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Clinical chorioamnionitis at term V: umbilical cord plasma cytokine profile in the context of a systemic maternal inflammatory response

Abstract

Objective: Microbial invasion of the fetus due to intra-amniotic infection can lead to a systemic inflammatory response characterized by elevated concentrations of cytokines in the umbilical cord plasma/serum. Clinical chorioamnionitis represents the maternal syndrome often associated with intra-amniotic infection, although other causes of this syndrome have been recently described. The objective of this study was to characterize the umbilical cord plasma cytokine profile in neonates born to mothers with clinical chorioamnionitis at term, according to the presence or absence of bacteria and/or intra-amniotic inflammation.

Materials and methods: A cross-sectional study was conducted, including patients with clinical chorioamnionitis at term (n=38; cases) and those with spontaneous term labor without clinical chorioamnionitis (n=77; controls). Women with clinical chorioamnionitis were classified according to the results of amniotic fluid culture, broad-range polymerase chain reaction coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) and amniotic fluid interleukin (IL)-6 concentration into three groups: 1) no intra-amniotic inflammation; 2) intra-amniotic inflammation without detectable microorganisms; or 3) microbial-associated intra-amniotic inflammation. A

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fetal inflammatory response syndrome (FIRS) was defined as an umbilical cord plasma IL-6 concentration >11 pg/mL. The umbilical cord plasma concentrations of 29 cytokines were determined with sensitive and specific V-PLEX immunoassays. Nonparametric statistical methods were used for analysis, adjusting for a false discovery rate of 5%.

Results: 1) Neonates born to mothers with clinical chorioamnionitis at term (considered *in toto*) had significantly higher median umbilical cord plasma concentrations of IL-6, IL-12p70, IL-16, IL-13, IL-4, IL-10 and IL-8, but significantly lower interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF)-α concentrations than neonates born to mothers with spontaneous term labor without clinical chorioamnionitis; 2) neonates born to mothers with clinical chorioamnionitis at term but without intra-amniotic inflammation had higher concentrations of IL-6, IL-12p70, IL-13, IL-4, IL-5, and IL-8, but lower IFN-γ, than neonates not exposed to clinical chorioamnionitis, suggesting that maternal fever in the absence of intra-amniotic inflammation leads to a change in the fetal cytokine network; 3) there were significant, positive correlations between maternal and umbilical cord plasma IL-6 and IL-8 concentrations (IL-6: Spearman correlation=0.53; P<0.001; IL-8: Spearman correlation=0.42; P<0.001), consistent with placental transfer of cytokines; 4) an elevated fetal plasma IL-6 (>11 pg/mL), the diagnostic criterion for FIRS, was present in 21% of cases (8/38), and all these neonates were born to mothers with proven intra-amniotic infection; and 5) FIRS was associated with a high concentration of umbilical cord plasma IL-8, IL-10 and monocyte chemoattractant protein (MCP)-1.

Conclusions: Neonates born to mothers with clinical chorioamnionitis at term had higher concentrations of umbilical cord plasma cytokines than those born to mothers without clinical chorioamnionitis. Even neonates exposed to clinical chorioamnionitis but not to intra-amniotic inflammation had elevated concentrations of multiple cytokines, suggesting that intrapartum fever alters the fetal immune response.

Keywords: Biomarker; chemokine; fetal inflammatory response syndrome (FIRS); funisitis; interleukin-6; interleukin-8; intra-amniotic infection/inflammation; monocyte chemoattractant protein (MCP)-1; neonatal sepsis; umbilical cord plasma.

Introduction

Clinical chorioamnionitis is a syndrome characterized by a maternal systemic inflammatory response [1] and often

attributed to intra-amniotic infection [2–14]. Neonates born to mothers with clinical chorioamnionitis are at risk for sepsis [15–25], meconium aspiration syndrome [15, 26–30], neonatal encephalopathy [31–35], long-term neurodevelopmental disabilities including cognitive impairment [36–39] and cerebral palsy [22, 33, 40–46], as well as neonatal death [24, 47–50].

A growing body of evidence suggests that a maternal systemic inflammatory response can have powerful effects on the fetus [51–54]. For example, maternal systemic inflammation (sterile or induced by microorganisms) during critical windows of pregnancy may predispose to serious adverse infant outcomes [51–92] including autism spectrum disorders [52, 73, 74, 77, 79, 81–83, 85–89, 92–94] and schizophrenia [55, 56, 58, 60–64, 66, 67, 69–74, 76–78, 80, 85, 92]. Intrapartum fever is associated with an increased risk for cerebral palsy (odds ratio=9.3; 95% confidence interval: 2.7–31) [41]. Moreover, recent evidence suggests that maternal systemic inflammatory response can affect other target organs, such as the lung [95–103]. The emerging picture is that early exposure to maternal systemic inflammation may predispose to multiple organ injury, whose clinical manifestations may only occur in infancy or adulthood.

Clinical chorioamnionitis at term (a state of maternal systemic inflammation [1]), offers a unique opportunity to examine the relationship between maternal systemic inflammation (with or without intra-amniotic inflammation) and the fetal systemic immune response as reflected by the peripheral concentrations of cytokines in humans. The purpose of this study was to determine the cytokine profile in the fetal peripheral circulation after exposure to systemic maternal inflammation.

Materials and methods

Study population

A cross-sectional study was conducted including patients with clinical chorioamnionitis at term (n=38; cases) and those with spontaneous term labor without clinical chorioamnionitis (n=77; controls). Inclusion and exclusion criteria for the study population were reported previously [1, 3]. These patients have been included in previous communications focusing on clinical chorioamnionitis at term [2, 3]. The number of cases is slightly different among the studies. This is due to the availability of samples.

All patients provided written informed consent, and the use of biological specimens, as well as clinical and ultrasound data for research purposes, were approved by the Institutional Review Boards of NICHD, Wayne State University and the Sótero del Río Hospital, Santiago, Chile.

The clinical definitions, microbiological studies and the determination of cytokines/chemokines have been described previously [1–3, 13]. The fetal inflammatory response syndrome (FIRS) is defined as an umbilical cord blood IL-6 concentration >11 pg/mL [104–113].

Sample collection and cytokine immunoassays

Umbilical cord blood samples were collected immediately after delivery in both cases and controls, and then placed into tubes containing ethylenediaminetetraacetic acid (EDTA), centrifuged for 10 min at 4°C and stored at –70°C. Laboratory personnel were blinded to the clinical diagnosis. The umbilical cord plasma concentrations of the following 29 cytokines were determined with sensitive and specific V-PLEX immunoassays (Meso Scale Discovery, Gaithersburg, MD, USA): [Pro-inflammatory cytokines: interferon gamma (IFN-γ), interleukin (IL)-1α, IL-1β, IL-2, IL-6, IL-7, IL-12p70, IL-12/IL-23p40, IL-15, IL-16, IL-17α, tumor necrosis factor (TNF)-α, TNF-β, vascular endothelial growth factor (VEGF), granulocyte macrophage colony-stimulating factor (GM-CSF); anti-inflammatory cytokines: IL-4, IL-5, IL-10, IL-13; and chemokines: IL-8, thymus and activation-regulated chemokine (TARC), eotaxin, eotaxin-3, macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemoattractant protein (MCP)-1, MCP-4, C-X-C motif chemokine 10 (CXCL-10) or IFN-γ-induced protein 10 (IP-10)].

