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Clinical chorioamnionitis at term IX: *in vivo* evidence of intra-amniotic inflammasome activation

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Abstract

Background: The inflammasome has been implicated in the mechanisms that lead to spontaneous labor at term. However, whether the inflammasome is activated in the amniotic cavity of women with clinical chorioamnionitis at term is unknown. Herein, by measuring extracellular ASC [apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (CARD)], we investigated whether there is *in vivo* inflammasome activation in amniotic fluid of patients with clinical chorioamnionitis at term with sterile intra-amniotic inflammation and in those with intra-amniotic infection.

Methods: This was a retrospective cross-sectional study that included amniotic fluid samples collected from 76 women who delivered after spontaneous term labor with diagnosed clinical chorioamnionitis. Intra-amniotic inflammation was defined as an elevated amniotic fluid interleukin (IL)-6 concentration ≥2.6 ng/mL, and intra-amniotic infection was diagnosed by the presence of microbial invasion of the amniotic cavity (MIAC) accompanied by intra-amniotic inflammation. Patients were classified into the following groups: (1) women without intra-amniotic inflammation or infection (n = 16); (2) women with MIAC but without intra-amniotic inflammation (n = 5); (3) women with sterile intra-amniotic inflammation (n = 15); and (4) women with intra-amniotic infection (n = 40). As a readout of *in vivo* inflammasome activation, extracellular ASC was measured in

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amniotic fluid by enzyme-linked immunosorbent assay. Acute inflammatory responses in the amniotic fluid and placenta were also evaluated.

Results: In clinical chorioamnionitis at term: (1) amniotic fluid concentrations of ASC (extracellular ASC is indicative of *in vivo* inflammasome activation) and IL-6 were greater in women with intra-amniotic infection than in those without intra-amniotic inflammation, regardless of the presence of MIAC; (2) amniotic fluid concentrations of ASC and IL-6 were also higher in women with sterile intra-amniotic inflammation than in those without intra-amniotic inflammation, regardless of the presence of MIAC; (3) amniotic fluid concentrations of IL-6, but not ASC, were more elevated in women with intra-amniotic infection than in those with sterile intra-amniotic inflammation; (4) a positive and significant correlation was observed between amniotic fluid concentrations of ASC and IL-6; (5) no differences were observed in amniotic fluid ASC and IL-6 concentrations between women with and without MIAC in the absence of intra-amniotic inflammation; (6) women with intra-amniotic infection had elevated white blood cell counts and reduced glucose levels in amniotic fluid compared to the other three study groups; and (7) women with intra-amniotic infection presented higher frequencies of acute maternal and fetal inflammatory responses in the placenta than those with sterile intra-amniotic inflammation.

Conclusion: The intra-amniotic inflammatory response, either induced by alarmins or microbes, is characterized by the activation of the inflammasome – as evidenced by elevated amniotic fluid concentrations of extracellular ASC – in women with clinical chorioamnionitis at term. These findings provide insight into the intra-amniotic inflammatory response in women with clinical chorioamnionitis at term.

Keywords: alarmins; amniotic fluid; ASC; cytokine; DAMPs; danger signals; interleukin 1β (IL-1β); interleukin-6 (IL-6); intra-amniotic infection; microbial invasion of the amniotic cavity (MIAC); neutrophils; NLRP3; parturition; PYCARD; sterile intra-amniotic inflammation.

Introduction

Clinical chorioamnionitis is the most common infection-related diagnosis made in labor and delivery units worldwide [1–4]. The standard clinical definition refers to the presence of maternal fever associated with clinical signs (foul-smelling discharge, uterine tenderness, maternal and fetal tachycardia) as well as laboratory abnormalities (i.e. leukocytosis [5, 6]). Clinical chorioamnionitis at term is associated with adverse maternal events [2, 7, 8] and increased maternal admission to the intensive care unit [2, 9]. Importantly, neonates born to mothers with clinical signs of chorioamnionitis have a high risk of neonatal mortality [4] in addition to short- and long-term complications such as neonatal sepsis [10–14], meconium aspiration syndrome [15–18], stillbirth [19, 20], and neurodevelopmental disorders including cerebral palsy [21–31].

Clinical chorioamnionitis was originally thought to occur as a result of the inflammation initiated by microbial invasion of the amniotic cavity (MIAC) [32–39]. However, recent studies have shown that only 60% of patients with the diagnosis of clinical chorioamnionitis at term have proven intra-amniotic infection using culture or molecular microbiologic techniques; the remaining patients have a maternal systemic inflammatory response (fever) either in the absence of intra-amniotic inflammation, MIAC without intra-amniotic inflammation, or intra-amniotic inflammation without demonstrable microorganisms [40], which is referred to as sterile intra-amniotic inflammation [41–45].

