

CLINICAL RESEARCH ARTICLE



Placental clearance not synthesis tempers exaggerated pro-inflammatory cytokine response in neonates exposed to chorioamnionitis

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BACKGROUND: The source and clearance of cytokines in the fetal circulation in term pregnancies complicated by chorioamnionitis remains unclear as are the contributions of placental transport, synthesis, and clearance. The objectives of the study were to determine (1) fetal and/or placental contributions to synthesis and/or clearance of inflammatory and anti-inflammatory cytokines in term pregnancies complicated by chorioamnionitis and (2) whether this differs in pregnancies further complicated by fetal hypoxia.

METHODS: Prospective cohort study of pregnancies >37 weeks gestational age that included: Group 1, uncomplicated cesarean delivery without labor ($n = 20$); Group 2, uncomplicated vaginal delivery ($n = 30$); Group 3, pregnancies complicated by chorioamnionitis ($n = 10$); Group 4, complicated by chorioamnionitis + fetal hypoxia ($n = 10$). Umbilical arterial (UmA) and venous (UmV) blood were assayed for IL-1 β , IL-2, IL-6, IL-8, TNF α , and IL-10.

RESULTS: IL-6 and IL-8 were below assay detection in UmA and UmV blood in Group 1 and increased in Group 2 ($P < 0.01$), UmA»UmV ($P < 0.01$). Their concentrations increased further in Groups 3 and 4 ($P = 0.003$), UmA»UmV. Placental clearance was concentration dependent that approaches saturation in the presence of chorioamnionitis.

CONCLUSIONS: Marked increases in fetal synthesis of IL-6 and IL-8 occur in chorioamnionitis. Synthesis increase further when complicated by fetal hypoxia. Cytokine removal occurs via placental concentration-dependent mechanisms, potentially contributing to adverse fetal effects.

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IMPACT:

- The source and role of the placenta in synthesis and/or clearance of inflammatory mediators in term pregnancies complicated by clinical chorioamnionitis are unclear; however, conventional wisdom suggests the placenta is their source.
- This is the first study demonstrating that circulating concentrations of fetal IL-6 and IL-8 in clinical chorioamnionitis \pm birth asphyxia in term pregnancies are of fetal origin.
- Circulating fetal inflammatory cytokines are cleared by concentration-dependent placental mechanisms that are nearly saturated in chorioamnionitis \pm fetal hypoxia.
- These observations provide additional insight into understanding the fetal immune response in term pregnancies complicated by clinical chorioamnionitis.

INTRODUCTION

Clinical chorioamnionitis is the most common infection related diagnosis made in labor and delivery units worldwide and is traditionally diagnosed by the presence of maternal fever and at least two of the following criteria: maternal tachycardia, maternal leukocytosis, uterine tenderness, fetal tachycardia, and foul-smelling amniotic fluid.¹ One to 5% of neonates >35 weeks gestational age (GA) are born to women with a diagnosis of clinical chorioamnionitis.^{2,3} These neonates are at increased risk for early-onset sepsis within 72 h of birth,^{4–6} meconium aspiration

syndrome,^{4,7–9} neonatal asphyxia and encephalopathy,^{10–12} increased neonatal death,^{13–15} and long-term neurodevelopmental disabilities, including cognitive impairment^{16–19} and cerebral palsy.^{20–23} The mechanism(s) whereby clinical chorioamnionitis with or without fetal hypoxia leads to the development of neonatal morbidities, including brain injury, is not well understood.

Infection-induced fetal inflammation with the production of multiple cytokines/chemokines is thought to underlie many of the complications associated with exposure to inflammatory stresses

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in utero, including maternal chorioamnionitis.^{24,25} Fetal systemic inflammation in the form of markedly elevated pro-inflammatory cytokines and chemokines in umbilical cord blood in pregnancies complicated by clinical chorioamnionitis in near-term and term pregnancies has been reported.^{10,25} However, the source of these cytokines, i.e., whether fetal, placental, or maternal derived, is frequently unclear. If the primary source is either transplacental transport from the maternal circulation to the fetal circulation or direct placental synthesis and release into the fetal circulation, concentrations in the fetal umbilical venous (UmV) blood will exceed those in the umbilical arterial (UmA) blood. If, however, the fetus is the primary source of cytokines found in the fetal compartment and the placenta is the site of clearance, UmA concentrations will exceed levels in the UmV. These aspects of fetal-placental synthesis and clearance have been observed in intact maternal-fetal sheep following fetal infusions of dehydroepiandrosterone, the precursor for placental estrogen synthesis.²⁶ To date, circulating fetal cytokine concentrations have predominantly been measured in mixed umbilical arterial-venous blood^{10,25} and rarely in the UmA, UmV, or both.²⁷ Thus, the contributions of the fetus and/or placenta to cytokine concentrations in circulating fetal blood and their site of removal in clinical chorioamnionitis with or without fetal hypoxia are often unclear.