Briefly, 50 µL of each umbilical cord blood sample were dispensed into separate wells of the plates and incubated for 2 h with vigorous shaking at room temperature. The samples and calibrators were discarded, and the plates were washed three times with phosphate-buffered saline and 0.05% Tween-20 (Meso Scale Discovery), followed by an addition of 25 µL of the 1x Detection Antibody Solution (Meso Scale Discovery) into each well. Plates were then incubated for 2 h with vigorous shaking at room temperature. The detection antibody was removed and the plates were washed three times. To each well were added 150 µL of 2x Read Buffer T (Meso Scale Discovery), and the signals were read by the SECTOR® Imager 2400 (Meso Scale Discovery). Standard curves were generated, and the assay values of the samples were interpolated from the curves. The assay characteristics are described in the Supplementary Table. The coefficient of variation was <15% for 19 of the 29 analytes. For samples with concentrations below the limits of detection, missing values were replaced with 99% of the lowest detectable concentration.

Statistical analysis

For demographic data analysis, the Kolmogorov-Smirnov test was used to test whether the distribution of continuous variables was normal. Chi-square and Fisher's exact tests were used for comparisons of proportions. Kruskal-Wallis and Mann-Whitney *U*-tests were used to compare median concentrations of analytes between and among groups. Statistical analysis of demographics data was performed using SPSS 19 (IBM Corp, Armonk, NY, USA). A P-value <0.05 was considered statistically significant.

Comparison of analyte concentrations determined by multiplex assay was restricted to the analytes that were detected in a number of samples larger than one-half of the size of the smallest group. Statistical analysis was performed using the Wilcoxon rank-sum test and R statistical environment [114]. Nominal P-values were adjusted using the Benjamini and Hochberg method [115], controlling the false discovery rate at 5%.

Results

Characteristics of the study population

A total of 38 cases (patients with clinical chorioamnionitis at term) and 77 controls (patients with spontaneous term labor without clinical chorioamnionitis) were included in the study. Descriptive characteristics of the study population are displayed in Table 1. The patients in this study represent a subset included in previous reports [1, 3]. A description of the microorganisms identified in the amniotic fluid [2], and the concentration of cytokines in maternal plasma [1] and amniotic fluid [3], have been reported elsewhere.

When classified according to the presence or absence of intra-amniotic inflammation and microorganisms [by amniotic fluid cultures and PCR/ESI-MS (broad-range PCR coupled with electrospray ionization mass spectrometry)], 57.9% (22/38) of cases had microbial-associated intra-amniotic inflammation, 18.4% (7/38)

Table 1: Characteristics of the study population.

	Term in labor (n=77)	Clinical chorioamnionitis at term (n=38)	P-value
Maternal age (years)	25 (20.5–30.5)	20.5 (18–25)	0.003
Body mass index (kg/m²)	23.9 (21.0–25.9)	23.8 (21.6–24.8)	0.96
Amniotic fluid glucose (mg/dL)	NA	9 (9–9)	
Amniotic fluid white blood cell (cell/mm³)	NA	41.5 (5–468.7)	
Gestational age at amniocentesis and delivery (weeks)	39.6 (38.9–40.5)	39.9 (38.9–40.8)	0.46
Birthweight (g)	3400 (3175–3650)	3500 (3210–3775)	0.34
Suspected neonatal sepsis	1.3% (1/77)	34.2% (13/38)	<0.001
Fetal inflammatory response syndrome (FIRS)	NA	21.1% (8/38)	–
Acute inflammatory lesion of placenta	0% (0/0)	55.3% (21/38)	–

Data presented as % (n), median (interquartile range). Acute inflammatory lesions of placenta: acute histologic chorioamnionitis and/or acute funisitis. NA=results were not available.

had intra-amniotic inflammation without detectable microorganisms and 23.7% (9/38) had no evidence of intra-amniotic inflammation. About half of the patients with clinical chorioamnionitis at term [55.3% (21/38)] had acute inflammatory lesions of the placenta, whereas no patients in the control group had such lesions. The frequency of suspected neonatal sepsis was significantly higher in the group with clinical chorioamnionitis than in controls [34.2% (13/38) vs. 1.3% (1/77); P<0.001]. Approximately 70% (9/13) of neonates with suspected neonatal sepsis were born to mothers with intra-amniotic inflammation. All neonates with suspected sepsis had negative blood cultures. The diagnosis of neonatal sepsis was based on clinical signs and laboratory tests such as white blood cell count and C-reactive protein (CRP). Of the patients with clinical chorioamnionitis at term, 21% (8/38) had neonates with FIRS. All of these neonates were exposed to microbial-associated intra-amniotic inflammation (also termed "intra-amniotic infection").

Umbilical cord plasma cytokine concentrations

Clinical chorioamnionitis vs. spontaneous term labor without clinical chorioamnionitis

The median (interquartile range: IQR) cytokine concentrations in umbilical cord plasma between cases and controls are displayed in Table 2. Neonates born to mothers

Table 2: Umbilical cord plasma cytokine and chemokine concentrations in term in labor vs. clinical chorioamnionitis at term.

Analytes (pg/mL)	Term in labor median (IQR) (n=77)	Clinical chorioamnionitis at term median (IQR) (n=38)	Fold change	Adjusted P-value
Pro-inflammatory cytokines				
IL-12p70	**0.02 (0.02–0.1)**	**0.12 (0.1–0.16)**	**6.00**	**0.00001**
IL-6	**0.55 (0.32–0.82)**	**1.92 (1.16–3.55)**	**3.48**	**<0.00001**
IL-16[a]	**288.61 (223.88–406.54)**	**402.6 (294.1–610.95)**	**1.39**	**0.02**
IFN-γ	**2.9 (2.26–3.65)**	**1.89 (1.48–2.64)**	**0.65**	**0.00004**
TNF-β	**0.23 (0.16–0.33)**	**0.17 (0.12–0.25)**	**0.72**	**0.04**
IL-1β	0.38 (0.18–0.79)	0.38 (0.12–1.4)	1.01	0.97
IL-17α	1.32 (1.07–1.71)	1.34 (0.1–1.75)	1.02	0.95
TNF-α	2.8 (2.4–3.1)	2.88 (2.49–3.32)	1.04	0.73
IL-2	0.06 (0.01–0.14)	0.12 (0.01–0.18)	1.92	0.26
IL-12/IL-23p40	645.63 (537.97–815.11)	618.83 (480.67–871.85)	0.96	0.77
IL-15	1.23 (0.92–1.52)	1.43 (0.87–2.67)	1.16	0.1
IL-1α	0.92 (0.73–1.15)	0.84 (0.69–1.1)	0.91	0.72
GM-CSF	0.15 (0.09–0.22)	0.05 (0.02–0.24)	0.33	0.26
IL-7	1.24 (0.73–2.21)	1.13 (0.75–3.58)	0.91	0.95
VEGF	45.16 (18.52–103.61)	45.91 (20.21–98.51)	1.02	0.77
Anti-inflammatory cytokines				
IL-13	**0.17 (0.17–0.54)**	**0.87 (0.55–1.21)**	**5.14**	**<0.00001**
IL-4	**0.01 (0–0.02)**	**0.03 (0.02–0.04)**	**2.50**	**0.00001**
IL-10	**0.28 (0.2–0.38)**	**0.42 (0.27–1.04)**	**1.48**	**0.009**
IL-5	0.51 (0.37–0.65)	0.58 (0.34–0.72)	1.13	0.47
Chemokines				
IL-8	**2.50 (1.63–3.93)**	**6.69 (3.84–10.48)**	**2.67**	**<0.00001**
MIP-1α	11.97 (9.83–14.37)	11.47 (10–13.6)	0.96	0.97
Eotaxin	64.73 (48.03–81.72)	56.93 (39.19–70.37)	0.88	0.26
MCP-4	51.94 (42.32–73.82)	49.11 (27.56–89.94)	0.95	0.88
MCP-1	60 (44.78–67.54)	58.78 (48.06–80.69)	0.98	0.76
MDC	1124.23 (931.33–1311.54)	1028.62 (834.1–1347.12)	0.91	0.33
Eotaxin-3	17.8 (12.25–22.92)	14.27 (6.27–27.13)	0.8	0.68
CXCL-10 (IP-10)	84.82 (66.27–114.91)	109.82 (66.68–151.54)	1.29	0.31
TARC	98.97 (64.29–194.61)	113.93 (70.44–276.82)	1.15	0.24
MIP-1β	94.77 (73.29–120.46)	76.39 (63.93–112.93)	0.81	0.26

