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## A role for CXCL13 (BCA-1) in pregnancy and intra-amniotic infection/inflammation

Chia-Ling Nhan-Chang, MD<sup>1,2</sup>, Roberto Romero, MD<sup>1,3</sup>, Juan Pedro Kusanovic, MD<sup>1</sup>, Francesca Gotsch, MD<sup>1</sup>, Samuel S. Edwin, PhD<sup>1,3</sup>, Offer Erez, MD<sup>2</sup>, Pooja Mittal, MD<sup>1,2</sup>, Chong Jai Kim, MD, PhD<sup>1,4</sup>, Mi Jeong Kim, MD<sup>1,3</sup>, Jimmy Espinoza, MD<sup>1,2</sup>, Lara A. Friel, MD<sup>1,2</sup>, Edi Vaisbuch, MD<sup>1</sup>, Nandor Gabor Than, MD, PhD<sup>1</sup>, Shali Mazaki-Tovi, MD<sup>1,2</sup>, and Sonia S. Hassan, MD<sup>1,2</sup>

<sup>1</sup>Perinatology Research Branch, Intramural Division, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NICHD/NIH/DHHS, Hutzel Women's Hospital, Bethesda, MD, and Detroit, MI

<sup>2</sup>Department of Obstetrics and Gynecology, Wayne State University/Hutzel Women's Hospital, Detroit, MI

<sup>3</sup>Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI

<sup>4</sup>Department of Pathology, Wayne State University School of Medicine, Detroit, MI

### Abstract

**Objective**—CXCL13 is a potent chemokine, produced by mature and recently recruited macrophages to sites of inflammation, which has anti-microbial and anti-angiogenic properties. The purpose of this study was to determine whether CXCL13 is present in maternal serum, umbilical cord blood and amniotic fluid (AF); if AF concentration changes with intra-amniotic infection/inflammation (IAI); and localize the production of CXCL13 in chorio-amniotic membranes and umbilical cord.

**Study design**—A cross-sectional study on maternal serum was performed including patients in the following groups: 1) non-pregnant women (n=20); 2) normal pregnant women (n=49); 3) patients at term not in labor (n=30); and 4) patients in spontaneous labor at term (n=29). Umbilical cord blood was collected from term neonates with (n=30) and without labor (n=28). Amniotic fluid was attained from patients in the following groups: 1) midtrimester (n=65); 2) term not in labor (n=22); 3) term in labor (n=47); 4) preterm labor (PTL) with intact membranes leading to term delivery (n=70); and 5) PTL leading to preterm delivery with IAI (n=79) and without IAI (n=60). CXCL13 concentrations were determined by ELISA. Chorio-amniotic membranes and umbilical cords were examined with immunohistochemistry. Non-parametric statistics were used for analysis.

**Results**—1) CXCL13 was present in 100% of serum and cord blood samples, and 99% of AF samples (339/343); 2) Serum CXCL13 concentration was significantly higher in pregnant women when compared to non-pregnant women [median 313.3 pg/mL (IQR: 197.2–646.9) vs. 40.5 pg/mL (IQR: 29.5–93.5), respectively; p<0.001]; 3) Serum CXCL13 concentration decreases with advancing gestational age (Spearman's rho = -0.424; p<0.001); 4) There were no significant differences in the median serum CXCL13 concentration between women at term with and without labor [371.6 pg/mL (IQR: 194.3–614.3) vs. 235.1 pg/mL (IQR: 182.8–354.7), respectively];

p=0.6]; 5) The concentration of CXCL13 in AF did not change with gestational age (p=0.11); 6) Patients with PTL and delivery with IAI had a significantly higher median concentration of CXCL13 than those without IAI [median 513.2 pg/mL (199.7–2505.5) vs 137.3 pg/mL (96.7–209.6), respectively; p<0.001] and those who delivered at term [133.7 pg/mL (97.8–174.8); p<0.001]; 7) Spontaneous labor did not result in a change in the median AF concentration of CXCL13 [labor: 86.9 pg/mL (55.6–152) vs no labor: 77.8 pg/mL (68–98); p=0.75]; 8) CXCL13 was immunolocalized to macrophages in fetal membranes and umbilical vein.

**Conclusions**—1) We report for the first time the presence of CXCL13 in AF; 2) AF CXCL13 concentrations are dramatically increased in intra-amniotic infection/inflammation; 3) Unlike other chemokines, AF and serum CXCL13 concentrations did not change with spontaneous parturition.

### Keywords

amniotic fluid; BLC; chorioamnionitis; preterm delivery; preterm labor

## INTRODUCTION

The preterm parturition syndrome can be caused by multiple pathologic processes, however, the only process that has been proven to be causal is intra-uterine infection/inflammation.<sup>1–5</sup> The understanding of the specific roles and the identification of all the key components of the innate inflammatory defense system is still a work in progress, and preterm labor and its related adverse consequences are on the increase.<sup>6</sup>

Successful pregnancy requires tolerance to the fetal allograft as well as effective protection against infection. The mechanisms responsible for immune tolerance during pregnancy have been the subject of investigation for more than 30 years and remain controversial. On the other hand, a complex system exists to protect the conceptus and mother from infection. This system involves physical barriers to infection such as the mucous plug,<sup>7–11</sup> placenta,<sup>12</sup> fetal membranes,<sup>13,14</sup> and components of the innate and adaptive immune system such as neutrophils, macrophages, natural killers cells and trophoblasts.<sup>12,15–19</sup> Many of these cells require recruitment to the site of inflammation which is accomplished by chemokines.