The purpose of this study was to determine: (1) the contributions of the fetus and/or placenta to the serum concentrations in the fetal circulation and gain a better understanding of the synthesis, metabolism and/or clearance of inflammatory and anti-inflammatory cytokines, and (2) whether this differs in term human pregnancies complicated by clinical chorioamnionitis in the absence and presence of fetal hypoxia. Addressing these objectives will better define the role of the placenta in the modulation of circulating fetal cytokines in term pregnancies complicated by clinical chorioamnionitis with or without fetal hypoxia.

METHODS

Study design

This prospective cohort study included 70 pregnant women at term gestation, who were admitted to the Labor and Delivery Services at Parkland Hospital, Dallas, TX between June 2016 and June 2017. We divided pregnancies into four groups: Group 1 included uncomplicated term births delivered by repeat cesarean-section in the absence of labor ($n=20$); Group 2 were term births by spontaneous vaginal delivery without complications ($n=30$); Group 3 included women with clinical chorioamnionitis as defined earlier ($n=10$); and Group 4 were women with clinical chorioamnionitis plus evidence of non-reassuring fetal heart pattern and meconium-stained amniotic fluid that suggest fetal hypoxia prior to birth ($n=10$). Women delivering at term gestation were selected if they were >37 completed weeks GA and had singleton pregnancies, no complications during pregnancy, including hypertension, pre-eclampsia, diabetes, and there were no evidence of fetal anomalies or growth restriction. Patients included in the Groups 1 and 2 were included in a previous report.²⁸

Blood collection and cytokine analysis

Immediately after delivery of the neonate and before delivery of the placenta, i.e., with an intact placental circulation, a 20–25 cm segment of umbilical cord was double clamped and resected, and blood was obtained from both the UmA and UmV with separate sterile 5-ml plastic syringes within 1–2 min of birth. Blood samples were centrifuged for 10 min at 10,000 rpm and serum samples were brought to the laboratory and stored at -80°C until analyzed. As per routine hospital policy, 0.5 ml of UmA was analyzed for blood gases.

At the time of assay, paired UmA/UmV serum samples were thawed and analyzed in duplicate for measurement of pro-inflammatory cytokines interleukin (IL)-1 β , IL-2, IL-6, IL-8, and tumor necrosis factor- α (TNF α) using a multiplex immunoassay kit (Bio-Rad Laboratories, Hercules, CA). The anti-inflammatory cytokine IL-10 was measured in a separate assay in duplicate using randomly selected paired UmA/UmV serum samples with sufficient

volume from Group 1 ($n=10$), Group 2 ($n=10$), Group 3 ($n=10$), and Group 4 ($n=10$). The lower limits for measurement of the selected cytokines in pg/ml are: IL-1 β 0.14, IL-2 0.8, IL-6 0.7, IL-8 0.5, and TNF α 0.9. The coefficient of variation for all assays was <15% for all the analyses.

Placental tissue collection and histopathological analysis

Fresh samples of placental tissue were obtained from four quadrants of placenta, placed in sterile phosphate-buffered saline, immediately brought to the laboratory, and either frozen in liquid nitrogen and stored at -80°C or fixed in 10% formalin. All placental tissue samples underwent histopathological analyses by two perinatal pathologists, who were blinded to all patient data. Increases in placental contents of pro-inflammatory cytokines in the presence of UmA>UmV concentrations would support the concept of placental clearance of pro-inflammatory cytokines and localize the site of uptake, transfer, or metabolism. Increased placental levels of IL-10 in the absence of clearance, reflected by the presence of UmA<UmV differences, suggest that the placenta is a site of synthesis. To address this, 30–40 mg of placental tissue collected from Groups 1 ($n=40$), 2 ($n=7$), 3 ($n=7$), and 4 ($n=7$) were thawed, homogenized by Ripa buffer (Sigma, R0278), 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 , we assayed supernatants for the cytosolic contents of human IL-6, IL-8, and IL-10 by enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN) according to the manufacturer's instructions. Results are presented as pg/mg of placental tissue.

