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Chronic Inflammatory Lesions of the Placenta are Associated with an Up-regulation of Amniotic Fluid CXCR3: a Marker of Allograft Rejection

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Abstract

Objective—The objective of this study is to determine whether the amniotic fluid (AF) concentration of soluble CXCR3 and its ligands CXCL9 and CXCL10 changes in patients whose placentas show evidence of chronic chorioamnionitis or other placental lesions consistent with maternal anti-fetal rejection.

Methods—This retrospective case-control study included 425 women with 1) preterm delivery (n=92); 2) term in labor (n=68); and 3) term not in labor (n=265). Amniotic fluid CXCR3, CXCL9, and CXCL10 concentrations were determined by ELISA.

Results—1) Amniotic fluid concentrations of CXCR3 and its ligands CXCL9 and CXCL10 are higher in patients with preterm labor and maternal anti-fetal rejection lesions than in those without these lesions [CXCR3: preterm labor and delivery with maternal anti-fetal rejection placental lesions (median, 17.24 ng/mL; IQR, 6.79–26.68) vs. preterm labor and delivery without these placental lesions (median 8.79 ng/mL; IQR, 4.98–14.7; p=0.028)]; 2) patients with preterm labor and chronic chorioamnionitis had higher AF concentrations of CXCL9 and CXCL10, but not

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CXCR3, than those without this placental lesion [CXCR3: preterm labor with chronic chorioamnionitis (median, 17.02 ng/mL; IQR, 5.57–26.68) vs. preterm labor and delivery without chronic chorioamnionitis (median, 10.37 ng/mL; IQR 5.01–17.81; $p=0.283$); 3) patients with preterm labor had a significantly higher AF concentration of CXCR3 than those in labor at term regardless of the presence or absence of placental lesions.

Conclusion—Our findings support a role for maternal anti-fetal rejection in a subset of patients with preterm labor.

Keywords

chronic chorioamnionitis; CXCL9; CXCL10; maternal anti-fetal rejection; preterm labor; T cell

Introduction

The fetus and placenta are semi-allografts that express paternal antigens, and tolerance of such antigens is considered a central mechanism for reproductive success [1–9]. A breakdown of maternal-fetal tolerance has been implicated in the mechanisms of disease responsible for a subset of recurrent pregnancy loss [10], fetal death [11, 12], preterm labor [13–18], preterm prelabor rupture of the membranes (preterm PROM) [15], and other obstetrical complications [19–24].

The most common pathologic placental lesion in late spontaneous preterm labor and birth is chronic chorioamnionitis [22, 25], which is considered a manifestation of maternal anti-fetal rejection. In this lesion, maternal CD8⁺ T cells infiltrate the chorioamniotic membranes; thus, lymphocytes from the host (i.e. mother) infiltrate the semi-allograft fetus. There is evidence that CD8⁺ cytotoxic T cells can establish direct contact with trophoblast cells in the chorion laeve and induce apoptosis [11, 25]. Damage of the trophoblast in the chorion laeve is considered a mechanism of disease for preterm labor and preterm PROM [25], which reflects a clinical manifestation of rejection.

The proposed mechanisms whereby maternal CD8⁺ T cells infiltrate the chorioamniotic membranes involve the generation of a T-cell chemokine gradient from the amniotic cavity [13]. Increased concentrations of the T-cell chemokines CXCL9, CXCL10, and CXCL11 are thought to be responsible for the chemotaxis of T cells from the peripheral circulation into the decidua and, subsequently, the chorioamniotic membranes [13, 25]. The receptor for CXCL9, CXCL10, and CXCL11 is CXCR3 [26–30]. The importance of CXCR3 in the mechanisms of allograft rejection is demonstrated by the observation that either gene deletion or protein neutralization protects against the pancreatic islets and cardiac allograft rejection [31, 32].

