-3) precoated with capture antibodies specific for each cytokine and uses the sensitivity of fluorescence detection by flow cytometry to measure soluble cytokines in a particle-based immunoassay. Each bead provides a capture surface for a specific cytokine and is analogous to an individually coated well in an ELISA plate. Briefly, 50 µl of mixed beads coated with cytokine-specific capture antibodies were added to 50 µl of patient plasma and incubated for 1.5 h at room temperature. After washing, 50 µL of phycoerythrin-conjugated anti-human inflammatory cytokine antibodies were added. Simultaneously, 50 µl of standards for each cytokine (0 to 5000 pg/ml) were treated likewise to generate a standard curves. Two-color flow cytometric analysis was performed using a FACSCalibur® flow cytometer (BD biosciences). Data were acquired and analyzed using BD CBA software. Forward vs. side scatter gating was employed to exclude any sample particles other than the 7.5 µm polystyrene beads. Flow cytometric analysis was performed and analyzed by a single operator and cytokine concentrations were determined based on the standard curves using the CBA software. The lower limit of detection for the various cytokines evaluated ranged from 2-10 pg/mL. For results above the upper limit of detection, serial dilution of the sample was performed to accurately determine cytokine levels. A level of ≤ 0.1 pg/mL was regarded as non-detectable. Intraassay coefficents of variation (CV) for each of the individual cytokines are stated on www.bdbiosciences.com.

Plasma concentrations of the respective cytokines from umbilical cord blood and from neonatal blood at 6, 24 and 72 h of postnatal age were used to calculate an area under curve (AUC) as an assessment of cytokine burden over time in each subject. AUC was calculated according to the trapezium rule (329). AUC was only calculated in subjects with three or more valid plasma samples (N = 69). Calculated AUC was adjusted for total sampling period, being either 66 or 72 h, thus achieving a weighted average level over time.

Quantitative analyses of IGF-I, IGFBP-3 and IGFBP-1

Blood samples were collected in a Vacutainer tube (Becton Dickinson (BD) biosciences, San Jose, CA, U.S.A.), put on ice and delivered within 30 minutes to the local chemical laboratory where the plasma and serum in the respective samples were stored in a freezer (-70°C) until analyzed in one batch.

Analyses of samples in paper III

IGF-I, IGFBP-3 and lp/hpIGFBP-1 were determined in plasma samples. IGF-I was analyzed using enzyme linked immunosorbent assay kit (DSL-10-5600, Diagnostic Systems Laboratories, Inc, Webster, TX, U.S.A). Intra- and inter-assay coefficients of variations were 4.5-7.1% and 4.8-8.8%, respectively, and the detection limit was 5 μg/L. IGFBP-3 was measured by immunofluorometric assay (330). Intra- and inter-assay coefficients of variation were 3.6-6.2% and 4.9-11%, respectively, and the detection limit was 0.3 μg/L.

IGFBP-1 was analysed using two monoclonal antibody based immunoenzymometric assays detecting different phosphoisoforms of IGFBP-1 (Medix Biochemica, Kauniainen, Finland) (331). The intra-assay coefficients of variations were 3.0% and 4.6%, respectively, and the inter-assay coefficients of variations were 6.8% and 6.4% respectively. The detection limits were 0.25 μg/L and 0.3 μg/L respectively.

Analysis of samples in paper IV

IGF-I and IGFBP-3 in neonatal serum samples and in plasma samples from batch of administered FFP were analyzed using IGFBP-blocked RIA and a specific RIA (Mediagnost GmbH, Tübingen, Germany) (332). The IGF-I samples were diluted 1:50 and the IGFBP-3 samples were diluted 1:300. The intra-assay coefficients of variations (CV) for IGF-I were 18, 11 and 7% at concentrations of 9, 33, and 179 μg/L and for IGFBP-3 10, 7 and 6% at concentrations of 716, 1750 and 3929 μg/L, respectively.

Pharmacokinetics of IGF-I

Estimation of pharmacokinetic parameters of IGF-I was performed by population analysis using nonlinear mixed effects modeling in the computer program NONMEM. Calculation of half-life ($t_{1/2}$) was based on the equation $t_{1/2}$ = ln (2) * Volume of distribution (Vd) / Clearance (Cl).

Cerebral ultrasound

In paper I, II and III repeated ultrasound examinations of the brain were performed by two of the investigators (I H-P, DL) using an Acuson XP 512 (7.5 MHz) or Acuson Sequoia (8.5 MHz) (Mountain View, CA, U.S.A.) at 1, 3 and 7 postnatal days, at 6 weeks and at 40 gestational weeks. The images were reviewed by a pediatric radiologist. Images with suspected abnormalities where reassessed by another pediatric radiologist blinded to the clinical history. Cerebral hemorrhage was classified as subependymal hemorrage (grade I), intraventricular hemorrhage (IVH) (grade II-III) or as parenchymal hemorrhagic infarction (PHI). White matter brain damage was defined in the presence of periventricular echodensities persisting for more than 7 days or periventricular cysts.

Blood pressure measurements

Values of mean, systolic, diastolic arterial BP, oxygen saturation and heart rate were monitored continuously and were digitally stored every 15 minutes during the first 72 h (Hewlett-Packard M3153A, Viridia Surveillance Center, U.S.A.). In the infants without an indwelling arterial line (N=9), non-invasive BP (Neonatal blood pressure cuff, Hewlett Packard, U.S.A.) was obtained every 60 minutes and documented in the protocol. Average

systolic, diastolic and MABP were calculated as the mean of aggregated data for each subject.

Neurological examination

Neurological assessment was performed by one examiner (I H-P) using the Hammersmith Infant Neurological examination. This is a standardized method for neurological assessment that has been used from 2 months of age and has been validated in both preterm and term infants up to 18 months of age. The method is based on 26 items where each item is scored separately. The item scores can be added, thus achieving a Neurologic Optimality Score (NOS) with a maximum score of 78. A score below the 10th percentile was defined as suboptimal which was defined as NOS <74 in infants at 18 months of age (333, 334).

CP was defined using the definitions adopted for European classification of CP (335). Children presenting previously not recognized symptoms of CP at follow-up at 2 years of corrected age were re-evaluated by a pediatric neurologist. Although the diagnosis of CP has been stated to be reliably diagnosed at 24 months of age a secondary interrogation of the regional CP registry was carried out to identify any additional children being diagnosed between 2 and 3 years of corrected age (336). No additional cases were identified.

Bayley Scales of Infant Development

Developmental outcome was assessed by one psychologist (AH) using the Bayley Scales of Infant Development (BSID-II) which consist of three separate scales (the Mental scale, the Motor scale and the Behaviour rating scale). Only the Mental and the Motor scale were used in the assessment. Raw scores of both scales are converted to a Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) both with a mean of 100 and SD of 15. A subnormal development was defined by an index score of 1 SD below the normative mean (MDI or PDI <85) and a developmental delay was defined by an index score of 2 SD below the normative mean (MDI or PDI <70). Infants with

MDI or PDI scores of 50 or below were assigned a score of 50 according to the manual (337).

Clinical data

Preeclampsia was defined as blood pressure ≥ 140/90 mm Hg and albuminuria > 0.3 g/l/day and PROM as a rupture of membranes before the onset of labour. Suspected maternal infection was defined in the case of elevated maternal C-reactive protein > 5 mg/l and/or fever > 38°C. Clinical chorioamnionitis was defined when two of the following criteria were present: maternal fever > 38°C, maternal tachycardia, fetal tachycardia, malodorous amniotic fluid and uterine tenderness. The infants were defined as small for gestational age (SGA) at birth if the deviation of birth weight (BW) was more than 2 SD below the gestational age-related mean of the population (338). Retinopathy of prematurity was defined according to the international classification (339).

Statistics

Statistical analysis was performed using the Statistical Package for Social Sciences software for Microsoft Windows (SPSS Inc., Chicago, IL). Levels of cytokines, IGFBP-1 and calculated AUC were logarithmically transformed before statistical evaluation to obtain a more normal distribution of values (papers I-III).

Correlations between cytokines were assessed with the Spearman Rank correlation (paper I) and correlations between MDI, PDI and NOS were assessed with the Pearson correlation coefficient (paper II). Differences between paired samples were assessed using the Wilcoxon rank sum test (paper I and III) and paired *t*-test (paper IV). Univariate relationships between categorical variables and other categorical or continuous variables were assessed with Chi-square test, Fischers exact test, one sample *t*-test or the Mann-Whitney U-test as appropriate (paper I-IV). Logistic regression was used to obtain odds ratios (95% CI) for each of the comparisons (papers I and II).

Multivariate analysis with adjustment for other variables was performed by using logistic regression analysis (stepwise backward and forward procedures, log-likelihood ratio) or

multiple linear regression analysis as appropriate (papers I-IV). Levels of cytokines, IGF-I or IGFBP-3 exhibiting significant univariate relationships with outcome variables at specific time-points were re-evaluated with adjustment for multiple comparisons using ANOVA for repeated measures (papers II and IV).

Ethical considerations

All studies were approved by the Regional Committé for Research Ethics, in Lund. Parental informed consent from both the father and mother was the rule. Information was given prior to delivery aiming to allow for sufficient time for parental consideration concerning study participation. The studies are hypothesis driven and descriptive and will not directly benefit the participating infant. The results obtained in these studies have to be viewed in a wider perspective where increased knowledge may serve as a base for continued research with the aim of developing therapeutic strategies with the purpose to improve neuro-developmental outcome in infants with very preterm birth.

Results and comments

Temporal profiles of cytokines in very preterm infants (I)

Pro-inflammatory and modulatory cytokines were analysed in cord blood and at 6, 24 and 72 h of postnatal age in 74 preterm infants with a mean GA of 27.1 (2.0) weeks.

Antenatal variables and cytokines

Infants delivered after PROM had higher levels of IL-2, IFN-γ and TNF-α at birth and at 6 h. Further, infants born after maternal infection had higher levels of IL-6 in cord blood (p=0.003) and infants born after clinical chorioamnionits had higher levels of IL-1β, IL-6, IL-8 and TNF-α in cord blood, p=0.011, 0.001, 0.012 and 0.045 respectively, whereas infants born after maternal preeclampsia had lower levels of IL-6 in cord blood (p=0.001) as compared to infants without the respective maternal condition. All associations remained significant after adjustment for GA and gender.

In the absence of placental histology, clinical risk variables (PROM, suspected maternal infection and clinical chorioamnionitis) were used as markers of antenatal inflammation. All these risk variables were associated with higher concentrations of pro-inflammatory cytokines in cord blood or at 6 h, where PROM was associated with an increase in primarily Th1 cytokines (IL-2, IFN-γ and TNF-α). A Th1 predominance has earlier been described in maternal blood and in cord blood in women with PROM at term (340, 341). Preeclampsia was associated with *lower* levels of IL-6 in cord blood, with similar observations described previously (56, 342).

Temporal decrease of cytokines

Concentrations of IL-6, IL-8 and IL-10 decreased significantly from those at birth and at 6h to levels at 72 h of age. These findings are in line with another study of extremely preterm infants showing a temporal decrease of pro-inflammatory cytokines during the first postnatal week (110). The associations between clinical markers of inflammation and pro-inflammatory activity in cord blood as well as the temporal decrease of several

cytokines following birth, suggests that inflammation was initiated *in utero* in the majority of studied infants.

Temporal profile of IL-10

The modulatory cytokine IL-10 was observed to have a very homogenous increase at 6 h in almost all studied infants, with a subsequent decrease in concentrations thereafter (Fig 3). Placental IL-10 modulates the inflammatory process initiated by labour (94, 124). The birth process is associated with very high levels of catecholamines, described to exert anti-inflammatory effects and increase production of IL-10 (217, 343). Adenosine is a metabolite with anti-inflammatory properties that interferes with TLR mediated cytokine production and potentiates LPS stimulated production of IL-10 (100). Adenosine increase in response to hypoxia and stress, due to ATP degradation and higher levels have been described after vaginal delivery (344). Thus, the consistent increase of IL-10 after birth may be related to the parturition process involving catecholamines and/or adenosine. Dopamine can also increase production of IL-10, but the influence of dopamine seems less likely since only a minority (24%) of the infants were treated with dopamine during the first 6 postnatal hours (217).

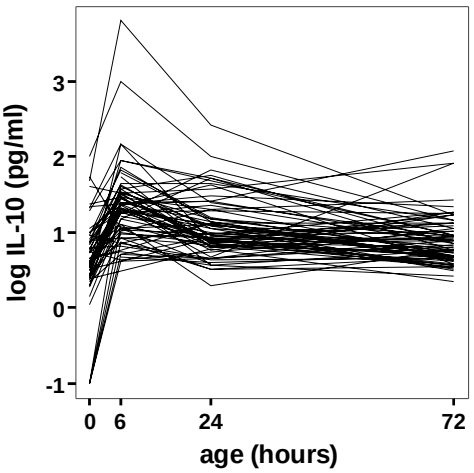

Figure 3*. Individual plasma levels of IL-10 (pg/ml) at birth and at 6, 24 and 72 h postnatal age in preterm infants (N=74). Adapted from paper I with permission.*

Temporal profiles of cytokines and arterial hypotension

Concentrations of IL-6 and IL-8 at 6 and at 24 h as well as AUC IL-6 and AUC IL-8 displayed strong inverse correlations with MABP during the first 72 postnatal hours. AUC IL-1β correlated inversely but less strongly than IL-6 and IL-8 with MABP. All described associations remained significant after adjustment for GA, gender and treatment with hydrocortisone (0-72 h).

A strong inverse correlation between increased cord blood concentrations of IL-6 and BP parameters has previously been described in preterm infants, however the cord values were related to a single non-invasive BP measurement between 2-4 h of postnatal age (214). Since short-term variability of BP is common, continuous BP-monitoring is warranted. We calculated from continuous monitoring the mean of 15 min BP medians for the 72 h period with omitted artefacts, but might have obscured some true short-lasting extreme values. Evaluation with analysis of BP during shorter time epochs (6 h) in relation to cytokines, demonstrated however the same relationships as described above (data not shown). We did not observe any relationships between BP and cytokines in cord blood samples, whereas postnatal blood samples at 6 and 24 h exhibited strong correlations with BP. Thus infants exhibiting a postnatal increase in pro-inflammation were those at risk for arterial hypotension.

In the study by Yanowitz et al mentioned above, histologic chorioamnionits was associated with a decrease in mean and diastolic BP, which is in line with earlier described relationship between funisitis and arterial hypotension described in another study by the same authors (345). We did not observe any association between maternal variables associated with antenatal inflammation (PROM, clinical CA, maternal infection) and BP parameters, which is consistent with the absent relationship between cord levels of cytokines and MABP.

The majority of infants receiving dopamine treatment (0-72 h) for arterial hypotension (N=25) had a distinct increase in concentrations of IL-6 from birth up to 6 h postnatal age, as compared to infants not requiring dopamine treatment who expressed highest

concentrations in cord blood. This is shown in Fig. 4. Dopamine has been ascribed to stimulate production of IL-6 and might therefore have caused the early postnatal increase in IL-6 (217). However, dopamine treatment initiated after 6 h postnatal age was not followed by an increase in IL-6 and no differences in concentrations of IL-6 at 6 h were seen between infants receiving dopamine before 6 h of age and infants receiving dopamine after 6 h of age (data not shown).

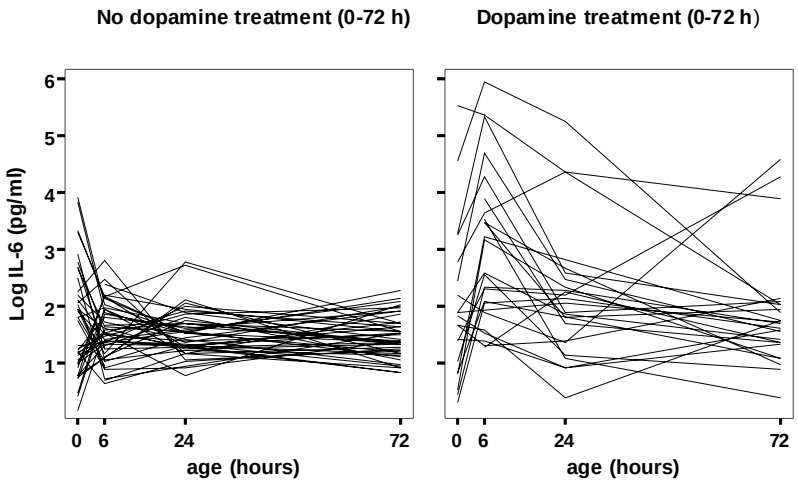

Figure 4. *Individual concentrations of IL-6 (log pg/ml) at birth and at 6, 24 and 72 h postnatal age in infants receiving dopamine treatment for arterial hypotension (N=25) and in those not receiving dopamine treatment (N=49). Adapted from paper I with permission.*

Temporal profiles of cytokines and cerebral hemorrhage

Five infants developed IVH grade III and one infant developed parenchymal hemorrhage which were associated with increases in AUC IL-6 and AUC IL-8 were associated with severe cerebral hemorrhage with associations remaining significant after adjustment for GA and gender. MABP was not associated with ultrasound abnormalities and did not affect the relationship between AUC IL-6 and AUC IL-8 and severe cerebral hemorrhage.

Previous studies have found similar relationships between increased concentrations of IL-6/IL-8 in cord blood or in early neonatal blood and cerebral hemorrhage (211, 346).

