Placenta 69 (2018) 1–8

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

# Fetal-placental crosstalk occurs through fetal cytokine synthesis and placental clearance

Imran N. Mir[a,*], Lina F. Chalak[a], Jie Liao[a], Sarah Johnson-Welch[c], Larry S. Brown[d], Christopher Longoria[a], Rashmin C. Savani[a,b], Charles R. Rosenfeld[a]

[a] Division of Neonatal-Perinatal Medicine, University of Texas Southwestern Medical Center, USA
[b] The Center for Pulmonary & Vascular Biology, University of Texas Southwestern Medical Center, USA
[c] Department of Pediatrics, Department of Pathology, University of Texas Southwestern Medical Center, USA
[d] Parkland Health and Hospital Systems, USA

ARTICLE INFO

*Keywords:*
Parturition
IL-6
IL-8
IL-10
Chorioamnionitis
Maternal neutrophils

ABSTRACT

*Background:* Cytokines modulate fetal well-being and contribute to parturition. Their origin in fetal blood, whether maternal, placental or fetal, at the time of parturition remains unclear.
*Objective:* To determine fetal and placental contributions to circulating fetal cytokines by measuring umbilical arterial (UmA) and venous (UmV) concentration differences in uncomplicated term pregnancies in the absence and presence of labor.
*Methods:* Term uncomplicated pregnancies were assessed: *Group 1* were not in labor and delivered by elective cesarean section (n = 20); *Group 2* delivered vaginally following uncomplicated pregnancy and labor (n = 30). UmA and UmV blood was collected before delivery of the placenta to measure circulating cytokines. Placental tissue was collected for histology and to determine cytokine contents and localization.
*Results: Group 1* UmA and UmV IL-10 concentrations were similar (504 ± 15 and 468 ± 16 pg/ml, respectively; $P \geq 0.1$); other cytokines were below level of detection. During labor, IL-10 concentrations increased 15–34%, but placental contents decreased. *Group 2* UmA IL-6 and IL-8 concentrations increased ($P < 0.001$) to 16.7 ± 1.6 and 18.4 ± 4.3 pg/ml, respectively, but were less ($P < 0.001$) in UmV, 0.29 ± 0.2 and 0.74 ± 0.3 pg/ml, respectively, demonstrating placental clearances ≥97%. This was associated with > 6-fold increases in placental IL-6/IL-8 contents ($P < 0.001$) and chorioamniotic infiltration of activated maternal neutrophils. IL-6 and IL-10 were localized to villous syncytiotrophoblasts.
*Conclusions:* In uncomplicated term pregnancies fetal circulating IL-10 is likely of placental origin, whereas IL-6/IL-8 are derived from the fetus, increase during parturition, and circulating levels are modulated by non-saturable placental clearance, revealing a novel pathway for fetal-placental crosstalk and signaling.

## 1. Introduction

The mechanisms underlying the onset and progression of spontaneous labor in women are not fully understood. The transition from uterine quiescence to activation and parturition is believed to involve inflammatory processes [1]. This is supported by the observation that increased levels of pro-inflammatory cytokines are found in the amniotic fluid, maternal and fetal blood, and cervical-vaginal secretions of term and preterm laboring women [2–11]. There is substantial evidence that these cytokines contribute to several aspects of parturition [12–17]. For example, they activate the transcription factor NF-κB in myometrial cells, which in turn induces expression of genes known to promote the progression of labor and delivery, including oxytocin receptors, COX-2 and CX-43 [18–20]. In addition, placental expression and contents of the anti-inflammatory cytokine IL-10 decline before the onset of labor and remain low throughout parturition [21]. This withdrawal is believed to facilitate and enhance activation of inflammatory processes associated with labor. However, the sources, clearance and/or metabolism of these diverse cytokines are poorly understood and their contribution to the mechanisms that facilitate crosstalk between the fetus, placenta and mother remain unclear.