[a]IL-16 has pro- and anti-inflammatory properties. The units of all analytes are pg/mL. CXCL-10=C-X-C motif chemokine 10, GM-CSF=granulocyte macrophage colony-stimulating factor, IFN-γ=interferon gamma, IL=interleukin, IP-10=interferon gamma-induced protein 10, IQR=interquartile range, MDC=macrophage-derived chemokine, MIP=macrophage inflammatory protein, MCP=monocyte chemoattractant protein, TARC=thymus and activation-regulated chemokine, TNF=tumor necrosis factor, VEGF=vascular endothelial growth factor. Values in bold font indicate that the results are significant.

with clinical chorioamnionitis at term had significantly higher median umbilical cord plasma concentrations of IL-6, IL-12p70, IL-16, IL-13, IL-4, IL-10 and IL-8 than those with spontaneous term labor without clinical chorioamnionitis, with a fold-change difference in median concentrations that ranged from 1.39 to 6.0. IL-12p70 was the cytokine with the highest fold change (fold change=6). Median umbilical cord plasma IFN-γ and TNF-β concentrations were significantly lower in patients with clinical chorioamnionitis at term than in controls [fold differences in median: IFN-γ=0.65 (P=0.00004), TNF-β=0.72 (P=0.04)] (Table 2).

Clinical chorioamnionitis without intra-amniotic inflammation

Neonates born to mothers with clinical chorioamnionitis but without intra-amniotic inflammation had significantly higher median umbilical plasma concentrations of IL-12p70, IL-6, IL-4, IL-5, IL-8 and IL-13 than neonates born to mothers with spontaneous labor at term and without clinical chorioamnionitis (Figure 1). The fold-change difference in their median concentrations ranged from 1.9 to 6 (Table 3). In contrast, the median umbilical cord plasma concentrations of IFN-γ and MIP-1β were significantly lower.

Clinical chorioamnionitis with intra-amniotic inflammation without demonstrable bacteria

Neonates born to mothers with clinical chorioamnionitis at term and intra-amniotic inflammation (amniotic fluid IL-6 concentration ≥2.6 ng/mL) without detectable microorganisms had significantly higher median umbilical cord plasma concentrations of IL-12p70 (fold change 6), IL-6 (fold change 3.8) and IL-8 (fold change 2.7) but lower IFN-γ concentrations than neonates not exposed to clinical chorioamnionitis (Table 3).

Clinical chorioamnionitis with microbial-associated intra-amniotic inflammation

Neonates born to mothers with intra-amniotic infection had significantly higher median umbilical cord plasma concentrations of IL-12p70, IL-6, IL-16, IL-13, IL-4, IL-10 and IL-8 than neonates not exposed to clinical chorioamnionitis. The fold change ranged from 1.46 to 6 (Table 3).

Clinical chorioamnionitis with and without FIRS

All neonates with FIRS were born to mothers who had microbial-associated intra-amniotic inflammation or intra-amniotic infection. The median umbilical cord plasma concentrations of IL-6, IL-8, IL-10 and MCP-1 were significantly higher in neonates with FIRS than in those without FIRS, with fold-change differences in the median that ranged from 2.02 to 6.69 (Table 4 and Figure 2). Umbilical cord plasma IL-6 and IL-8 had the highest fold-change differences in the median [6.69 and 4.33, respectively (Table 4)].

The relationship between the concentrations of umbilical cord and maternal plasma IL-6
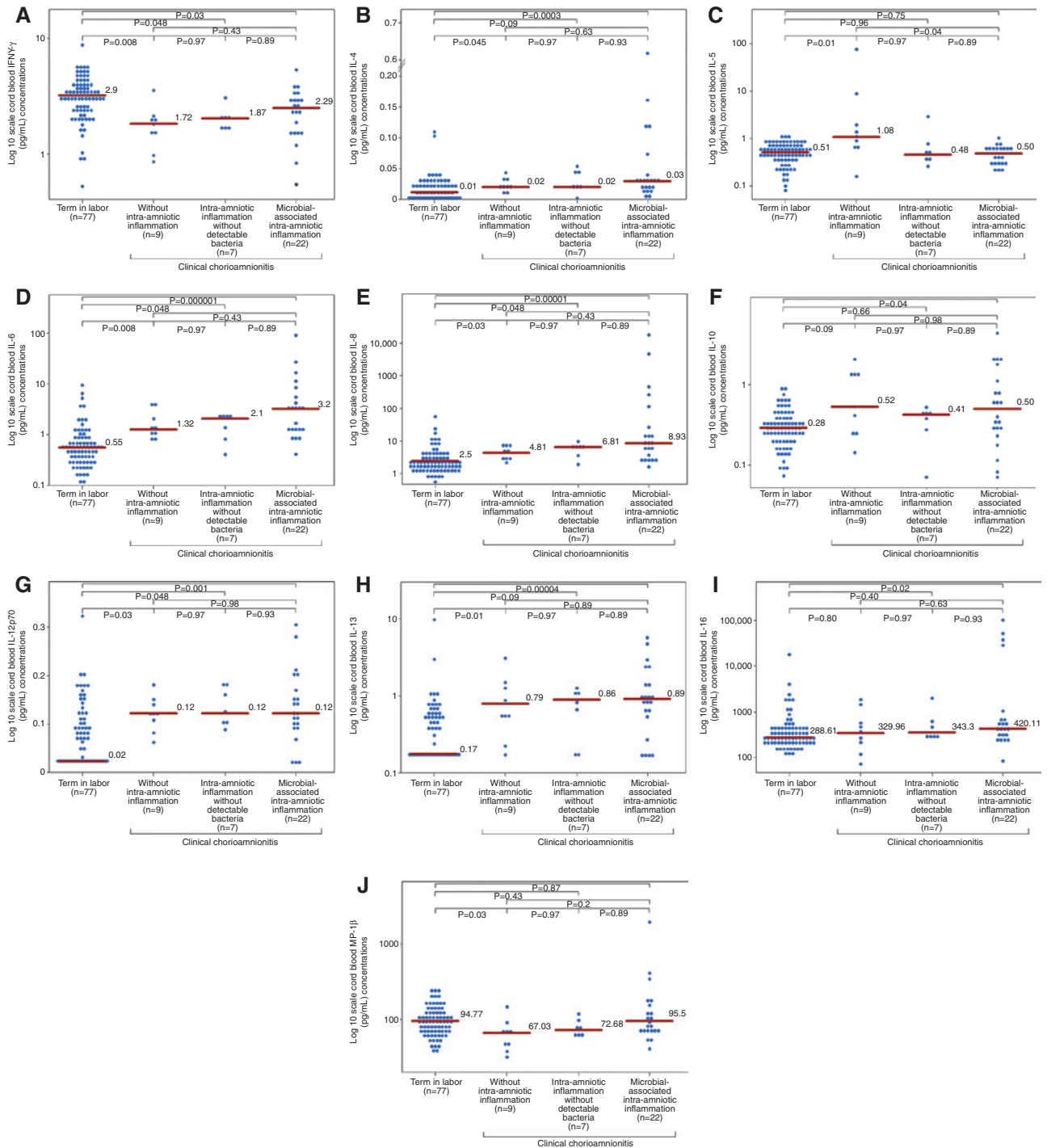
IL-6, a major cytokine involved in the host response against infection and tissue injury, is believed to cross the placenta [116]; therefore, we examined the relationship of IL-6 between the maternal and umbilical cord circulations. Median (IQR) IL-6 concentrations were significantly higher in maternal than umbilical cord plasma for both cases and controls [control: maternal plasma: 2.53 (1.64–4.08) pg/mL vs. umbilical cord plasma: 0.55 (0.32–0.82) pg/mL; P≤0.0001; cases: maternal plasma: 10.94 (5.36–22.08) pg/mL vs. umbilical cord plasma: 1.92 (1.16–3.55) pg/mL; P≤0.001]. There was a positive correlation between maternal and umbilical cord plasma IL-6 and IL-8 concentrations (IL-6: Spearman correlation=0.53; P<0.001; IL-8 Spearman correlation=0.42; P<0.001) (Figures 3 and 4).