Among patients with clinical chorioamnionitis at term, the inflammatory responses in the amniotic fluid [46], cord blood [47] and maternal circulation [48] are similar between those with sterile intra-amniotic inflammation and those with intra-amniotic infection. These data suggest that similar to microbes, danger signals or alarmins, which are mediators initiating sterile inflammation [49–51], can activate similar inflammatory pathways in the amniotic cavity. Our recent studies have shown that, in the context of preterm and term labor, the mechanisms that lead to both sterile intra-amniotic inflammation and intra-amniotic infection involve the inflammasome [52–57]. However, whether the inflammasome is activated in the amniotic cavity of women with clinical chorioamnionitis at term is unknown.

Inflammasomes are cytoplasmic multiprotein complexes that, upon activation, induce the autocatalytic cleavage of pro-caspase-1 into its active form which, in turn, can cleave the inflammatory cytokines pro-interleukin (IL)-1β and pro-IL-18 into their mature and secreted bioactive forms [58–72]. Inflammasome activation can be measured by the detection of the adaptor protein ASC [apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (CARD)] in the extracellular space [73–75]. Therefore, the detection of extracellular ASC in biological fluids (e.g. amniotic fluid)

is considered an *in vivo* determination of inflammasome activation [56, 57].

In the study herein, we measured extracellular ASC to investigate whether there is *in vivo* inflammasome activation in amniotic fluid of patients with clinical chorioamnionitis at term with sterile intra-amniotic inflammation and in those with intra-amniotic infection.

Materials and methods

Study design

This was a retrospective cross-sectional study conducted by querying our clinical database and bank of biological samples of the Perinatology Research Branch, an intramural program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), National Institutes of Health, U.S. Department of Health and Human Services. The Institutional Review Boards of Wayne State University, NICHD, and the Sótero del Rio Hospital in Santiago, Chile, approved the use of samples and biological specimens as well as the use of clinical data for research purposes.

Clinical definitions

Clinical chorioamnionitis was diagnosed by the presence of maternal fever (temperature >37.8°C) accompanied by two or more of the following criteria: (1) uterine tenderness, (2) foul-smelling amniotic fluid, (3) fetal tachycardia (heart rate >160 beats/min), (4) maternal tachycardia (heart rate >100 beats/min), and (5) maternal leukocytosis (leukocyte count >15,000 cells/mm^3) [5, 6, 32, 33, 76, 77].

Microbial invasion of the amniotic cavity was defined as a positive amniotic fluid culture and/or polymerase chain reaction with electrospray ionization mass spectrometry (PCR/ESI-MS) (Ibis® Technology-Athogen, Carlsbad, CA, USA) test result [40, 78–81]. Intra-amniotic inflammation was defined as an amniotic fluid IL-6 concentration ≥2.6 ng/mL [82–84]. Sterile intra-amniotic inflammation was defined as an amniotic fluid IL-6 concentration ≥2.6 ng/mL [82] without microorganisms detected by culture or PCR/ESI-MS [40, 41, 43–48, 85, 86]. Intra-amniotic infection (or microbial-associated intra-amniotic inflammation) was defined as the presence of MIAC together with intra-amniotic inflammation [40, 41, 43–48, 85–89].

Study population

This study included amniotic fluid samples collected from 76 women who delivered after spontaneous term labor with diagnosed clinical chorioamnionitis. Patients were classified into the following groups (Table 1): (1) women without intra-amniotic inflammation or infection (n = 16); (2) women with MIAC but without intra-amniotic inflammation (n = 5); (3) women with sterile intra-amniotic inflammation (n = 15); and (4) women with intra-amniotic infection (n = 40).

Amniotic fluid sample collection

Amniotic fluid samples were transported to the clinical laboratory in a sterile capped syringe and cultured for aerobic and anaerobic bacteria, including genital mycoplasmas. The clinical tests also included the determination of amniotic fluid white blood cell (WBC) count [90], glucose concentration [91], Gram stain [92], and IL-6 concentration [82].

Table 1: Demographic and clinical characteristics of the study population.