Interestingly, no relationship was observed between MABP and cerebral hemorrhage, which is in line with another study, where arterial hypotension during the first 3 postnatal days did not predict cerebral ultrasound abnormalities (74). On the other hand, low CBF after birth as estimated by NIRS or low superior vena cava flow has been associated with IVH in preterm infants (347, 348). As previously mentioned, both preserved and reduced CBF as estimated by NIRS have been described during hypotensive periods in preterm infants (75, 77).

The relationship of IL-6 and IL-8 to circulatory impairment *and* to cerebral hemorrhage, suggests that pro-inflammation is the common factor associated with both of these conditions. A local decrease in blood flow and inflammatory induced endothelial activation, is followed by up-regulation of inducible NO-synthase and adhesion of leukocytes that releases proteases and ROS, which can damage the vessel wall and surrounding tissue (106, 349). Further, ROS can inactivate catecholamines thus decreasing vessel wall tone (215).

Temporal profiles of cytokines and WMD

Eight (11%) infants developed cystic or diffuse WMD, as defined by cerebral ultrasound. Concentrations of IFN-γ at all individual time points as well as AUC IFN-γ were higher in infants with WMD (Fig. 5). In addition levels of IL-2 in cord blood, IL-12 at 6 and 24 h and AUC IL-12 were increased in infants developing WMD. Associations remained significant after adjustment for gender, gestational age and MABP 0-72 h.

These findings show that pro-inflammatory cytokines linked to the Th1 subsets of cytokines were associated with WMD, with IFN-γ being the cytokine most consistently showing associations at all individual time points. An increased expression of the Th1 cytokines IFN-γ, TNF-α and of IL-2 has been observed in human brains with PVL (160, 161, 163). In the brain, IFN-γ is produced by astrocytes and preferentially induces damage to oligodendrocyte progenitors cells that express the IFN-γ receptor, (160, 184). IFN-γ seems to damage immature OLs principally by induced NO-production and thereby RNS (184, 350). IL-12 has not been described as a cytokine that is directly involved in mechanisms associated with WMD, but IL-12 is a strong inducer of Th1 cytokines and

stimulates production of IFN-γ (90). AUC IL-12 correlated with AUC IFN-γ, which may suggest that the association between IL-12 and WMD was due to a covariation with IFN-γ.

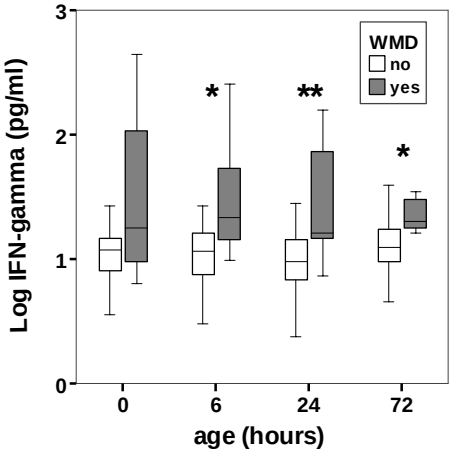

Figure 5. *Plasma concentrations of interferon-gamma (IFN-γ) (pg/ml) at birth and at 6, 24 and 72 h of age in infants developing white matter damage (WMD) (N=8) compared with infants without WMD (N=66) * p<0.05, ** p<0.01. Adapted from paper I with permission.*

Temporal profiles of cytokines and developmental outcome in very preterm infants (II)

Proinflammatory and modulatory cytokines were analysed in cord blood and at 6, 24 and 72 h postnatal age and related to neurological and developmental outcome in 67 very preterm children with a mean GA of 27.1 (0.53) weeks at 2 years of corrected age. Four different outcome measures were assessed: Neurological optimality score (NOS), Mental Developmental Index (MDI), Psychomotor Developmental Index (PDI) and CP.

Outcome measures

The median (range) scores of NOS, MDI and PDI at assessment were 71 (24-78), 86 (50-118) and 96 (50-117), respectively. Forty seven children (70%) had suboptimal NOS (<74), 31 children (46%) had a subnormal MDI (<85) and 20 children (30%) had subnormal PDI (<85). All infants with subnormal PDI also had suboptimal NOS. Both PDI and NOS primarily identify motor impairment. The high proportion of children with

suboptimal NOS may be related to the age of assessed infants. The NOS has been standardized in preterm infants up to 18 months of age and some items may be difficult to assess in older infants (333).

Outcome measures and clinical risk variables

In the studied cohort 16 (24%) children had IVH I-III, 5 (8%) children had IVH III, and 7(10%) children had WMD as defined by cerebral ultrasound. IVH grade III was associated with suboptimal NOS, subnormal PDI and with CP. This supports previous findings that IVH with ventricular dilatation is a strong risk factor for adverse outcome (6, 14).

In contrast, associations between WMD and outcome measures were restricted to CP where all 3 infants with *cystic* WMD but no infants with *diffuse* WMD developed CP. Both positive and negative relationships between transient periventricular echodensities identified by cerebral ultrasound and outcome have been described previously (21, 351, 352). Diffuse WMD seems to be better described by MRI at term (23, 24). All five (7%) children developing CP had abnormalities on cerebral ultrasound. One child had focal WMD (PVL), two children had focal WMD (PVL) and IVH grade III, one child had IVH grade III and one child had IVH grade II and signs of cerebral atrophy.

Several antenatal and neonatal clinical risk factors were univariately associated with outcome measures, which was of relevance for the subsequent evaluation between cytokines and outcome. Lower GA was associated with suboptimal NOS and subnormal PDI which was expected since GA is probably the single most important predictive factor for impaired long-term outcome (47, 49). Clinical maternal infection was associated with suboptimal NOS, subnormal PDI and with CP. A low Apgar Score (< 7 at 5 min) was associated with subnormal MDI/PDI and with CP.

PROM was unexpectedly protective against subnormal MDI, with the association remaining after adjustment for confounders by multivariate analysis. Other studies have rather considered PROM being a risk factor of adverse developmental outcome (199, 353). This discrepancy in effects of PROM on later outcome may be due to the timing of

an inflammatory insult that precedes preterm birth, where the interval between the first and secondary insult determines if tolerance or sensitization occurs (158, 159).

A subnormal MDI (< 85) was neither associated with GA nor with cerebral ultrasound abnormalities. However, developmental delay (MDI< 70) (N=8) did relate to GA and cerebral abnormalities, $p<0.001$ and $p=0.011$, respectively. MDI in BSID-II version has been demonstrated to have low stability as well as low positive predictive validity when performed in younger infants, especially in infants without any neuro-sensory impairment (354, 355). Additionally these studies described an influence of socio-demographic factors on the predictive validity of this subscale. As in many other studies, we observed that a higher maternal or paternal educational level were associated with a lower risk for a subnormal MDI.

Cytokines and outcome measures

Significant univariate associations between concentrations of cytokines and outcome measures were exclusively present in cord blood and at 6 h and were confined to concentrations of TNF-α, IL-6 and IL-8 and to the outcome measures PDI< 85 and CP. A trend towards an association between TNF-α at 6 h and NOS <74 was also present, $p=0.06$.

These findings indicate that a fetal inflammatory response initiated *in utero* is predictive for adverse developmental outcome. TNF-α, IL-6 and IL-8 exhibited highest concentrations in cord blood or at 6 h, followed by a subsequent decline with many individuals presenting a very short half-life of IL-6 and IL-8. The half-life of especially IL-8 appears considerable shorter as compared to temporal profiles of cytokines in cord blood from *in vitro* studies, showing a persistent up-regulation of IL-8 after an inflammatory stimulus (181). The absence of associations between cytokines sampled at later time points than 6 h and outcome may explain why a previous retrospective study with sampling of neonatal blood at a median age of 2.3 days failed to show an association with CP in preterm infants (213).

To our knowledge, only one other study has *prospectively* evaluated the association between cytokine concentrations in cord blood samples and developmental outcome in preterm infants. This study did not demonstrate any significant relationships between cytokines and outcome (56). However, as the inclusion criteria were defined on BW, the mean BW was significantly lower (770 g vs 1007 g) despite similar GA. This implies inclusion of more growth restricted infants and possibly a smaller proportion of infants with antenatal inflammation.

Surprisingly, IFN-γ did not show any relationship with outcome measures, although we previously observed that increased concentrations of IFN-γ were related to WMD. Further, WMD was not associated with outcome except with CP, where all 3 infants with focal WMD (cystic PVL) developed CP. Consequently, the poor relationship between WMD and outcome was accompanied by absence in associations between IFN-γ and outcome variables. There is a great deal of support in experimental studies for the role of IFN-γ in the pathogenesis of white matter injury (160). Further, clinical studies have suggested a role of IFN-γ in the development of cystic PVL and of CP (225, 356).

Cytokine and outcome measures-multivariate analysis

Cytokines with univariate relationships with each respective outcome measure were evaluated in multivariate analyses together with other clinical risk variables exhibiting corresponding univariate associations. Models of variables with the highest combined predictive capacity for each respective outcome measure were obtained. A PDI <85 was best predicted by the combination of increased concentration of TNF-α in cord blood and decreasing GA. CP was best predicted by the combination of increased concentration of IL-8 in cord blood, Apgar score <7 and severe brain damage. MDI <85 was best predicted by the combination of PROM and Apgar Score < 7 at 5 min. NOS < 74 was best predicted by the combination of decreasing GA and any cerebral hemorrhage.

TNF-α was the cytokine most frequently associated with deviant outcome at 2 years of age. Importantly, increased concentrations of TNF-α were neither associated with IVH nor with WMD. In addition, TNF-α remained associated with subnormal PDI after adjustment for severe brain damage. TNF-α is a key cytokine in experimental models of

brain injury and induces injury to both neural cells and to oligodendrocyte precursor cells, as compared to IFN-γ that mediates injury preferentially in immature oligodendrocytes in white matter (170-172). Up-regulation of TNF-α is rapid following an inflammatory or ischemic insult with a relatively short half life obscuring its detection in the circulation (169, 180). The frequently low or undetectable concentrations of TNF-α in this study in contrast to some of the other pro-inflammatory cytokines, suggest that the highest levels of TNF-α were present before birth.

The weak relationships between TNF-α and WMD and between WMD and outcome may also be attributed to the use of cerebral ultrasound for the definition of WMD. Ultrasound has been demonstrated to be less reliable than MRI in predicting diffuse WMD (23, 24). Increased cord blood concentrations of TNF-α and IL-1β was recently shown to be associated with early PVL when defined by MRI (209).

Increased cord blood concentrations of the chemokine IL-8 were predictive of CP together with low Apgar score and cerebral damage (IVH grade III/WMD). Additionally, TNF-α displayed an univariate association with CP. The contribution of inflammation as well as low Apgar score to later development of CP is in line with the theory of sensitization where previous inflammation may sensitize the brain to a subsequent HI insult. In a previous clinical study, the *combination* of placental perfusion defect and histological CA predicted abnormal neurological outcome. These findings together support the hypothesis of hypoxia and inflammation as being the two most important factors preceding white matter damage (45, 56).

Although a positive association between IL-8 in cord blood and CP has been described previously, there is little evidence of a *direct* causal contribution of IL-8 in the mechanisms mediating cell damage in the immature brain (207). Concentrations of IL-8 are most probably a readily detected marker of an initiated fetal inflammatory response and thus of value for detection of associations in clinical study.

Cytokines and the IGF-system at birth in very preterm infants (III)

Concentrations of IGF-I, IGFBP-3 and lp/hp IGFBP-1 were analysed in cord blood and at 72 h postnatal age in 74 preterm infants. Twenty infants (27%) were born SGA. The evaluation was performed separately according to BW for GA (AGA/SGA).

The IGF-system and GA, SGA/AGA

Significant relationships between IGF-I and IGFBPs in cord blood and GA were restricted to the AGA infants, where concentrations of IGF-I and IGFBP-3 were positively related and concentrations of IGFBP-1 were negatively related to GA. No significant associations were observed between gender and IGF-I and IGFBPs in cord blood.

Infants with BW SGA had lower concentrations of IGF-I and a tendency towards higher concentrations of lpIGFBP-1 ($p=0.06$) in cord blood as compared to infants born AGA, which has been described previously (252, 253). Fetal concentrations of IGF-I and IGFBPs are dependent of maternal nutrition and placental supply (243, 246). The decrease in IGF-I and increase in IGFBP-1 in infants with BW restriction is considered to be a physiological response, with the purpose to restrict fetal growth when placental nutrient supply is limited.

Temporal changes in the IGF-system

Median concentrations of IGF-I (Fig. 6), IGFBP-3 and lpIGFBP-1 demonstrated a significant decrease from birth up to 72 h postnatal age The decrease was highly proportionate to the initial concentrations in cord blood, *ie* infants with the highest concentrations in cord blood had the highest absolute decrease up to 72 h, whereas those with the lowest concentrations had the lowest absolute decrease. The proportionate decrease in components of the IGF-system suggests that endogenous production of IGF-I and IGFBPs after birth is very low or even absent during the immediate postnatal period after very preterm birth. Levels of IGF-I continue to be low during the first postnatal weeks in very preterm infants, whereas levels of IGF-I in term infants show a restitution within the first postnatal days (305, 306). Persistent low postnatal levels of

IGF-I in very preterm infants have been associated with decreased postnatal growth velocity, development of ROP and with other neonatal morbidity (321, 322).

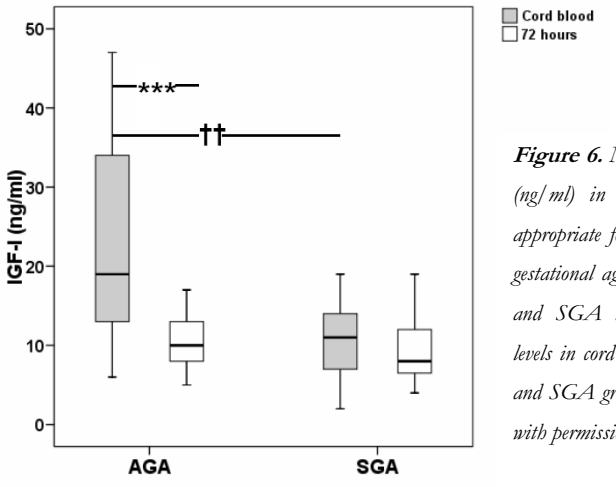

Figure 6. *Median and interquartile ranges of IGF-I (ng/ml) in cord blood and at 72 h in infants appropriate for gestational age (AGA) and small for gestational age (SGA). †= differences between AGA and SGA infants. Adapted *=differences between levels in cord blood and at 72 h of age within AGA and SGA groups respectively. Adapted from paper III with permission. ***p<0.001, ††p<0.01*

The IGF-system in relation to nutrional intake and fresh frozen plasma

Enteral and nutritional intake (0-72 h) did not influence the decrease of IGF-I and IGFBPs from birth up to 72 h of age and was not related to concentrations of IGF-I and IGFBPs at 72 h of age, although the estimated nutritional intakes were significantly lower as compared to intrauterine requirements (data not shown). An influence of postnatal nutrition on the IGF-system has previously been described in preterm infants, but during a significantly longer observational period (307, 311).

Due to clinical purposes, 32 (43%) infants received FFP during the first 72 postnatal hours. The accumulated administered median (range) volume of FFP was 20 (9-68) ml/kg. Concentrations of IGF-I and IGFBP-3 at 72 h correlated positively with accumulated volume (ml/kg) of administered FFP, $r=0.57$, $p<0.001$ and $r=0.72$, $p<0.001$ respectively (Fig. 7). This suggests, that exogenous administration of IGF components by administration of FFP from adult donors has the potential of increasing circulating levels in the preterm infant.

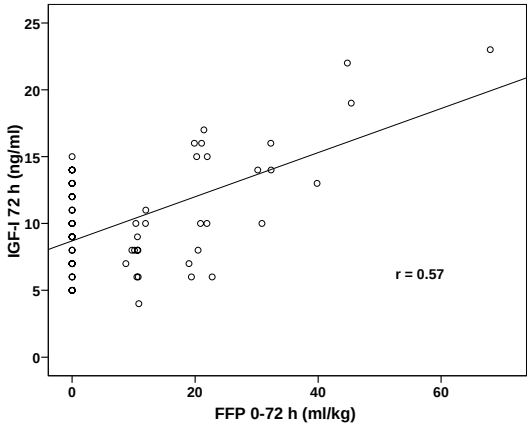

Figure7. *Relationship between volume of administered fresh frozen plasma (FFP) (mL/kg) during 0-72 h and levels of IGF-I (ng/mL) at 72 h of age (N=70). The figure has only previously been published in abstract form.*

The IGF-system and cytokines

Higher concentrations of IL-6 and IL-8 in cord blood correlated negatively with IGF-I and higher concentrations of IL-6, IL-8 and TNF-α correlated positively with lp/hpIGFBP-1, in infants with BW AGA (Fig. 8)

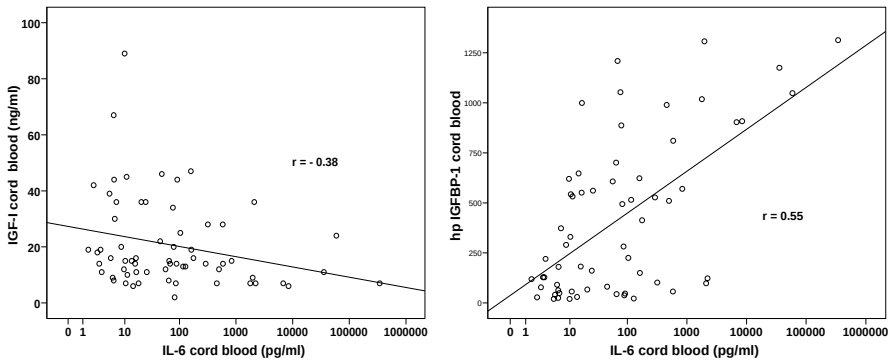

Figure 8. Relationships between plasma concentrations of IL-6 (pg/mL) and those of IGF-I (ng/mL) and high phosporylated insulin-like growth factor I (hpIGFBP-1), respectively, in cord blood in preterm infants with birth weight appropriate for gestational age (N=47). Adapted from paper III with permission

These findings suggest that inflammation at birth defined as increased levels of pro-inflammatory cytokines in cord blood is associated with a decreased capacity of the IGF-system. IGF-I mediates protective and anti-apoptotic effects in immature oligodendrocytes in cerebral white matter after induced hypoxia, as well as after induced inflammation *in vivo* and *in vitro* (280, 281, 284). Very preterm birth is often accompanied by both of these insults and consequently the neuro-protective effects of the IGF-system may be of substantial relevance.