CXCR3 is involved in the chemotaxis of activated T cells, dendritic cells, and natural killer (NK) cells [33, 34] as well as angiogenesis [34–39]. This chemokine receptor is mainly expressed on T helper (Th)1 cells [40–43] and is up-regulated in activated lymphocytes recruited to the site of inflammation [34, 37]. The expression of CXCR3 and its ligands (CXCL9, CXCL10, and CXCL11) is increased during allograft rejection [44–62]. CXCR3 is also expressed by the placenta (villous cytotrophoblasts and syncytiotrophoblasts) [63, 65],

the fetal membranes, and the choriodecidua of women who deliver term or preterm [64, 65]. In addition, CXCL10 concentration in the amniotic fluid is greater in women who undergo spontaneous preterm labor with chronic chorioamnionitis than in those without this placental lesion [13, 66]. CXCL10 concentration also increases in the amniotic fluid during the mid-trimester of pregnancy in patients who undergo spontaneous preterm birth after 32 weeks of gestation compared to those who deliver at term [67]. Therefore, it is likely that CXCR3 plays a role in the recruitment of T cells in chronic inflammatory lesions of the placenta, which represents maternal anti-fetal rejection.

The objective of this study is to determine whether the amniotic fluid concentration of CXCR3 and its ligands changes in patients whose placentas had chronic chorioamnionitis or other placental lesions consistent with maternal anti-fetal rejection (villitis of unknown etiology and chronic deciduitis with plasma cells).

Materials and Methods

Characteristics of the study population

The retrospective case-control study included 425 patients in the following groups: 1) spontaneous preterm labor and delivery (n=92); 2) term in labor (n=68); and 3) term not in labor (n=265). All samples were obtained from the Bank of Biological Materials at Wayne State University, the Detroit Medical Center, and the Perinatology Research Branch of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) (Detroit, Michigan). The inclusion criteria were: 1) singleton gestation; 2) transabdominal amniocentesis performed for microbiological studies in patients with the diagnosis of preterm labor at less than 36 weeks of gestation [68]; 3) intraoperative transabdominal amniocentesis for research purposes in patients at term with and without labor; 4) a live-born fetus with available neonatal outcomes; and 5) an available placental pathology report. Patients were excluded if chromosomal or structural fetal anomalies or placenta previa were present.

All patients provided written informed consent, and the use of biological specimens and clinical data for research purposes was approved by the Institutional Review Boards of Wayne State University and NICHD.

Biological samples and analysis

Amniotic fluid samples were transported to the clinical laboratory in capped sterile syringes and cultured for aerobic and anaerobic bacteria, including genital *Mycoplasmas*. Evaluation of the white blood cell (WBC) count, glucose concentration, and Gram stain of the amniotic fluid samples was performed shortly after collection. Amniotic fluid samples were centrifuged at $1300 \times g$ for 10 minutes at 4°C shortly after collection and stored at -70°C until analysis. Amniotic fluid concentrations of CXCL9, CXCL10, and CXCR3 were measured using enzyme-linked immunosorbent assays (ELISA; CXCL9 and CXCL10 ELISA kits from R&D Systems, Minneapolis, MN, USA, and a CXCR3 ELISA kit from Cloud Clone, Houston, TX, USA). Immunoassays were performed following the manufacturers' instructions. CXCL9 and CXCL10 were captured by the specific pre-coated

monoclonal antibodies and then detected by enzyme-linked polyclonal antibodies that were also specific to the same targets, respectively. The color developed from the substrate was proportional to the amount of target, and the target concentrations (pg/mL) in the samples were interpolated from the standard curve. The CXCR3 kit utilized a pre-coated antibody and a biotin-conjugated antibody specific to CXCR3. The CXCR3 concentrations (ng/mL) in the samples were interpolated from the standard curve. Sensitivity and intra- and inter-assay coefficients of variations for each assay are displayed in Table 1.

Clinical definitions

Gestational age was determined by the last menstrual period and confirmed by ultrasound examination, or by ultrasound examination alone, if the sonographic determination of gestational age was not consistent with menstrual dating. Preterm labor was diagnosed by the presence of at least two regular uterine contractions every 10 minutes in association with cervical changes in patients with a gestational age between 20 and 36 6/7 weeks, which led to preterm delivery (defined as birth prior to the 37th week of gestation).

Study groups

Patients with spontaneous preterm labor and delivery and term delivery with and without labor were classified according to the presence or absence of chronic chorioamnionitis into the following study groups: 1) without chronic chorioamnionitis [spontaneous preterm labor (n=51), term in labor (n=48), and term not in labor (n=187)]; and 2) with chronic chorioamnionitis [preterm delivery (n=41), term in labor (n=20), and term not in labor (n=78)].

