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Cytokine profiles of seventeen cytokines, growth factors and chemokines in cord blood and its relation to perinatal clinical findings

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ABSTRACT

Few papers have investigated the cytokine profiles of multiple cytokines in cord blood. We obtained cord blood samples from 224 infants admitted to our neonatal intensive care unit. Cytokine profiles of 17 cytokines were investigated using cytometric bead array technology. We found a wide variety of cytokines of various levels which ranged from 0.59 pg/ml (in Interleukin (IL)-4) to 222.0 pg/ml (in macrophage inflammatory protein-1β. Pro-inflammatory cytokines were highly correlated with each other and with granulocyte-colony stimulating factor and IL-8. On the contrary, IL-5, IL-13, and IL-17 did not show any significant correlation with other cytokines. Several maternal factors were strongly related to several cytokines in cord blood. IL-6, IL-8 and monocyte chemotactic protein-1 were closely related to certain neonatal diseases in preterm neonates. Some cytokines may be regulated independently of each other, while others appear to work as a network affecting physiological and pathological conditions in the fetus.

1. Introduction

The fetus and newborn are required to face a complex set of immunological demands, including protection against infection, avoidance of harmful inflammatory immune responses leading to preterm delivery, and a safe transition from a sterile intrauterine environment to a hostile outside world rich in foreign antigens [1]. It was initially thought that the neonatal immune response was impaired or depressed because it lacked T helper (Th) 1 cytokines [1–3]. Production of Interleukin (IL)-6 and IL-8 from neonatal mononuclear cells, however, is higher than adult mononuclear cells [4,5], and evidence of the clinical significance of cytokines in perinatal medicine has been accumulating.

Infection-induced production of pro-inflammatory cytokines, including tumor-necrosis factor (TNF) and IL-1β, are associated with premature labor and preterm delivery [1,6]. IL-6 and IL-8 in cord blood are related to chorioamnionitis (CAM) [7,8]. Pro-inflammatory cytokines in cord blood are also related to the preterm brain white matter damage [9–13] and chronic lung disease in preterm infants [14]. Cytokine production is not only related to pathologic risk but also is affected by the delivery mode itself [15].

An important aspect of cytokine biology is that cytokines work as a network and highly overlap in source, target cell, and function. This fact makes it difficult to identify which individual cytokines are "more important" in specific settings. There have been only a few papers demonstrating cytokine profiles, including many kinds of cytokines in cord blood [12,14–16]. Furthermore, no study has investigated the relationships between individual cytokines in cord blood. It is also important to know the serum level of each cytokine in cord blood.

In the present study, we investigated a profile composed of 17 cytokines, including pro-inflammatory cytokines, Th cytokines, growth factors and chemokines in 224 cord blood samples. We analyzed not only the relationship between each cytokine level but also the relationship between cytokine levels and any perinatal or neonatal findings. We hope to determine serum levels of cytokines and their clinical role in the perinatal period.

2. Materials and methods

2.1. Subjects

Two hundred and sixty-one infants were consecutively admitted to the Neonatal Intensive Care Unit (NICU) in Jichi Medical University (JMU) Hospital (JMUH) between September 2005 and February 2006 and between July 2007 and September 2007. Twenty cases with serious congenital anomalies were excluded from the study. Parents of those infants were informed of the study design. Written informed consent was obtained from parents of 224 infants. Cord blood samples from those infants were included in the study.

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2.2. Prenatal data

Antenatal data were obtained from maternal hospital records. The data included preexisting maternal medical conditions, delivery mode, singleton or twin delivery, and existence of maternal and prenatal complications. Threatened early labor was presumed if maternal administration of tocolytic agents was recorded just before delivery. Non-reassuring fetal status (NRFS) was defined by fetal heart rate monitoring. Pregnancy-induced hypertension (PIH) was defined as blood pressure >140/90 mm Hg and associated clinical conditions. Fetal growth restriction was defined as fetal body weight below 10 percentile of fetal growth curve. Clinical CAM was diagnosed by presence of maternal fever, maternal leukocytosis, elevation of maternal serum C-reactive protein (CRP), uterine tenderness, and/or foul-smelling cloudy amniotic fluid. Histologic CAM was defined as the presence of polymorphonuclear leukocytes in the tissues of fixed placenta. Premature rupture of membrane (PROM) was determined as more than 24 h from a rupture of membranes to the delivery. Placental abruption and bleeding from placenta previa was defined when these findings were the primary reason for emergency caesarean section for the delivery.