Experimental and human studies have described that an increase in pro-inflammatory cytokines, is followed by a reduction in levels of IGF-I and an up-regulation in levels of IGFBP-1 (292, 303, 304). In addition pro-inflammation may further decrease the capacity of the IGF-system by inducing GH-insensitivity *in vivo* (298). In preterm infants GH-insensitivity appears less important since expression of GH receptors are low in the fetus (240). Inverse relationships between IL-6 and IGF-I have previously been shown in children with systemic juvenile idiopathic arthritis, as well as in stable growing preterm infants (291, 357). However, a previous study in moderately preterm and term infants did not display any correlation between IL-6, IGF-I and IGFBP-1 in cord blood (358).

The associations between cytokines and the IGF components were only observed in the subgroup of infants born AGA. This is an expected finding, since infants born SGA have lower concentrations of IGF-I and a tendency to increased concentrations of IGFBP-1 at birth, as compared to infants with BW AGA. Infants with BW SGA are also less likely to have fetal inflammation. Very preterm infants with vascular placental defects have been observed to have lower levels of IL-6 and IL-8, as compared to infants with histologic CA (56).

IGF-I is down regulated whereas IGFBP-1 and pro-inflammatory cytokines are up-regulated by fetal birth stress, which may infer an influence on our described associations between cytokines and the IGF-system (198, 261). Adjusting for Apgar score had no influence on the association between cytokines and IGF-I or IGFBP-1. Cord blood pH was obtained in about half of the eligible infants (N=36) and was not associated with

IGF-I, IGFBP-3 or IGFBP-1 which may however be due to the small group of analysed infants.

IGFBP-1 and cerebral hemorrhage

Infants with IVH grade III (all with BW AGA) had higher levels of lp/hpIGFBP-1, as compared to infants without IVH or with subependymal hemorrhage. Further, increased levels of lp/hpIGFBP-1 in cord blood related univariately to subnormal PDI at 2 y of age, p=0.008 and p=0.047 respectively. Increased expression of IGFBP-1 has been described to interfere with brain development in mice (274, 275). Our previous finding showing a relationship between elevated IL-6 and IL-8 and severe cerebral hemorrhage (study I), suggests that the described association between IGFBP-1 and IVH may be due to pro-inflammation. There is to date no data supporting a causal relationship between increase in IGFBP-1 and development of IVH.

Effects of fresh frozen plasma on the IGF-system in extremely preterm infants (IV)

The previous findings in study III, where administered amount of FFP (ml/kg) during the first 72 postnatal hours correlated positively with concentrations of IGF-I and IGFBP-3 at 72 h of age, formed the basis for a continued systematic evaluation of FFP as an exogenous source of IGF-I and IGFBP-3 during the catabolic period immediately following preterm birth.

Concentrations of IGF-I and IGFBP-3 were analyzed in 20 extremely preterm infants who received FFP from adult donors due to clinical purposes. Analyses were performed from the portion of administered FFP as well as from neonatal blood, immediately before initiation of transfusion, directly after and at 6, 12, 24 and 48 h after completed FFP transfusion.

Administration of FFP and effects on levels of IGF-I and IGFBP-3

FFP was administered with a mean (SD) volume of 11 (3.1) ml/kg during median (range) 120 (90-240) min at a postnatal age of median (range) 2 (1-7) days. Base-line

concentrations of IGF-I in the infants before study entry were significantly lower than previously described fetal concentrations at similar GA and corresponded well with concentrations at 72 h of age previously described in study III (250, 253, 254).

Directly after administration of FFP concentrations of IGF-I and of IGFBP-3 displayed a marked increase where concentrations of IGF-I more than doubled in all infants and achieved fetal physiological levels in the majority of infants (Fig 9). The significant elevation in concentrations was achieved with a mean administered dose of IGF-I of 1.4 μg/kg. As expected preterm infants required a much lower dose to achieve physiological concentrations than those reported from studies in adults (359, 360).

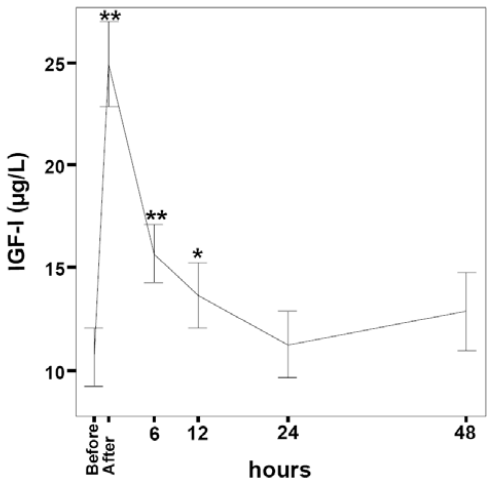

Figure 9. *Mean concentrations of insulin-like growth factor I (IGF-I) (μg/L) before and after fresh frozen plasma (FFP) and at 6, 12, 24 and 48 hours after FFP. Error bars show ±1 SE.*

Pharmacokinetic aspects

Significant correlations were observed between administered amounts (μg) of IGF-I and IGFBP-3 and those (μg) remaining in the circulation after completed FFP transfusion. The latter was calculated by approximating plasma volume in each infant to 50 ml/kg. The ratio between administered amount and that remaining after completed FFP transfusion ranged between 40 and 103 % for IGF-I. We found that GA at birth correlated significantly to this ratio. This correlation remained significant after adjustment for postnatal age at FFP transfusion, duration of FFP transfusion and BW for GA. This

suggests, that decreased GA at birth was associated with increased loss of administered IGF-I from the circulation during the course of transfusion. GA did not display any correlation with IGF-I concentrations or relative changes thereof during the study period.

A rapid decline in concentrations of IGF-I and IGFBP-3 was observed following completed FFP transfusion and at 24 h concentrations of both proteins did not differ from base-line concentrations. Calculated half-life ($t_{1/2}$) of IGF-I for a neonate of 1 kg was 3.5 h based on typical estimates. Half life for IGF-I was thus considerably shorter than that described in healthy adults receiving rhIGF-I (360). A large proportion of IGF-1 in administrated FFP from adult donors is hypothetically present in either binary or ternary complexes bound to IGFBP-3 and ALS. The circulating $t_{1/2}$ of IGF-I would therefore be expected to have been considerably longer (233).

The endogenous production of IGF-I and IGFBP-3 in very preterm infants is probably very low or absent during the immediate postnatal period. Moreover, extremely preterm infants are in a catabolic state during the first weeks which may further impair the capacity of the nutritionally regulated IGF-system (312). In study III we observed that inflammation at birth was associated with a decrease in levels of IGF-I. Catabolism and pro-inflammation may contribute to increased proteolytic degradation of IGFBP-3, thereby causing a severe decrease in circulating $t_{1/2}$ of IGF-I.

Due to clinical indications five infants had received FFP during the last 24 h before initiation of study and four infants received additional FFP between study sampling at 12 h and 24 h. This was accompanied by higher mean concentrations of IGF-I and IGFBP-3 at initiation of study and at 24 h, respectively, as compared to remaining infants.

Although levels of IGF-I were doubled on average, we did not observe any effects on plasma glucose levels. Increasing levels of IGF-I to those of the healthy fetus following preterm birth does therefore not appear to be associated with hypoglycemia. FFP includes other proteins that may interfere with the IGF-system. Transferrin and plasminogen interfere with the binding affinity of IGFBP-3 (361-363). High cytokine levels in plasma have been associated with febrile transfusion reactions (364). The risk of blood-borne

infections is very small but still not negligible. Therefore, continued evaluation of IGF-I /IGFBP-3 administration in preterm infants should preferably be based on the use of recombinant IGF-I/IGFBP-3.

General discussion and future perspectives

This thesis aimed to describe the effects of inflammation at very preterm birth on subsequent morbidity, as well as on the neuro-protective IGF-system. The relationships have been evaluated in prospective clinical studies where temporal changes in levels of cytokines have been chosen as markers of an induced inflammatory response.

The use of antenatal recruitment in papers I-III may well have biased selection of subjects towards pregnancies hospitalized before birth and included proportionally less rapid unexpected deliveries. The latter may be associated with a high prevalence of antenatal infection. By retrieving data from non-included infants born during the same time period some differences were observed between the study cohort and non-participants. Prevalence of WMD and IVH I-II was lower in those not included for study. This may be attributed to different diagnostic sensitivity of ultrasound examinations performed within or without of study. It could importantly be ascertained that neither mortality nor severe brain damage were increased in infants not recruited to study as compared to those within the study.

Early inflammation, low Apgar score at birth, as well as cerebral morphology were related primarily to motor outcome, but not to cognitive outcome (paper II). The follow-up was performed at a corrected age of 2 years, an age commonly used in many follow-up studies. The predictive value of developmental outcome at 24 months of age appears sufficiently reliable regarding diagnosis of CP, whereas cognitive impairment is not reliably tested until school age (336, 365). Continued follow-up at higher ages is currently being performed for the cohort of infants in paper II.

Repeated measurements of modulatory and pro-inflammatory cytokines over time enabled us to assess time-frames of inflammatory response and their relationships to morbidity. However, the analysis of 9 cytokines at 4 time points raises the statistical issue of multiple comparisons. Adjustment was performed for repeated measures in papers II

and IV. Several of the analysed cytokines had no hypothetical relationship to evaluated outcomes of these studies and could therefore have been omitted. As negative results carry a value we included all measured cytokines in analysis. However, in studies with smaller samples of individuals, the number of tests performed should be carefully considered.

Prospective clinical studies performed at one single perinatal center do not have the possibility of recruiting a large number of infants within a reasonable time period as in multi-center studies. On the other hand, the single center can perform a study at a different level of detail and ensure consistent data retrieval. Ideally, results from detailed single-center studies lead to a new definition of a research question which can be applied in subsequent multi-center study. Our results have shown that sampling of inflammatory markers in cord blood and at 6 h is of relevance when performing association studies with early and late morbidity. This information is of great value for design of continued multi-center study.

Associations between pro-inflammation and *late* morbidity at 2 years of age in paper II and between cytokines and the IGF-system in paper III were confined to *cord blood* samples after adjustment for other risk variables. If pro-inflammation is causal in brain damage our results would suggest that an essential part of injury takes place *before* birth implicating that neuro-protective strategies initiated towards the fetus have the greatest potential for success. Timing of delivery based on fetal inflammatory markers is a possible strategy, as yet, without proven benefits.

The strong relationship shown between early postnatal inflammation (best described by increase in IL-6) and severe IVH and arterial hypotension respectively suggests that pro-inflammation is the common factor associated with both of these conditions. We and others have failed to demonstrate associations between arterial hypotension and either cerebral damage or later developmental outcome. Continued study should focus on concomitant biochemical evaluation of inflammation and hemodynamic assessment including CBF. Disturbance in cerebral blood flow in *combination* with endothelial activation may be additive risk factors for development of IVH. Cerebral hemorrhage takes place during the immediate postnatal period and is readily detected by bed-side

ultrasound. Thus the time-period of intervention is much more clearly defined than *eg* that required for intervention directed at putative insults causing WMD.

The source of cytokines measured in cord blood in papers I-III was either fetal or placental. An inflammatory response produced by the fetus may be defined as presence of fetal vasculitis accompanied by signs of endothelial activation. Since we did not perform any placental histology or microbiology, we do not know if the inflammatory reaction defined by circulatory elevations of cytokines actually was induced by an antenatal infection or a hypoxic insult or a combination of these events. Cellular source of measured circulating cytokines can only be speculated upon. Subjects with high circulating levels of IFN-γ would be expected to have an activated adaptive immunity with signs of activation in lymphocytic subsets.

The observation of the homogenous increase in circulating levels of the anti-inflammatory cytokine IL-10 following very preterm birth has formed hypothesis for continued study. The hypothesis of endogenous stress causing down-regulation of pro-inflammation and up-regulation of modulatory cytokines, and its potential in neuro-protection is currently being evaluated in an experimental setting.

A systematic evaluation of the temporal decrease in components of the IGF-system in a larger cohort of preterm infants during the transition from fetal to neonatal life has not been performed previously (paper III). The decrease in IGF-I is most probably caused by insufficient endogenous production after interruption of placental supply and suggests that not only components of the IGF-system but also that other proteins essential for the developing brain may decrease in the same manner. The decrease may be further aggravated by the catabolic situation with deficit in protein intake that occurs after very preterm birth. Enhanced nutrition during the first days may theoretically have positive effects on the IGF-system but will probably not be sufficient to achieve fetal physiological levels which is indicated by our findings where early nutritional intake did not influence components of the IGF-system. Early exogenous administration of IGF-I may therefore be essential.

In paper IV we describe, for the first time, pharmacokinetic aspects of exogenous administration of IGF-I from adult donor plasma to extremely preterm infants. The findings where concentrations of IGF-I could be elevated up to fetal physiological levels without any side effects, supports continued studies with administration of recombinant IGF-I during a longer time period accompanied by concomitant evaluation of clinical variables related to IGF-I deficiency. It should however be emphasized that the biological activity of IGF-I in the tissues is regulated by a complicated network of different mechanisms and is not only determined by the secretion rate of IGF-I and the total circulatory IGF-concentration (227). Further it is not clear whether IGF-I administered to the circulation can reach the target organ *ie* the immature brain. The possible beneficial effects of supplementary and systemic treatment with IGF-I on brain development or on severe ROP can only be evaluated by future randomized trials of intervention in preterm infants.

The studies in this thesis have attempted to highlight how inflammation present at very preterm birth relates to different aspects of morbidity. Time windows were identified where future interventions may be possible. The first time window is present before birth where antenatal markers of fetal inflammation could be of help in the decision of an optimal time point of delivery. The other time window is present shortly after birth where continued study should address the interaction between pro-inflammation, hemodynamic impairment and development of severe IVH.

Conclusions

Very preterm birth is frequently accompanied by a circulating inflammatory response in the infant defined as an increase in both pro-inflammatory and modulatory cytokines. Temporal profiles of cytokines show that pro-inflammation is mainly initiated *in utero*. Time point of induced inflammatory response appears of importance for type of subsequent neonatal morbidity.

Inflammation at birth is associated with impaired developmental outcome at 2 years of age where TNF-α seems to be a key cytokine for subsequent brain injury. Markers of fetal inflammation may help in optimizing time point of delivery thereby reducing fetal exposure to damaging influence.

Presence of inflammation at birth is associated with changes in the IGF-system. Impairment of the endogenous IGF-system may aggravate the consequences of inflammation and hypoxia at birth on the immature brain.

An immediate postnatal decline in levels of IGF-I occurs after very preterm birth. Adminstration of exogenous IGF-I from adult donor plasma elevates low endogenous postnatal levels of IGF-I in extremely preterm infants without any overt side effects. Future studies with continuous administration of recombinant IGF-I during a longer time period deserve evaluation.

En bok skall vara som en bro:
den skall ha sina grundvalar i verklighetens mark
men höja sig över den i en vid båge
- Frank Heller 1886-1947

Sammanfattning på svenska (Swedish summary)

I Sverige föds cirka 0,3% av alla levande födda barn före 28 fulla graviditetsveckor Överlevnaden för dessa höggradigt prematurfödda barn har under de senaste 15 åren förbättrats dramatiskt. Samtidigt uppvisar en stor andel av barnen vid senare uppföljningsundersökningar ett flertal svårigheter både avseende motorik, inlärning, beteende samt uppmärksamhet. Dessa svårigheter anses orsakade av skador som uppstår i den omogna hjärnan vid en prematur förlossning samt under den efterföljande nyföddhetsperioden.

Orsakerna till en uppkommen hjärnskada efter en mycket prematur födelse är flera. Under de sista 3 månaderna av graviditeten sker en mycket snabb tillväxt och utmognad av hjärnan vilken gör att hjärnan under denna tidsperiod är extra känslig för påverkan av yttre faktorer.