The supplementary material contains scatterplots of maternal plasma concentrations of cytokines among different groups (Supplementary Figures 1–5).

Discussion

Principal findings of the study

1. Neonates born to mothers with clinical chorioamnionitis at term (considered *in toto*) had significantly higher median umbilical cord plasma concentrations of IL-6, IL-12p70, IL-16, IL-13, IL-4, IL-10 and IL-8, but significantly lower IFN-γ and TNF-α concentrations than neonates born to mothers with spontaneous term labor without clinical chorioamnionitis.

2. Neonates born to mothers with clinical chorioamnionitis but without intra-amniotic inflammation had higher concentrations of IL-6, IL-12p70, IL-13, IL-4, IL-5, and IL-8, but lower IFN-γ, than neonates not exposed to clinical chorioamnionitis, suggesting that maternal fever in the absence of intra-amniotic inflammation leads to a change in the fetal cytokine network.

3. There were significant, positive correlations between maternal and umbilical cord plasma IL-6 and IL-8 concentrations (IL-6: Spearman correlation=0.53; P<0.001; IL-8: Spearman correlation=0.42; P<0.001), consistent with the placental transfer of cytokines.

4. An elevated fetal plasma IL-6 (FIRS; IL-6 >11 pg/mL), the diagnostic criterion for FIRS, was present in 21%

Figure 1: The umbilical cord plasma concentrations of cytokines and chemokines in patients at term in labor (control) (n=77) with clinical chorioamnionitis without intra-amniotic inflammation (n=9), with clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria (n=7) and with clinical chorioamnionitis with microbial-associated intra-amniotic inflammation (n=22). (A) The median umbilical cord plasma concentrations of interferon (IFN)-γ are 2.9 pg/mL (term in labor), 1.72 pg/mL (clinical chorio-amnionitis without intra-amniotic inflammation), 1.87 pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria) and 2.29 pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflammation). (B) The median umbilical cord plasma concentrations of interleukin (IL)-4 are 0.01 pg/mL (term in labor), 0.02 pg/mL (clinical chorioamnionitis without intra-amniotic inflammation), 0.02 pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria) and 0.03 pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflammation). (C) The median umbilical cord plasma concentrations of interleukin (IL)-5 are 0.51 pg/mL (term in labor), 1.08 pg/mL (clinical chorioamnionitis without intra-amniotic inflam-mation), 0.48 pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria) and 0.50 pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflammation). (D) The median umbilical cord plasma concentra-tions of interleukin (IL)-6 are 0.55 pg/mL (term in labor), 1.32 pg/mL (clinical chorioamnionitis without intra-amniotic inflammation), 2.1 pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria) and 3.2 pg/mL (clinical chorioam-nionitis with microbial-associated intra-amniotic inflammation). (E) The median umbilical cord plasma concentrations of interleukin (IL)-8 are 2.5 pg/mL (term in labor), 4.81 pg/mL (clinical chorioamnionitis without intra-amniotic inflammation), 6.81 pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria), and 8.93 pg/mL (clinical chorioamnionitis with micro-bial-associated intra-amniotic inflammation). (F) The median umbilical cord plasma concentrations of interleukin (IL)-10 are 0.28 pg/mL (term in labor), 0.52 pg/mL (clinical chorioamnionitis without intra-amniotic inflammation), 0.41 pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria) and 0.50 pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflammation). (G) The median umbilical cord plasma concentrations of interleukin (IL)-12p70 are 0.02 pg/mL (term in labor), 0.12 pg/mL (clinical chorioamnionitis without intra-amniotic inflammation), 0.12 pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria) and 0.12 pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflam-mation). (H) The median umbilical cord plasma concentrations of interleukin (IL)-13 are 0.17 pg/mL (term in labor), 0.79 pg/mL (clinical chorioamnionitis without intra-amniotic inflammation), 0.86 pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria) and 0.89 pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflammation). (I) The median umbilical cord plasma concentrations of interleukin (IL)-16 are 288.61 pg/mL (term in labor), 329.96 pg/mL (clinical chorioamnionitis without intra-amniotic inflammation), 343.3 pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria) and 420.11 pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflammation). (J) The median umbilical cord plasma concentrations of macrophage inflammatory protein (MIP)-1β are 94.77 pg/mL (term in labor), 67.03 pg/mL (clinical cho-rioamnionitis without intra-amniotic inflammation), 72.68 pg/mL (clinical chorioamnionitis with intra-amniotic inflammation without detectable bacteria) and 95.5 pg/mL (clinical chorioamnionitis with microbial-associated intra-amniotic inflammation).

(8/38), of patients with clinical chorioamnionitis at term, and all these neonates were born to mothers with proven intra-amniotic infection.