Chemokines are classified into four families by the structure of the conserved cysteine-motif in the amino-terminus of the proteins.<sup>20</sup> Less than half of recognized chemokines have been identified in amniotic fluid (AF), and several have been associated with preterm birth, intra-amniotic infection or inflammation including CCL2(MCP-1),<sup>17</sup> CCL3(MIP-1 $\alpha$ ), CCL5(RANTES),<sup>21</sup> CXCL1(GRO- $\alpha$ ),<sup>22</sup> and CXCL8(IL-8)<sup>23</sup> among others.<sup>24–29</sup>

CXCL13, a chemokine previously named B-lymphocyte chemoattractant (BLC) in mice<sup>30</sup> or B cell-attracting chemokine-1 (BCA-1) in humans,<sup>31</sup> was first identified in 1998 in liver and lymph nodes. CXCL13 is a potent chemokine secreted by monocytes, lymphocytes and dendritic cells<sup>32</sup> and is detected in serum,<sup>33</sup> normal lymphoid tissue and in acute and chronically inflamed tissue.<sup>34</sup> Its role in the inflammatory response is to attract B and T lymphocytes to areas of infection and inflammation.<sup>35</sup> CXCL13 is involved in normal chemotaxis in response to routine physiological stress in the lymph nodes during development.<sup>36</sup> In other tissues, parasitic and microbial exposure induces local production of CXCL13 leading to the formation of ectopic germinal centers,<sup>37</sup> which serve as tertiary lymphoid organs in inflamed and infected tissues.

In pregnancy, the identification of CXCL13 has been reported only in cases of placental malaria.<sup>38</sup> Present in both acute and chronic placental malaria, CXCL13 expression in placenta was up-regulated >130 fold in active malaria and >1,000 fold in cases of massive

malarial infection. In addition, CXCL13 has been observed to form lymphoid-like aggregates similar to ectopic germinal centers in other tissues.

The purpose of this study was to determine if: 1) CXCL13 is present in maternal serum and the amniotic cavity in normal pregnancy; 2) concentrations of CXCL13 in AF and maternal serum change with increasing gestational age and in the presence of spontaneous labor at term; 3) CXCL13 concentrations in AF is affected by the presence of intra-amniotic infection/inflammation; 4) CXCL13 is present in cord blood of term neonates; and 5) if the production of CXCL13 can be localized in chorio-amniotic membranes and umbilical cord.

## MATERIALS AND METHODS

### Study design and population

A cross-sectional study was conducted by searching our clinical database and bank of biological samples including patients in the following groups: 1) non-pregnant women in the secretory phase of their cycle (n=20); 2) normal pregnant women (n=49); 3) patients at term not in labor (n=30); and 4) patients in spontaneous labor at term (n=29). Umbilical cord blood from term neonates was collected from mothers with (n=30) and without labor (n=28).

For the analysis of CXCL13 in AF, a cross-sectional study was conducted including patients in the following groups: 1) normal women in the mid-trimester of pregnancy (16–18 weeks) who underwent amniocentesis for genetic indications and delivered normal neonates at term (n=65); 2) normal pregnancies at term ( $\geq 37$  weeks) with (n=47) and without (n=22) spontaneous labor; and 3) women with an episode of spontaneous preterm labor (PTL) and intact membranes who were further subdivided into three groups: a) PTL who delivered at term without intra-amniotic infection/inflammation (IAI) (n=70); b) PTL who delivered preterm without IAI (n=60); and c) PTL who delivered preterm with IAI (n=79).

All patients provided written informed consent prior to the collection of samples. The collection and utilization of samples for research purposes was approved by the Institutional Review Boards of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD/NIH/DHHS, Bethesda, MD) and participating institutions. These samples have been previously utilized in published studies of other inflammatory markers and antimicrobial peptides in normal and abnormal pregnancies.

### Definitions

Patients were considered to have a normal pregnancy if they did not have any medical, obstetrical, or surgical complications, and delivered a term neonate ( $\geq 37$  weeks) of appropriate birth weight for gestational age.<sup>39,40</sup> Spontaneous preterm labor was defined by the presence of at least two regular uterine contractions every 10 minutes associated with cervical changes before 37 completed weeks of gestation, requiring admission to the hospital. Intra-amniotic infection was defined as a positive AF culture for microorganisms. Intra-amniotic inflammation was defined as AF interleukin-6 (IL-6) concentration  $>2.6$  ng/mL.<sup>41</sup>

### Amniotic fluid collection

Amniotic fluid samples were obtained by transabdominal amniocentesis performed for genetic indications, evaluation of microbial status of the amniotic cavity, or assessment of fetal lung maturity in patients approaching term. The AF samples were transported to the laboratory in a sterile capped syringe. AF samples from patients with PTL were cultured for aerobic/anaerobic bacteria and genital mycoplasmas. White blood cell (WBC) count, glucose concentration and Gram-stain were also performed after collection as previously

described.<sup>41–45</sup> The results of these tests were used for subsequent clinical management. In addition, IL-6 concentrations were determined from the AF of PTL patients the results were used only for research purposes. AF not utilized for clinical assessment was centrifuged for 10 minutes at 4°C and the supernatant was aliquoted and stored at –70°C until analysis.

### Human CXCL13 immunoassays

Concentrations of CXCL13 in AF, maternal and umbilical cord serum were determined using specific and sensitive enzyme-linked immunosorbent assays obtained from R&D Systems, Inc. (Minneapolis, MN, USA) as per manufacturer's instructions. AF CXCL13 assays were validated in our laboratory prior to the conduction of this study. Validation included spike and recovery experiments, which produced parallel curves indicating that AF matrix constituents did not interfere with antigen-antibody binding in this assay system. The calculated inter-assay coefficients of variation for serum and AF in our laboratory were 7.2% and 3.9%, respectively. The calculated intra-assay coefficients of variation for serum and AF were 4.9% and 3.0%, respectively. The sensitivity was calculated to be 3.7 pg/mL for serum and 2.8 pg/mL for AF.

### Immunofluorescence staining

Two frozen specimens with chorioamnionitis and funisitis and one control case with no evidence of inflammation were selected to determine the immuno-localization of CXCL13 in fetal membranes and umbilical cord. Immunofluorescence staining was performed on 5 µ-thick frozen sections after fixation with 4% paraformaldehyde. Following permeabilization with 0.25% TritonX-100 and blocking, sections were incubated with a rabbit polyclonal anti-CXCL13 antibody (ProteinTech Group, IL, USA). Alexa Fluor 594-labeled donkey anti-rabbit Ig (Invitrogen, CA, USA) was used as the secondary antibody. For double staining, sections were further incubated with a murine monoclonal anti-HLA-DR antibody (Dako, Glostrup, Denmark), and Alexa Fluor 488-labeled goat anti-mouse Ig (Invitrogen). The slides were mounted in ProLong Gold antifade reagent with DAPI (Invitrogen), and images were taken using a Leica TCS SP5 spectral confocal system (Leica Microsystems GmbH, Wetzlar, Germany).