Den främsta orsaken till mycket för tidig förlossning är infektioner som uppkommer hos modern och sedan eventuellt fortplantas till fostret. Dessa infektioner kan ofta förekomma under lång tid utan att ge några symptom. Om infektionen passerar över till fostret reagerar fostret med att initiera ett inflammatoriskt svar. Detta inflammatoriska svar är ett sätt för fostret att försvara sig mot en infektion men samtidigt vet man att ett inflammatoriskt svar kan vara skadligt för flera olika organ inte minst hjärnan. Cytokiner är proteiner som produceras av immunologiska celler vid en infektion. Dessa cytokiner fungerar som budbärare för immunförsvaret och reglerar intensitet och varaktighet i det immunologiska svaret. Cytokiner kan både stimulera och nedreglera ett inflammatoriskt svar.

Fostrets tillväxt regleras under graviditeten av ett flertal olika tillväxtfaktorer. Nivåerna av dessa tillväxtfaktorer hos fostret är starkt kopplade till moderns och fostrets näringsstatus och regleras genom ett utbyte mellan fostret och moderkakan. Vid en för tidig förlossning upphör detta utbyte abrupt och barnet måste själv producera dessa tillväxtfaktorer. Insulin-like growth factor I (IGF-I) är en av de viktigaste tillväxtfaktorerna under slutet av graviditeten och har stor betydelse för hjärnans mognad och tillväxt. IGF-I har också visat sig ha en skyddande effekt vid en etablerad hjärnskada i djurexperiment.

Hos mycket för tidigt födda barn har man visat att låga nivåer av IGF-I kan bestå under flera veckor efter födelsen. Man tror att de låga nivåerna av IGF-I efter för tidig födelse bland annat beror på en för låg protein produktion som kan vara en följd av otillräcklig protein tillförsel. Även om man med dagens nyföddhetsvård strävar efter att optimera näringstillförseln så kan man idag inte fullt ut ersätta den näringstillförsel som skett via moderkakan.

Det övergripande syftet med detta projekt var att undersöka hur inflammation påverkar den omogna hjärnan och IGF-systemet vid en mycket för tidig förlossning. Projektet baseras på kliniska studier av prematura barn födda i Lund (i delstudie IV även barn födda vid Drottning Silvias Barnsjukhus, Göteborg) under en 5-års period (2001-2006) och som ligger till grund för 4 olika delarbeten. Samtliga studier har godkänts av Regionala Etikprövningsnämnden i Lund.

I det första delarbetet ingick 74 barn som föddes i Lund under 2001-2003 med en graviditetslängd på mindre än 32 veckor. Vi undersökte nivåer av 9 olika cytokiner i blodet vid 4 olika tidpunkter från födelsen och fram till 3 dagars ålder. Under samma tidsperiod uppmättes blodtryck. Ultraljud av hjärnan gjorde vid flera tillfällen från födelsen fram till dess att barnet var fullgånget. Vi fann att förhöjda nivåer av de cytokiner som initierar inflammation relaterade till lågt blodtryck samt till utveckling av hjärnblödning och skador i hjärnans vita substans. Detta stödjer hypotesen att etablerad inflammation vid förlossningen kan ha betydelse för uppkomst av tidig sjuklighet hos det prematurfödda barnet. Tidpunkten för när det inflammatoriska svaret initieras i

förhållande till förlossningen tros också ha betydelse för vilken typ och grad av skada som uppstår.

Samtliga överlevande barn som undersöktes i det första delarbetet rekryterades till en uppföljningsundersökning vid 2 års korrigerad ålder där 67 barn deltog under 2003-2005. Vid uppföljningen utfördes dels en neurologisk undersökning och dels en utvecklingsbedömning med ett standardiserat test instrument (Bayley Scales of Developmental Index). Syftet var att se om det fanns några samband mellan inflammation vid födelsen (förhöjda cytokinnivåer) och senare utveckling. Vi fann att förhöjda nivåer av två cytokiner (som båda inducerar inflammation) relaterade till försenad motorisk utveckling och till cerebral pares. Detta stödjer hypotesen att en tidig inflammation vid födelsen kan ha betydelse för utvecklingen även på längre sikt.

I delarbete III undersöktes samma barn som i delarbete I. Vi undersökte nivåer av IGF-I vid födelsen och vid 72 timmars ålder och analyserade även cytokiner vid motsvarande tidpunkter. Vi fann att nivåerna av IGF-I minskade drastiskt från födelsen och fram till 72 timmars ålder. Vi fann också att inflammation vid födelsen (förhöjda cytokinnivåer) relaterade till lägre nivåer av IGF-I. Dessa resultat visar att uppkomst av låga nivåer av IGF-I efter en för tidig förlossning ytterligare kan förvärras vid en samtidigt pågående inflammation.

Som ett delfynd i delarbete III fann vi att barn som på klinisk indikation behandlades med färskfrusen plasma hade högre nivåer av IGF-I vid 72 timmars ålder jämfört med de barn som inte hade behandlats med färskfrusen plasma. Prematura barn behandlas ofta med färskfrusen plasma från vuxna blodgivare, främst vid etablerade blodtrycksproblem. Denna plasma innehåller koncentrationer av IGF-I som är cirka 10 gånger högre jämfört med koncentrationer i blodet hos mycket prematura barn. Dessa fynd var en anledning till att genomföra det sista delarbetet (IV), där vi systematiskt studerade effekter av färskfrusen plasma på nivåer av IGF-I hos 20 barn födda före 28 graviditetsveckor under 2005-2006.

Barnen inkluderades i studien om de hade kliniska behov av transfusion med färskfrusen plasma före 1 veckas ålder. Vi undersökte nivåer av IGF-I före transfusion av färskfrusen plasma och sedan direkt efter transfusion och vid ytterligare 4 tillfällen fram till 48 timmar efter transfusion. Vi fann att nivåerna av IGF-I mer än dubblerades efter transfusionen och att man uppnådde fysiologiska nivåer d.v.s. de nivåer som barnet skulle ha haft under en fortsatt graviditet. Inga övriga negativa effekter kunde konstateras.

Sammanfattningsvis har detta projekt inneburit en ökad kunskap om effekterna av pågående inflammation vid mycket för tidig förlossning. Inflammation vid födelsen förefaller ha ett inflytande på såväl tidig som sen sjuklighet och påverkar dessutom tillväxtfaktorer med betydelse för hjärnans utveckling. Projektet har också för första gången kunnat visa att man genom tillförsel av tillväxtfaktorn IGF-I utifrån kan påverka de låga nivåerna av IGF-I hos prematura barn. Dessa resultat kan innebära en bas för framtida studier där IGF-I ges under längre tidsperiod. I framtiden är det viktigt att försöka identifiera de foster som är utsatta för en inflammatorisk påverkan före födelsen för att på så sätt försöka optimera tidpunkten för förlossningen och för att terapeutiskt kunna motverka en skadlig inflammation.

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References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371(9606):75-84.

2. Wilson-Costello D, Friedman H, Minich N, Fanaroff AA, Hack M. Improved survival rates with increased neurodevelopmental disability for extremely low birth weight infants in the 1990s. Pediatrics 2005;115(4):997-1003.

3. Hamrick SE, Miller SP, Leonard C, Glidden DV, Goldstein R, Ramaswamy V, et al. Trends in severe brain injury and neurodevelopmental outcome in premature newborn infants: the role of cystic periventricular leukomalacia. J Pediatr 2004;145(5):593-9.

4. Platt MJ, Cans C, Johnson A, Surman G, Topp M, Torrioli MG, et al. Trends in cerebral palsy among infants of very low birthweight (<1500 g) or born prematurely (<32 weeks) in 16 European centres: a database study. Lancet 2007;369(9555):43-50.

5. Vohr BR, Wright LL, Poole WK, McDonald SA. Neurodevelopmental outcomes of extremely low birth weight infants <32 weeks' gestation between 1993 and 1998. Pediatrics 2005;116(3):635-43.

6. Ment LR, Allan WC, Makuch RW, Vohr B. Grade 3 to 4 intraventricular hemorrhage and Bayley scores predict outcome. Pediatrics 2005;116(6):1597-8.

7. Wilson-Costello D, Friedman H, Minich N, Siner B, Taylor G, Schluchter M, et al. Improved neurodevelopmental outcomes for extremely low birth weight infants in 2000-2002. Pediatrics 2007;119(1):37-45.

8. Wilson-Costello D. Is there evidence that long-term outcomes have improved with intensive care? Semin Fetal Neonatal Med 2007;12(5):344-54.

9. Marlow N, Wolke D, Bracewell MA, Samara M. Neurologic and developmental disability at six years of age after extremely preterm birth. N Engl J Med 2005;352(1):9-19.

10. Sweet MP, Hodgman JE, Pena I, Barton L, Pavlova Z, Ramanathan R. Two-year outcome of infants weighing 600 grams or less at birth and born 1994 through 1998. Obstet Gynecol 2003;101(1):18-23.

11. Laptook AR, O'Shea TM, Shankaran S, Bhaskar B. Adverse neurodevelopmental outcomes among extremely low birth weight infants with a normal head ultrasound: prevalence and antecedents. Pediatrics 2005;115(3):673-80.

12. Paneth N. Classifying brain damage in preterm infants. J Pediatr 1999;134(5):527-9.

13. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr 1978;92(4):529-34.

14. Kuban K, Sanocka U, Leviton A, Allred EN, Pagano M, Dammann O, et al. White matter disorders of prematurity: association with intraventricular hemorrhage and ventriculomegaly. The Developmental Epidemiology Network. J Pediatr 1999;134(5):539-46.

15. Leviton A, Kuban K, Paneth N. Intraventricular haemorrhage grading scheme: time to abandon? Acta Paediatr 2007;96(9):1254-6.

16. de Vries LS, Eken P, Dubowitz LM. The spectrum of leukomalacia using cranial ultrasound. Behav Brain Res 1992;49(1):1-6.

17. Pierrat V, Duquennoy C, van Haastert IC, Ernst M, Guilley N, de Vries LS. Ultrasound diagnosis and neurodevelopmental outcome of localised and extensive cystic periventricular leucomalacia. Arch Dis Child Fetal Neonatal Ed 2001;84(3):F151-6.

18. De Vries LS, Van Haastert IL, Rademaker KJ, Koopman C, Groenendaal F. Ultrasound abnormalities preceding cerebral palsy in high-risk preterm infants. J Pediatr 2004;144(6):815-20.
19. Leijser LM, de Vries LS, Cowan FM. Using cerebral ultrasound effectively in the newborn infant. Early Hum Dev 2006;82(12):827-35.
20. Anderson NG, Laurent I, Woodward LJ, Inder TE. Detection of impaired growth of the corpus callosum in premature infants. Pediatrics 2006;118(3):951-60.
21. Horsch S, Muentjes C, Franz A, Roll C. Ultrasound diagnosis of brain atrophy is related to neurodevelopmental outcome in preterm infants. Acta Paediatr 2005;94(12):1815-21.
22. O'Shea TM, Counsell SJ, Bartels DB, Dammann O. Magnetic resonance and ultrasound brain imaging in preterm infants. Early Hum Dev 2005;81(3):263-71.
23. Inder TE, Anderson NJ, Spencer C, Wells S, Volpe JJ. White matter injury in the premature infant: a comparison between serial cranial sonographic and MR findings at term. AJNR Am J Neuroradiol 2003;24(5):805-9.
24. Maalouf EF, Duggan PJ, Counsell SJ, Rutherford MA, Cowan F, Azzopardi D, et al. Comparison of findings on cranial ultrasound and magnetic resonance imaging in preterm infants. Pediatrics 2001;107(4):719-27.
25. Counsell SJ, Allsop JM, Harrison MC, Larkman DJ, Kennea NL, Kapellou O, et al. Diffusion-weighted imaging of the brain in preterm infants with focal and diffuse white matter abnormality. Pediatrics 2003;112(1 Pt 1):1-7.
26. Inder TE, Wells SJ, Mogridge NB, Spencer C, Volpe JJ. Defining the nature of the cerebral abnormalities in the premature infant: a qualitative magnetic resonance imaging study. J Pediatr 2003;143(2):171-9.
27. Maalouf EF, Duggan PJ, Rutherford MA, Counsell SJ, Fletcher AM, Battin M, et al. Magnetic resonance imaging of the brain in a cohort of extremely preterm infants. J Pediatr 1999;135(3):351-7.
28. Ajayi-Obe M, Saeed N, Cowan FM, Rutherford MA, Edwards AD. Reduced development of cerebral cortex in extremely preterm infants. Lancet 2000;356(9236):1162-3.
29. Inder TE, Warfield SK, Wang H, Huppi PS, Volpe JJ. Abnormal cerebral structure is present at term in premature infants. Pediatrics 2005;115(2):286-94.
30. Huppi PS, Murphy B, Maier SE, Zientara GP, Inder TE, Barnes PD, et al. Microstructural brain development after perinatal cerebral white matter injury assessed by diffusion tensor magnetic resonance imaging. Pediatrics 2001;107(3):455-60.
31. Miller SP, Vigneron DB, Henry RG, Bohland MA, Ceppi-Cozzio C, Hoffman C, et al. Serial quantitative diffusion tensor MRI of the premature brain: development in newborns with and without injury. J Magn Reson Imaging 2002;16(6):621-32.
32. Nagae LM, Hoon AH, Jr., Stashinko E, Lin D, Zhang W, Levey E, et al. Diffusion tensor imaging in children with periventricular leukomalacia: variability of injuries to white matter tracts. AJNR Am J Neuroradiol 2007;28(7):1213-22.
33. Isaacs EB, Edmonds CJ, Chong WK, Lucas A, Morley R, Gadian DG. Brain morphometry and IQ measurements in preterm children. Brain 2004;127(Pt 12):2595-607.
34. Kehrer M, Blumenstock G, Ehehalt S, Goelz R, Poets C, Schoning M. Development of cerebral blood flow volume in preterm neonates during the first two weeks of life. Pediatr Res 2005;58(5):927-30.
35. Nagy Z, Westerberg H, Skare S, Andersson JL, Lilja A, Flodmark O, et al. Preterm children have disturbances of white matter at 11 years of age as shown by diffusion tensor imaging. Pediatr Res 2003;54(5):672-9.
36. Nosarti C, Giouroukou E, Healy E, Rifkin L, Walshe M, Reichenberg A, et al. Grey and white matter distribution in very preterm adolescents mediates neurodevelopmental outcome. Brain 2008;131(Pt 1):205-17.

37. Peterson BS, Vohr B, Staib LH, Cannistraci CJ, Dolberg A, Schneider KC, et al. Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. Jama 2000;284(15):1939-47.
38. Volpe JJ. Neurobiology of periventricular leukomalacia in the premature infant. Pediatr Res 2001;50(5):553-62.
39. Haynes RL, Folkerth RD, Keefe RJ, Sung I, Swzeda LI, Rosenberg PA, et al. Nitrosative and oxidative injury to premyelinating oligodendrocytes in periventricular leukomalacia. J Neuropathol Exp Neurol 2003;62(5):441-50.
40. Inder TE, Huppi PS, Warfield S, Kikinis R, Zientara GP, Barnes PD, et al. Periventricular white matter injury in the premature infant is followed by reduced cerebral cortical gray matter volume at term. Ann Neurol 1999;46(5):755-60.
41. Back SA, Luo NL, Borenstein NS, Levine JM, Volpe JJ, Kinney HC. Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. J Neurosci 2001;21(4):1302-12.
42. Back SA, Gan X, Li Y, Rosenberg PA, Volpe JJ. Maturation-dependent vulnerability of oligodendrocytes to oxidative stress-induced death caused by glutathione depletion. J Neurosci 1998;18(16):6241-53.
43. Baud O, Haynes RF, Wang H, Folkerth RD, Li J, Volpe JJ, et al. Developmental up-regulation of MnSOD in rat oligodendrocytes confers protection against oxidative injury. Eur J Neurosci 2004;20(1):29-40.
44. Haynes RL, Baud O, Li J, Kinney HC, Volpe JJ, Folkerth DR. Oxidative and nitrative injury in periventricular leukomalacia: a review. Brain Pathol 2005;15(3):225-33.
45. Khwaja O, Volpe JJ. Pathogenesis of cerebral white matter injury of prematurity. Arch Dis Child Fetal Neonatal Ed 2008;93(2):F153-61.
46. Volpe JJ. Cerebral white matter injury of the premature infant-more common than you think. Pediatrics 2003;112(1 Pt 1):176-80.
47. Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. Jama 2002;288(6):728-37.
48. Hack M, Fanaroff AA. Outcomes of children of extremely low birthweight and gestational age in the 1990s. Semin Neonatol 2000;5(2):89-106.
49. Valcamonico A, Accorsi P, Sanzeni C, Martelli P, La Boria P, Cavazza A, et al. Mid- and long-term outcome of extremely low birth weight (ELBW) infants: an analysis of prognostic factors. J Matern Fetal Neonatal Med 2007;20(6):465-71.
50. Vohr BR, Wright LL, Dusick AM, Mele L, Verter J, Steichen JJ, et al. Neurodevelopmental and functional outcomes of extremely low birth weight infants in the National Institute of Child Health and Human Development Neonatal Research Network, 1993-1994. Pediatrics 2000;105(6):1216-26.
51. Elsmen E, Hansen Pupp I, Hellstrom-Westas L. Preterm male infants need more initial respiratory and circulatory support than female infants. Acta Paediatr 2004;93(4):529-33.
52. Hack M, Wilson-Costello D, Friedman H, Taylor GH, Schluchter M, Fanaroff AA. Neurodevelopment and predictors of outcomes of children with birth weights of less than 1000 g: 1992-1995. Arch Pediatr Adolesc Med 2000;154(7):725-31.
53. Stevenson DK, Verter J, Fanaroff AA, Oh W, Ehrenkranz RA, Shankaran S, et al. Sex differences in outcomes of very low birthweight infants: the newborn male disadvantage. Arch Dis Child Fetal Neonatal Ed 2000;83(3):F182-5.
54. Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. Jama 2004;292(19):2357-65.