5. FIRS was associated with a high concentration of umbilical cord plasma IL-8, IL-10 and MCP-1.

Maternal systemic inflammation: an underappreciated cause of developmental disorders

Normal pregnancy is a state of physiologic intravascular inflammation in which there is activation of the innate immune system and suppression of the adaptive immune response to paternal antigens [117–123]. The latter is thought to promote a tolerogenic state favoring the sur-vival of the placental and fetal semi-allograft [121, 124–132]. Activation of the innate limb of the immune response is thought to protect the mother and fetus against infec-tion [118]. Infection during pregnancy is known to lead to an exaggerated systemic intravascular inflammatory response, with increased concentrations of cytokines [118, 133, 134]. This occurs in the context of both bacte-rial and viral infections. It is now clear that mothers who have infections during pregnancy are at increased risk for maternal death [135–138]. This has been the case in the pandemics of influenza [139–149] and more recently Ebola [150–153] and has been attributed to a cytokine storm that occurs when pregnant women are exposed to microorgan-isms [154–156].

Maternal inflammation during pregnancy can also have an effect on fetal brain development [51–89, 92, 157]. A solid body of evidence now indicates that infants of mothers who experienced viral infections during preg-nancy are at increased risk of both schizophrenia [55, 56, 58, 60–64, 66, 67, 69–72, 74, 76–78, 80, 85, 92] and autism spectrum disorders [52, 73, 74, 77, 79–83, 85–87, 92, 93]. Systemic infection is thought to induce the pro-duction of cytokines, and in particular IL-6, which has direct effects on the placenta by inducing the activation of Janus kinase-signal transducer and activator of transcrip-tion 3 (JAK-STAT3) [158]. IL-6 can cross the placenta [116,

Table 3: Umbilical cord plasma cytokines and chemokines concentrations in the subgroups of clinical chorioamnionitis and term in labor.

Analytes (pg/mL)	Term in labor (controls) (n=77) Median (IQR)	Without intra-amniotic inflammation (n=9)			With intra-amniotic inflammation without dedetectable microorganisms (n=7)				With microbial-associated intra-amniotic inflammation (n=22)				
		Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value[a] (compared to term in labor)	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to without intra-amniotic inflammation)	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to without intra-amniotic inflammation)	Adjusted P-value (compared to with intra-amniotic inflammation without demonstrable microorganisms)
Pro-inflammatory cytokines													
IL-12p70	0.02 (0.02–0.1)	0.12 (0.11–0.14)	6.00	0.03	0.12 (0.1–0.17)	6.00	0.048	0.97	0.12 (0.09–0.17)	6.00	0.001	0.98	0.93
IL-6	0.55 (0.32–0.82)	1.32 (1.12–2.09)	2.40	0.008	2.1 (1.17–2.25)	3.82	0.048	0.97	3.2 (1.33–5.37)	5.81	<0.00001	0.43	0.89
IFN-γ	2.9 (2.26–3.65)	1.72 (1.37–1.81)	0.59	0.008	1.87 (1.62–1.95)	0.64	0.048	0.97	2.29 (1.48–2.73)	0.79	0.03	0.43	0.89
IL-16	288.61 (223.88–406.54)	329.96 (232.68–517.24)	1.14	0.80	343.3 (313.84–503.11)	1.19	0.40	0.97	420.11 (304.97–637.82)	1.46	0.02	0.63	0.93
TNF-α	2.78 (2.39–3.14)	3.06 (1.8–3.33)	1.10	0.94	2.72 (2.7–3.3)	0.98	0.76	0.97	2.91 (2.45–3.19)	1.04	0.73	0.98	0.89
IL-2	0.06 (0.01–0.14)	0.16 (0.11–0.28)	2.67	0.07	0.14 (0.06–0.15)	2.33	0.76	0.97	0.1 (0.01–0.15)	1.58	0.73	0.43	0.89
IL-15	1.23 (0.92–1.52)	2.48 (1.34–2.74)	2.02	0.08	1.49 (0.92–2.18)	1.21	0.70	0.97	1.4 (0.8–2.37)	1.14	0.41	0.83	0.93
IL-17α	1.32 (1.07–1.71)	1.34 (0.82–1.45)	1.02	0.45	1.27 (1.16–1.54)	0.96	0.96	0.97	1.43 (0.93–1.9)	1.08	0.73	0.63	0.89
TNF-β	0.23 (0.16–0.33)	0.17 (0.15–0.21)	0.74	0.12	0.14 (0.13–0.21)	0.61	0.40	0.97	0.18 (0.12–0.31)	0.78	0.38	0.98	0.93
IL-12/IL-23p40	645.63 (537.97–815.11)	515.93 (474.91–782.58)	0.80	0.53	627.35 (583.53–965.06)	0.97	0.76	0.97	651.52 (336.43–933.05)	1.01	0.73	0.98	0.89
IL-1α	0.92 (0.73–1.15)	0.78 (0.7–0.88)	0.85	0.50	0.83 (0.73–0.94)	0.9	0.76	0.97	0.89 (0.7–1.22)	0.97	1.00	0.65	0.89
GM-CSF	0.15 (0.09–0.22)	0.02 (0.02–0.23)	0.13	0.50	0.04 (0.02–0.11)	0.27	0.31	1.00	0.08 (0.02–0.28)	0.50	0.66	0.87	0.89
IL-7	1.24 (0.73–2.21)	0.82 (0.73–1.39)	0.66	0.50	1.12 (0.72–2.58)	0.9	0.89	0.97	1.35 (0.81–8.13)	1.09	0.50	0.43	0.89
IL-1β	0.38 (0.18–0.79)	0.14 (0.06–0.38)	0.37	0.12	0.22 (0.19–0.76)	0.58	0.89	0.97	0.59 (0.15–2.76)	1.55	0.41	0.39	0.89
VEGF	45.16 (18.52–103.61)	45.4 (14.01–59.87)	1.00	0.80	30.72 (22.41–63.07)	0.68	0.89	1.00	57.93 (28.19–138.75)	1.28	0.47	0.63	0.89

Table 3 (continued)