### Statistical analysis

Amniotic fluid and serum CXCL13 concentrations were not normally distributed. Kruskal-Wallis with post-hoc analysis and Mann-Whitney U tests were used for the evaluation of continuous variables. Spearman's Rho was utilized to assess correlations. Among patients with PTL and intact membranes, receiver operating characteristic (ROC) curve analysis was employed for the identification of patients who had IAI. A survival analysis with Kaplan-Meier was performed to examine the amniocentesis-to-delivery interval according to AF CXCL13 concentrations. A *p*-value of <0.05 was considered statistically significant. The statistical package utilized was SPSS v.12.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Detection of CXCL13 in serum

Serum CXCL13 concentrations were detected in all patients. The median serum CXCL13 concentration was significantly higher in pregnant women when compared to non-pregnant women [median 313.3 pg/mL (interquartile range [IQR]: 197.2–646.9) vs. median 40.5 pg/mL (IQR: 29.5–93.5), respectively; *p*<0.001] (Figure 1). In normal pregnancy, maternal serum CXCL13 concentration decreases with advancing gestational age from 8 to 40.7 weeks of gestation (Spearman's rho = –0.424; *p*<0.001). There were no significant differences in the median serum CXCL13 concentration between women at term with and

without labor [median 371.6 pg/mL (IQR: 194.3–614.3) vs. median 235.1 pg/mL (IQR: 182.8–354.7), respectively;  $p=0.6$ ].

### Detection of CXCL13 in amniotic fluid

Table 1 displays the demographic and clinical characteristics of patients of the study groups. Amniotic fluid CXCL13 was detected in 98.8% (339/343) of patients. Four patients had CXCL13 concentrations below the limit of detection (3 patients in the mid-trimester group and 1 in the term in labor group). All four patients delivered at term without complication. The median AF concentration of CXCL13 was not different between women in the midtrimester of pregnancy and women at term not in labor [median 63.7 pg/mL (IQR: 42.9–94.9) vs. median 77.8 pg/mL (IQR: 68–98), respectively;  $p=0.11$ ] (Figure 2a). Similarly, no differences were found in the median AF CXCL13 concentration between women not in labor at term and those with spontaneous labor [median 77.8 pg/mL (IQR: 68–98) vs. median 86.9 pg/mL (IQR: 55.6–152), respectively;  $p=0.8$ ] (Figure 2b).

### AF CXCL13 in preterm labor

Table 2 displays the demographic and clinical characteristics of patients with spontaneous preterm labor with intact membranes. Among patients with spontaneous PTL with intact membranes, those with IAI had a significantly earlier gestational age at amniocentesis and delivery than those without IAI. In addition, patients with PTL with IAI had a significantly higher median AF concentration of CXCL13 than those without IAI who delivered preterm [median 513.2 pg/mL (IQR: 199.7–2505.5) vs. median 137.3 pg/mL (96.7–209.6), respectively;  $p<0.001$ ]. Similarly, patients with PTL and IAI had significantly higher median AF concentrations of CXCL13 than those with an episode of PTL who delivered at term [median 133.7 pg/mL (IQR: 97.8–174.8),  $p<0.001$ ] (Figure 3a). The median AF CXCL13 concentration in patients with PTL without evidence of IAI were not different between those who subsequently deliver preterm and at term ( $p=0.8$ ).

### AF CXCL13 in preterm labor with evidence of histological chorioamnionitis

In patients with PTL who delivered within 72 hours of amniocentesis, placental pathology was available in 100% of them. Patients with histological chorioamnionitis and/or funisitis had higher median AF CXCL13 concentrations than those without [median 1201.1 pg/mL (IQR: 246.5–4643.8), vs median 216.0 pg/mL (IQR: 113.4–430.3), respectively;  $p=0.01$ ] (Figure 3b).

### Diagnostic cutoff and amniocentesis-to-delivery interval of an elevated AF CXCL13

The diagnostic efficacy of CXCL13 concentration in AF was assessed among patients with PTL and intact membranes. A cutoff of 260.2 pg/mL was derived from the ROC curve analysis for the identification of patients who had IAI (sensitivity 70.9%, specificity 89.2%; area under ROC curve 84%;  $p<0.001$ ) (Figure 4a). Survival analysis was employed to assess the relationship between IAI defined by CXCL13 concentration and the duration of the amniocentesis-to-delivery interval. Spontaneous labor and delivery was entered in the model as the event of interest and patients delivered for fetal or maternal indications were censored. The median amniocentesis-to-delivery interval was significantly shorter in patients whose AF CXCL13 concentrations were above 260.2 pg/mL (median amniocentesis-to-delivery interval: 4 days vs. 41 days;  $p<0.001$ ) (Figure 4b).

### Immunohistochemistry of fetal membranes and umbilical cord

Immunohistochemical analysis of chorioamniotic membranes with histological inflammation demonstrated that CXCL13 was present only in HLA-DR positive macrophages in both chorion and decidua of fetal membranes (Figure 5). In cases of

funisitis, fetal macrophages in the umbilical vein were also immunoreactive to CXCL13 (Figure 6a–d). Control sections of umbilical vein without funisitis also demonstrated evidence of CXCL13 immunoreactivity (Figure 6e–h), but was qualitatively diminished in comparison to cases with funisitis. In all cases, CXCL13 was localized mainly in the cytoplasm of the macrophages.

### Detection of CXCL13 in umbilical cord blood

In term neonates, CXCL13 was detected in all umbilical cord samples. The median concentration of CXCL13 in neonates born to mothers with and without labor was not significantly different [median 79.0 pg/mL (IQR: 62.5–103.8), vs. median 66.7 pg/mL (IQR: 58.5–88.0), respectively;  $p=0.2$ ].

## DISCUSSION

### Principal findings of this study

1) serum CXCL13 is increased with pregnancy and decreases as a function of gestational age, but did not change with spontaneous term labor; 2) CXCL13 was detectable in 98.8% of amniotic fluid samples; 3) CXCL13 concentration in AF was significantly increased in the presence of intra-amniotic infection and/or inflammation; 4) no changes were observed in AF CXCL13 concentration with advancing gestation or in the presence of spontaneous labor at term; 5) CXCL13 is present in chorioamniotic, decidual and umbilical cord macrophages, and the intracellular immunoreactivity of macrophages in the umbilical cord is increased in cases of histologic funisitis compared to those without evidence of histologic funisitis; and 6) CXCL13 was present in the umbilical cord sera of neonates at term, and its concentration does not change with labor.