The presence of placental lesions associated with maternal anti-fetal rejection was examined as a secondary outcome in patients with spontaneous preterm delivery and term delivery with and without labor.

Sonographic assessment of the cervix

Transvaginal ultrasound examinations were performed using commercially available ultrasound systems (Acuson Sequoia, Siemens Medical Systems, Mountain View, CA, USA; Voluson 730 Expert™ or Voluson E8, GE Healthcare, Milwaukee, WI, USA) equipped with endovaginal transducers with frequency ranges of 5–7.5 MHz and 5–9 MHz, respectively. All sonographic examinations of the cervix were performed using a previously described technique [69, 70].

Placental examination

Placentas were collected in dry plastic containers with airtight lids and labeled by research personnel. The umbilical cord was cut at the place of insertion into the chorionic plate. Membrane rolls and pieces of the umbilical cord and placental disc (selected using a random-sequence generator for the purposes of placental sampling) were obtained within 30 minutes of delivery and placed in formalin. After overnight fixation in formalin, the tissue samples were embedded in paraffin and were further processed for histologic examination. A five micron-thick Hematoxylin and Eosin (H&E) section was taken from each paraffin block. The H&E sections of the chorioamniotic membrane roll (n = 2), umbilical cord (n =

2) and placental disc (n = 2) were examined by pathologists who were blinded to the clinical diagnoses and outcomes.

The diagnosis of acute histologic chorioamnionitis was based on the presence of acute inflammatory changes in the extra-placental chorioamniotic membrane roll and/or the chorionic plate of the placenta, using previously described criteria [71, 72]. The grading and staging of placental lesions consistent with amniotic fluid infection is defined according to the Amniotic Fluid Infection Nosology Committee of the Perinatal Section of the Society for Pediatric Pathology as reported by Redline et al [73]. Chronic placental inflammatory lesions included: 1) chronic chorioamnionitis; 2) villitis of unknown etiology; and 3) chronic deciduitis. Chronic chorioamnionitis was diagnosed when lymphocytic infiltration into the chorionic trophoblast layer or chorioamniotic connective tissue was observed [13, 25]. Villitis of unknown etiology was defined as the presence of lymphohistiocytic infiltration of varying proportions of the placental villous tree [19, 74, 75]. Chronic deciduitis was defined as the presence of lymphocytic infiltration into the decidua of the basal plate [76].

Placental lesions consistent with maternal anti-fetal rejection proposed by our group included chronic chorioamnionitis, villitis of unknown etiology, or chronic deciduitis with plasma cells [14, 19].

Statistical Analysis

All analyses were performed using the R software package [77]. The two-sided, two-sample Wilcoxon test (also known as the Mann-Whitney U test) was used to compare differences between the groups, and the obtained p-values were adjusted, for multiple comparisons, using the Holm-Bonferroni method. A p-value cut-off of 0.05 was used to determine significance. The Fisher's exact test was used for categorical variables. A linear model was used to compare the groups while adjusting for gestational age at amniocentesis.

Results

Clinical characteristics of the study population

The demographic and clinical characteristics are shown in Table 2. As expected, gestational age at amniocentesis and at delivery as well as birthweight were significantly lower in patients with spontaneous preterm delivery than in those who delivered at term ($p < 0.001$ for each).

In the preterm labor group, the median interval from amniocentesis to delivery was one day (range: 0 to 21 days).

Amniotic fluid CXCR3, CXCL9, and CXCL10 concentrations are higher in spontaneous preterm deliveries with placental lesions consistent with maternal anti-fetal rejection

Among patients presenting with spontaneous preterm labor who delivered preterm, the median of amniotic fluid CXCR3, CXCL9, and CXCL10 concentrations was significantly higher in those who had placental lesions consistent with maternal anti-fetal rejection compared to those without these lesions [CXCR3, CXCL9, and CXCL10; preterm delivery with placental lesions consistent with maternal anti-fetal rejection: median (IQR) 17.24

(ng/mL) (6.79–26.68); 0.35 (ng/mL) (0.16–0.86); and 3.12 (ng/mL) (1.46–4.4) versus preterm delivery without placental lesions consistent with maternal anti-fetal rejection: median (IQR) 8.79 (ng/mL) (4.98–14.7); 0.10 (ng/mL) (0.07–0.18); and 1.12 (ng/mL) (0.65–1.47); $p=0.028$, $p<0.001$, and $p<0.001$, respectively] (Figure 1).