Statistical analyses

Percent placental clearance was calculated using the following equation:

$$(\text{UmA concentration} - \text{UmV concentration}) / \text{UmA concentration} \times 100.$$

A descriptive analysis of the cytokines and other variables was conducted using frequency distributions and differences between groups were assessed by t test or Mann-Whitney test. The relationship or association between categorical variables and Group was determined by the χ^2 analysis, Fisher exact tests, or analysis of variance (ANOVA) for multiple groups with subsequent pair-wise comparison. When placental clearance occurred, the limits of placental clearance and saturation were assessed over the range of concentrations identified for IL-6 and IL-8 using linear regression analyses. Statistical analyses were performed using the statistical package SPSS version 19. Data are presented as means \pm standard error of the mean unless otherwise note.

Study approval

The study met the Health Insurance Portability Accountability Act requirements and was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center and Parkland Health and Hospital Systems. Patient consent was not required.

RESULTS

The patients studied are predominantly White Hispanic, mirroring the delivery population at Parkland Hospital. The maternal and neonatal characteristics in Groups 1 and 2, i.e., uncomplicated term cesarean and vaginal deliveries do not differ from each other (Table 1). They also do not differ from pregnancies complicated by the presence of maternal chorioamnionitis (Group 3) except for greater maternal age, gravidity, and parity. Notably, when compared to Group 3, Group 4 has a greater maternal age, parity and gravidity, and lower Apgar score at 1 min. Further, Group 3 women have lower age than all other groups.

Concentration differences of pro-inflammatory cytokines in UmA and UmV in clinical chorioamnionitis with or without fetal hypoxia

Serum concentrations of all pro-inflammatory cytokines measured in paired UmA and UmV samples in Group 1 are below the level of detection of the immunoassays. This demonstrates the absence of a fetal and placental inflammatory response in uncomplicated

Table 1. Comparison of maternal and neonatal characteristics in uncomplicated pregnancies delivered at term by cesarean delivery without labor and by vaginal delivery and pregnancies complicated by chorioamnionitis in the absence and presence of birth asphyxia.

	Term cesarean delivery (n = 20)	Term vaginal delivery (n = 30)	Clinical chorioamnionitis (n = 10)	Clinical chorioamnionitis + birth asphyxia (n = 10)	P value ^d
Maternal age (years)	29 ± 5 ^a	28 ± 4	20 ± 2	28 ± 5	<0.01
Gestational age (weeks)	39.3 ± 0.5	40 ± 1	39.7 ± 1	39.8 ± 1	0.34
Maternal gravidity	3 ± 1	2 ± 1	1 ± 0.3	3 ± 2	<0.01
Maternal parity	3 ± 1	2 ± 1	1 ± 0.3	2 ± 1	<0.01
Race/ethnicity					0.20
White Hispanic	18 (90) ^b	27 (90)	9 (90)	8 (89)	
Black	0	3 (10)	1 (11)	1 (10)	
White non- Hispanic	2 (1)	0	0	1 (10)	
Pregnancy complications					0.2
HTN/pre-eclampsia	0	0	1 (10)	2 (20)	
Diabetes	0	0	0	0	
Other complications	0	0	1 (10)	1 (10)	
Male gender	12 (60)	18 (60)	3 (30)	4 (40)	0.12
Birth weight (g)	3464 ± 407	3388 ± 371	3402 ± 281	3816 ± 608	0.06
Fetal growth pattern					
<10th centile	0	0	0	0	NS
10th–90th centile	15 (75)	23 (67)	10 (100)	4 (40)	NS
>90th centile	5 (25)	7 (23)	0	6 (60)	0.03
Apgar scores					
1 min	8 (8, 9) ^c	8 (8, 9)	8 (8, 9)	6 (3, 8)	0.01
5 min	9 (9, 9)	9 (9, 9)	9 (9, 9)	8 (8, 9)	0.1
Umbilical arterial blood gases analysis					
pH	7.23 ± 0.04	7.27 ± 0.1	7.27 ± 0.1	7.20 ± 0.2	0.3
P _a CO ₂ (mmHg)	60 ± 7	47.8 ± 8.8	48 ± 8	54 ± 19	0.07
P _a O ₂	<29	<29	<29	<29	1
Base deficit	−6 ± 2	−6.2 ± 3.4	−6 ± 2	−9 ± 8	0.06

^aData are presented as means ± SD.

^bNumbers in parenthesis are percent of total in each column.