Analytes (pg/mL)	Term in labor (controls) (n=77)	Without intra-amniotic inflammation (n=9)			With intra-amniotic inflammation without dedetectable microorganisms (n=7)				Clinical chorioamnionitis at term (n=38) With microbial-associated intra-amniotic inflammation (n=22)				
	Median (IQR)	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value[a] (compared to term in labor)	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to without intra-amniotic inflammation)	Median (IQR)	Fold change (compared to term in labor)	Adjusted P-value (compared to term in labor)	Adjusted P-value (compared to without intra-amniotic inflammation)	Adjusted P-value (compared to with intra-amniotic inflammation without demonstrable microorganisms)
Anti-inflammatory cytokines													
IL-13	**0.17** (0.17–0.54)	**0.79** (0.54–1.22)	**4.69**	**0.01**	0.86 (0.41–1.05)	5.11	0.09	0.97	**0.89** (0.6–1.48)	**5.26**	**0.00004**	0.89	0.89
IL-4	**0.01** (0–0.02)	**0.02** (0.02–0.03)	**2.00**	**0.045**	0.02 (0.02–0.04)	2	0.09	0.97	**0.03** (0.02–0.04)	**3**	**0.0003**	0.63	0.93
IL-5	**0.51** (0.37–0.65)	**1.08** (0.72–1.89)	**2.12**	**0.01**	0.48 (0.39–0.64)	0.94	0.96	0.97	**0.50** (0.31–0.65)	0.97	0.75	**0.04**	0.89
IL-10	0.28 (0.2–0.38)	0.52 (0.25–1.38)	1.86	0.09	0.41 (0.32–0.44)	1.46	0.66	0.97	**0.50** (0.27–1.04)	**1.77**	**0.04**	0.98	0.89
Chemokines													
IL-8	**2.50** (1.63–3.93)	**4.81** (3.82–6.56)	**1.92**	**0.03**	**6.81** (5.16–7.19)	**2.72**	**0.048**	0.97	**8.93** (3.57–26.09)	**3.57**	**0.00001**	0.43	0.89
MIP-1β	**94.77** (73.29–120.46)	**67.03** (46.18–70.18)	**0.71**	**0.03**	72.68 (62.24–89.07)	0.77	0.43	0.97	95.5 (68.24–141.3)	1.01	0.87	0.20	0.89
CXCL-10 (IP-10)	84.82 (66.27–114.91)	107.04 (37.79–141.01)	1.26	0.99	117.05 (78.85–135.4)	1.38	0.66	0.97	98.99 (70.17–155.14)	1.17	0.41	0.63	0.93
MCP-1	60 (44.78–67.54)	59.63 (33.22–79.21)	0.99	0.64	53.25 (51.52–62.79)	0.89	0.83	1.00	61.62 (50.68–127.63)	1.03	0.41	0.43	0.89
Eotaxin-3	17.8 (12.25–22.92)	12.57 (10.95–22.5)	0.71	0.53	19.5 (16.12–23.37)	1.1	0.89	0.97	13.42 (4.25–31.77)	0.75	0.66	0.98	0.89
MIP-1α	11.97 (9.83–14.37)	11.59 (9.84–13.34)	0.97	0.80	10.84 (9.51–12.34)	0.91	0.76	0.97	12.08 (10.23–13.97)	1.01	0.67	0.77	0.89
TARC	98.97 (64.29–194.61)	164.06 (75.37–212)	1.66	0.53	196.14 (100.6–283.75)	1.98	0.55	0.97	108.64 (68.33–419.86)	1.1	0.41	0.98	0.89
MDC	1124.23 (931.33–1311.54)	959.09 (864.9–1227.79)	0.85	0.53	1156.54 (953.28–1262.82)	1.03	0.96	0.97	981.61 (834.1–1397.83)	0.87	0.41	0.98	0.89
MCP-4	51.94 (42.32–73.82)	29.51 (27.53–35.29)	0.57	0.11	61.01 (24.07–76.94)	1.17	0.9	1.00	57.87 (42.57–99.73)	1.11	0.56	0.43	0.89
Eotaxin	64.73 (48.03–81.72)	61.54 (50.91–69.26)	0.95	0.53	52.82 (46.64–69.6)	0.82	0.76	0.97	56.49 (37.92–78.75)	0.87	0.41	0.98	0.93

aIL-16 has pro- and anti-inflammatory properties. The units of all analytes are pg/mL. CXCL-10=C-X-C motif chemokine 10, GM-CSF=granulocyte macrophage colony-stimulating factor, IFN-γ=interferon gamma, IL=interleukin, IP-10=interferon gamma-induced protein 10, IQR=interquartile range, MDC=macrophage-derived chemokine, MIP=macrophage inflammatory protein, MCP=monocyte chemoattractant protein, TARC=thymus and activation-regulated chemokine, TNF=tumor necrosis factor, VEGF=vascular endothelial growth factor. Values in bold font indicate that the results are significant.

Table 4: Umbilical cord plasma cytokines and chemokines concentrations in neonates with FIRS and without FIRS born to mothers with clinical chorioamnionitis at term.

Analytes (pg/mL)	Neonates born without FIRS median (IQR) (n=30)	Neonates born with FIRS median (IQR) (n=8)	Fold change	Adjusted P-value
Pro-inflammatory cytokines				
IL-6	**1.46 (1.12–2.27)**	**9.77 (5.37–20.49)**	**6.69**	**0.002**
IL-12p70	0.12 (0.1–0.15)	0.14 (0.1–0.17)	1.17	0.76
IL-16[a]	394.71 (299.01–620.64)	435.23 (266.18–9422.55)	1.1	0.96
IFN-γ	1.78 (1.47–2.35)	2.61 (2.03–2.93)	1.47	0.38
TNF-β	0.15 (0.12–0.23)	0.26 (0.18–0.32)	1.7	0.38
IL-1β	0.21 (0.09–0.64)	1.35 (0.49–7.88)	6.43	0.15
IL-17α	1.35 (1.07–1.74)	1.36 (0.81–1.8)	1.004	0.96
TNF-α	2.82 (2.44–3.29)	2.97 (2.58–3.28)	1.05	0.82
IL-2	0.1 (0.01–0.16)	0.18 (0.11–0.26)	1.8	0.38
IL-12/IL-23p40	610.32 (502.52–813.43)	677.61 (285.39–901.52)	1.11	0.96
IL-15	1.4 (0.87–2.53)	2 (1.24–6.76)	1.43	0.51
IL-1α	0.84 (0.7–1.11)	0.85 (0.54–1.52)	1.01	0.96
GM-CSF	0.02 (0.02–0.2)	0.15 (0.04–0.36)	7.58	0.38
IL-7	1.1 (0.73–1.59)	6.98 (0.96–16.33)	6.34	0.38
VEGF	43.03 (18.97–66.87)	87.71 (25.05–454.06)	2.04	0.46
Anti-inflammatory cytokines				
IL-10	**0.37 (0.24–0.6)**	**1.47 (0.66–2.1)**	**3.96**	**0.04**
IL-13	0.79 (0.28–1.01)	1.47 (0.78–3.39)	1.85	0.33
IL-4	0.02 (0.02–0.03)	0.03 (0.03–0.08)	1.5	0.38
IL-5	0.57 (0.33–0.89)	0.52 (0.36–0.66)	0.9	0.68
Chemokines				
IL-8	**5.02 (3.22–8.51)**	**21.76 (11.02–1283.3)**	**4.33**	**0.02**
MCP-1	**53.92 (44.95–61.82)**	**108.77 (72.23–172.57)**	**2.02**	**0.047**
MIP-1α	11.22 (9.84–13.32)	13.34 (11.73–17.32)	1.19	0.37
Eotaxin	56.13 (44.74–69.38)	53.86 (32.5–81.94)	0.96	0.96
MCP-4	43.25 (27.53–72.92)	90.88 (59.82–236.68)	2.1	0.38
MDC	989.07 (864.9–1227.79)	979.36 (764.73–1606.51)	0.99	0.96
Eotaxin-3	14.11 (10.95–25.39)	13.33 (4.17–133.17)	0.94	0.96
CXCL-10 (IP-10)	107.04 (65.12–150.1)	116.8 (72.7–465.04)	1.09	0.43
TARC	108.32 (68.8–212)	373.74 (99.85–637.92)	3.45	0.43
MIP-1β	70.18 (61.17–98.05)	111.01 (70.43–200.1)	1.58	0.33

[a]IL-16 has pro- and anti-inflammatory properties. The units of all analytes are pg/mL. CXCL-10=C-X-C motif chemokine 10, FIRS=fetal inflammatory response syndrome, GM-CSF=granulocyte macrophage colony-stimulating factor, IFN-γ=interferon gamma, IL=interleukin, IP-10=interferon gamma-induced protein 10, IQR=interquartile range, MDC=macrophage-derived chemokine, MIP=macrophage inflammatory protein, MCP=monocyte chemoattractant protein, TARC=thymus and activation-regulated chemokine, TNF=tumor necrosis factor, VEGF=vascular endothelial growth factor. Values in bold font indicate that the results are significant.