### What is CXCL13?

Chemokines are a subgroup of chemo-attracting cytokines that include approximately 50 ligands involved in normal homeostasis in many organs and in the pathophysiology of infectious and inflammatory disease.<sup>46</sup> Chemokines are classified into four structural families according to the structure of the cysteine residues in the amino terminus (C, CC, CXC, CX<sub>3</sub>C). CXCL13 belongs to the CXC family of chemokines, all of whose genes are located on chromosome 4.<sup>47</sup> The only known receptor for CXCL13 is CXCR5, which exclusively binds CXCL13<sup>48</sup> and is expressed by mature B cells,<sup>49</sup> a subset of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, in secondary lymphoid tissue follicles<sup>50</sup> and immature dendritic cells.<sup>51</sup>

Originally thought to be involved only in B cell chemotaxis, CXCL13 was previously known as BLC and BCA-1. CXCL13 assists in B cell trafficking to high endothelial venules (HEVs) in mesenteric lymph nodes, Peyer's patches,<sup>52</sup> inducible bronchus-associated lymphoid tissues (iBALT),<sup>53</sup> and various mucosa-associated lymphoid tissues (MALT).<sup>54</sup> CXCL13 knockout mice have been used in various investigations involving lymph node development, and appear to have absent or reduced numbers of lymph nodes,<sup>55</sup> absence of B1 cells in the peritoneal and pleural cavities,<sup>56</sup> structurally abnormal nasal-associated lymphoid tissue that lack follicular dendritic cells<sup>57</sup> and altered B cell adhesion in lymph nodes and Peyer's patches.<sup>54</sup> Lymph nodes that are present in these animals, albeit mostly abnormal, are capable of mounting an immune response to microbial challenge although B cell migration into dendritic-rich zones of lymph nodes is critically impaired. In addition, CXCL13 does not appear to be essential for survival or reproduction since the CXCL13<sup>-/-</sup> mice used in these studies were able to mate and produce litters of pups without any reproductive complications although the litter sizes or percentage of resorbed embryos were not reported. The presence of CXCL13 in both endothelium and epithelium-associated

lymphoid tissues suggests an important role of CXCL13 on B cell function and the primary human defense response.

Recent studies have reported that CXCL13 also participates in T cell homing to germinal centers of lymphoid tissue,<sup>58</sup> induction of migration of immature dendritic cells<sup>51</sup> and maintenance of epithelial cell angiostatic activity.<sup>59</sup> In a study using human umbilical vein endothelial cells, CXCL13 interfered with angiogenesis through competitive binding to fibroblast growth factor (FGF)-2 receptors on endothelium and through the formation of CXCL13-FGF-2 heterodimers.<sup>60</sup>

### Role of CXCL13 in infection and inflammation

CXCL13 is implicated in chronic infectious diseases and is found in both the serum and tissues of infected individuals. CXCL13 expressing dendritic cells are found in high concentrations in granulomas in Cat Scratch Disease (*Bartonella henselae* infections).<sup>61</sup> In neuroborreliosis, CXCL13 production is significantly correlated with spirochete load<sup>62</sup> and measurement of serum CXCL13 has been proposed as a putative diagnostic or therapeutic marker for infection.<sup>63</sup> In relapsing fever from *Borrelia* species, serum concentrations of CXCL13 have also been considered as a diagnostic marker for the disease.<sup>64</sup> Serum concentrations of CXCL13 are elevated with human immunodeficiency virus (HIV) infection, and strongly correlate with inducible protein-10 (IP-10) and moderately correlate with viral loads.<sup>33</sup> In addition, there is a down-regulation of CXCR5 on naïve B-cells in patients infected with HIV, which impairs the host's immune system to mount a proper response to the virus despite the elevated concentrations of CXCL13.<sup>65</sup>

In several chronic infectious and inflammatory diseases, lymphoid neogenesis occurs in ectopic germinal centers in non-lymphoid tissue mediated by CXCL13.<sup>66</sup> These organized centers of local interaction of B cells, T cells and dendritic cells serve to produce a local supply of immunoglobulin and chemokines. Ectopic germinal centers are located in bowel mucosa in inflammatory bowel disease,<sup>34</sup> synovial membranes in rheumatoid arthritis,<sup>67,68</sup> neural tissues in Lyme disease neuroborreliosis,<sup>62</sup> vascular endothelium of the CNS in lymphoma,<sup>69</sup> kidney in lupus nephritis,<sup>70</sup> salivary glands of patients with Sjogren's disease,<sup>71,72</sup> and lung in tuberculosis.<sup>73</sup>

While it is advantageous for lymphoid neogenesis to occur near the infection site in order to assist the host immune response in antigen presentation and antibody production, the resultant inflammation may exacerbate symptoms of the disease. For instance, in Lyme disease, the hallmark of chronic infection is marked tissue inflammation with resultant tissue damage. Patients frequently suffer from myositis<sup>74,75</sup> and tissue biopsies of diseased skeletal muscles demonstrate increased expression of CXCL13.<sup>76</sup>

There is paucity of literature about the role of CXCL13 in pregnancy. Muehlenbachs et al<sup>38</sup> investigated placentas from women with active *Plasmodium falciparum* infection and identified aggregates of B cells resembling ectopic germinal centers. The maternal macrophages in the intervillous space of malaria-positive placentas were positive for CXCL13, suggesting that up-regulation of CXCL13 was from the maternal inflammatory response. This local concentration of CXCL13 attracts naïve B cells, plasma cells and facilitates antibody production that results in extensive inflammatory intervillous infiltration that can occlude the maternal circulation. In these placentas, the pathologic finding of massive chronic intervillitis is hypothesized to cause adverse fetal outcomes.<sup>77</sup>

### CXCL13 in normal pregnancy

We report for the first time, the presence of CXCL13 in the AF of normal pregnancy. In addition, serum CXCL13 concentration is increased in normal pregnancy when compared to

the non-pregnant state, and decreases as a function of gestational age. CXCL13 is also detected in umbilical cord blood of term neonates. In all compartments, CXCL13 is detected in the majority of samples, and at term, the concentration does not change with spontaneous labor.