^cMedian (25th and 75th centile) of each study population

^dValues across rows compared by ANOVA with subsequent pair-wise comparison.

term pregnancies in the absence of labor.²⁸ In contrast, UmA concentrations of IL-6 and IL-8 in Group 2 increased to 16.7 ± 1.6 and 18.4 ± 4.3 pg/ml, respectively ($P < 0.001$; $n = 20$). Simultaneous UmV concentrations were 0.29 ± 0.2 and 0.74 ± 0.3 pg/ml, respectively, and were significantly lower than UmA levels ($P < 0.001$), demonstrating placental clearance rates of 98 ± 0.6 and 97 ± 1.3%, respectively.

Serum concentrations of all pro-inflammatory cytokines measured in Group 3 ($n = 10$) increased further compared to Group 2 and are significantly higher in UmA vs. UmV (IL-6: 94.3 ± 42 vs. 45.9 ± 28, $P = 0.02$; IL-8: 320 ± 117 vs. 72.8 ± 22, $P = 0.003$; TNF α : 57.9 ± 6.8 vs. 35.5 ± 4.9, $P = 0.01$; IL-1 β : 7.8 ± 3.1 vs. 2.5 ± 1.1 pg/ml, respectively, $P = 0.05$), demonstrating the fetus as the source of each cytokine as well as evidence of placental clearance (Fig. 1a). Placental clearance of these cytokines is concentration dependent and decreases with increasing serum concentrations, e.g., IL-6 and IL-8 clearance rates decrease 45 and 77%, respectively, at the highest serum concentrations. Group 4 also exhibits higher concentrations of most of the pro-inflammatory cytokines in UmA vs. UmV (IL-6: 1445 ± 614 vs. 487 ± 212, $P = 0.001$; IL-8: 987 ± 242 vs. 255 ± 54, $P = 0.002$; TNF α : 26 ± 9.4 vs. 20 ± 5.8, $P = 0.12$; IL-1 β : 41.4 ± 5.9 vs. 10.7 ± 5.2 pg/ml, $P = 0.003$, respectively),

providing further evidence of fetal synthesis and placental clearance (Fig. 1b). Serum concentrations of IL-2 were below detection levels in all groups. In addition, serum concentrations of pro-inflammatory cytokines in paired UmA and UmV samples from Group 4 ($n = 10$) are significantly higher than Group 3 ($P < 0.05$), demonstrating an exaggerated fetal pro-inflammatory cytokine response in the presence of fetal hypoxia. The placental clearance of these cytokines is also saturated at elevated serum concentrations, resulting in clearance rates that decreased 66 and 74% for IL-6 and IL-8, respectively, at the highest serum concentrations (Table 2).

Comparison of serum concentrations of pro-inflammatory cytokines IL-6 and IL-8 in UmA blood in all study groups

In order to determine any differences in the fetal inflammatory responses elicited in the four study Groups, we compared UmA serum concentrations of all pro-inflammatory cytokines using ANOVA for multiple groups. Serum concentrations of all pro-inflammatory cytokines in Group 1 are below the level of detection for the immunoassay. In contrast, UmA concentrations of IL-6 and IL-8 in Group 2 increase to 16.7 ± 7.5 and 18.4 ± 20 pg/ml, respectively ($P < 0.01$). The UmA serum concentrations of IL-6