Amniotic fluid CXCL9 and CXCL10, but not CXCR3, concentrations are higher in spontaneous preterm deliveries with chronic chorioamnionitis

The median amniotic fluid CXCL9 and CXCL10, but not CXCR3, concentrations were significantly higher in patients with spontaneous preterm labor and delivery whose placentas had chronic chorioamnionitis than in those without chronic chorioamnionitis [CXCR3, CXCL9, and CXCL10 (median and interquartile range; IQR): preterm delivery with chronic chorioamnionitis: 17.02 (ng/mL) (5.57–26.68); 0.34 (ng/mL) (0.16–0.67); and 2.94 (ng/mL) (1.52–4.37) versus preterm delivery without chronic chorioamnionitis: 10.37 (ng/mL) (5.01–17.81); 0.12 (ng/mL) (0.07–0.27); and 1.19 (ng/mL) (0.66–1.63); $p=0.283$, $p=0.006$, and $p<0.001$, respectively] (Figure 2).

CXCR3, CXCL9, and CXCL10 concentrations in the amniotic fluid of patients in labor at term

Median CXCR3, CXCL9, and CXCL10 concentrations in the amniotic fluid were not significantly different in patients with labor at term whose placenta had chronic chorioamnionitis compared to those without chronic chorioamnionitis, those whose placental lesions were consistent with maternal anti-fetal rejection and to those without placental lesions consistent with maternal anti-fetal rejection (Table 3).

Amniotic fluid CXCR3 concentration is higher in patients with spontaneous preterm labor and chronic chorioamnionitis

Among patients with chronic chorioamnionitis, the median amniotic fluid concentration of CXCR3, but not of CXCL10 or CXCL9, was significantly higher in patients with spontaneous preterm labor compared to those with labor at term [CXCR3, CXCL10, CXCL9, median (IQR): preterm delivery with chronic chorioamnionitis: 17.02 (ng/mL) (5.57–26.68); 2.94 (ng/mL) (1.52–4.37); and 0.34 (ng/mL) (0.16–0.67) versus term in labor with chronic chorioamnionitis 2.57 (ng/mL) (0.37–5.38); 1.55 (ng/mL) (0.25–2.14); 0.16 (ng/mL) (0.03–0.27); $p<0.001$ for CXCR3 and not significant for CXCL10 and for CXCL9 after adjusting for gestational age] (Figure 3A).

Similar results were found in patients whose placentas were without chronic chorioamnionitis [CXCR3, CXCL9, CXCL10, median (IQR): spontaneous preterm labor and delivery without chronic chorioamnionitis 10.37 (ng/mL) (5.01–17.81); 1.19 (ng/mL) (0.66–1.63); and 0.12 (ng/mL) (0.07–0.27) versus term in labor without chronic chorioamnionitis: 1.39 (ng/mL) (0.51–3.27); 0.5 (ng/mL) (0.27–0.74); and 0.1 (ng/mL) (0.07–0.15); $p<0.01$ for CXCR3 and not significant for CXCL9 and CXCL10 after adjusting for gestational age] (Figure 3B).

Amniotic fluid CXCR3 and CXCL9 concentrations are higher in patients with spontaneous preterm labor and placental lesions consistent with maternal anti-fetal rejection

Among patients presenting with spontaneous preterm labor who subsequently delivered preterm, the median amniotic fluid concentrations of CXCR3 and CXCL9, but not CXCL10, were significantly higher in those who had placental lesions consistent with maternal anti-fetal rejection than in those with labor at term who had similar lesions [preterm labor and delivery with placental lesions consistent with maternal anti-fetal rejection: median (IQR) 17.24 (ng/mL) (6.79–26.68); 0.35(ng/mL) (0.16–0.86); 3.12 (ng/mL) (1.46–4.4) versus term in labor with these placental lesions: median (IQR) 2.57 (ng/mL) (0.37–5.48); 0.13(ng/mL) (0.06–0.25); and 0.76 (ng/mL) (0.32–1.85); $p<0.001$ for CXCR3 and $p=0.003$ for CXCL9 and not significant for CXCL10 after adjusting for gestational age] (Figure 4a).