The precise biological action exerted by CXCL13 in the amniotic cavity is not clear because this is not a site of lymphopoiesis. However, the finding of dramatic up-regulation of CXCL13 in IAI does not preclude its importance in the role of maternal or fetal immunity or in maternal tolerance of the fetal allograft. Regulatory T (Treg) cells have been implicated in autoimmune disease,<sup>78</sup> allergies,<sup>79</sup> transplant rejection<sup>80</sup> and pregnancy.<sup>81–83</sup> Regulatory T cells express CXCR5, the receptor for CXCL13. Blockade of CXCL13 with an antibody prevents migration of regulatory T cells into transplanted organs and may favor rejection.<sup>84</sup> Under normal circumstances, there is up-regulation of CXCL13 in the organ shortly after transplantation. This suggests that anti-rejection T cells are homed to the allograft in the initial host response. In addition, the evaluation of renal transplant biopsies as a function of time reveal that CXCL13 expression is 27-fold higher in biopsies (with well formed aggregates of B cells) from acutely rejected transplants and below the limit of detection in non-rejected transplants.<sup>85</sup> CXCL13 is also highly expressed in severe transplant vasculopathy.<sup>86</sup> These findings suggest that there is a sensitive balance of CXCL13 expression and regulatory T cell chemotaxis in the maintenance of transplanted organs. The interaction between CXCL13 in the transplant and CXCR5 on the T cells plays an important role in allogeneic organ acceptance.

The rise in maternal serum CXCL13 concentration with pregnancy and the subsequent decrease throughout gestation parallels the chemokine changes that occur with successful transplanted allografts.<sup>80,84,85</sup> The presence of CXCL13 in AF early in gestation and its stable concentration throughout pregnancy suggests that CXCL13 may also contribute to the homeostatic balance that it may be important in the maintenance of a normal gestation.

### CXCL13 in preterm labor

CXCL13 is expressed in biological fluids such as cerebrospinal fluid (CSF),<sup>64</sup> plasma<sup>64</sup> and serum,<sup>33</sup> and tissues in infection and inflammation.<sup>38,61,62,73,74,76,87,88</sup> The findings of our study reported herein indicate that this can also occur in the amniotic cavity in cases of intra-amniotic infection. While microbial invasion of the amniotic cavity is an important cause of preterm labor, inflammation has also been implicated in the etiology of preterm labor and is associated with an equal rate of adverse fetal outcomes when compared with microbial invasion of the amniotic cavity.<sup>41</sup> Numerous inflammatory peptides have been identified and proposed to be used as diagnostic markers<sup>27,89</sup> of increased risk of IAI and PTL. Of note, epithelial cell-derived neutrophil-activating peptide (ENA)-78,<sup>25</sup> IL-6,<sup>90</sup> and macrophage inhibitory factor<sup>91</sup> are all pro-inflammatory proteins found to be elevated in IAI. Strong support of the involvement of CXCL13 in the inflammatory response is the stimulation of its production by TNF- $\alpha$  in the murine model.<sup>92</sup>

Lipopolysaccharide (LPS) can induce CXCL13 production by dendritic cells.<sup>93</sup> This microbial product has been previously found in the amniotic cavity and decidua, which contain dendritic cells.<sup>94</sup> It is possible that extra-amniotic infection stimulates the production of CXCL13 in the decidua. However, the precise mechanism responsible for the increased CXCL13 in amniotic fluid in cases of intra-amniotic infection is unknown. We have demonstrated the presence of CXCL13 in the cytoplasm of macrophages in the fetal membranes and the umbilical cord. It is possible that microbial products or bacteria stimulate the production of CXCL13 by resident macrophages in the amniotic cavity, in the fetal membranes, or in the fetus. Indeed, there is evidence that LPS can stimulate monocytes and macrophages to produce CXCL13.<sup>34</sup> The same study indicated that macrophages secrete



more CXCL13 than recently recruited monocytes or dendritic cells. It is possible that CXCL13 may be an important link between innate and adaptive immune responses because this chemokine would participate in the recruitment of B cells, which could produce antibodies.

What is unique about CXCL13 is that unlike other pro-inflammatory chemokines, such as IL-8<sup>23</sup> and CCL5/RANTES,<sup>21</sup> its concentration in AF does not change with normal term parturition and does not decrease with advancing gestation. Increased CXCL13 concentration in AF is specific to PTL with evidence of IAI. Indeed, the increase in AF CXCL13 is not only a marker for a disease process, but may also initiate a pro-inflammatory response to IAI that leads to the activation of preterm parturition pathways.

### Origin of CXCL13 in the amniotic fluid

In placental malaria, maternal macrophages in the intervillous space were hypothesized to be the source of CXCL13 production.<sup>38</sup> However, the identification of CXCL13 in the maternal compartment does not explain its presence in AF. Evaluation of cord blood demonstrates that the fetus mounts a response to infection by activation of monocytes and neutrophils.<sup>95</sup> Specifically, elevations in fetal umbilical cord blood cytokines have been identified in IAI,<sup>96</sup> periventricular leukomalacia,<sup>97,98</sup> and a fetal systemic inflammatory response syndrome.<sup>99</sup> Indeed, the leading hypothesis for the source of inflammatory cytokines in AF has been of fetal origin, from the fetal intravascular compartment<sup>100</sup> or from fetal membranes.<sup>101–103</sup> It is known that CXCL13 is present in normal umbilical endothelium<sup>104</sup> and CXCL13 expression has been identified in fetal spleen and vascular smooth muscle as early as 14 weeks of gestation.<sup>105</sup> Therefore, the source of the increase in CXCL13 concentrations in the AF with IAI may be a fetal response to IAI.