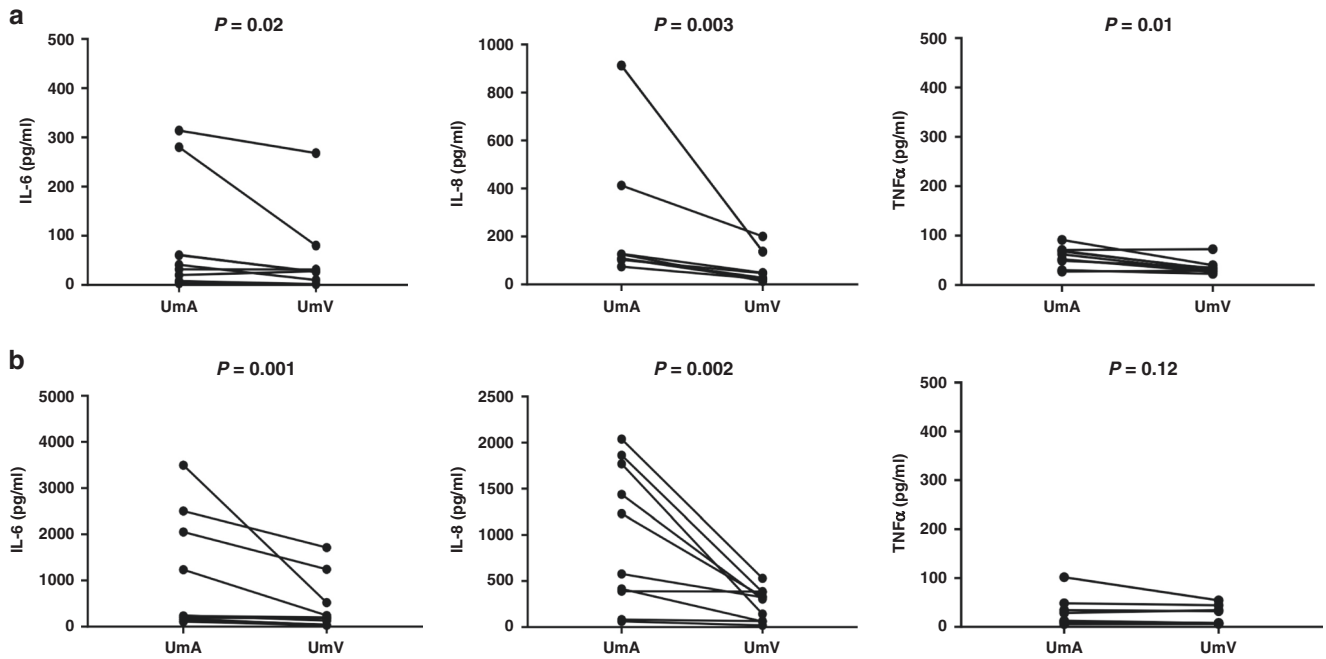


Fig. 1 Proinflammatory cytokines in UmA and UmV in clinical chorioamnionitis with or without fetal hypoxia. Comparison of concentration differences for IL-6, IL-8, and TNF α in paired umbilical arterial (UmA) and venous (UmV) blood samples from term pregnancies complicated by clinical chorioamnionitis (**a**, $n = 10$) and clinical chorioamnionitis with fetal hypoxia (**b**, $n = 10$). Data were analyzed using paired t test.

Table 2. Comparison of the relative placental clearance of IL-6 and IL-8 in uncomplicated pregnancies delivered at term by vaginal delivery (Group 2) and pregnancies complicated by chorioamnionitis in the absence (Group 3) and presence (Group 4) of fetal hypoxia.

	Group 2 ($n = 30$)	Group 3 ($n = 10$)	Group 4 ($n = 10$)
IL-6	98 \pm 0.6	45 \pm 12	66 \pm 12
IL-8	97 \pm 1.3	77 \pm 11	74 \pm 10.9

Data are presented as mean percent clearance \pm SD calculated from: $\text{UmA} - \text{UmV} \div \text{UmA} \times 100$.

and IL-8 increased further (Fig. 2) in Groups 3 and 4 (IL-6: 94.3 \pm 42 and 1445 \pm 614, $P = 0.02$; IL-8: 320 \pm 117 and 987.2 \pm 465.3, $P = 0.03$, respectively). Since serum concentrations of IL-6 and IL-8 are elevated during uncomplicated vaginal delivery at term, we examined the effect of route of delivery in Groups 3 and 4. The route of delivery has no significant effect on the serum concentrations of the cytokines measured in either Group with chorioamnionitis. It is notable that all pregnancies in Group 4 were in labor and delivered by cesarean section due to non-reassuring fetal heart rate pattern or non-progressing labor.

Concentration difference of anti-inflammatory cytokine IL-10 in UmA in all groups

Overall, serum concentrations of IL-10 are low in UmA and UmV in all the four study groups, and there are no significant UmA and UmV serum concentration differences: Group 1: 0.81 vs. 0.81, $P = 1.0$; Group 2: 0.81 vs. 0.99, $P = 0.7$; Group 3: 4.8 vs. 5.2, $P = 0.78$; and Group 4: 13.8 vs. 16.3 pg/ml, $P = 0.6$, respectively. Thus, there is no evidence of either placental synthesis or clearance of this anti-inflammatory cytokine. Notably, UmA concentrations of IL-10 are greater in term pregnancies complicated by clinical chorioamnionitis without and with fetal hypoxia vs. Groups 1 and 2 (Fig. 3).

Placental histopathology and tissue cytokine concentrations of IL-6, IL-8, and IL-10

There are no histopathologic lesions or evidence of neutrophilic infiltration in placentas obtained from Group 1 pregnancies ($n = 20$). In contrast, 53% (16/30) of Group 2 placentas have evidence of histologic acute chorioamnionitis, defined by neutrophilic infiltration of chorioamnionic membrane. All placental samples from Group 3 ($n = 10$) and Group 4 ($n = 10$) had evidence of histologic acute chorioamnionitis.