Similarly, among patients presenting with spontaneous preterm labor who subsequently had a preterm delivery, the median amniotic fluid CXCR3, but not CXCL9 and CXCL10, concentration was significantly higher in those who did not have placental lesions consistent with maternal anti-fetal rejection than in patients with labor at term without these lesions [CXCR3, CXCL9, and CXCL10; preterm labor and delivery without placental lesions consistent with maternal anti-fetal rejection: median (IQR) 8.79 (ng/mL) (4.98–14.7); 0.10 (ng/mL) (0.07–0.18); 1.12 (ng/mL) (0.65–1.47) versus term in labor without these placental lesions: median (IQR) 0.99 (ng/mL) (0.51–2.98); 0.1(ng/mL) (0.06–0.15); and 0.44 (ng/mL) (0.26–0.75); $p<0.001$ for CXCR3 and not significant for CXCL9 and CXCL10 after adjusting for gestational age] (Figure 4B).

CXCR3, CXCL9, and CXCL10 concentrations in the amniotic fluid of patients at term with and without labor

Median CXCR3, CXCL9, and CXCL10 concentrations in the amniotic fluid were not significantly different between patients at term with and without labor, regardless of the presence or absence of chronic chorioamnionitis and placental lesions consistent with maternal anti-fetal rejection.

Discussion

Principal findings of the study

1) CXCR3 is present in human amniotic fluid at the third trimester of pregnancy; 2) patients with spontaneous preterm labor who deliver preterm and who have placental lesions consistent with maternal anti-fetal rejection have higher median amniotic fluid CXCR3, CXCL9, and CXCL10 concentrations than those without these placental lesions; 3) among patients with placental lesions consistent with maternal anti-fetal rejection, those with spontaneous preterm labor and delivery have higher median CXCR3 and CXCL9 concentrations in the amniotic fluid compared to those with labor at term; 4) among patients with the absence of placental lesions consistent with maternal anti-fetal rejection, those with spontaneous preterm labor and delivery have a higher median CXCR3 concentration in the amniotic fluid than those with labor at term. These findings strongly suggest that the concentrations of chemokine ligands and their soluble receptors reflect pathologic chronic inflammation; and 5) spontaneous labor at term is not associated with a change in the

amniotic fluid concentrations of CXCR3, CXCL9, and CXCL10, indicating that physiologic labor at term does not increase the concentration of these chemokines or their receptors in the amniotic cavity.

CXCR3 functions and its ligands

The CXCR3 receptor is a transmembrane G protein-coupled receptor that belongs to the CXC chemokine family [34, 37], and its gene is located on chromosome Xq13 [78]. This receptor is expressed on several immune cells, including activated T cells [40–43], dendritic cells [79, 80], and NK cells [81], but not on resting T cells, B cells, monocytes, or granulocytes [34]. Non-immune cells such as fibroblasts and endothelial, epithelial, and smooth-muscle cells also express this receptor [38, 82].

CXCR3-deficient mice show a normal phenotype [32, 83, 84]; however, these mice display a diminished recruitment of mononuclear cells and CD8⁺ T cells in the meninges in a model of lymphocytic choriomeningitis virus infection [83]. Similar results were observed in a model of bleomycin-induced lung injury where CXCR3-deficient mice showed a decreased recruitment of CD8⁺ T cells, NK cells, and natural killer T (NKT) cells in the lung and liver [85]. These observations suggest that CXCR3 is required for inflammation-induced recruitment of CD8⁺ T cells, NK cells, and NKT cells.