The fetal and/or placental contributions to the production and release of inflammatory cytokines is unclear, but may be related to alterations in the fetal endocrine milieu [2,3,5], distension of the fetal

* Corresponding author. The University of Texas Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, TX, 75390-9063, USA.
*E-mail address:* imran.mir@utsouthwestern.edu (I.N. Mir).

https://doi.org/10.1016/j.placenta.2018.07.006
Received 16 February 2018; Received in revised form 13 June 2018; Accepted 9 July 2018
0143-4004/ Published by Elsevier Ltd.

membranes and maternal cervix [5], alterations in placental perfusion and oxygenation [5,22], fetal hypoxia-acidemia [23], or exposure to infectious agents [3,5]. The overall effect of these stimuli is to increase the circulating levels of inflammatory cytokines within the fetal-placental compartment and thus the levels measured in umbilical cord blood. If the placenta is the primary source of these circulating cytokines, assuming fetal metabolism and/or clearance, or they are derived from the maternal circulation via transplacental transport, fetal umbilical venous (UmV) concentrations should exceed concentrations in the umbilical arterial blood (UmA). If, however, the fetus is the source of these cytokines and the placenta is the site of clearance or removal, as previously reported for angiotensin II and dehyroepiandrosterone [24,25], UmA concentrations will exceed levels in the UmV. To date, circulating fetal cytokine concentrations have predominantly been measured in mixed umbilical arterial-venous blood and rarely in the UmA, UmV or both. In the few instances where this has been explored, limited cytokines were examined and there was no mention of either placental metabolism or clearance [10,11]. Thus, the contributions of the fetus and/or placenta to the fetal levels of circulating cytokines and their site of their removal are unclear. Moreover, the effects of uncomplicated term parturition on the sites of cytokine synthesis and clearance are unknown. Thus, the purpose of this study was to determine: 1) the contribution of the fetus and/or placenta to the synthesis, metabolism and/or clearance of inflammatory and anti-inflammatory cytokines and 2) if this is different in the absence and presence of labor in uncomplicated term human pregnancies. Addressing these objectives would not only define the role of the placenta in the modulation of circulating fetal cytokines, but also improve our understanding of potential avenues for fetal-placental crosstalk.

## 2. Methods

### 2.1. Study design

We conducted a prospective cohort study that included 50 randomly chosen, uncomplicated, term pregnant women admitted to the 'Low Risk' Delivery Service at Parkland Hospital between June 2015 and April 2016. They were divided into two groups: *Group 1* included non-laboring women admitted for repeat elective cesarean delivery in the absence of any pregnancy complications and labor (n = 20); *Group 2* included laboring women who underwent spontaneous vaginal delivery (n = 30) in the absence of any pregnancy or intrapartum or neonatal complications. Inclusion criteria also included completion of at least 37wks of gestation, singleton pregnancy, no evidence of fetal anomalies or fetal growth restriction, and absence of fetal asphyxia or compromise.

### 2.2. Blood collection

Immediately after delivery of the neonate and before delivery of the placenta, i.e., with an intact placental circulation, a segment of intact umbilical cord was double clamped (~25 cm), resected, and blood withdrawn within 1–2min from the UmA and UmV with separate sterile 5 ml plastic syringes. Thus, the blood samples represent the intact fetal-placental circulation. Samples were allowed to clot and then centrifuged for 10 min at 10,000 rpm; serum samples were brought to the laboratory and stored at −80 °C until analyzed. UmA blood (0.5 ml) was also used to immediately measure fetal blood gases.

### 2.3. Serum cytokine analyses

At the initiation of the study, paired UmA/UmV blood samples from both Groups (20/group) were thawed and 50 μl of serum analyzed in duplicate for measurement of the proinflammatory cytokines IL-1β, IL-2, IL-6, IL-8, and TNFα using a multiplexed immunoassay kit (Bio-Rad Laboratories, Hercules, CA). The anti-inflammatory cytokine IL-10 was subsequently measured in a separate assay in duplicate using randomly selected paired UmA/UmV serum samples with sufficient volume from *Groups 1* (n = 10) and *2* (n = 10) by immunoassay (Bio-Rad Laboratories, Hercules, CA). All paired UmA/UmV samples were measured in the same assay. The lower limits for measurement of the selected cytokines in pg/ml are: IL-1β 0.14, IL-2 0.8, IL-6 0.7, IL-8 0.5, and TNFα 0.9. The coefficient of variation for all assays was < 15% for all the analysts.