Tissue contents of IL-6 and IL-8 in placental homogenates from Group 2 are 8- and 15-fold greater than Group 1 (0.65 \pm 0.2 vs. 0.10 \pm 0.02 and 31.6 \pm 1.3 vs. 0.22 \pm 0.10 pg/mg, respectively, $P = 0.04$). Furthermore, placental contents of IL-6, IL-8, and IL-10 in Groups 1 and 2 are significantly less than that measured in placentas from Groups 3 and 4 (Table 3, $P < 0.01$). However, tissue contents of IL-10 are significantly greater in Group 3 vs. Group 4.

DISCUSSION

The goal of the present study was to examine the acute fetal inflammatory responses in term pregnancies complicated by clinical chorioamnionitis in the absence and presence of evidence of fetal hypoxia prior to delivery. We observed that the presence of maternal clinical chorioamnionitis at term is associated with a robust pro-inflammatory response in fetal umbilical artery. Moreover, serum concentrations of pro-inflammatory cytokines are even higher in pregnancies complicated by clinical chorioamnionitis and fetal hypoxia. Furthermore, the UmA concentrations of pro-inflammatory cytokines exceed UmV concentrations, demonstrating that they are of fetal origin. This concentration difference also provides additional evidence that the placenta is a site for the clearance of pro-inflammatory cytokines and this clearance mechanism is a concentration-dependent mechanism and nearly saturated in the presence of clinical chorioamnionitis without and with fetal hypoxia.²⁸ Importantly, the placental clearance of IL-6 and IL-8 is associated with increases in the placental tissue contents, thus demonstrating placental removal. Thus, the present

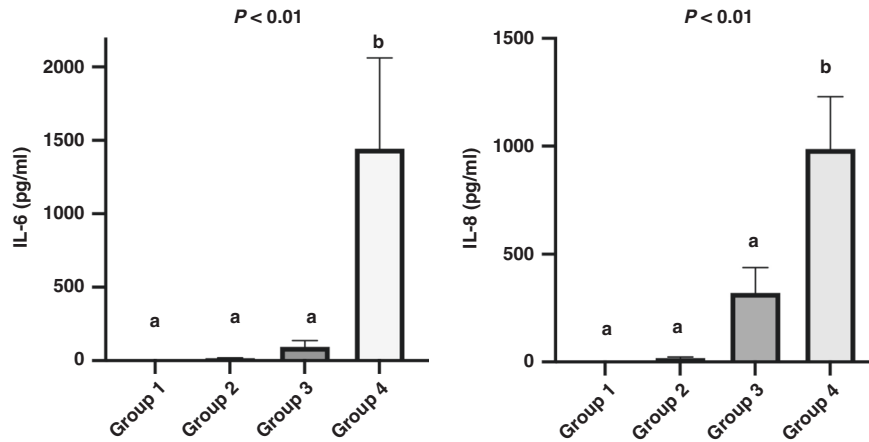


Fig. 2 Comparison of IL-6 and IL-8 concentrations in umbilical arterial (UmA) blood between pregnancies with uncomplicated term cesarean delivery in the absence of labor (Group 1, $n = 20$), uncomplicated term vaginal delivery (Group 2, $n = 30$), clinical chorioamnionitis (Group 3, $n = 10$), and clinical chorioamnionitis with evidence of fetal hypoxia (Group 4, $n = 10$). Data are means \pm SEM and analyzed by ANOVA for multiple groups; different letters (a, b) signify significant differences between groups, $P < 0.01$.

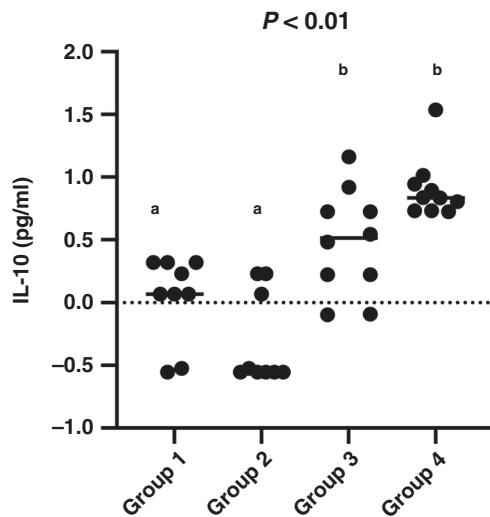


Fig. 3 Comparison of concentration differences for IL-10 in umbilical arterial blood in pregnancies with uncomplicated term delivery by cesarean section without labor (Group 1, $n = 10$), uncomplicated term vaginal delivery (Group 2, $n = 10$), clinical chorioamnionitis (Group 3, $n = 10$), and clinical chorioamnionitis with fetal hypoxia (Group 4, $n = 10$). Data are analyzed by ANOVA for multiple groups after transforming to logarithmic scale (\log_{10}); different letters (a, b) signify significant differences between groups, $P < 0.01$. Solid horizontal line represents the mean.