CXCR3 has three splice variants: 1) CXCR3A, 2) CXCR3B, and 3) CXCR3-alt [34, 38]. CXCR3A is mainly expressed on most cells, including leukocytes [33, 78], while endothelial cells express CXCR3B [63]. There are five chemokines known as CXCR3 ligands: 1) CXCL4 (platelet factor 4), 2) CXCL4L1 (platelet factor 4 variant), 3) CXCL9 (monokine induced by IFN- γ or MIG), 4) CXCL10 (IFN- γ -induced protein 10 or IP-10), and 5) CXCL11 (IFN- γ -inducible T-cell α chemoattractant or I-TAC) [34]. CXCL9, CXCL10, and CXCL11 are also recognized as IFN- γ -inducible CXCR3 ligands, as they are induced by this cytokine and others, such as tumor necrosis factor (TNF)- α [27, 29, 86, 87]. In contrast, CXCL4 and CXCL4L1 are produced by activated platelets and are not induced by IFN- γ [88, 89]. All of these chemokines bind to both CXCR3A and CXCR3B, with the exception of CXCL11, which binds to CXCR3-alt [27, 33, 63, 80, 90].

The CXCR3 receptor-ligand system plays a central role in both the chemotaxis of immune cells [34, 37, 91] and angiogenesis [34–39]. CXCR3A activation induces chemotactic and proliferative responses [38]; whereas, CXCR3B activation mediates anti-proliferative and angiostatic effects on endothelial cells [38]. The binding of IFN- γ -inducible CXCR3 ligands results in the regulation of T-cell trafficking [26], increased production of Th1 cytokines and diminished synthesis of Th2 cytokines [37, 41, 43, 92]. However, the binding of platelet-derived CXCR3 ligands induces angiostasis [34, 93]. Due to the pleiotropic nature of CXCR3, the involvement of this receptor has been described in inflammatory, autoimmune, and angiogenesis-related disorders such as inflammatory arthritis [94–97], systemic sclerosis [97–100], type I diabetes [97, 101, 102], transplant rejection [44–62], and cancer [36, 38, 103–105].

The role of CXCR3 in transplant rejection

There is compelling evidence that CXCR3 and its ligands (CXCL9, CXCL10, and CXCL4) are implicated in the pathogenesis of transplant rejection [34, 36, 44–62, 106]. For example, an increase in the concentration of CXCR3 ligands and the infiltration of CXCR3+ T cells has been demonstrated in biopsies from rejected solid organ transplants, including lungs [44], kidneys [107], skin [54], and endomyocardial tissue [46]. Further, the significance of CXCR3 ligands (CXCL9 and CXCL10) and the recruitment of CXCR3+ T cells in transplant rejection have been shown in several *in vivo* models of allograft rejection, such as the lungs [49], heart [32], and small intestine [108]. Given the chemotactic roles of CXCR3 on T cells, the blockage of CXCR3 can reduce an inflammatory response, and this could be of benefit in controlling allograft rejection. For example, in a model of cardiac transplantation, CXCR3-deficient mice showed an increased tolerance to the development of acute allograft rejection [32]. These findings are consistent with previous reports demonstrating that CXCR3 blockage can delay rejection time and inhibit acute and chronic allograft rejection in murine models of cardiac and pancreatic islet transplants [109–111]. In humans, during acute renal allograft rejection, the expression of CXCR3 on peripheral CD4⁺ T cells increases after three days and remains elevated for two weeks [112]. In addition, the mRNA abundance of CXCR3 and CXCL10 in the urinary cells is higher in patients with acute renal allograft rejection than in healthy individuals [113]. Therefore, it has been suggested that the expression of CXCR3 and CXCL10 could be used as a urinary biomarker to predict acute renal allograft rejection [113]. Collectively, CXCR3 and its ligands play a role in the development of transplant rejection. For a more comprehensive review on the role of CXCR3 ligands (CXCL9 and CXCL10) in transplant rejection, the reader is referred to specific reviews [51, 59, 106].

A role for CXCR3, CXCL9, and CXCL10 in the placental lesions consistent with maternal anti-fetal rejection

Herein, we report that the median amniotic fluid concentrations of CXCR3 as well as its ligands (CXCL9 and CXCL10) are higher in patients with placental lesions consistent with maternal anti-fetal rejection (villitis of unknown etiology, chronic deciduitis, and chronic chorioamnionitis) than in those without these placental lesions. Patients who undergo spontaneous preterm labor have an increase in these chemokines in the amniotic fluid compared to those with labor at term.