### Conclusion

The chemokine CXCL13 is a physiologic constituent of AF. Unlike other known chemokines, CXCL13 concentration does not increase with advancing gestation or with spontaneous labor at term. Concentrations of this protein are significantly increased in the presence of intra-amniotic infection/inflammation, indicating an acute response to microbial products in the amniotic cavity. Our findings suggest that CXCL13 may contribute to the innate immune response against intra-amniotic infection and/or inflammatory response. Further investigations are needed to elucidate the origin and biological actions of CXCL13 in normal and abnormal pregnancy.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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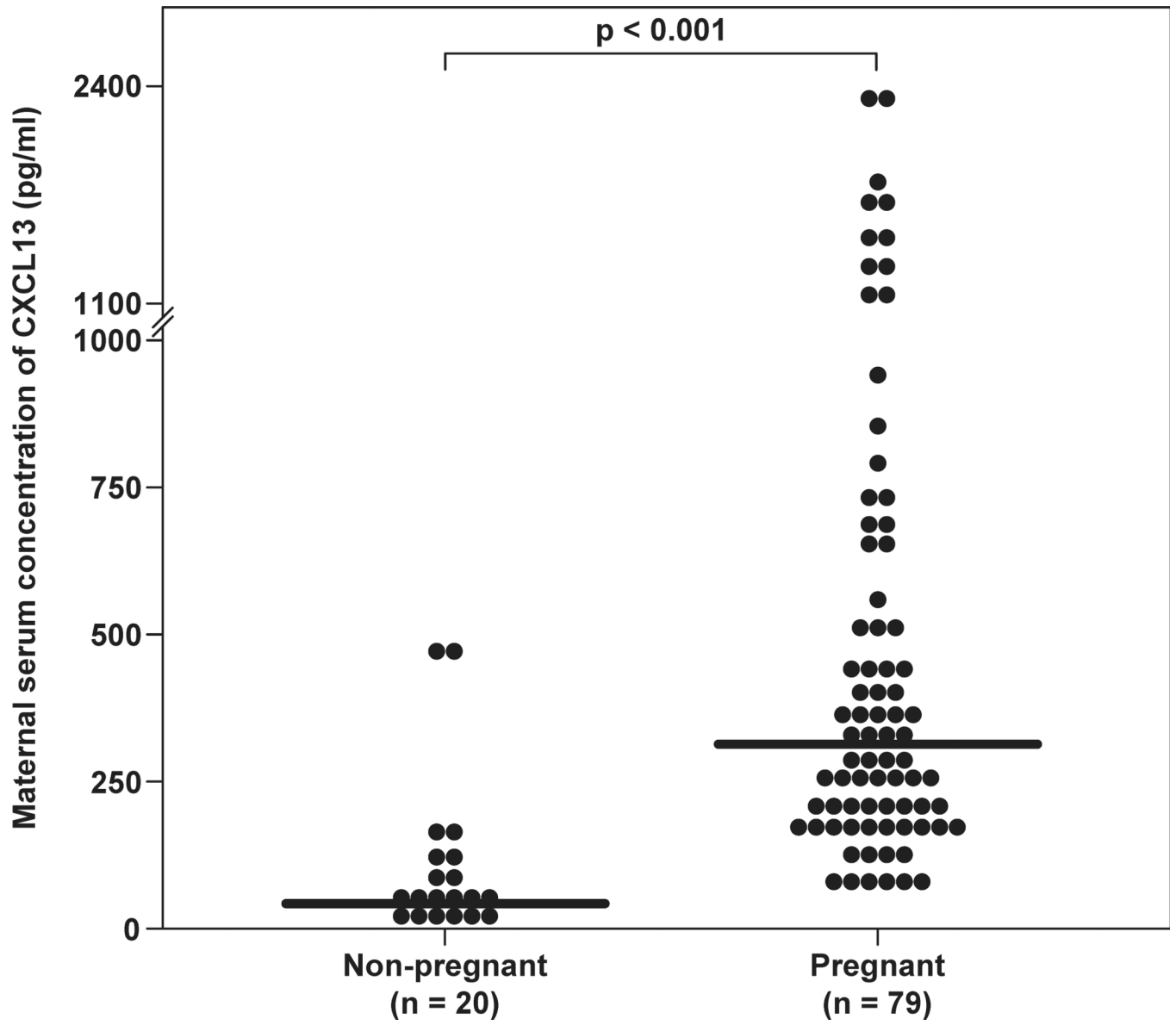


Figure 1.