	Without intra-amniotic inflammation or infection (n = 16)	With MIAC but without intra-amniotic inflammation (n = 5)	Sterile intra-amniotic inflammation (n = 15)	Intra-amniotic infection (n = 40)	P-value
Maternal age, years[a]	21 (18–25)	25 (21–28)	23 (20–26)	22 (19–26)	0.4
Body mass index, kg/m[a]	29.8 (26–39.3)	36.4 (23.8–41.7)	26.1 (22.8–33.7)	26.3 (23.3–32.8)	0.4
Ethnicity[b]					0.3
African American	50 (8/16)	60 (3/5)	13.3 (2/15)	50 (20/40)	
Hispanic	37.5 (6/16)	40 (2/5)	66.7 (10/15)	40 (16/40)	
Caucasian	6.3 (1/16)	0 (0/0)	13.3 (2/15)	5 (2/40)	
Asian	0 (0/0)	0 (0/0)	0 (0/0)	2.5 (1/40)	
Other	6.3 (1/16)	0 (0/0)	6.7 (1/15)	2.5 (1/40)	
Gestational age at amniocentesis, weeks[a]	38.8 (38.2–39.5)	40.4 (40.1–40.6)	39.4 (38.7–39.8)	39.9 (38.8–40.6)	0.01
Delivery route[b]					0.7
Vaginal	68.8 (11/16)	60 (3/5)	60 (9/15)	72.5 (29/40)	
Cesarean section	31.2 (5/16)	40 (2/5)	40 (6/15)	27.5 (11/40)	
Gestational age at delivery, weeks[a]	38.9 (38.3–39.8)	40.6 (40.1–40.6)	39.6 (38.8–39.9)	39.9 (38.9–40.6)	0.05
Birth weight, g[a]	3487.5 (3071.3–3687.5)	3610 (3135–3815)	3545 (3230–3790)	3440 (3145–3670)	0.8

Data are given as median (interquartile range) and percentage (number/Number). [a]Kruskal-Wallis test. [b]Fisher's exact test. MIAC, microbial invasion of the amniotic cavity.

Determination of IL-6 in amniotic fluid

Amniotic fluid concentrations of IL-6 were determined by using a sensitive and specific enzyme immunoassay obtained from R&D Systems (Minneapolis, MN, USA). The IL-6 concentrations were determined by interpolation from the standard curves. The inter- and intra-assay coefficients of variation for IL-6 were 8.7% and 4.6%, respectively. The detection limit of the IL-6 assay was 0.09 pg/mL. The IL-6 concentrations in amniotic fluid were determined for clinical purposes.

Determination of extracellular ASC in the amniotic fluid

Concentrations of extracellular ASC in the amniotic fluid were determined using a sensitive and specific enzyme-linked immunosorbent assay (ELISA) kit obtained from LifeSpan Biosciences (Seattle, WA, USA). This ELISA kit was initially validated in our laboratory prior to the execution of this study. Amniotic fluid concentrations of ASC were obtained by interpolation from the standard curve. The inter- and intra-assay coefficients of variation were 5.0% and 8.6%, respectively. The sensitivity of the assay was 0.131 ng/mL.

Placental histopathological examination

Sampling of the placentas was conducted according to established protocols by the Perinatology Research Branch. Five-µm-thick sections of formalin-fixed, paraffin-embedded tissue specimens were cut and mounted on SuperFrost™ Plus microscope slides (Erie Scientific LLC, Portsmouth, NH, USA). After deparaffinization, slides were rehydrated and stained with hematoxylin-eosin. A minimum of five full-thickness sections of chorionic plate, three sections of umbilical cord, and three chorioamniotic membrane rolls from each case were examined by placental pathologists who were blinded to clinical histories and additional testing results. Acute inflammatory lesions of the placenta (maternal and fetal inflammatory responses) were diagnosed according to established criteria, including staging and grading [93–96].

Statistical analysis

Statistical analyses were performed using the R statistical language and environment (www.r-project.org) and SPSS v19 software (SPSS Inc., IBM Corporation, Armonk, NY, USA). For patient demographics, the Fisher's exact test was used to compare proportions among groups and the Kruskal-Wallis test was used for the comparison of continuous variables among groups. A P-value of <0.05 was considered statistically significant. Experimental data was compared between groups using unpaired Wilcoxon tests, and P-values were adjusted across comparisons and the two analytes (IL-6 and ASC) to control the false discovery rate. An adjusted P-value (i.e. q-value) <0.05 was considered a significant result. The magnitude of differences was expressed as the difference in means after $\log_2$ transformation of the data, to obtain $\log_2$ fold changes in concentration. The correlation between ASC and IL-6 levels was assessed via Spearman correlation tests.

Results

Characteristics of the study population

The demographic and clinical characteristics of the study population are shown in Table 1. There were no differences in maternal age, body mass index, ethnicity, delivery route or neonatal birth weight among the study groups (Table 1). Gestational age at amniocentesis was different between the study groups, and such a difference was mostly driven by the study group of MIAC without intra-amniotic inflammation that included women who delivered after 40 weeks of gestation (Table 1).