55. Wheater M, Rennie JM. Perinatal infection is an important risk factor for cerebral palsy in very-low-birthweight infants. Dev Med Child Neurol 2000;42(6):364-7.
56. Kaukola T, Herva R, Perhomaa M, Paakko E, Kingsmore S, Vainionpaa L, et al. Population cohort associating chorioamnionitis, cord inflammatory cytokines and neurologic outcome in very preterm, extremely low birth weight infants. Pediatr Res 2006;59(3):478-83.
57. Zupan V, Gonzalez P, Lacaze-Masmonteil T, Boithias C, d'Allest AM, Dehan M, et al. Periventricular leukomalacia: risk factors revisited. Dev Med Child Neurol 1996;38(12):1061-7.
58. Cheng SW, Chou HC, Tsou KI, Fang LJ, Tsao PN. Delivery before 32 weeks of gestation for maternal pre-eclampsia: neonatal outcome and 2-year developmental outcome. Early Hum Dev 2004;76(1):39-46.
59. Kaukola T, Rasanen J, Herva R, Patel DD, Hallman M. Suboptimal neurodevelopment in very preterm infants is related to fetal cardiovascular compromise in placental insufficiency. Am J Obstet Gynecol 2005;193(2):414-20.
60. Gray PH, Jones P, O'Callaghan MJ. Maternal antecedents for cerebral palsy in extremely preterm babies: a case-control study. Dev Med Child Neurol 2001;43(9):580-5.
61. Murphy DJ, Sellers S, MacKenzie IZ, Yudkin PL, Johnson AM. Case-control study of antenatal and intrapartum risk factors for cerebral palsy in very preterm singleton babies. Lancet 1995;346(8988):1449-54.
62. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 2006;117(4):1253-61.
63. Shinwell ES, Karplus M, Reich D, Weintraub Z, Blazer S, Bader D, et al. Early postnatal dexamethasone treatment and increased incidence of cerebral palsy. Arch Dis Child Fetal Neonatal Ed 2000;83(3):F177-81.
64. Doyle LW, Halliday HL, Ehrenkranz RA, Davis PG, Sinclair JC. Impact of postnatal systemic corticosteroids on mortality and cerebral palsy in preterm infants: effect modification by risk for chronic lung disease. Pediatrics 2005;115(3):655-61.
65. Redline RW, Wilson-Costello D, Borawski E, Fanaroff AA, Hack M. The relationship between placental and other perinatal risk factors for neurologic impairment in very low birth weight children. Pediatr Res 2000;47(6):721-6.
66. Skidmore MD, Rivers A, Hack M. Increased risk of cerebral palsy among very low-birthweight infants with chronic lung disease. Dev Med Child Neurol 1990;32(4):325-32.
67. Dyet LE, Kennea N, Counsell SJ, Maalouf EF, Ajayi-Obe M, Duggan PJ, et al. Natural history of brain lesions in extremely preterm infants studied with serial magnetic resonance imaging from birth and neurodevelopmental assessment. Pediatrics 2006;118(2):536-48.
68. Tran U, Gray PH, O'Callaghan MJ. Neonatal antecedents for cerebral palsy in extremely preterm babies and interaction with maternal factors. Early Hum Dev 2005;81(6):555-61.
69. Woodward LJ, Anderson PJ, Austin NC, Howard K, Inder TE. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. N Engl J Med 2006;355(7):685-94.
70. Bax M, Tydeman C, Flodmark O. Clinical and MRI correlates of cerebral palsy: the European Cerebral Palsy Study. Jama 2006;296(13):1602-8.
71. Evans N, Kluckow M. Early determinants of right and left ventricular output in ventilated preterm infants. Arch Dis Child Fetal Neonatal Ed 1996;74(2):F88-94.
72. Weindling AM, Kissack CM. Blood pressure and tissue oxygenation in the newborn baby at risk of brain damage. Biol Neonate 2001;79(3-4):241-5.

73. Dammann O, Allred EN, Kuban KC, Van Marter LJ, Pagano M, Sanocka U, et al. Systemic hypotension and white-matter damage in preterm infants. Dev Med Child Neurol 2002;44(2):82-90.

74. Limperopoulos C, Bassan H, Kalish LA, Ringer SA, Eichenwald EC, Walter G, et al. Current definitions of hypotension do not predict abnormal cranial ultrasound findings in preterm infants. Pediatrics 2007;120(5):966-77.

75. Munro MJ, Walker AM, Barfield CP. Hypotensive extremely low birth weight infants have reduced cerebral blood flow. Pediatrics 2004;114(6):1591-6.

76. Tyszczuk L, Meek J, Elwell C, Wyatt JS. Cerebral blood flow is independent of mean arterial blood pressure in preterm infants undergoing intensive care. Pediatrics 1998;102(2 Pt 1):337-41.

77. Wardle SP, Yoxall CW, Weindling AM. Determinants of cerebral fractional oxygen extraction using near infrared spectroscopy in preterm neonates. J Cereb Blood Flow Metab 2000;20(2):272-9.

78. Soul JS, Hammer PE, Tsuji M, Saul JP, Bassan H, Limperopoulos C, et al. Fluctuating pressure-passivity is common in the cerebral circulation of sick premature infants. Pediatr Res 2007;61(4):467-73.

79. Borch K, Greisen G. Blood flow distribution in the normal human preterm brain. Pediatr Res 1998;43(1):28-33.

80. Greisen G, Borch K. White matter injury in the preterm neonate: the role of perfusion. Dev Neurosci 2001;23(3):209-12.

81. Dordelmann M, Kerk J, Dressler F, Brinkhaus MJ, Bartels DB, Dammann CE, et al. Interleukin-10 high producer allele and ultrasound-defined periventricular white matter abnormalities in preterm infants: a preliminary study. Neuropediatrics 2006;37(3):130-6.

82. Harding D, Brull D, Humphries SE, Whitelaw A, Montgomery H, Marlow N. Variation in the interleukin-6 gene is associated with impaired cognitive development in children born prematurely: a preliminary study. Pediatr Res 2005;58(1):117-20.

83. Baier RJ. Genetics of perinatal brain injury in the preterm infant. Front Biosci 2006;11:1371-87.

84. Hartel C, Schultz C, Herting E, Gopel W. Genetic association studies in VLBW infants exemplifying susceptibility to sepsis--recent findings and implications for future research. Acta Paediatr 2007;96(2):158-65.

85. Fleer A, Krediet TG. Innate immunity: toll-like receptors and some more. A brief history, basic organization and relevance for the human newborn. Neonatology 2007;92(3):145-57.

86. Li M, Carpio DF, Zheng Y, Bruzzo P, Singh V, Ouaaz F, et al. An essential role of the NF-kappa B/Toll-like receptor pathway in induction of inflammatory and tissue-repair gene expression by necrotic cells. J Immunol 2001;166(12):7128-35.

87. Aagaard-Tillery KM, Silver R, Dalton J. Immunology of normal pregnancy. Semin Fetal Neonatal Med 2006;11(5):279-95.

88. Marodi L. Innate cellular immune responses in newborns. Clin Immunol 2006;118(2-3):137-44.

89. Goldsby R. Immunology. 5th ed. New York Basingstoke: W. H. Freeman ; Palgrave Macmillan; 2002.

90. Romagnani S. T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol 2000;85(1):9-18; quiz 18, 21.

91. de Jong EC, Vieira PL, Kalinski P, Schuitemaker JH, Tanaka Y, Wierenga EA, et al. Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells in vitro with diverse th cell-polarizing signals. J Immunol 2002;168(4):1704-9.

92. Hessle CC, Andersson B, Wold AE. Gram-positive and Gram-negative bacteria elicit different patterns of pro-inflammatory cytokines in human monocytes. Cytokine 2005;30(6):311-8.

93. Plata-Salaman CR, Ilyin SE, Gayle D, Flynn MC. Gram-negative and gram-positive bacterial products induce differential cytokine profiles in the brain: analysis using an integrative molecular-behavioral in vivo model. Int J Mol Med 1998;1(2):387-97.

94. Robertson SA, Skinner RJ, Care AS. Essential role for IL-10 in resistance to lipopolysaccharide-induced preterm labor in mice. J Immunol 2006;177(7):4888-96.

95. El-Shazly S, Makhseed M, Azizieh F, Raghupathy R. Increased expression of pro-inflammatory cytokines in placentas of women undergoing spontaneous preterm delivery or premature rupture of membranes. Am J Reprod Immunol 2004;52(1):45-52.

96. Makhseed M, Raghupathy R, El-Shazly S, Azizieh F, Al-Harmi JA, Al-Azemi MM. Pro-inflammatory maternal cytokine profile in preterm delivery. Am J Reprod Immunol 2003;49(5):308-18.

97. Forster-Waldl E, Sadeghi K, Tamandl D, Gerhold B, Hallwirth U, Rohrmeister K, et al. Monocyte toll-like receptor 4 expression and LPS-induced cytokine production increase during gestational aging. Pediatr Res 2005;58(1):121-4.

98. Sadeghi K, Berger A, Langgartner M, Prusa AR, Hayde M, Herkner K, et al. Immaturity of infection control in preterm and term newborns is associated with impaired toll-like receptor signaling. J Infect Dis 2007;195(2):296-302.

99. Angelone DF, Wessels MR, Coughlin M, Suter EE, Valentini P, Kalish LA, et al. Innate immunity of the human newborn is polarized toward a high ratio of IL-6/TNF-alpha production in vitro and in vivo. Pediatr Res 2006;60(2):205-9.

100. Levy O, Coughlin M, Cronstein BN, Roy RM, Desai A, Wessels MR. The adenosine system selectively inhibits TLR-mediated TNF-alpha production in the human newborn. J Immunol 2006;177(3):1956-66.

101. Hasko G, Szabo C, Nemeth ZH, Kvetan V, Pastores SM, Vizi ES. Adenosine receptor agonists differentially regulate IL-10, TNF-alpha, and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. J Immunol 1996;157(10):4634-40.

102. Dammann O, Phillips TM, Allred EN, O'Shea TM, Paneth N, Van Marter LJ, et al. Mediators of fetal inflammation in extremely low gestational age newborns. Cytokine 2001;13(4):234-9.

103. Schultz C, Rott C, Temming P, Schlenke P, Moller JC, Bucsky P. Enhanced interleukin-6 and interleukin-8 synthesis in term and preterm infants. Pediatr Res 2002;51(3):317-22.

104. Schultz C, Temming P, Bucsky P, Gopel W, Strunk T, Hartel C. Immature anti-inflammatory response in neonates. Clin Exp Immunol 2004;135(1):130-6.

105. Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. Am J Obstet Gynecol 1998;179(1):194-202.

106. Cohen J. The immunopathogenesis of sepsis. Nature 2002;420(6917):885-91.

107. Gotsch F, Romero R, Kusanovic JP, Mazaki-Tovi S, Pineles BL, Erez O, et al. The fetal inflammatory response syndrome. Clin Obstet Gynecol 2007;50(3):652-83.

108. Romero R, Espinoza J, Goncalves LF, Gomez R, Medina L, Silva M, et al. Fetal cardiac dysfunction in preterm premature rupture of membranes. J Matern Fetal Neonatal Med 2004;16(3):146-57.

109. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labour and delivery. Semin Fetal Neonatal Med 2006;11(5):317-26.

110. Dammann O, Leviton A. Inflammatory brain damage in preterm newborns--dry numbers, wet lab, and causal inferences. Early Hum Dev 2004;79(1):1-15.

111. D'Alquen D, Kramer BW, Seidenspinner S, Marx A, Berg D, Groneck P, et al. Activation of umbilical cord endothelial cells and fetal inflammatory response in preterm infants with chorioamnionitis and funisitis. Pediatr Res 2005;57(2):263-9.

112. Steel JH, O'Donoghue K, Kennea NL, Sullivan MH, Edwards AD. Maternal origin of inflammatory leukocytes in preterm fetal membranes, shown by fluorescence in situ hybridisation. Placenta 2005;26(8-9):672-7.

113. Sampson JE, Theve RP, Blatman RN, Shipp TD, Bianchi DW, Ward BE, et al. Fetal origin of amniotic fluid polymorphonuclear leukocytes. Am J Obstet Gynecol 1997;176(1 Pt 1):77-81.

114. De Felice C, Toti P, Laurini RN, Stumpo M, Picciolini E, Todros T, et al. Early neonatal brain injury in histologic chorioamnionitis. J Pediatr 2001;138(1):101-4.

115. Cornette L. Fetal and neonatal inflammatory response and adverse outcome. Semin Fetal Neonatal Med 2004;9(6):459-70.

116. Mittendorf R, Montag AG, MacMillan W, Janeczek S, Pryde PG, Besinger RE, et al. Components of the systemic fetal inflammatory response syndrome as predictors of impaired neurologic outcomes in children. Am J Obstet Gynecol 2003;188(6):1438-4.

117. Yoon BH, Romero R, Park JS, Kim CJ, Kim SH, Choi JH, et al. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. Am J Obstet Gynecol 2000;182(3):675-81.

118. Goncalves LF, Chaiworapongsa T, Romero R. Intrauterine infection and prematurity. Ment Retard Dev Disabil Res Rev 2002;8(1):3-13.

119. Hitti J, Tarczy-Hornoch P, Murphy J, Hillier SL, Aura J, Eschenbach DA. Amniotic fluid infection, cytokines, and adverse outcome among infants at 34 weeks' gestation or less. Obstet Gynecol 2001;98(6):1080-8.

120. Shim SS, Romero R, Hong JS, Park CW, Jun JK, Kim BI, et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. Am J Obstet Gynecol 2004;191(4):1339-45.

121. Romero R, Gomez R, Ghezzi F, Yoon BH, Mazor M, Edwin SS, et al. A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. Am J Obstet Gynecol 1998;179(1):186-93.

122. Holst RM, Laurini R, Jacobsson B, Samuelsson E, Savman K, Doverhag C, et al. Expression of cytokines and chemokines in cervical and amniotic fluid: Relationship to histological chorioamnionitis. J Matern Fetal Neonatal Med 2007;20(12):885-93.

123. Murtha AP, Sinclair T, Hauser ER, Swamy GK, Herbert WN, Heine RP. Maternal serum cytokines in preterm premature rupture of membranes. Obstet Gynecol 2007;109(1):121-7.

124. Simpson KL, Keelan JA, Mitchell MD. Labor-associated changes in interleukin-10 production and its regulation by immunomodulators in human choriodecidua. J Clin Endocrinol Metab 1998;83(12):4332-7.

125. Aaltonen R, Heikkinen T, Hakala K, Laine K, Alanen A. Transfer of proinflammatory cytokines across term placenta. Obstet Gynecol 2005;106(4):802-7.

126. Zaretsky MV, Alexander JM, Byrd W, Bawdon RE. Transfer of inflammatory cytokines across the placenta. Obstet Gynecol 2004;103(3):546-50.

127. Holcberg G, Amash A, Sapir O, Sheiner E, Levy S, Huleihel M. Perfusion with lipopolysaccharide differently affects the secretion of tumor necrosis factor-alpha and interleukin-6 by term and preterm human placenta. J Reprod Immunol 2007;74(1-2):15-23.

128. Hahn-Zoric M, Hagberg H, Kjellmer I, Ellis J, Wennergren M, Hanson LA. Aberrations in placental cytokine mRNA related to intrauterine growth retardation. Pediatr Res 2002;51(2):201-6.