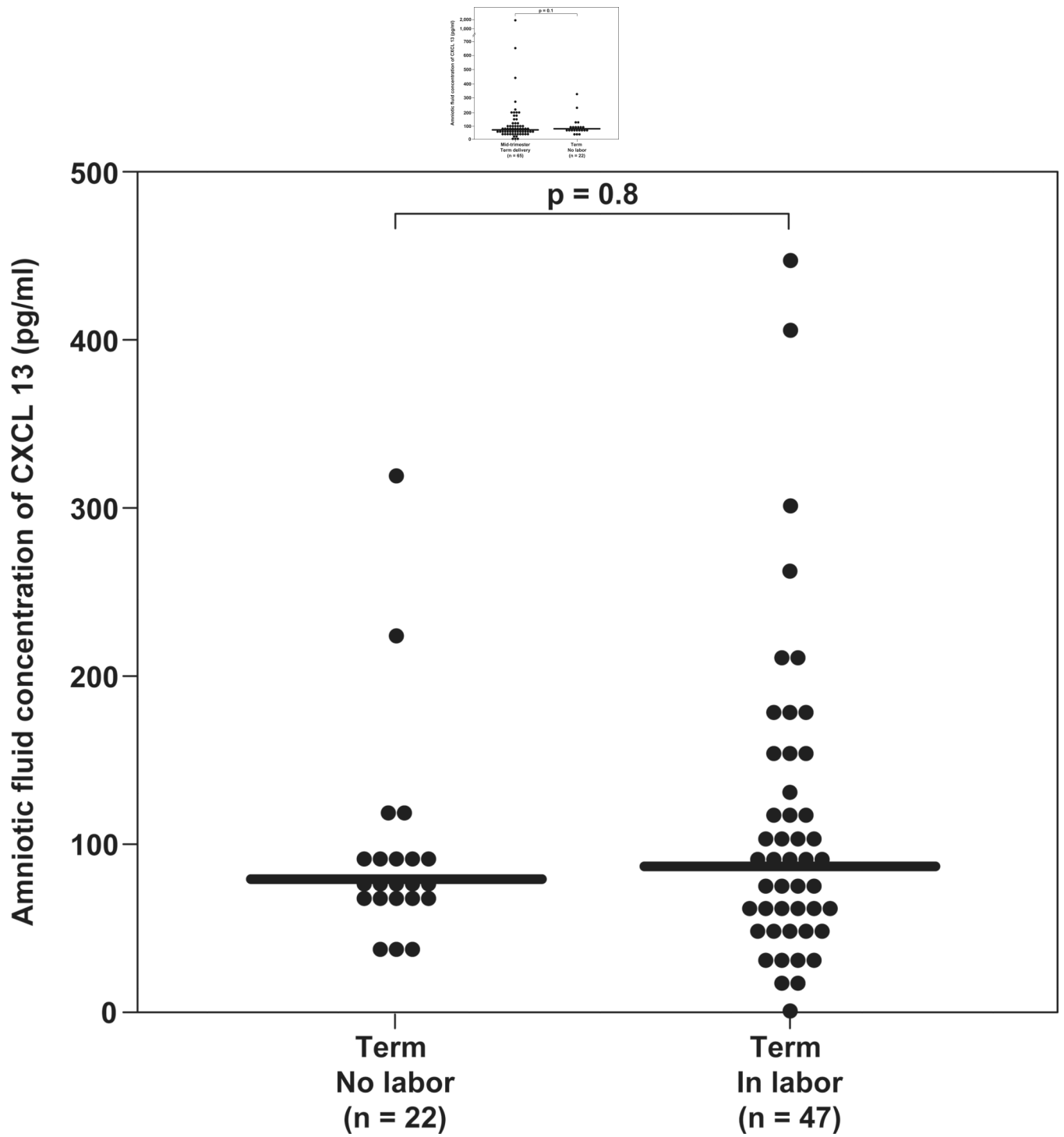


Figure 2.

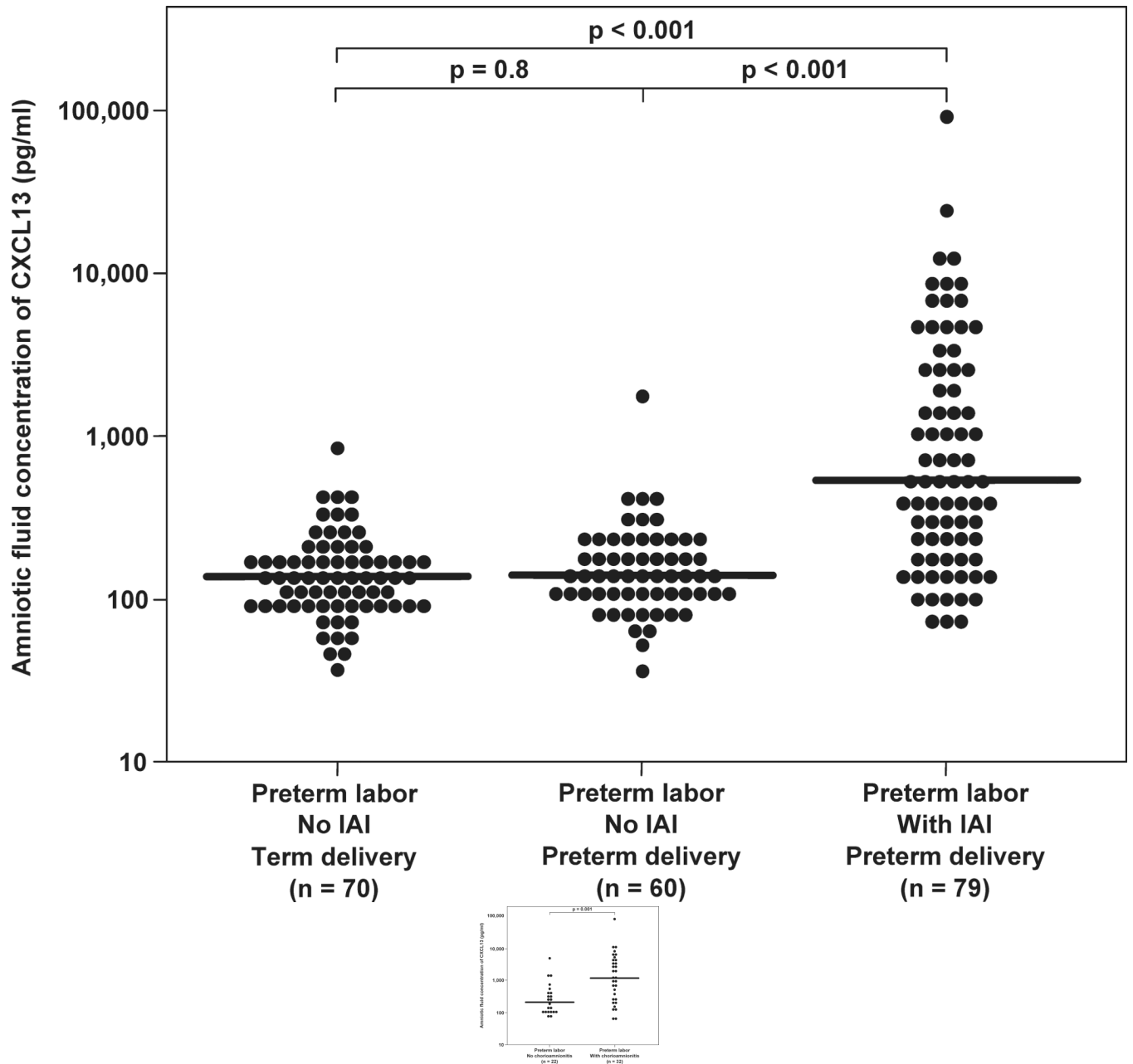


Figure 3.

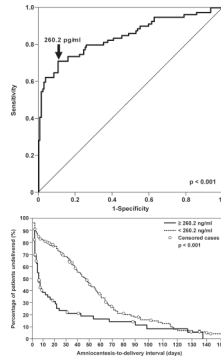


Figure 4.