158–161], have direct effects in multiple target organs of the fetus, including the brain [73, 158, 162, 163] and can induce microglial activation, astrogliosis and synaptic pruning [164–166]. This is thought to be the basis for the predisposition to schizophrenia and autism. Even short-lived fever, such as intrapartum fever or acute histologic chorioamnionitis, has been associated with an increased risk for cerebral palsy at the age of 3 in both term and preterm neonates [167]. The mechanism whereby intrapartum fever leads to the development of brain injury [31, 40, 41, 43, 45, 101, 167–181] during labor has been a subject of recent investigation. Fever is mediated by pyrogenic cytokines [182–184] [i.e. IL-1 [184–192], IL-2 [193–195], IL-6 [196–200] and TNF [195, 201–204] and can induce the production of multiple other cytokines/chemokines, as well as generate a strong pro-inflammatory state. The effects of maternal systemic inflammation in the human fetal immune system have not been adequately studied. Clinical chorioamnionitis at term represents a unique model to examine the effect of maternal systemic inflammation in the presence or absence of intra-amniotic inflammation with or without bacteria. This study aimed to examine the
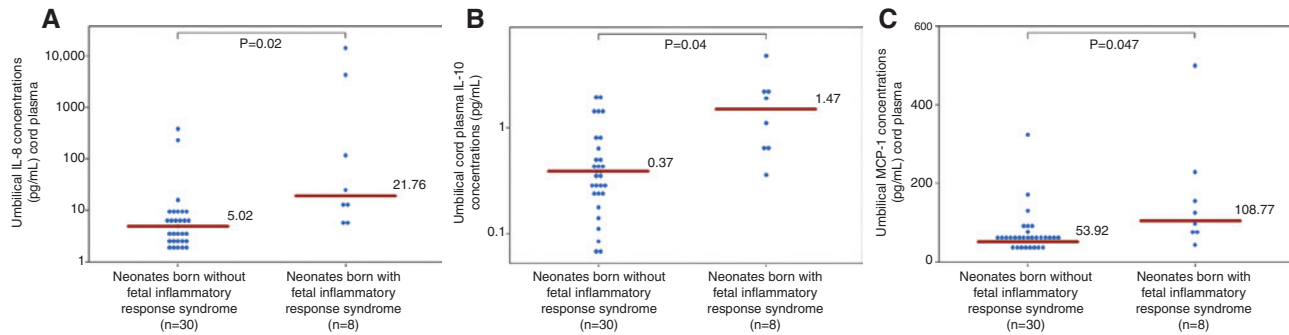
Figure 2: The umbilical cord plasma concentrations of cytokines and chemokines in neonates born with (n=8) and without (n=30) fetal inflammatory response syndrome.
(A) The median umbilical cord plasma concentrations of interleukin (IL)-8 are 5.02 pg/mL in neonates born without fetal inflammatory response syndrome and 21.76 pg/mL in neonates born with fetal inflammatory response syndrome. (B) The median umbilical cord plasma concentrations of interleukin (IL)-10 are 0.37 pg/mL in neonates born without fetal inflammatory response syndrome and 1.47 pg/mL in neonates born with fetal inflammatory response syndrome. (C) The median umbilical cord plasma concentrations of monocyte chemoattractant protein (MCP)-1 are 53.92 pg/mL in neonates born without fetal inflammatory response syndrome and 108.77 pg/mL in neonates born with fetal inflammatory response syndrome.
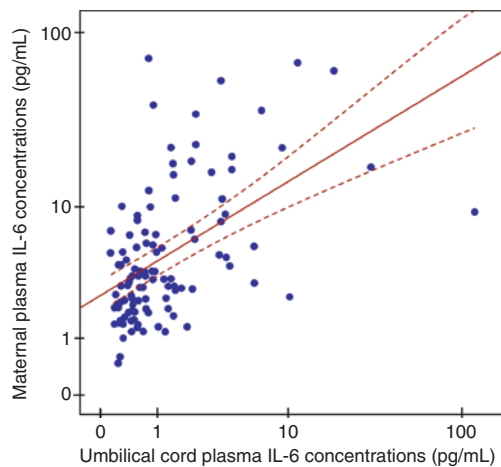
Figure 3: Umbilical cord and maternal plasma interleukin-6 (IL-6) scatter diagram with linear regression line (red line).
Dashed line indicates confidence interval of mean. There is a positive correlation between maternal and umbilical cord plasma IL-6 concentrations (Spearman correlation=0.53; P<0.001).
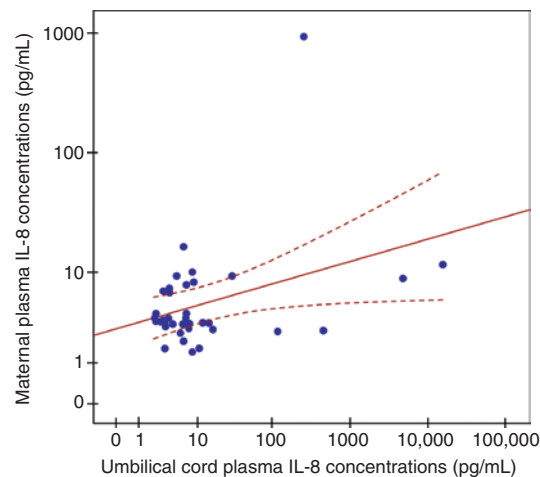
Figure 4: Umbilical cord and maternal plasma interleukin-8 (IL-8) scatter diagram with linear regression line (red line).
Dashed line indicates confidence interval of mean. There is a positive correlation between maternal and umbilical cord plasma IL-8 concentrations (Spearman correlation=0.42; P<0.001).

effect of such maternal systemic inflammatory response on the fetal inflammatory response gauged by the concentrations of circulating fetal cytokines.

The human fetal cytokine profile after exposure to maternal systemic inflammation

The clinical signs of chorioamnionitis at term represent evidence of a systemic maternal inflammatory response [1]. In this study, IL-12p70 and IL-13 had a fold-change difference in the median of >5. IL-12p70 is mainly produced by dendritic cells [205–217], monocytes [205, 211–214, 216–218], macrophages [205, 211, 212, 214–218] and neutrophils [213, 219–221]. This pro-inflammatory cytokine can induce the production of IFN-γ [207, 212, 214, 215, 222–228]. An elevation of circulating IL-12p70 has been reported in patients with sepsis [229–231] and preterm delivery <35 weeks with severe histologic chorioamnionitis [228]. Our findings are consistent with these results.

IL-13 is produced by activated Th2 cells [232–234], mast cells [233, 235], natural killer cells [233, 236], dendritic cells [237] and alveolar macrophages [238, 239]. This cytokine has anti-inflammatory properties that are

thought to result from downregulation of the expression of pro-inflammatory cytokines such as IL-1α, IL-1β, TNF-α, IFN-γ, IL-6, IL-8, IL-10 and IL-12 [232, 233, 239–242]. In murine models of sepsis, IL-13 reduces the inflammatory response by suppression of pro-inflammatory cytokines and chemokines in tissues such as the liver, lung and kidney [239, 242–246]. In addition, an increase in circulating IL-13 concentrations is associated with sepsis in both adults [234, 247] and children [248, 249]. Therefore, it is not surprising that clinical chorioamnionitis at term is associated with a significant elevation in the umbilical cord plasma concentrations of IL-12p70 and IL-13.