129. Holcberg G, Huleihel M, Sapir O, Katz M, Tsadkin M, Furman B, et al. Increased production of tumor necrosis factor-alpha TNF-alpha by IUGR human placentae. Eur J Obstet Gynecol Reprod Biol 2001;94(1):69-72.
130. Trudinger B, Wang J, Athayde N, Beutler L, Wang X. Association of umbilical placental vascular disease with fetal acute inflammatory cytokine responses. J Soc Gynecol Investig 2002;9(3):152-7.
131. Lehnardt S, Lachance C, Patrizi S, Lefebvre S, Follett PL, Jensen FE, et al. The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. J Neurosci 2002;22(7):2478-86.
132. Wang X, Rousset CI, Hagberg H, Mallard C. Lipopolysaccharide-induced inflammation and perinatal brain injury. Semin Fetal Neonatal Med 2006;11(5):343-53.
133. Singh AK, Jiang Y. How does peripheral lipopolysaccharide induce gene expression in the brain of rats? Toxicology 2004;201(1-3):197-207.
134. Yan E, Castillo-Melendez M, Nicholls T, Hirst J, Walker D. Cerebrovascular responses in the fetal sheep brain to low-dose endotoxin. Pediatr Res 2004;55(5):855-63.
135. Stolp HB, Dziegielewska KM, Ek CJ, Habgood MD, Lane MA, Potter AM, et al. Breakdown of the blood-brain barrier to proteins in white matter of the developing brain following systemic inflammation. Cell Tissue Res 2005;320(3):369-78.
136. Pang Y, Cai Z, Rhodes PG. Disturbance of oligodendrocyte development, hypomyelination and white matter injury in the neonatal rat brain after intracerebral injection of lipopolysaccharide. Brain Res Dev Brain Res 2003;140(2):205-14.
137. Cai Z, Pang Y, Lin S, Rhodes PG. Differential roles of tumor necrosis factor-alpha and interleukin-1 beta in lipopolysaccharide-induced brain injury in the neonatal rat. Brain Res 2003;975(1-2):37-47.
138. Briscoe T, Duncan J, Cock M, Choo J, Rice G, Harding R, et al. Activation of NF-kappaB transcription factor in the preterm ovine brain and placenta after acute LPS exposure. J Neurosci Res 2006;83(4):567-74.
139. Duncan JR, Cock ML, Scheerlinck JP, Westcott KT, McLean C, Harding R, et al. White matter injury after repeated endotoxin exposure in the preterm ovine fetus. Pediatr Res 2002;52(6):941-9.
140. Duncan JR, Cock ML, Suzuki K, Scheerlinck JP, Harding R, Rees SM. Chronic endotoxin exposure causes brain injury in the ovine fetus in the absence of hypoxemia. J Soc Gynecol Investig 2006;13(2):87-96.
141. Mallard C, Welin AK, Peebles D, Hagberg H, Kjellmer I. White matter injury following systemic endotoxemia or asphyxia in the fetal sheep. Neurochem Res 2003;28(2):215-23.
142. Peebles DM, Miller S, Newman JP, Scott R, Hanson MA. The effect of systemic administration of lipopolysaccharide on cerebral haemodynamics and oxygenation in the 0.65 gestation ovine fetus in utero. Bjog 2003;110(8):735-43.
143. Dalitz P, Harding R, Rees SM, Cock ML. Prolonged reductions in placental blood flow and cerebral oxygen delivery in preterm fetal sheep exposed to endotoxin: possible factors in white matter injury after acute infection. J Soc Gynecol Investig 2003;10(5):283-90.
144. Ashdown H, Dumont Y, Ng M, Poole S, Boksa P, Luheshi GN. The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia. Mol Psychiatry 2006;11(1):47-55.
145. Hutton LC, Castillo-Melendez M, Smythe GA, Walker DW. Microglial activation, macrophage infiltration, and evidence of cell death in the fetal brain after uteroplacental administration of lipopolysaccharide in sheep in late gestation. Am J Obstet Gynecol 2008;198(1):117 e1-11.
146. Cai Z, Pan ZL, 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(1):64-72.

147. Rousset CI, Chalon S, Cantagrel S, Bodard S, Andres C, Gressens P, et al. Maternal exposure to LPS induces hypomyelination in the internal capsule and programmed cell death in the deep gray matter in newborn rats. Pediatr Res 2006;59(3):428-33.

148. Nitsos I, Rees SM, Duncan J, Kramer BW, Harding R, Newnham JP, et al. Chronic exposure to intra-amniotic lipopolysaccharide affects the ovine fetal brain. J Soc Gynecol Investig 2006;13(4):239-47.

149. Wang X, Hagberg H, Zhu C, Jacobsson B, Mallard C. Effects of intrauterine inflammation on the developing mouse brain. Brain Res 2007;1144:180-5.

150. Bell MJ, Hallenbeck JM, Gallo V. Determining the fetal inflammatory response in an experimental model of intrauterine inflammation in rats. Pediatr Res 2004;56(4):541-6.

151. Elovitz MA, Mrinalini C, Sammel MD. Elucidating the early signal transduction pathways leading to fetal brain injury in preterm birth. Pediatr Res 2006;59(1):50-5.

152. Poggi SH, Park J, Toso L, Abebe D, Roberson R, Woodard JE, et al. No phenotype associated with established lipopolysaccharide model for cerebral palsy. Am J Obstet Gynecol 2005;192(3):727-33.

153. Borrell J, Vela JM, Arevalo-Martin A, Molina-Holgado E, Guaza C. Prenatal immune challenge disrupts sensorimotor gating in adult rats. Implications for the etiopathogenesis of schizophrenia. Neuropsychopharmacology 2002;26(2):204-15.

154. Grether JK, Nelson KB. Maternal infection and cerebral palsy in infants of normal birth weight. Jama 1997;278(3):207-11.

155. Hagberg H, Dammann O, Mallard C, Leviton A. Preconditioning and the developing brain. Semin Perinatol 2004;28(6):389-95.

156. Mallard C, Hagberg H. Inflammation-induced preconditioning in the immature brain. Semin Fetal Neonatal Med 2007;12(4):280-6.

157. Gustavsson M, Mallard C, Vannucci SJ, Wilson MA, Johnston MV, Hagberg H. Vascular response to hypoxic preconditioning in the immature brain. J Cereb Blood Flow Metab 2007;27(5):928-38.

158. Eklind S, Mallard C, Arvidsson P, Hagberg H. Lipopolysaccharide induces both a primary and a secondary phase of sensitization in the developing rat brain. Pediatr Res 2005;58(1):112-6.

159. Xu DX, Wang H, Zhao L, Ning H, Chen YH, Zhang C. Effects of low-dose lipopolysaccharide (LPS) pretreatment on LPS-induced intra-uterine fetal death and preterm labor. Toxicology 2007;234(3):167-75.

160. Folkerth RD, Keefe RJ, Haynes RL, Trachtenberg FL, Volpe JJ, Kinney HC. Interferon-gamma expression in periventricular leukomalacia in the human brain. Brain Pathol 2004;14(3):265-74.

161. Kadhim H, Tabarki B, De Prez C, Rona AM, Sebire G. Interleukin-2 in the pathogenesis of perinatal white matter damage. Neurology 2002;58(7):1125-8.

162. Kadhim H, Tabarki B, De Prez C, Sebire G. Cytokine immunoreactivity in cortical and subcortical neurons in periventricular leukomalacia: are cytokines implicated in neuronal dysfunction in cerebral palsy? Acta Neuropathol (Berl) 2003;105(3):209-16.

163. Kadhim H, Tabarki B, Verellen G, De Prez C, Rona AM, Sebire G. Inflammatory cytokines in the pathogenesis of periventricular leukomalacia. Neurology 2001;56(10):1278-84.

164. Deguchi K, Mizuguchi M, Takashima S. Immunohistochemical expression of tumor necrosis factor alpha in neonatal leukomalacia. Pediatr Neurol 1996;14(1):13-6.

165. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol 2003;3(9):745-56.

166. Jara JH, Singh BB, Floden AM, Combs CK. Tumor necrosis factor alpha stimulates NMDA receptor activity in mouse cortical neurons resulting in ERK-dependent death. J Neurochem 2007;100(5):1407-20.

167. Stellwagen D, Malenka RC. Synaptic scaling mediated by glial TNF-alpha. Nature 2006;440(7087):1054-9.

168. Shohami E, Ginis I, Hallenbeck JM. Dual role of tumor necrosis factor alpha in brain injury. Cytokine Growth Factor Rev 1999;10(2):119-30.

169. Prabhakar U, Conway TM, Murdock P, Mooney JL, Clark S, Hedge P, et al. Correlation of protein and gene expression profiles of inflammatory proteins after endotoxin challenge in human subjects. DNA Cell Biol 2005;24(7):410-31.

170. Ben-Hur T, Ben-Menachem O, Furer V, Einstein O, Mizrachi-Kol R, Grigoriadis N. Effects of proinflammatory cytokines on the growth, fate, and motility of multipotential neural precursor cells. Mol Cell Neurosci 2003;24(3):623-31.

171. Downen M, Amaral TD, Hua LL, Zhao ML, Lee SC. Neuronal death in cytokine-activated primary human brain cell culture: role of tumor necrosis factor-alpha. Glia 1999;28(2):114-27.

172. Feldhaus B, Dietzel ID, Heumann R, Berger R. Effects of interferon-gamma and tumor necrosis factor-alpha on survival and differentiation of oligodendrocyte progenitors. J Soc Gynecol Investig 2004;11(2):89-96.

173. 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 U S A 1995;92(24):11294-8.

174. Rosenberg GA, Estrada EY, Dencoff JE, Stetler-Stevenson WG. Tumor necrosis factor-alpha-induced gelatinase B causes delayed opening of the blood-brain barrier: an expanded therapeutic window. Brain Res 1995;703(1-2):151-5.

175. Arnett HA, Mason J, Marino M, Suzuki K, Matsushima GK, Ting JP. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. Nat Neurosci 2001;4(11):1116-22.

176. Correale J, Villa A. The neuroprotective role of inflammation in nervous system injuries. J Neurol 2004;251(11):1304-16.

177. Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. J Neuroimmunol 2007;184(1-2):53-68.

178. Quintana A, Molinero A, Florit S, Manso Y, Comes G, Carrasco J, et al. Diverging mechanisms for TNF-alpha receptors in normal mouse brains and in functional recovery after injury: From gene to behavior. J Neurosci Res 2007;85(12):2668-85.

179. Nawashiro H, Tasaki K, Ruetzler CA, Hallenbeck JM. TNF-alpha pretreatment induces protective effects against focal cerebral ischemia in mice. J Cereb Blood Flow Metab 1997;17(5):483-90.

180. Rosenzweig HL, Minami M, Lessov NS, Coste SC, Stevens SL, Henshall DC, et al. Endotoxin preconditioning protects against the cytotoxic effects of TNFalpha after stroke: a novel role for TNFalpha in LPS-ischemic tolerance. J Cereb Blood Flow Metab 2007;27(10):1663-74.

181. Dembinski J, Behrendt D, Martini R, Heep A, Bartmann P. Modulation of pro- and anti-inflammatory cytokine production in very preterm infants. Cytokine 2003;21(4):200-6.

182. Buntinx M, Moreels M, Vandenabeele F, Lambrichts I, Raus J, Steels P, et al. Cytokine-induced cell death in human oligodendroglial cell lines: I. Synergistic effects of IFN-gamma and TNF-alpha on apoptosis. J Neurosci Res 2004;76(6):834-45.

183. Baerwald KD, Popko B. Developing and mature oligodendrocytes respond differently to the immune cytokine interferon-gamma. J Neurosci Res 1998;52(2):230-9.

184. Molina-Holgado E, Vela JM, Arevalo-Martin A, Guaza C. LPS/IFN-gamma cytotoxicity in oligodendroglial cells: role of nitric oxide and protection by the anti-inflammatory cytokine IL-10. Eur J Neurosci 2001;13(3):493-502.
185. Felderhoff-Mueser U, Sifringer M, Polley O, Dzietko M, Leineweber B, Mahler L, et al. Caspase-1-processed interleukins in hyperoxia-induced cell death in the developing brain. Ann Neurol 2005;57(1):50-9.
186. Hu X, Nesic-Taylor O, Qiu J, Rea HC, Fabian R, Rassin DK, et al. Activation of nuclear factor-kappaB signaling pathway by interleukin-1 after hypoxia/ischemia in neonatal rat hippocampus and cortex. J Neurochem 2005;93(1):26-37.
187. Felderhoff-Mueser U, Schmidt OI, Oberholzer A, Buhrer C, Stahel PF. IL-18: a key player in neuroinflammation and neurodegeneration? Trends Neurosci 2005;28(9):487-93.
188. Hedtjarn M, Leverin AL, Eriksson K, Blomgren K, Mallard C, Hagberg H. Interleukin-18 involvement in hypoxic-ischemic brain injury. J Neurosci 2002;22(14):5910-9.
189. Hedtjarn M, Mallard C, Arvidsson P, Hagberg H. White matter injury in the immature brain: role of interleukin-18. Neurosci Lett 2005;373(1):16-20.
190. Ng PC, Li K, Wong RP, Chui K, Wong E, Li G, et al. Proinflammatory and anti-inflammatory cytokine responses in preterm infants with systemic infections. Arch Dis Child Fetal Neonatal Ed 2003;88(3):F209-13.
191. Froen JF, Munkeby BH, Stray-Pedersen B, Saugstad OD. Interleukin-10 reverses acute detrimental effects of endotoxin-induced inflammation on perinatal cerebral hypoxia-ischemia. Brain Res 2002;942(1-2):87-94.
192. Rodts-Palenik S, Wyatt-Ashmead J, Pang Y, Thigpen B, Cai Z, Rhodes P, et al. Maternal infection-induced white matter injury is reduced by treatment with interleukin-10. Am J Obstet Gynecol 2004;191(4):1387-92.
193. Pang Y, Rodts-Palenik S, Cai Z, Bennett WA, Rhodes PG. Suppression of glial activation is involved in the protection of IL-10 on maternal E. coli induced neonatal white matter injury. Brain Res Dev Brain Res 2005;157(2):141-9.
194. Pang Y, Fan LW, Zheng B, Cai Z, Rhodes PG. Role of interleukin-6 in lipopolysaccharide-induced brain injury and behavioral dysfunction in neonatal rats. Neuroscience 2006;141(2):745-55.
195. Vedder H, Schreiber W, Yassouridis A, Gudewill S, Galanos C, Pollmacher T. Dose-dependence of bacterial lipopolysaccharide (LPS) effects on peak response and time course of the immune-endocrine host response in humans. Inflamm Res 1999;48(2):67-74.
196. Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF, et al. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. J Clin Invest 1998;101(2):311-20.
197. Dembinski J, Behrendt D, Heep A, Dorn C, Reinsberg J, Bartmann P. Cell-associated interleukin-8 in cord blood of term and preterm infants. Clin Diagn Lab Immunol 2002;9(2):320-3.
198. Yektaei-Karin E, Moshfegh A, Lundahl J, Berggren V, Hansson LO, Marchini G. The stress of birth enhances in vitro spontaneous and IL-8-induced neutrophil chemotaxis in the human newborn. Pediatr Allergy Immunol 2007;18(8):643-51.
199. Dammann O, Allred EN, Veelken N. Increased risk of spastic diplegia among very low birth weight children after preterm labor or prelabor rupture of membranes. J Pediatr 1998;132(3 Pt 1):531-5.
200. Dammann O, Drescher J, Veelken N. Maternal fever at birth and non-verbal intelligence at age 9 years in preterm infants. Dev Med Child Neurol 2003;45(3):148-51.
201. Jacobsson B, Hagberg G, Hagberg B, Ladfors L, Niklasson A, Hagberg H. Cerebral palsy in preterm infants: a population-based case-control study of antenatal and intrapartal risk factors. Acta Paediatr 2002;91(8):946-51.

202. Perlman JM, Risser R, Broyles RS. Bilateral cystic periventricular leukomalacia in the premature infant: associated risk factors. Pediatrics 1996;97(6 Pt 1):822-7.

203. Verma U, Tejani N, Klein S, Reale MR, Beneck D, Figueroa R, et al. Obstetric antecedents of intraventricular hemorrhage and periventricular leukomalacia in the low-birth-weight neonate. Am J Obstet Gynecol 1997;176(2):275-81.

204. Wu YW. Systematic review of chorioamnionitis and cerebral palsy. Ment Retard Dev Disabil Res Rev 2002;8(1):25-9.

205. Duggan PJ, Maalouf EF, Watts TL, Sullivan MH, Counsell SJ, Allsop J, et al. Intrauterine T-cell activation and increased proinflammatory cytokine concentrations in preterm infants with cerebral lesions. Lancet 2001;358(9294):1699-700.

206. Goepfert AR, Andrews WW, Carlo W, Ramsey PS, Cliver SP, Goldenberg RL, et al. Umbilical cord plasma interleukin-6 concentrations in preterm infants and risk of neonatal morbidity. Am J Obstet Gynecol 2004;191(4):1375-81.

207. Huang HC, Wang CL, Huang LT, Chuang H, Liu CA, Hsu TY, et al. Association of cord blood cytokines with prematurity and cerebral palsy. Early Hum Dev 2004;77(1-2):29-36.

208. Minagawa K, Tsuji Y, Ueda H, Koyama K, Tanizawa K, Okamura H, et al. Possible correlation between high levels of IL-18 in the cord blood of pre-term infants and neonatal development of periventricular leukomalacia and cerebral palsy. Cytokine 2002;17(3):164-70.

209. Tsukimori K, Komatsu H, Yoshimura T, Hikino S, Hara T, Wake N, et al. Increased inflammatory markers are associated with early periventricular leukomalacia. Dev Med Child Neurol 2007;49(8):587-90.

210. Yoon BH, Romero R, Yang SH, Jun JK, Kim IO, Choi JH, 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(5):1433-40.

211. Heep A, Behrendt D, Nitsch P, Fimmers R, Bartmann P, Dembinski J. Increased serum levels of interleukin 6 are associated with severe intraventricular haemorrhage in extremely premature infants. Arch Dis Child Fetal Neonatal Ed 2003;88(6):F501-4.

212. Grether JK, Nelson KB, Walsh E, Willoughby RE, Redline RW. Intrauterine exposure to infection and risk of cerebral palsy in very preterm infants. Arch Pediatr Adolesc Med 2003;157(1):26-32.

213. Nelson KB, Grether JK, Dambrosia JM, Walsh E, Kohler S, Satyanarayana G, et al. Neonatal cytokines and cerebral palsy in very preterm infants. Pediatr Res 2003;53(4):600-7.

214. Yanowitz TD, Jordan JA, Gilmour CH, Towbin R, Bowen A, Roberts JM, et al. Hemodynamic disturbances in premature infants born after chorioamnionitis: association with cord blood cytokine concentrations. Pediatr Res 2002;51(3):310-6.