Chronic chorioamnionitis is characterized by the infiltration of maternal CD8⁺ T lymphocytes into the chorioamniotic membranes [25, 114–116], which can induce trophoblast apoptosis [11, 25]. The proposed chemotactic signals responsible for the migration of T cells from the decidua into the chorioamniotic membranes are the CXCR3 ligands, such as amniotic fluid CXCL10 and the up-regulation of CXCL9, CXCL10, and CXCL11 in the chorioamniotic membranes [13, 25, 66]. Therefore, the observations from the current study are consistent with previous studies demonstrating that these chemokines, including their receptor (CXCR3), play a role in the pathogenesis of chronic chorioamnionitis.

The placental lesions associated with maternal anti-fetal rejection are chronic chorioamnionitis [11, 13, 14, 20–22, 117], villitis of unknown etiology [19, 21, 23, 24, 118, 119], and chronic deciduitis with plasma cells [21] as evidenced by the presence of cell- and antibody-mediated immune responses. For an in-depth appraisal on this topic, please refer to the review by Kim et al [25].

A role for CXCR3, CXCL9, and CXCL10 in preterm labor

CXCR3 is expressed by the placenta (villous cytotrophoblasts and syncytiotrophoblasts) [63, 65] and fetal membranes [64, 65]. This receptor has been proposed to play a role in preterm [65] and term [64] labor processes. Specifically, CXCR3A protein expression is high in spontaneous preterm labor; whereas, CXCR3B protein expression is up-regulated in spontaneous term delivery [65]. In addition, CXCR3 mRNA abundance in the choriodecidual leukocytes is increased in spontaneous term labor [64].

It has been reported that the activation of CXCR3 signaling may impair maternal-fetal tolerance and predispose to spontaneous preterm labor [65] and *Listeria monocytogenes*-induced fetal death [120]. Also, there is an association between a fetal CXCR3 polymorphism (rs2280964) and spontaneous preterm birth [odds ratio: 0.52; 95% confidence interval (CI): 0.32–0.86] [65]. This specific polymorphism is associated with increased CXCL9 concentrations in the umbilical cord blood of newborns who were delivered preterm [65]. In an intra-peritoneal lipopolysaccharide model of spontaneous preterm birth, CXCR3-deficient mice have shown a decrease in interleukin (IL)-6 and CCL2 (also known as MCP-1) concentrations in the amniotic fluid compared to wild-type mice [65]. In addition, mice that receive a CXCR3 blocking agent or are CXCR3-deficient were protected against *Listeria monocytogenes*-induced fetal death [120]. This effect is potentially mediated by a reduction in the expression of CXCL9 by innate immune cells and/or a diminished influx of fetal-specific CD8⁺ T cells into the maternal-fetal interface [120, 121]. These observations suggest that the CXCL9/CXCR3 pathway regulates the infiltration of maternal immune cells (fetal-specific cytotoxic T cells) into the maternal-fetal interface, which can participate in maternal-fetal tolerance during pregnancy. A disruption of this pathway may be implicated in the mechanisms that lead to fetal death and premature labor.

A role for T cells in pregnancy

During pregnancy, maternal T cells recognize fetal antigens through interactions with antigen-presenting cells [122, 123]. Fetal antigen-specific Tregs (regulatory T cells) maintain maternal-fetal tolerance throughout pregnancy [3, 4, 124]. In contrast, effector T cells infiltrate into the maternal-fetal interface and are implicated in the processes of term [64, 125–127] and preterm [17, 25, 128–130] parturition.

Recently, we provided evidence that effector CD4⁺ T cells are involved in the process of parturition at term [64], and that activation of T cells by injecting a monoclonal antibody against the CD3 complex can induce preterm labor and birth [130]. Effector T cells are preferentially recruited into the zone rupture of the fetal membranes in spontaneous labor at term, a process mediated by CXCL10 and CCL5 [64, 126, 131]. Specifically, we have provided evidence that decidual CD4⁺ T cells are more abundant in spontaneous labor at

term than in preterm and term gestations without labor [64]. These T cells express CD45RO, but not CD45RA [64], which suggests that they are memory T cells generated early in pregnancy when fetal-antigen presentation occurs [4, 122, 132]. The fact that decidual CD4⁺ T cells express IL-1 β , TNF- α , and MMP-9 (i.e. labor mediators [133–142]) during spontaneous labor at term [64], as well as activation markers such as CD25 [143], suggests that the adaptive limb of the immune system participates in the process of parturition.