study extends our earlier reports of fetal synthesis of pro-inflammatory cytokines and their removal by the placenta in pregnancies complicated by chorioamnionitis rather than a maternal and/or placental source. In addition, we now show that this pathway of removal is concentration dependent.²⁸ Thus, the limitation to the removal of inflammatory cytokines from the fetal circulation may be contribute to the potential adverse effects of these cytokines.

Several investigators have reported an exaggerated pro-inflammatory response in the fetal blood of term pregnancies complicated by maternal clinical chorioamnionitis without or with evidence of fetal hypoxia.^{10,25,29–36} However, it has been unclear whether these cytokines are maternal, placental, or fetal in origin.³⁷ The conventional wisdom has been that clinical chorioamnionitis is associated with maternal systemic inflammation that results in the

transplacental transport of cytokines and chemokines to the fetus or that these maternal cytokines, neutrophils, or macrophages exert a direct effect in the placenta and modulate the placental inflammatory response and the release of cytokines into the fetal circulation.³⁸ Unfortunately, circulating fetal cytokine concentrations have predominantly been measured in samples of mixed umbilical arterial-venous blood^{10,25} and rarely in the UmA, UmV, or importantly, in both.²⁷ We previously reported that UmA concentrations of IL-6 and IL-8 were more than eightfold higher during uncomplicated vaginal delivery compared to term deliveries without labor and that the placenta was responsible for modulating the concentrations of these pro-inflammatory cytokines in fetal blood via placental clearance.²⁸ Recently, Zarate et al. exposed fetal sheep to intra-amniotic injections of lipopolysaccharide (LPS), a Toll-like receptor agonist, in order to study the fetal responses to a pro-inflammatory stimulus (an established animal model to study clinical chorioamnionitis).³⁹ They noted that the fetus mounted an early and robust pro-inflammatory innate immune response in the form of increased pro-inflammatory cytokines, with most activation occurring in the fetal liver and some in the fetal lung and skin within an hour after exposure to LPS.³⁹ Furthermore, they observed that LPS-induced hepatic nuclear factor- κ B activation contributed to this response. They also reported that hepatic macrophages isolated from the fetal sheep responded robustly to LPS exposure, providing evidence that this cell might contribute to the fetal innate immune response.³⁹ Notably, they did not observe a placental immune response,³⁹ which differs from other reports suggesting that the placenta contributes to the upregulation of pro-inflammatory genes and immune cell infiltration in clinical and experimental models of chorioamnionitis.^{40–44} Nonetheless, the source of the fetal immune response has remained unclear since others have not observed a hepatic innate immune response/activation following intra-amniotic injections of LPS or described hepatic inflammation and injury in fetuses exposed to an inflammatory challenge.^{43,44} However, these studies either focused on the hepatic response days or weeks after LPS exposure and after preterm delivery^{45,46} or did not address/observe changes in the transcriptional machinery response.⁴⁷ The present data provide evidence for the fetal origin of pro-inflammatory cytokines in the umbilical cord blood in human pregnancies complicated by clinical chorioamnionitis without and with fetal hypoxia and demonstrate that the placenta is a site of concentration-dependent clearance of these cytokines rather than maternal-fetal transport or placental synthesis.

In the present study, the clearance of pro-inflammatory cytokines in term pregnancies complicated by clinical

Table 3. Comparison of placental tissue contents (in pg/mg of tissue) of IL-6, IL-8, and IL-10 in uncomplicated pregnancies delivered at term by cesarean delivery without labor (Group 1) and by vaginal delivery (Group 2) and pregnancies complicated by chorioamnionitis in the absence (Group 3) and presence (Group 4) of fetal hypoxia.