### 2.4. Placental tissue collection and analyses

Placental tissue samples were obtained from 4 quadrants of each placenta, placed in sterile PBS, immediately brought to the laboratory and either frozen in liquid nitrogen and stored at −80 °C or fixed in 10% formalin. All placental tissues samples from *Groups 1* (n = 20) and *2* (n = 30) underwent histopathologic analyses by a perinatal pathologist, who was blinded to all patient data, after staining with hematoxylin-eosin. The Redline classification for major placental histology was used to assess the tissues [26]. After noting the presence of neutrophilic invasion in the chorioamniotic membrane in several placentas within *Group 2*, we randomly collected an additional 10 *Group 2* placentas for confirmation, resulting in n = 30. Because of the neutrophil infiltration, we randomly selected placental samples from 6 male neonates with histologic evidence of acute chorioamnionitis in order to determine if their origin was maternal or fetal. To do this, we used fluorescence *in situ* hybridization (FISH) for XY/XX chromosomes. The presence of neutrophils with XX chromosomes in the male fetuses would demonstrate they are maternal in origin. Samples analyzed had to contain > 5 leukocytes per high power field at 100× magnification and the reader was blinded as to the fetal gender. In addition, we sought to determine if the neutrophils seen in the histologic evaluation were active. In order to do this, randomly selected fixed tissues from *Group 1* without neutrophilic invasion (n = 3) and *Group 2* with neutrophilic invasion (n = 6) were analyzed for myeloperoxidase (MPO) by immunofluorescence using anti-MPO antibody diluted 1:50 (Cat. # ab9535, Abcam, Cambridge, MA). Two blinded, independent observers counted MPO- and DAPI-positive cells per high-power field to quantify absolute and percent neutrophil accumulation. Each observer counted five random fields within one slide section and used sections from different placental quadrants to ensure adequate sampling. In addition, all sections were observed under low-power magnification to ensure that all areas in a section were included. Good concordance was found between observers.

Increases in placental contents of IL-6 and IL-8 would support the concept of placental clearance of proinflammatory cytokines and localize the site of uptake, transfer or metabolism. Increased placental levels of IL-10 in the absence of clearance would suggest the placental is a site of synthesis. To address this, 1 mg placental samples from *Groups 1* and *2* (n = 20/Group) were thawed, homogenized by plastic pellet pestle (Sigma-Aldrich, St. Louis, MO) and lysed in 10 μL RIPA buffer (25 mM Tris-HCl, 0.1% sodium dodecyl sulfate, 1% TritonX-100, 1% sodium deoxycholate, 0.15 M NaCl, 1 mM EDTA; Sigma, St. Louis, MO). After centrifugation at 13.2 K rpm for 60 min at 4 °C, supernatants were assayed for the cytosolic contents of human IL-6, IL-8 and IL-10 by ELISA (R & D systems, Minneapolis, MN) according to the manufacturer's instructions. Results are presented as pg/ml.

In order to determine placental localization of IL-6 and IL-10, immunofluorescence studies were performed in placental slices with anti-IL-6 (1:50 dilution; Cat. # ab6672, Abcam, Cambridge, MA) and anti-IL-10 (1:100 dilution; Cat. # ab34843, Abcam, Cambridge, MA). Endogenous fluorescence was blocked with separate exposure to sodium borohydrate (1 g/10 ml PBS, 3min x 2) and 1 M glycine in PBS for 30min. Non-specific sites were blocked with 100% goat serum/0.02% azide incubated overnight at 4 °C. Sections were incubated with rabbit anti-human IL-6 and IL-10 overnight at 4 °C. After washing, the slides were incubated with goat anti-rabbit FITC-conjugated secondary