Another effector CD4⁺ T-cell subset that infiltrates the human decidua comprises the Th17 cells [144]. The tissue density of Th17 cells is higher in cases with acute chorioamnionitis than in cases without this placental lesion [145]. This finding further supports our hypothesis that pro-inflammatory effector T cells at the maternal-fetal interface are implicated in the pathophysiology of chronic chorioamnionitis, a lesion associated with preterm labor and birth [25].

Cytotoxic T cells (CD8⁺ T cells, or CTLs) are present at the maternal-fetal interface in term gestations in the absence of labor, where they express perforin and granzyme B [146–148]. In the placenta, CTLs are abundant in cases with villitis of unknown etiology and express T-cell chemokine receptors (CXCR3 and CCR5) [19]. In the peripheral circulation, CD300a⁺ CTLs have an effector-memory phenotype, and their proportions are higher in women with chronic chorioamnionitis than in women without this placental lesion [117]. Taken together, these data suggest that CTLs may participate in the chronic pathological inflammatory response associated with term and preterm labor.

Conclusion

CXCR3 is detectable in the amniotic fluid, and elevated amniotic fluid CXCR3, CXCL9, and CXCL10 concentrations are associated with the presence of placental lesions consistent with maternal anti-fetal rejection. These findings indicate that CXCR3 concentration in the amniotic fluid may serve as a potential marker of maternal anti-fetal rejection in a subset of patients with spontaneous preterm delivery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Figure 2.

Figure 3A.

Figure 3B.

Figure 4A.

Figure 4B.

Table 1

Assay characteristics for amniotic fluid CXCL3, CXCL9, and CXCL10

Analytes	Sensitivity (pg/mL)	Intra-assay coefficients of variations (%)	Inter-assay coefficients of variations (%)
CXCR3	146	7.95	19.3
CXCL9	14.9	5.34	4.29
CXCL10	3.29	5.96	5.50

Table 2

Demographic and clinical characteristics of the study population

	Spontaneous preterm labor (n=92)	P-value*	Term delivery without labor (n=265)	Term delivery with labor (n=68)	P-value
Maternal age (years)	23 (21–28)	0.17	26 (23–30)	25.5 (21.25–29.0)	0.04
Nulliparity (%)	26.1% (24)	1	7.2% (19)	25% (17)	<0.001
Gestational age at amniocentesis (weeks)	32.7 (29.4–34.3)	<0.001	39 (38.9–39.3)	39 (38.4–40.3)	0.38
Gestational age at delivery (weeks)	33.4 (30.0–35.3)	<0.001	39 (38.9–39.3)	39 (38.45–40.3)	0.38
Birth weight (grams)	2,003 (1,243–2,468)	<0.001	3,375 (3,130–3,655)	3,323 (3,110–3,685)	0.76

Data are presented as median (interquartile range) or % (n);

* Comparison between spontaneous preterm labor and term delivery with labor.

Table 3

Amniotic fluid CXCR3, CXCL9, and CXCL10 concentrations in patients with spontaneous term labor according to the presence or absence of chronic chorioamnionitis/placental lesions consistent with maternal anti-fetal rejection

Analytes	Term Labor			Term Labor		
	Without chronic chorioamnionitis (n=48)	With chronic chorioamnionitis (n=20)	P-value	Without placental lesions consistent with maternal anti-fetal rejection (n=39)	With placental lesions consistent with maternal anti-fetal rejection (n=29)	P-value
	Median (IQR) (ng/mL)	Median (IQR) (ng/mL)		Median (IQR) (ng/mL)	Median (IQR) (ng/mL)	
CXCR3	1.39 (0.51–3.27) *	2.57 (0.37–5.38)	1	0.99 (0.51–2.98)	2.57 (0.37–5.48) *	1
CXCL9	0.1 (0.007–0.15)	0.16 (0.03–0.27)	1	0.1 (0.06–0.15)	0.13 (0.06–0.25)	1
CXCL10	0.5 (0.27–0.74)	1.55 (0.25–2.14)	0.334	0.44 (0.26–0.75)	0.76 (0.32–1.85)	0.463

* Data are not available for one case; IQR: interquartile range