215. Pleiner J, Mittermayer F, Schaller G, Marsik C, MacAllister RJ, Wolzt M. Inflammation-induced vasoconstrictor hyporeactivity is caused by oxidative stress. J Am Coll Cardiol 2003;42(9):1656-62.

216. Carr L, Tucker A, Fernandez-Botran R. In vivo administration of L-dopa or dopamine decreases the number of splenic IFN gamma-producing cells. J Neuroimmunol 2003;137(1-2):87-93.

217. Uusaro A, Russell JA. Could anti-inflammatory actions of catecholamines explain the possible beneficial effects of supranormal oxygen delivery in critically ill surgical patients? Intensive Care Med 2000;26(3):299-304.

218. Banks WA, Kastin AJ, Durham DA. Bidirectional transport of interleukin-1 alpha across the blood-brain barrier. Brain Res Bull 1989;23(6):433-7.

219. de Vries HE, Blom-Roosemalen MC, van Oosten M, de Boer AG, van Berkel TJ, Breimer DD, et al. The influence of cytokines on the integrity of the blood-brain barrier in vitro. J Neuroimmunol 1996;64(1):37-43.
220. Wong D, Dorovini-Zis K, Vincent SR. Cytokines, nitric oxide, and cGMP modulate the permeability of an in vitro model of the human blood-brain barrier. Exp Neurol 2004;190(2):446-55.
221. Ellison VJ, Mocatta TJ, Winterbourn CC, Darlow BA, Volpe JJ, Inder TE. The relationship of CSF and plasma cytokine levels to cerebral white matter injury in the premature newborn. Pediatr Res 2005;57(2):282-6.
222. Viscardi RM, Muhumuza CK, Rodriguez A, Fairchild KD, Sun CC, Gross GW, et al. Inflammatory markers in intrauterine and fetal blood and cerebrospinal fluid compartments are associated with adverse pulmonary and neurologic outcomes in preterm infants. Pediatr Res 2004;55(6):1009-17.
223. Laborada G, Nesin M. Interleukin-6 and interleukin-8 are elevated in the cerebrospinal fluid of infants exposed to chorioamnionitis. Biol Neonate 2005;88(2):136-44.
224. Savman K, Blennow, M, Hagberg, H, Tarkowski, E, Thoresen, M, Whitelaw, A. Cytokine response in cerebrospinal fluid from preterm infants with posthaemorrhagic ventricular dilatation. Acta Paediatrica 2002;91(12):1357-1363.
225. Schmitz T, Heep A, Groenendaal F, Huseman D, Kie S, Bartmann P, et al. Interleukin-1beta, interleukin-18, and interferon-gamma expression in the cerebrospinal fluid of premature infants with posthemorrhagic hydrocephalus--markers of white matter damage? Pediatr Res 2007;61(6):722-6.
226. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995;16(1):3-34.
227. Holly J, Perks C. The role of insulin-like growth factor binding proteins. Neuroendocrinology 2006;83(3-4):154-60.
228. Boulware SD, Tamborlane WV, Rennert NJ, Gesundheit N, Sherwin RS. Comparison of the metabolic effects of recombinant human insulin-like growth factor-I and insulin. Dose-response relationships in healthy young and middle-aged adults. J Clin Invest 1994;93(3):1131-9.
229. Clemmons DR, Moses AC, McKay MJ, Sommer A, Rosen DM, Ruckle J. The combination of insulin-like growth factor I and insulin-like growth factor-binding protein-3 reduces insulin requirements in insulin-dependent type 1 diabetes: evidence for in vivo biological activity. J Clin Endocrinol Metab 2000;85(4):1518-24.
230. Underwood LE, Thissen JP, Lemozy S, Ketelslegers JM, Clemmons DR. Hormonal and nutritional regulation of IGF-I and its binding proteins. Horm Res 1994;42(4-5):145-51.
231. Fant ME, Weisoly D. Insulin and insulin-like growth factors in human development: implications for the perinatal period. Semin Perinatol 2001;25(6):426-35.
232. Arany E, Afford S, Strain AJ, Winwood PJ, Arthur MJ, Hill DJ. Differential cellular synthesis of insulin-like growth factor binding protein-1 (IGFBP-1) and IGFBP-3 within human liver. J Clin Endocrinol Metab 1994;79(6):1871-6.
233. Guler HP, Zapf J, Schmid C, Froesch ER. Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. Acta Endocrinol (Copenh) 1989;121(6):753-8.
234. Maile LA, Holly JM. Insulin-like growth factor binding protein (IGFBP) proteolysis: occurrence, identification, role and regulation. Growth Horm IGF Res 1999;9(2):85-95.
235. Timmins AC, Cotterill AM, Hughes SC, Holly JM, Ross RJ, Blum W, et al. Critical illness is associated with low circulating concentrations of insulin-like growth factors-I and -II, alterations in insulin-like growth factor binding proteins, and induction of an insulin-like growth factor binding protein 3 protease. Crit Care Med 1996;24(9):1460-6.

236. Maddux BA, Chan A, Mandarino LJ, Goldfine ID. IGF-binding protein-1 levels are related to insulin-mediated glucose disposal and are a potential serum marker of insulin resistance. Diabetes Care 2006;29(7):1535-7.
237. Lee PD, Giudice LC, Conover CA, Powell DR. Insulin-like growth factor binding protein-1: recent findings and new directions. Proc Soc Exp Biol Med 1997;216(3):319-57.
238. Westwood M, Gibson JM, White A. Purification and characterization of the insulin-like growth factor-binding protein-1 phosphoform found in normal plasma. Endocrinology 1997;138(3):1130-6.
239. Rajaram S, Baylink DJ, Mohan S. Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr Rev 1997;18(6):801-31.
240. Radetti G, Bozzola M, Paganini C, Valentini R, Gentili L, Tettoni K, et al. Growth hormone bioactivity and levels of growth hormone, growth hormone-binding protein, insulinlike growth factor I, and insulinlike growth factor-binding proteins in premature and full-term newborns during the first month of life. Arch Pediatr Adolesc Med 1997;151(2):170-5.
241. Bennett A, Wilson DM, Liu F, Nagashima R, Rosenfeld RG, Hintz RL. Levels of insulin-like growth factors I and II in human cord blood. J Clin Endocrinol Metab 1983;57(3):609-12.
242. Fowden AL. The insulin-like growth factors and feto-placental growth. Placenta 2003;24(8-9):803-12.
243. Gluckman PD, Pinal CS. Regulation of fetal growth by the somatotrophic axis. J Nutr 2003;133(5 Suppl 2):1741S-1746S.
244. McDonald TJ, Nijland MJ, Nathanielsz PW. The insulin-like growth factor system and the fetal brain: effects of poor maternal nutrition. Rev Endocr Metab Disord 2007;8(2):71-84.
245. Harding JE, Liu L, Evans PC, Gluckman PD. Insulin-like growth factor 1 alters feto-placental protein and carbohydrate metabolism in fetal sheep. Endocrinology 1994;134(3):1509-14.
246. Roberts CT, Owens JA, Carter AM, Harding JE, Austgulen R, Wlodek M. Insulin-like growth factors and foetal programming--a workshop report. Placenta 2003;24 Suppl A:S72-5.
247. Oliver MH, Harding JE, Breier BH, Evans PC, Gluckman PD. Glucose but not a mixed amino acid infusion regulates plasma insulin-like growth factor-I concentrations in fetal sheep. Pediatr Res 1993;34(1):62-5.
248. Koistinen R, Angervo M, Leinonen P, Hakala T, Seppala M. Phosphorylation of insulin-like growth factor-binding protein-1 increases in human amniotic fluid and decidua from early to late pregnancy. Clin Chim Acta 1993;215(2):189-99.
249. Westwood M, Gibson JM, Davies AJ, Young RJ, White A. The phosphorylation pattern of insulin-like growth factor-binding protein-1 in normal plasma is different from that in amniotic fluid and changes during pregnancy. J Clin Endocrinol Metab 1994;79(6):1735-41.
250. Langford K, Nicolaides K, Miell JP. Maternal and fetal insulin-like growth factors and their binding proteins in the second and third trimesters of human pregnancy. Hum Reprod 1998;13(5):1389-93.
251. Vatten LJ, Nilsen ST, Odegard RA, Romundstad PR, Austgulen R. Insulin-like growth factor I and leptin in umbilical cord plasma and infant birth size at term. Pediatrics 2002;109(6):1131-5.
252. Verhaeghe J, Van Herck E, Billen J, Moerman P, Van Assche FA, Giudice LC. Regulation of insulin-like growth factor-I and insulin-like growth factor binding protein-1 concentrations in preterm fetuses. Am J Obstet Gynecol 2003;188(2):485-91.
253. Giudice LC, de Zegher F, Gargosky SE, Dsupin BA, de las Fuentes L, Crystal RA, et al. Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. J Clin Endocrinol Metab 1995;80(5):1548-55.

254. Lassarre C, Hardouin S, Daffos F, Forestier F, Frankenne F, Binoux M. Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationships with growth in normal subjects and in subjects with intrauterine growth retardation. Pediatr Res 1991;29(3):219-25.

255. Vatten LJ, Odegard RA, Nilsen ST, Salvesen KA, Austgulen R. Relationship of insulin-like growth factor-I and insulin-like growth factor binding proteins in umbilical cord plasma to preeclampsia and infant birth weight. Obstet Gynecol 2002;99(1):85-90.

256. Laviola L, Perrini S, Belsanti G, Natalicchio A, Montrone C, Leonardini A, et al. Intrauterine growth restriction in humans is associated with abnormalities in placental insulin-like growth factor signaling. Endocrinology 2005;146(3):1498-505.

257. Bennet L, Oliver MH, Gunn AJ, Hennies M, Breier BH. Differential changes in insulin-like growth factors and their binding proteins following asphyxia in the preterm fetal sheep. J Physiol 2001;531(Pt 3):835-41.

258. Dinleyici EC, Tekin N, Colak O, Aksit MA. Cord blood IGF-1 and IGFBP-3 levels in asphyxiated term newborns. Neuro Endocrinol Lett 2006;27(6):745-7.

259. Huang ST, Vo KC, Lyell DJ, Faessen GH, Tulac S, Tibshirani R, et al. Developmental response to hypoxia. Faseb J 2004;18(12):1348-65.

260. Kajimura S, Aida K, Duan C. Insulin-like growth factor-binding protein-1 (IGFBP-1) mediates hypoxia-induced embryonic growth and developmental retardation. Proc Natl Acad Sci U S A 2005;102(4):1240-5.

261. Marchini G, Hagenas L, Kocoska-Maras L, Berggren V, Hansson LO. Insulin-like growth factor binding protein-1 and interleukin-6 are markers of fetal stress during parturition at term gestation. J Pediatr Endocrinol Metab 2005;18(8):777-83.

262. Scharf JG, Unterman TG, Kietzmann T. Oxygen-dependent modulation of insulin-like growth factor binding protein biosynthesis in primary cultures of rat hepatocytes. Endocrinology 2005;146(12):5433-43.

263. Price WA, Stiles AD, Moats-Staats BM, D'Ercole AJ. Gene expression of insulin-like growth factors (IGFs), the type 1 IGF receptor, and IGF-binding proteins in dexamethasone-induced fetal growth retardation. Endocrinology 1992;130(3):1424-32.

264. Ye P, D'Ercole AJ. Insulin-like growth factor actions during development of neural stem cells and progenitors in the central nervous system. J Neurosci Res 2006;83(1):1-6.

265. Popken GJ, Hodge RD, Ye P, Zhang J, Ng W, O'Kusky JR, et al. In vivo effects of insulin-like growth factor-I (IGF-I) on prenatal and early postnatal development of the central nervous system. Eur J Neurosci 2004;19(8):2056-68.

266. D'Ercole AJ, Ye P, Calikoglu AS, Gutierrez-Ospina G. The role of the insulin-like growth factors in the central nervous system. Mol Neurobiol 1996;13(3):227-55.

267. Mason JL, Xuan S, Dragatsis I, Efstratiadis A, Goldman JE. Insulin-like growth factor (IGF) signaling through type 1 IGF receptor plays an important role in remyelination. J Neurosci 2003;23(20):7710-8.

268. Wilson HC, Onischke C, Raine CS. Human oligodendrocyte precursor cells in vitro: phenotypic analysis and differential response to growth factors. Glia 2003;44(2):153-65.

269. Ye P, Li L, Richards RG, DiAugustine RP, D'Ercole AJ. Myelination is altered in insulin-like growth factor-I null mutant mice. J Neurosci 2002;22(14):6041-51.

270. D'Ercole AJ, Calikoglu AS. Editorial review: the case of local versus endocrine IGF-I actions: the jury is still out. Growth Horm IGF Res 2001;11(5):261-5.

271. Pan W, Kastin AJ. Interactions of IGF-1 with the blood-brain barrier in vivo and in situ. Neuroendocrinology 2000;72(3):171-8.

272. Reinhardt RR, Bondy CA. Insulin-like growth factors cross the blood-brain barrier. Endocrinology 1994;135(5):1753-61.

273. Hill DJ, Clemmons DR. Similar distribution of insulin-like growth factor binding proteins-1, -2, -3 in human fetal tissues. Growth Factors 1992;6(4):315-26.

274. Doublier S, Duyckaerts C, Seurin D, Binoux M. Impaired brain development and hydrocephalus in a line of transgenic mice with liver-specific expression of human insulin-like growth factor binding protein-1. Growth Horm IGF Res 2000;10(5):267-74.

275. Ni W, Rajkumar K, Nagy JI, Murphy LJ. Impaired brain development and reduced astrocyte response to injury in transgenic mice expressing IGF binding protein-1. Brain Res 1997;769(1):97-107.

276. Sizonenko SV, Bednarek N, Gressens P. Growth factors and plasticity. Semin Fetal Neonatal Med 2007;12(4):241-9.

277. Cheng CM, Reinhardt RR, Lee WH, Joncas G, Patel SC, Bondy CA. Insulin-like growth factor 1 regulates developing brain glucose metabolism. Proc Natl Acad Sci U S A 2000;97(18):10236-41.

278. Ness JK, Wood TL. Insulin-like growth factor I, but not neurotrophin-3, sustains Akt activation and provides long-term protection of immature oligodendrocytes from glutamate-mediated apoptosis. Mol Cell Neurosci 2002;20(3):476-88.

279. Cao Y, Gunn AJ, Bennet L, Wu D, George S, Gluckman PD, et al. Insulin-like growth factor (IGF)-1 suppresses oligodendrocyte caspase-3 activation and increases glial proliferation after ischemia in near-term fetal sheep. J Cereb Blood Flow Metab 2003;23(6):739-47.

280. Guan J, Bennet L, George S, Wu D, Waldvogel HJ, Gluckman PD, et al. Insulin-like growth factor-1 reduces postischemic white matter injury in fetal sheep. J Cereb Blood Flow Metab 2001;21(5):493-502.

281. Lin S, Fan LW, Pang Y, Rhodes PG, Mitchell HJ, Cai Z. IGF-1 protects oligodendrocyte progenitor cells and improves neurological functions following cerebral hypoxia-ischemia in the neonatal rat. Brain Res 2005;1063(1):15-26.

282. Wood TL, Loladze V, Altieri S, Gangoli N, Levison SW, Brywe KG, et al. Delayed IGF-1 administration rescues oligodendrocyte progenitors from glutamate-induced cell death and hypoxic-ischemic brain damage. Dev Neurosci 2007;29(4-5):302-10.

283. Ye P, D'Ercole AJ. Insulin-like growth factor I protects oligodendrocytes from tumor necrosis factor-alpha-induced injury. Endocrinology 1999;140(7):3063-72.

284. Ye P, Kollias G, D'Ercole AJ. Insulin-like growth factor-I ameliorates demyelination induced by tumor necrosis factor-alpha in transgenic mice. J Neurosci Res 2007;85(4):712-22.

285. Pang Y, Zheng B, Fan LW, Rhodes PG, Cai Z. IGF-1 protects oligodendrocyte progenitors against TNFalpha-induced damage by activation of PI3K/Akt and interruption of the mitochondrial apoptotic pathway. Glia 2007;55(11):1099-107.

286. Sizonenko SV, Sirimanne ES, Williams CE, Gluckman PD. Neuroprotective effects of the N-terminal tripeptide of IGF-1, glycine-proline-glutamate, in the immature rat brain after hypoxic-ischemic injury. Brain Res 2001;922(1):42-50.

287. Svedin P, Guan J, Mathai S, Zhang R, Wang X, Gustavsson M, et al. Delayed peripheral administration of a GPE analogue induces astrogliosis and angiogenesis and reduces inflammation and brain injury following hypoxia-ischemia in the neonatal rat. Dev Neurosci 2007;29(4-5):393-402.

288. Kelley KW. From hormones to immunity: the physiology of immunology. Brain Behav Immun 2004;18(2):95-113.

289. Tu W, Cheung PT, Lau YL. IGF-I increases interferon-gamma and IL-6 mRNA expression and protein production in neonatal mononuclear cells. Pediatr Res 1999;46(6):748-54.

290. Ye P, Price W, Kassiotis G, Kollias G, D'Ercole AJ. Tumor necrosis factor-alpha regulation of insulin-like growth factor-I, type 1 IGF receptor, and IGF binding protein expression in cerebellum of transgenic mice. J Neurosci Res 2003;71(5):721-31.

291. De Benedetti F, Alonzi T, Moretta A, Lazzaro D, Costa P, Poli V, et al. Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. J Clin Invest 1997;99(4):643-50.

