Cytokine 73 (2015) 101–107

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Constitutively high-level expression of TGFβ isoforms in cord blood and its relationship to perinatal findings

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ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form 17 January 2015
Accepted 23 January 2015
Available online 2 March 2015

Keywords:
TGFβ
Isoforms
Cord blood
Newborn infants
Cytokine profile

ABSTRACT

Background: The clinical significance of TGFβ isoforms in cord blood is not well understood.
Methods: We obtained cord blood samples from 37 term infants and 85 preterm infants who were born in several clinical settings. The serum levels of 3 TGFβ isoforms and of the other 17 cytokines in cord blood were investigated using cytometric bead array technology.
Results: Very high levels of TGFβ1 and TGFβ2 isoforms compared to the level of other cytokines were found; mean levels were 44,180 and 1871 pg/mL, respectively. The levels of all 3 isoforms of TGFβ were significantly correlated with birth weight, and the levels of TGFβ1 and TGFβ3 were correlated with gestational age. The levels of TGFβ1 and β2 isoforms were strongly correlated with each other, but not with levels of other cytokines. The levels of TGFβ1 and TGFβ2 were significantly higher in male infants and significantly lower in infants with fetal growth restriction. The prevalence of chronic lung disease was related to a low level of TGFβ1, and that of patent ductus arteriosus was related to a high level of TGFβ1 in preterm infants.
Conclusions: TGFβ1 and TGFβ2 appeared to play a significant role in physiological and pathological conditions in the fetus. TGFβ isoform levels appear to be regulated independently of those of other cytokines and do not appear to be influenced by inflammation in the fetal period. The role of TGFβ3 in cord blood and the postnatal chronological changes of the TGFβ isoforms should be investigated in the future.

1. Introduction

The transforming growth factor (TGF) β system, a superfamily of cytokines, is highly conserved in animal species. TGFβ is one of the few classes of proteins that can inhibit cell growth by halting mitosis at the G1 stage. TGFβ has a great variety of actions in angiogenesis, wound healing, growth, development and immunity [1]. Immunologically, TGFβ is a very potent cytokine and can function in both immune suppression and immune stimulation.

There are three isoforms of secreted TGFβ cytokines. TGFβ1 is the predominant TGFβ isoform that is produced by immune cells, whereas TGFβ2 is most abundant in breast milk [1–3]. TGFβ1 plays a critical role in controlling the immune system. TGFβ2 plays a role in embryonic development and in the suppression of tumor growth. TGFβ3 regulates cellular adhesion molecules and extracellular matrix formation, as well as lung and platelet development. TGFβ3 controls wound healing by regulating epidermal and dermal cell movement [1].

Abnormal regulation of TGFβ family members in the first trimester placenta is implicated in the pathophysiology of maternal complications [4,5]. However, TGFβ1 levels of cord blood were not consistent and very different in the past reports [6–9]. To our knowledge, there has been no previous report of identification of a relationship between the serum level of TGFβ in cord blood and the prevalence of neonatal complications. Furthermore, no reports have examined the levels of TGFβ2 and/or TGFβ3 isoforms in cord blood.

Abbreviations: NICU, Neonatal Intensive Care Unit; JMU, Jichi Medical University; JMUH, Jichi Medical University Hospital; PIH, pregnancy-induced hypertension; CAM, clinical chorioamnionitis; CRP, C-reactive protein; PROM, premature rupture of membrane; RDS, respiratory distress syndrome; CLD, chronic lung disease; PDA, patent ductus arteriosus; IVH, intraventricular hemorrhage; ROP, retinopathy of prematurity.

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http://dx.doi.org/10.1016/j.cyto.2015.01.024
1043-4666/

We previously reported cytokine profiles in cord blood, including 17 kinds of cytokines other than TGFβ, in several clinical settings including in preterm and full-term neonates [10]. In the present study, we investigated the serum concentration of three isoforms of TGFβ by using the same 122 cord blood samples among them. We also analyzed, not only the relationship between TGFβ and the other cytokine levels, but also the relationship between TGFβ levels and any perinatal or neonatal findings. We found that TGFβ1 and TGFβ2 in cord blood have a particular significance in perinatal clinical settings.

2. Materials and methods

2.1. Subjects

The subjects were newborn infants that were 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. Any infants with serious congenital anomalies were excluded from the study. Parents of infants were informed of the study design. Informed consent was obtained from the parents of 122 infants and cord blood samples from the 122 infants were included in the study.

2.2. Prenatal data

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

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. 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

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

2.5. Cytokine assays

Cytokine profiles were investigated using separated serum samples. Sera were separated by centrifugation and stored as aliquots at −80 °C until analysis. Serum cytokine levels were investigated using cytometric bead array technology, which combines the principles of the sandwich-based immunoassay with flow cytometry [10] using the BioPlex protein array (Bio-Rad, Hercules, CA, USA) and Luminex 100 (Mirai Bio, Alameda, CA, USA) systems according to the manufacturers' specifications. Two kinds of cytokine panels (Milliplex™ TGFβ1,2,3 MILLIPLEX KIT, Millipore, Billerica, MA, and BioPlex™ Human Cytokine17-Plex Panel, Bio-Rad, San Diego, CA) were used to detect cytokines (TGFβ1, β2, β3, and 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)-1α and macrophage inflammatory protein (MIP)-1β). For the TGFβ assay, serum samples required pretreatment with addition of 1.0 N HCl. The lower limits of cytokine detection were different for each cytokine and each experiment. The lower limits ranged between 0.01 and 1.0 pg/ml.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 20.0 Windows (SPSS Inc, Chicago, IL). Data of cytokine levels that were below the lower measurable limits were excluded from the statistical analysis. The rest of the cytokine data was subjected to logarithmic transformation, because those distributions were not parametric. Correlation coefficients were determined in order to assess the relationship between each cytokine. In order to assess the relationship between cytokine level and prenatal factors, the difference in cytokine levels between groups with or without each prenatal factor was investigated. These differences were tested using the Mann–Whitney test. Pearson's correlation coefficients were determined in order to assess the relationship between TGFβ levels and Apgar scores and neonatal laboratory data. In order to assess the relationship between each TGFβ level and the prevalence of neonatal complications, TGFβ levels were divided into four categories according to the 25th, 50th, and 75th percentile of each TGFβ level. The relationship between the categorized cytokine levels and the prevalence of neonatal complications in preterm neonates was 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. The parents of these infants were informed of the study design and written informed consent was obtained.

3. Results

3.1. Clinical features

Table 1 shows the clinical and demographic features of the 122 mothers and neonatal infants. The subjects consisted of 37 term infants and 85 preterm infants. The percentage of cesarean sections was high at 78.7%. Since the NICU of JMUH is a regional tertiary unit the incidence of preterm neonates and caesarean delivery mode was high. Of the 112 infants, 38 (31.1%) were twins.