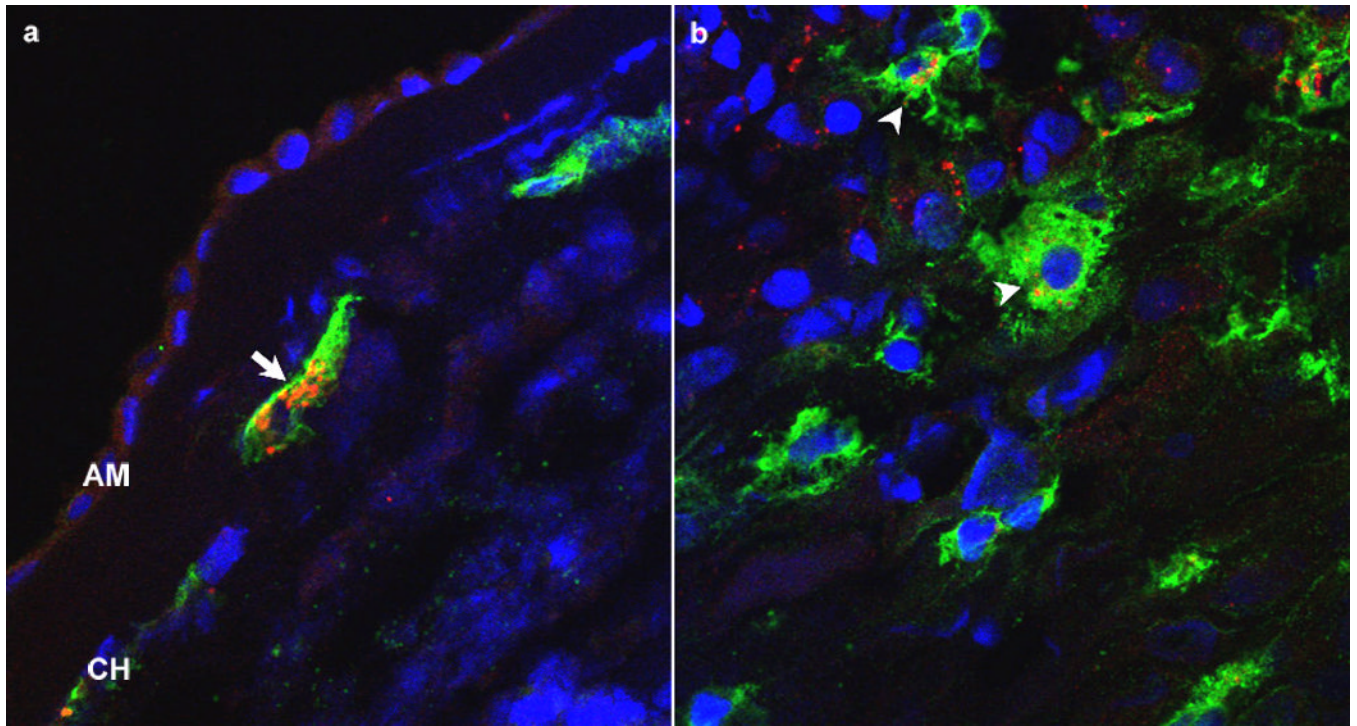
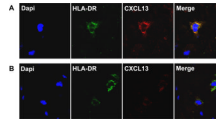


Figure 5.



**Figure 6.**

**Table 1**

Distribution of maternal characteristics in the study categories.

	<b>Mid-trimester (n = 65)</b>	<b>Term not in labor (n = 22)</b>	<b>Term in labor (n = 47)</b>	<b>Preterm labor (n = 209)</b>
<b>Maternal age (years)</b>	37 (35–38)	28.5 (21.8–32)	23 (20–28)	23.9 (20–27)
<b>Gravity</b>	3 (2–3)	3 (2–4)	2 (1–3.3)	3 (2–4)
<b>Gestational age at amniocentesis (weeks)</b>	16 (16–17)	39.3 (38.4–40)	39 (38–40)	28.5 (24.7–32.4)
<b>Amniotic fluid sample storage time (years)</b>	11.7 (10.9–12.2)	16.6 (16.5–16.6)	16.6 (16.6–16.7)	5.2 (4.7–6.1)
<b>Gestational age at delivery (weeks)</b>	39 (38–40)	39.3 (38.4–40)	39 (38–40)	33.1 (28.7–37.7)
<b>Birth weight (grams)</b>	3345 (3144–3632)	3405 (3130–3688)	3220 (3090–3680)	2044 (1110–2760)

Values are expressed as median (interquartile range).

**Table 2**

Demographic and clinical characteristics of the preterm labor groups

	<b>PTL with Term Delivery (n=70)</b>	<b>PTD without IAI (n=88)</b>	<b>p<sup>†</sup></b>	<b>PTD with IAI (n=51)</b>	<b>p<sup>*</sup></b>
<b>Maternal age (years)</b>	23 (19–26)	22 (1–27)	NS	23 (20–28)	NS
<b>Gravity</b>	3 (1–4)	3 (2–5)	NS	3 (2–4)	NS
<b>Race</b>	African American: 97% Caucasian: 3% Other: 0%	African American: 82% Caucasian: 7% Other: 12%	NS	African American 86% Caucasian: 13% Other: 1%	NS
<b>Gestational age at amniocentesis (weeks)</b>	31.2 (26.4–32.9)	30.8 (26.9–32.3)	<0.05	25.9 (24–32.3)	<0.05
<b>Amniotic Fluid Sample Storage time (years)</b>	4.9 (4.6–5.4)	5.2 (4.8–6.1)	NS	5.7 (4.9–6.2)	NS
<b>Gestational age at delivery (weeks)</b>	38.3 (37.1–39.2)	34.3 (32.3–35.3)	<0.05	27.0 (24.4–32.4)	<0.01
<b>Birthweight (grams)</b>	2970 (2725–3255)	1810 (1715–2382)	<0.01	1040 (634–1752)	<0.01

Values are expressed as medians (inter-quartile range).

PTD: preterm delivery; IAI: intra-amniotic infection and/or inflammation; PTL: preterm labor; NS: not significant.

p<sup>†</sup>: Comparison between patients with spontaneous PTL without IAI who delivered preterm and those who subsequently delivered at term.

p<sup>\*</sup>: Comparison between patients with spontaneous PTL without IAI and those with spontaneous PTL with IAI.