292. De Benedetti F, Meazza C, Oliveri M, Pignatti P, Vivarelli M, Alonzi T, et al. Effect of IL-6 on IGF binding protein-3: a study in IL-6 transgenic mice and in patients with systemic juvenile idiopathic arthritis. Endocrinology 2001;142(11):4818-26.

293. Lieskovska J, Guo D, Derman E. IL-6-overexpression brings about growth impairment potentially through a GH receptor defect. Growth Horm IGF Res 2002;12(6):388-98.

294. Fan J, Char D, Bagby GJ, Gelato MC, Lang CH. Regulation of insulin-like growth factor-I (IGF-I) and IGF-binding proteins by tumor necrosis factor. Am J Physiol 1995;269(5 Pt 2):R1204-12.

295. Fan J, Wojnar MM, Theodorakis M, Lang CH. Regulation of insulin-like growth factor (IGF)-I mRNA and peptide and IGF-binding proteins by interleukin-1. Am J Physiol 1996;270(3 Pt 2):R621-9.

296. Shalita-Chesner M, Katz J, Shemer J, Werner H. Regulation of insulin-like growth factor-I receptor gene expression by tumor necrosis factor-alpha and interferon-gamma. Mol Cell Endocrinol 2001;176(1-2):1-12.

297. Thissen JP, Verniers J. Inhibition by interleukin-1 beta and tumor necrosis factor-alpha of the insulin-like growth factor I messenger ribonucleic acid response to growth hormone in rat hepatocyte primary culture. Endocrinology 1997;138(3):1078-84.

298. Wang P, Li N, Li JS, Li WQ. The role of endotoxin, TNF-alpha, and IL-6 in inducing the state of growth hormone insensitivity. World J Gastroenterol 2002;8(3):531-6.

299. Wolf M, Bohm S, Brand M, Kreymann G. Proinflammatory cytokines interleukin 1 beta and tumor necrosis factor alpha inhibit growth hormone stimulation of insulin-like growth factor I synthesis and growth hormone receptor mRNA levels in cultured rat liver cells. Eur J Endocrinol 1996;135(6):729-37.

300. Andreozzi F, Laratta E, Cardellini M, Marini MA, Lauro R, Hribal ML, et al. Plasma interleukin-6 levels are independently associated with insulin secretion in a cohort of Italian-Caucasian nondiabetic subjects. Diabetes 2006;55(7):2021-4.

301. Benbassat CA, Lazarus DD, Cichy SB, Evans TM, Moldawer LL, Lowry SF, et al. Interleukin-1 alpha (IL-1 alpha) and tumor necrosis factor alpha (TNF alpha) regulate insulin-like growth factor binding protein-1 (IGFBP-1) levels and mRNA abundance in vivo and in vitro. Horm Metab Res 1999;31(2-3):209-15.

302. Lagathu C, Yvan-Charvet L, Bastard JP, Maachi M, Quignard-Boulange A, Capeau J, et al. Long-term treatment with interleukin-1beta induces insulin resistance in murine and human adipocytes. Diabetologia 2006;49(9):2162-73.

303. Pihl S, Carlsson-Skwirut C, Berg U, Ekstrom K, Bang P. Acute interleukin-6 infusion increases IGFBP-1 but has no short-term effect on IGFBP-3 proteolysis in healthy men. Horm Res 2006;65(4):177-84.
304. Samstein B, Hoimes ML, Fan J, Frost RA, Gelato MC, Lang CH. IL-6 stimulation of insulin-like growth factor binding protein (IGFBP)-1 production. Biochem Biophys Res Commun 1996;228(2):611-615.
305. Lineham JD, Smith RM, Dahlenburg GW, King RA, Haslam RR, Stuart MC, et al. Circulating insulin-like growth factor I levels in newborn premature and full-term infants followed longitudinally. Early Hum Dev 1986;13(1):37-46.
306. Saini JR, Morgan JB, Teale JD, Kovar IZ. Insulin-like growth factor-I in the parenterally fed low birth weight infant. Ann Nutr Metab 1989;33(2):88-91.
307. Engstrom E, Niklasson A, Wikland KA, Ewald U, Hellstrom A. The role of maternal factors, postnatal nutrition, weight gain, and gender in regulation of serum IGF-I among preterm infants. Pediatr Res 2005;57(4):605-10.
308. Kajantie E, Dunkel L, Rutanen EM, Seppala M, Koistinen R, Sarnesto A, et al. IGF-I, IGF binding protein (IGFBP)-3, phosphoisoforms of IGFBP-1, and postnatal growth in very low birth weight infants. J Clin Endocrinol Metab 2002;87(5):2171-9.
309. Lo HC, Tsao LY, Hsu WY, Chi CY, Tsai FA. Changes in serum insulin-like growth factors, not leptin, are associated with postnatal weight gain in preterm neonates. JPEN J Parenter Enteral Nutr 2005;29(2):87-92.
310. Cutfield WS, Regan FA, Jackson WE, Jefferies CA, Robinson EM, Harris M, et al. The endocrine consequences for very low birth weight premature infants. Growth Horm IGF Res 2004;14 Suppl A:S130-5.
311. Smith WJ, Underwood LE, Keyes L, Clemmons DR. Use of insulin-like growth factor I (IGF-I) and IGF-binding protein measurements to monitor feeding of premature infants. J Clin Endocrinol Metab 1997;82(12):3982-8.
312. Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 2001;107(2):270-3.
313. Yeung MY. Somatotropic axis derangement as an underlying factor in the genesis of retinopathy of prematurity. Acta Paediatr 2006;95(11):1334-40.
314. Lucas A, Morley R, Isaacs E. Nutrition and mental development. Nutr Rev 2001;59(8 Pt 2):S24-32; discussion S32-3.
315. Calikoglu A, Karayal A, D'Ercole A. Nutritional regulation of IGF-I expression during brain development in mice. Pediatr Res 2001;49(2):197-202.
316. Chowen JA, Goya L, Ramos S, Busiguina S, Garcia-Segura LM, Argente J, et al. Effects of early undernutrition on the brain insulin-like growth factor-I system. J Neuroendocrinol 2002;14(2):163-9.
317. Ye P, Lee KH, D'Ercole AJ. Insulin-like growth factor-I (IGF-I) protects myelination from undernutritional insult: studies of transgenic mice overexpressing IGF-I in brain. J Neurosci Res 2000;62(5):700-8.
318. Hayakawa M, Okumura A, Hayakawa F, Kato Y, Ohshiro M, Tauchi N, et al. Nutritional state and growth and functional maturation of the brain in extremely low birth weight infants. Pediatrics 2003;111(5 Pt 1):991-5.
319. Lofqvist C, Chen J, Connor KM, Smith AC, Aderman CM, Liu N, et al. IGFBP3 suppresses retinopathy through suppression of oxygen-induced vessel loss and promotion of vascular regrowth. Proc Natl Acad Sci U S A 2007;104(25):10589-94.
320. Lopez-Lopez C, LeRoith D, Torres-Aleman I. Insulin-like growth factor I is required for vessel remodeling in the adult brain. Proc Natl Acad Sci U S A 2004;101(26):9833-8.

321. Hellstrom A, Engstrom E, Hard AL, Albertsson-Wikland K, Carlsson B, Niklasson A, et al. Postnatal serum insulin-like growth factor I deficiency is associated with retinopathy of prematurity and other complications of premature birth. Pediatrics 2003;112(5):1016-20.

322. Lofqvist C, Engstrom E, Sigurdsson J, Hard AL, Niklasson A, Ewald U, et al. Postnatal head growth deficit among premature infants parallels retinopathy of prematurity and insulin-like growth factor-1 deficit. Pediatrics 2006;117(6):1930-8.

323. Clemmons DR, Sleevi M, Allan G, Sommer A. Effects of combined recombinant insulin-like growth factor (IGF)-I and IGF binding protein-3 in type 2 diabetic patients on glycemic control and distribution of IGF-I and IGF-II among serum binding protein complexes. J Clin Endocrinol Metab 2007;92(7):2652-8.

324. Camacho-Hubner C, Rose S, Preece MA, Sleevi M, Storr HL, Miraki-Moud F, et al. Pharmacokinetic studies of recombinant human insulin-like growth factor I (rhIGF-I)/rhIGF-binding protein-3 complex administered to patients with growth hormone insensitivity syndrome. J Clin Endocrinol Metab 2006;91(4):1246-53.

325. Chernausek SD, Backeljauw PF, Frane J, Kuntze J, Underwood LE. Long-term treatment with recombinant insulin-like growth factor (IGF)-I in children with severe IGF-I deficiency due to growth hormone insensitivity. J Clin Endocrinol Metab 2007;92(3):902-10.

326. Rosenbloom AL. Recombinant human insulin-like growth factor I (rhIGF-I) and rhIGF-I/rhIGF-binding-protein-3: new growth treatment options? J Pediatr 2007;150(1):7-11.

327. Kimble RM, Breier BH, Gluckman PD, Harding JE. Enteral IGF-I enhances fetal growth and gastrointestinal development in oesophageal ligated fetal sheep. J Endocrinol 1999;162(2):227-35.

328. Yu H, Mistry J, Nicar MJ, Khosravi MJ, Diamandis A, van Doorn J, et al. Insulin-like growth factors (IGF-I, free IGF-I and IGF-II) and insulin-like growth factor binding proteins (IGFBP-2, IGFBP-3, IGFBP-6, and ALS) in blood circulation. J Clin Lab Anal 1999;13(4):166-72.

329. Altman DG. Practical statistics for medical research. London: Chapman and Hall; 1991.

330. Koistinen H, Seppala M, Koistinen R. Different forms of insulin-like growth factor-binding protein-3 detected in serum and seminal plasma by immunofluorometric assay with monoclonal antibodies. Clin Chem 1994;40(4):531-6.

331. Rutanen EM, Karkkainen TH, Lehtovirta J, Uotila JT, Hinkula MK, Hartikainen AL. Evaluation of a rapid strip test for insulin-like growth factor binding protein-1 in the diagnosis of ruptured fetal membranes. Clin Chim Acta 1996;253(1-2):91-101.

332. Blum WF, Breier BH. Radioimmunoassays for IGFs and IGFBPs. Growth Regul 1994;4 Suppl 1:11-9.

333. Frisone MF, Mercuri E, Laroche S, Foglia C, Maalouf EF, Haataja L, et al. Prognostic value of the neurologic optimality score at 9 and 18 months in preterm infants born before 31 weeks' gestation. J Pediatr 2002;140(1):57-60.

334. Haataja L, Mercuri E, Regev R, Cowan F, Rutherford M, Dubowitz V, et al. Optimality score for the neurologic examination of the infant at 12 and 18 months of age. J Pediatr 1999;135(2 Pt 1):153-61.

335. Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. Surveillance of Cerebral Palsy in Europe (SCPE). Dev Med Child Neurol 2000;42(12):816-24.

336. Voss W, Neubauer AP, Wachtendorf M, Verhey JF, Kattner E. Neurodevelopmental outcome in extremely low birth weight infants: what is the minimum age for reliable developmental prognosis? Acta Paediatr 2007;96(3):342-7.

337. Bayley N. Bayley scales of Infant Development. Second edition.: The Psychological corporation USA; 1993.

338. Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996;85(7):843-8.

339. An international classification of retinopathy of prematurity. The Committee for the Classification of Retinopathy of Prematurity. Arch Ophthalmol 1984;102(8):1130-4.
340. Raghupathy R, Makhseed M, El-Shazly S, Azizieh F, Farhat R, Ashkanani L. Cytokine patterns in maternal blood after premature rupture of membranes. Obstet Gynecol 2001;98(1):122-6.
341. Yoneyama Y, Suzuki S, Sawa R, Otsubo Y, Miura A, Kuwabara Y, et al. Changes in the proportion of T helper 1 and T helper 2 cells in cord blood after premature rupture of membranes. Arch Gynecol Obstet 2003;267(4):217-20.
342. Kashlan F, Smulian J, Shen-Schwarz S, Anwar M, Hiatt M, Hegyi T. Umbilical vein interleukin 6 and tumor necrosis factor alpha plasma concentrations in the very preterm infant. Pediatr Infect Dis J 2000;19(3):238-43.
343. Lagercrantz H, Bistoletti P. Catecholamine release in the newborn infant at birth. Pediatr Res 1977;11(8):889-93.
344. Irestedt L, Dahlin I, Hertzberg T, Sollevi A, Lagercrantz H. Adenosine concentration in umbilical cord blood of newborn infants after vaginal delivery and cesarean section. Pediatr Res 1989;26(2):106-8.
345. Yanowitz TD, Baker RW, Roberts JM, Brozanski BS. Low blood pressure among very-low-birth-weight infants with fetal vessel inflammation. J Perinatol 2004;24(5):299-304.
346. Tauscher MK, Berg D, Brockmann M, Seidenspinner S, Speer CP, Groneck P. Association of histologic chorioamnionitis, increased levels of cord blood cytokines, and intracerebral hemorrhage in preterm neonates. Biol Neonate 2003;83(3):166-70.
347. Kluckow M, Evans N. Low superior vena cava flow and intraventricular haemorrhage in preterm infants. Arch Dis Child Fetal Neonatal Ed 2000;82(3):F188-94.
348. Meek JH, Tyszczuk L, Elwell CE, Wyatt JS. Low cerebral blood flow is a risk factor for severe intraventricular haemorrhage. Arch Dis Child Fetal Neonatal Ed 1999;81(1):F15-8.
349. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. Nat Rev Immunol 2007;7(10):803-15.
350. Gendron FP, Chalimoniuk M, Strosznajder J, Shen S, Gonzalez FA, Weisman GA, et al. P2X7 nucleotide receptor activation enhances IFN gamma-induced type II nitric oxide synthase activity in BV-2 microglial cells. J Neurochem 2003;87(2):344-52.
351. Lai FF, Tsou KY. Transient periventricular echodensities and developmental outcome in preterm infants. Pediatr Neurol 1999;21(5):797-801.
352. Ringelberg J, van de Bor M. Outcome of transient periventricular echodensities in preterm infants. Neuropediatrics 1993;24(5):269-73.
353. Wood NS, Costeloe K, Gibson AT, Hennessy EM, Marlow N, Wilkinson AR. The EPICure study: associations and antecedents of neurological and developmental disability at 30 months of age following extremely preterm birth. Arch Dis Child Fetal Neonatal Ed 2005;90(2):F134-40.
354. Hack M, Taylor HG, Drotar D, Schluchter M, Cartar L, Wilson-Costello D, et al. Poor predictive validity of the Bayley Scales of Infant Development for cognitive function of extremely low birth weight children at school age. Pediatrics 2005;116(2):333-41.
355. Harris SR, Megens AM, Backman CL, Hayes VE. Stability of the Bayley II Scales of Infant Development in a sample of low-risk and high-risk infants. Dev Med Child Neurol 2005;47(12):820-3.
356. Grether JK, Nelson KB, Dambrosia JM, Phillips TM. Interferons and cerebral palsy. J Pediatr 1999;134(3):324-32.

357. Ahmad I, Zaldivar F, Iwanaga K, Koeppel R, Grochow D, Nemet D, et al. Inflammatory and growth mediators in growing preterm infants. J Pediatr Endocrinol Metab 2007;20(3):387-96.
358. Lo HC, Tsao LY, Hsu WY, Chen HN, Yu WK, Chi CY. Relation of cord serum levels of growth hormone, insulin-like growth factors, insulin-like growth factor binding proteins, leptin, and interleukin-6 with birth weight, birth length, and head circumference in term and preterm neonates. Nutrition 2002;18(7-8):604-8.
359. Lieberman SA, Bukar J, Chen SA, Celniker AC, Compton PG, Cook J, et al. Effects of recombinant human insulin-like growth factor-I (rhIGF-I) on total and free IGF-I concentrations, IGF-binding proteins, and glycemic response in humans. J Clin Endocrinol Metab 1992;75(1):30-6.
360. Mizuno N, Kato Y, Iwamoto M, Urae A, Amamoto T, Niwa T, et al. Kinetic analysis of the disposition of insulin-like growth factor 1 in healthy volunteers. Pharm Res 2001;18(8):1203-9.
361. Oesterreicher S, Blum WF, Schmidt B, Braulke T, Kubler B. Interaction of insulin-like growth factor II (IGF-II) with multiple plasma proteins: high affinity binding of plasminogen to IGF-II and IGF-binding protein-3. J Biol Chem 2005;280(11):9994-10000.
362. Storch S, Kubler B, Honing S, Ackmann M, Zapf J, Blum W, et al. Transferrin binds insulin-like growth factors and affects binding properties of insulin-like growth factor binding protein-3. FEBS Lett 2001;509(3):395-8.
363. Weinzimer SA, Gibson TB, Collett-Solberg PF, Khare A, Liu B, Cohen P. Transferrin is an insulin-like growth factor-binding protein-3 binding protein. J Clin Endocrinol Metab 2001;86(4):1806-13.
364. Muylle L, Joos M, Wouters E, De Bock R, Peetermans ME. Increased tumor necrosis factor alpha (TNF alpha), interleukin 1, and interleukin 6 (IL-6) levels in the plasma of stored platelet concentrates: relationship between TNF alpha and IL-6 levels and febrile transfusion reactions. Transfusion 1993;33(3):195-9.
365. Vohr BR. Progress in predicting outcomes for extremely low birth weight infants: baby steps. Acta Paediatr 2007;96(3):331-2.