2.3. Neonatal clinical data

Neonatal data were obtained from the infants' hospital records. Birth weight, gestational age, sex, delivery mode, and Apgar score at 1 and 5 min after birth were obtained from the birth records. Respiratory distress syndrome (RDS) was diagnosed clinically. Chronic lung disease (CLD) was determined as those infants requiring oxygen supplementation at corrected 36 weeks of gestation. Patent ductus arteriosus (PDA) was determined as those infants requiring indomethacin or ligation to close the PDA. Periventricular leukomalacia (PVL) was diagnosed by ultrasound or magnetic resonance imaging of the brain. The patients with intraventricular hemorrhage (IVH) were counted as infants with grade 3 or 4 of Papile's categorization of IVH. Retinopathy of prematurity (ROP) was defined as those infants requiring photocoagulation.

2.4. Cord blood samples and laboratory data

Heparinized venous cord blood samples were obtained immediately after delivery. White blood cell count (WBC), granulocyte count, platelet count and serum level of CRP in cord blood were measured at the Laboratory of JMUH.

2.5. Cytokine assay

Cytokine profiles were investigated using separated serum samples. Serum was separated by centrifugation and stored in aliquots at −80 °C until analysis. Serum cytokine level was investigated using cytometric bead array technology which combines the principles of the sandwich-based immunoassay with flow cytometry [17] using the BioPlex protein array (Bio-Rad, Hercules, CA, USA) and Luminex 100 (Mirai Bio, Alameda, CA, USA) systems. The products were used according to the manufacturer specifications. Briefly, antibodies against specific cytokines covalently coupled to color-coded polystyrene beads, a biotinylated detection antibody specific to a different epitope on the cytokine, and streptavidin–phycoerythrin were used. The constituents of each well are drawn up into the flow-based BioPlex suspension array system, which identifies and quantifies each specific reaction based on bead color fluorescence. Unknown cytokine concentrations are automatically calculated by BioPlex Manager™ software using a standard curve derived from a recombinant cytokine standard. A cytokine panel (BioPlex™ Human Cytokine17-Plex Panel, Bio-Rad, San Diego, CA) detecting cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), IL-13, IL-17, Interferon (IFN) γ and TNFα), growth factors (IL-7, granulocyte-colony stimulating factor (G-CSF), granulocyte–macrophage-colony stimulating factor (GM-CSF)), and chemokines (IL-8, monocyte chemotactic protein (MCP)-1 and macrophage inflammatory protein (MIP)-1β) were used. The lower limits of detection for cytokines were different for each cytokine and each experiment. The lower limits ranged from 0.01 to 1.0 pg/mL.

2.6. Statistical analysis

Regarding cytokine levels, data below the lower measurable limits were excluded from the statistical analysis. The rest of the cytokine data underwent logarithmic transformation, because those distributions were not parametric. In order to assess the relationship between each cytokine, correlation coefficients were observed. In order to assess the relationship between cytokine level and prenatal factors, we investigated the difference of cytokine levels between groups with or without each prenatal factor. Those differences were tested using the Non-paired Student's *t*-test. In order to assess the relationship between cytokine levels and Apgar scores and neonatal laboratory data, correlation coefficients were determined. Correlation coefficient was also assessed between cytokine levels and gestational ages. Logistic regression analysis was performed to assess risks of prenatal factors including gestational age for increasing level of cytokines, if cytokine levels were related with gestational ages. In order to assess the relationship between each cytokine level and prevalence of neonatal complications, cytokine levels were divided into four categories according to the mean and standard deviation of each cytokine. The relationship between the categorized cytokine levels and prevalence of neonatal complications in preterm neonates were analyzed using the χ-square test. All *p*-values were two-tailed. The level of significance was set at $p < 0.05$.

2.7. Ethical approval

The study was approved by the ethics committee of JMU. Parents of those infants were informed of the study design. Written informed consent was obtained.

3. Results

3.1. Clinical features

Table 1 shows clinical and demographic features of the 224 mothers and neonatal infants. The subjects consisted of 66 term infants and 158 preterm infants. The percentage of cesarean sections was high at 79.0%. Since NICU of JMUH is a regional tertiary unit the incidence of preterm neonates and caesarean delivery mode was high. Of the 224 infants, 80 (35.7%) were infants of twins. Fifty infants (22.3%) showed NRFS and 42 infants (18.8%) showed fetal growth restriction. The percentage of mothers with PIH, PROM, histologic CAM, and clinical CAM were 21.0%, 17.0%, 10.2%, and 11.2%, respectively. The number of infants showing RDS, CLD, PDA, PVL, IVH ROP out of the 224 infants were 36 (16.1%), 20 (8.9%), 30 (13.4%), 1 (0.4%), 5 (2.2%), 6 (2.7%), respectively. Among the 224 infants, 10 died.

3.2. Distribution of each cytokine level in cord blood

Table 2 shows the serum level of each cytokine in the cord blood of the subjects. Since only nine neonates showed a measurable range of IL-2, we have excluded IL-2 from further investigation in the study. Fig. 1 shows the logarithmic distribution of serum levels for each cytokine. Among pro-inflammatory cytokines, IL-6 showed a relatively high serum level and 12 neonates showed more than 100.0 pg/ml of IL-6.