Cytokines (pg/mg)	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 7)	Group 4 (n = 7)	P value*
IL-6	0.10 ± 0.02 ^a	0.65 ± 0.2 ^b	14.24 ± 4.3 ^c	6.54 ± 3.23 ^c	<0.01
IL-8	0.22 ± 0.10 ^a	31.6 ± 1.3 ^b	54.8 ± 27.8 ^c	13.4 ± 5.6 ^c	<0.01
IL-10	0.15 ± 0.01 ^a	0.12 ± 0.01 ^b	0.27 ± 0.05 ^c	0.08 ± 0.03 ^b	<0.01

Data are presented as means ± SEM.

*Values across rows compared by ANOVA with subsequent pair-wise comparison. Different superscript letters (a–d) signify significant differences between groups.

chorioamnionitis is paralleled by substantial increases in the placental tissue contents of IL-6 and IL-8. The mechanism(s) of placental clearance of these cytokines is unclear but could involve transplacental transport from the fetal to the maternal circulation or metabolism within placenta. Transfer of IL-6 from the maternal compartment to the fetus has been reported both in vivo in intact pregnant rats⁴⁸ and in vitro in the doubly perfused human placental cotyledon.⁴⁹ However, IL-6 is also equally transferred from the fetal circulation into the mother and the clearance index does not differ.⁴⁹ We observed that placental clearance is concentration dependent and higher UmA cytokine concentrations are paralleled by increases in placental concentrations, suggesting that these cytokines may be partially transported across placenta into the maternal circulation. Although there are other potential transport mechanisms that could be involved, including receptor-mediated and trans-trophoblastic channels, they remain speculative and in need of further study.⁴⁸ Nonetheless, the lack of complete clearance of pro-inflammatory cytokines by placenta as seen with chorioamnionitis appears to result in increased the fetal exposure and thus potential adverse risks to the neonate, including alterations in neurodevelopment.

The umbilical cord blood levels of the anti-inflammatory cytokine, IL-10, were low in all groups. However, the concentrations were relatively higher in term pregnancies complicated by clinical chorioamnionitis without or with evidence of fetal hypoxia. Notably, there were no arterio-venous or venous–arterial concentration differences for IL-10 in the groups studied. IL-10 is expressed in human placenta,⁵⁰ and it modulates the effects of pro-inflammatory cytokines in GA-dependent manner.⁵⁰ Although speculative, our results suggest that local placental IL-10 synthesis and release is increased and maintained during active labor and clinical chorioamnionitis in order to protect the fetus.

Strengths and limitations

Our study has several strengths, including a well-characterized patient cohort with 70 term pregnancies including randomly selected control pregnancies delivered by different routes and multiple maternal and neonatal variables for analyses. In addition, we collected, prepared, and processed all the blood samples in a manner that minimized alterations in the cytokine measurements, e.g., the serum samples were thawed only at the time of immunoassay and stored as several aliquots. Limitations of the study design include the relatively small sample size of clinical chorioamnionitis without and with evidence of fetal hypoxia. We also were unable to assess the state of inflammation and/or infection in the amniotic cavity. Furthermore, the state of maternal systemic inflammation and any transplacental transport either from or to the mother could not be assessed in the absence of samples representative of maternal uterine arterial or venous blood. Notably, this has been addressed in the chronic maternal–fetal sheep model for several ligands, including fetal angiotensin II and placental estrogens.⁵¹

Summary

Neonates born at term gestation to women with evidence of clinical chorioamnionitis demonstrate a robust pro-inflammatory response in fetal UmA blood. These pro-inflammatory cytokines are of fetal origin as evidence by the significant UmA–UmV concentration differences, and the placenta appears to serve as the primary site for their clearance via concentration-dependent mechanisms that are nearly saturated in pregnancies complicated by clinical chorioamnionitis without and with fetal hypoxia. In contrast, the fetal serum concentrations of the anti-inflammatory cytokine IL-10 are generally low but may be relatively elevated in order to protect the fetus against the observed exaggerated pro-inflammatory cytokine response associated with clinical chorioamnionitis. These observations provide additional insight into understanding the fetal immune response in term pregnancies complicated by clinical chorioamnionitis.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

I.N.M. made substantial contributions to conception and design, acquisition of data, analysis, and interpretation of data. He drafted and submitted the final version of the manuscript after approval from all co-authors. L.F.C. and C.R.R. made substantial contributions to conception and design, analysis, and interpretation of data; revised the article for important intellectual content; and approved the final version of the manuscript. N.U., J.L., L.S.B., R.C.S., and R.L. made substantial contributions to data analysis and interpretation of data. They revised the manuscript critically for important intellectual content and approved its final version to be published.

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Consent was not required for this study, as we had obtained waiver of consent from IRB.

ADDITIONAL INFORMATION

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