Several studies have demonstrated the potential value of umbilical cord plasma concentrations of IL-6 [105, 250–277] and IL-8 [257, 260–262, 267, 268, 276, 278, 279] for the identification of intra-amniotic infection and early-onset neonatal sepsis. Our findings are consistent with those of Lencki et al., who reported that neonates born to mothers with clinical chorioamnionitis had higher umbilical cord plasma concentrations of IL-6, but not IL-1β and soluble IL-2 receptor, than those without this condition [280].

An increase in the concentrations of umbilical cord anti-inflammatory cytokines may reflect a host response mounted to counteract the effect of pro-inflammatory cytokines [281–283]. Collectively, clinical chorioamnionitis at term is associated with upregulation of several umbilical cord plasma pro- and anti-inflammatory cytokine/chemokine concentrations.

The fetal systemic cytokine profile after exposure to maternal systemic inflammation in the absence of intra-amniotic inflammation

An important finding of this study is that fetal exposure to a maternal systemic inflammatory response in the *absence* of intra-amniotic inflammation is associated with an elevation of multiple cytokines, consistent with a pro-inflammatory state in the human fetus. The precise mechanisms responsible for this observation remain to be elucidated. IL-6 can cross the placenta [116, 159, 161, 284] as well as induce an acute phase response in the fetus [104, 105, 111, 113, 285]; therefore, it is possible that some of the findings reported herein occur in response to the transplacental passage of cytokines and chemokines. However, some cytokines can exert a direct effect in the placenta and modulate its inflammatory response [158]. In mice, maternal immune activation via viral mimic (double stranded RNA) induces the production of IL-6 in

the placenta [158]. Maternally-derived IL-6 can engage the JAK/STAT3 pathway, which results in the upregulation of acute phase proteins, such as suppressor of cytokine signaling 3 (SOCS-3), and in the downregulation of placental growth hormone production [158]. These changes in endocrine factors could have effects on fetal development (including neurodevelopment). For example, we have reported changes in fetal plasma cortisol in patients with fetal systemic inflammation [286], and similar observations have been reported by Gravett et al. in the Rhesus monkey model of intrauterine infection [287, 288]. The effects of glucocorticoids in fetal programming of multiple organ systems (including the brain) are well-established [289–297]. Interestingly, maternal immune activation induces long-term changes in the composition of the gut microbiota and subsequent alteration in gastrointestinal physiology of the offspring [298–302].

Collectively, our findings, along with the observations from aforementioned experimental studies, support the view of maternal immune activation (even without intra-amniotic inflammation) and its subsequent effects on fetal immune development.

The fetal cytokine profile in the context of intra-amniotic inflammation with or without bacteria

Clinical chorioamnionitis with intra-amniotic inflammation with or without detectable bacteria in the amniotic cavity is characterized by elevated concentrations of several cytokines in the amniotic fluid [3] and maternal circulation [1]. In this study, a fetal systemic inflammatory response, reflected by changes in cytokine concentrations, was also observed. The main cytokines upregulated in the circulation of fetuses exposed to intra-amniotic inflammation were IL-12p70, IL-6, IL-13, IL-4 and IL-8. Umbilical cord plasma IL-6 and IL-8 concentrations were higher in neonates born to mothers with intra-amniotic inflammation with or without detectable bacteria than in neonates of mothers without clinical chorioamnionitis. Moreover, we and others have reported the occurrence of a fetal systemic inflammatory response in preterm labor with intact membranes [104, 113, 285, 303] as well as preterm PROM [108, 304].

Umbilical cord plasma cytokines could be of maternal, placental or fetal origin [305]. For example, IL-6 has been identified in neonatal blood mononuclear cells [254], trophoblasts [306, 307] and/or decidual cells [308, 309], as well as maternal cells that have crossed the placenta [310]. The observation that IL-6 concentrations are higher in the umbilical artery than in the umbilical vein is

consistent with fetal production of IL-6 [105, 255, 256]. The long-term consequences of early exposure of the human fetus to systemic inflammation need to be explored.

The cytokine profile of neonates born with a fetal systemic inflammatory response syndrome

In this study, *all* neonates with FIRS were born to mothers with microbial-associated intra-amniotic inflammation (intra-amniotic infection). Umbilical cord plasma IL-8, IL-10 and MCP-1 concentrations were significantly associated with FIRS. Our findings are consistent with those of Mestan et al., who used multiplex immunoassays and reported that, among the 27 cord blood biomarkers examined, the concentrations of IL-1β, IL-6 and IL-8 were associated with the presence of funisitis (the magnitude of association was stronger for IL-6 and IL-8 than for IL-1β) [268].

It is well established that FIRS is associated with the impending onset of labor [303], multi-systemic involvement and high risk of short- and long-term complications [104–107, 260, 276, 311–321]. This condition is defined by an elevation of the umbilical cord plasma concentration of IL-6 [104–113, 322], although changes in other cytokine concentrations, such as IL-10 [321, 323], granulocyte-colony-stimulating factor [111], IL-1β [313], soluble TNF receptors-1 and -2 [324, 325], TNF-α [313], IL-8 [107], IL-19 [326] and CRP [317], have been reported. An exaggerated and uncontrolled inflammatory response may be detrimental to the fetus by leading to multiple organ involvement including the skin [327–329], heart [304, 330, 331], lung [332–339], eyes [340], kidneys [341], adrenal glands [286], hematologic system [110, 111, 342], thymus [343–345] and central nervous system [167, 313, 314, 318, 346–355]. Reports from the laboratories of Newnham and Jobe have demonstrated that these changes can be experimentally produced after exposure to endotoxin [336, 356–361]. Altogether, these data suggest that neonates born to mothers with intra-amniotic infection or inflammation in the context of clinical chorioamnionitis at term are at increased risk for FIRS.

Strengths and limitations

The major strength of this study is that, by assessing the state of inflammation of the amniotic cavity, we could study the effect of maternal systemic inflammation in the presence or absence of intra-amniotic inflammation. We used both cultivation and molecular microbiologic techniques to identify microorganisms in the amniotic cavity;

therefore, the diagnosis of microbial invasion is based on state-of-the-art techniques. Limitations are related to the sample size in the three subgroups of patients with clinical chorioamnionitis at term. However, this is the only study that examines umbilical cord cytokines in term gestations with chorioamnionitis in reference to the microbial and inflammatory state of the amniotic cavity.

Conclusions

Neonates born to mothers with clinical chorioamnionitis at term had higher concentrations of umbilical cord plasma cytokines than those not exposed to clinical chorioamnionitis. Even neonates of mothers with clinical chorioamnionitis *without* intra-amniotic inflammation had elevated concentrations of multiple cytokines, suggesting that intrapartum fever alters the fetal immune response. In addition, intra-amniotic infection is associated with the presence of FIRS. Umbilical cord plasma IL-6, IL-8, IL-10 and MCP-1 are the major cytokines involved in FIRS in the context of clinical chorioamnionitis at term. The observations reported herein provide insight into the fetal immune response in patients with clinical chorioamnionitis at term.

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