Amniotic fluid ASC concentrations in women with clinical chorioamnionitis at term

Women with intra-amniotic infection had higher amniotic fluid concentrations of ASC than those without intra-amniotic inflammation [intra-amniotic infection: median 27.9 ng/mL [intraquartile range (IQR) 18.6–69.9 ng/mL] vs. without intra-amniotic inflammation: median 10.2 ng/mL

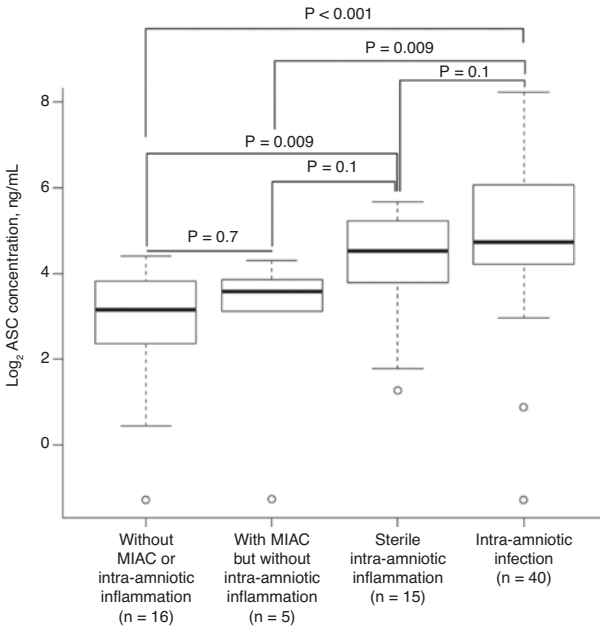

Figure 1: Amniotic fluid ASC concentrations in women with clinical chorioamnionitis at term.
Extracellular ASC (ng/mL) was measured in amniotic fluid of women with clinical chorioamnionitis at term: without intra-amniotic inflammation (n = 16), with microbial invasion of the amniotic cavity (MIAC) but without intra-amniotic inflammation (n = 5), with sterile intra-amniotic inflammation (n = 15), or with intra-amniotic infection (n = 40). Data are shown as $\log_2$ concentration (ng/mL).

(IQR 5.3–14.8 ng/mL); P < 0.001] (Figure 1). Women with intra-amniotic infection also had greater amniotic fluid concentrations of ASC than those with MIAC but without intra-amniotic inflammation [intra-amniotic infection: median 27.9 ng/mL (IQR 18.6–69.9 ng/mL) vs. MIAC without intra-amniotic inflammation: median 12.0 ng/mL (IQR 4.6–17.1 ng/mL); P = 0.009] (Figure 1). However, no differences were observed between women with intra-amniotic infection and those with sterile intra-amniotic inflammation [intra-amniotic infection: median 27.9 ng/mL (IQR 18.6–69.9 ng/mL) vs. sterile intra-amniotic inflammation: median 23.0 ng/mL (IQR 13.2–37.6 ng/mL); P = 0.1] (Figure 1).

Women with sterile intra-amniotic inflammation had higher amniotic fluid ASC concentrations compared to those without intra-amniotic inflammation [sterile intra-amniotic inflammation: median 23.0 ng/mL (IQR 13.2–37.6 ng/mL) vs. without MIAC or intra-amniotic inflammation: median 10.2 ng/mL (IQR 5.3–14.8 ng/mL); P = 0.009] (Figure 1). Although women with sterile intra-amniotic inflammation tended to have greater amniotic fluid ASC concentrations compared to women with MIAC but without intra-amniotic inflammation, no statistical differences were observed between groups [sterile intra-amniotic inflammation: median 23.0 ng/mL (IQR 13.2–37.6 ng/mL) vs. MIAC without intra-amniotic inflammation: median 12.0 ng/mL (IQR 4.6–17.1 ng/mL); P = 0.1] (Figure 1).

Amniotic fluid IL-6 concentrations in women with clinical chorioamnionitis at term

The amniotic fluid concentration of IL-6 was significantly greater in women with intra-amniotic infection compared to those without intra-amniotic inflammation [intra-amniotic infection: median 22,500 pg/mL (IQR 7634–57,150 pg/mL) vs. without intra-amniotic inflammation: median 531.8 pg/mL (IQR 250.6–1073 pg/mL); P < 0.001] (Figure 2). Women with intra-amniotic infection also had higher concentrations of amniotic fluid IL-6 than those with MIAC but without intra-amniotic inflammation [intra-amniotic infection: median 22,500 pg/mL (IQR 7634–57,150 pg/mL) vs. MIAC without intra-amniotic inflammation: median 1386 pg/mL (IQR 350.8–1789 pg/mL); P < 0.001] (Figure 2). Moreover, women with intra-amniotic infection had elevated amniotic fluid IL-6 concentrations compared to those with sterile intra-amniotic inflammation [intra-amniotic infection: median 22,500 pg/mL (IQR 7634–57,150 pg/mL) vs. sterile intra-amniotic inflammation: median 5470 pg/mL (IQR 3392–14,979 pg/mL); P < 0.001] (Figure 2).

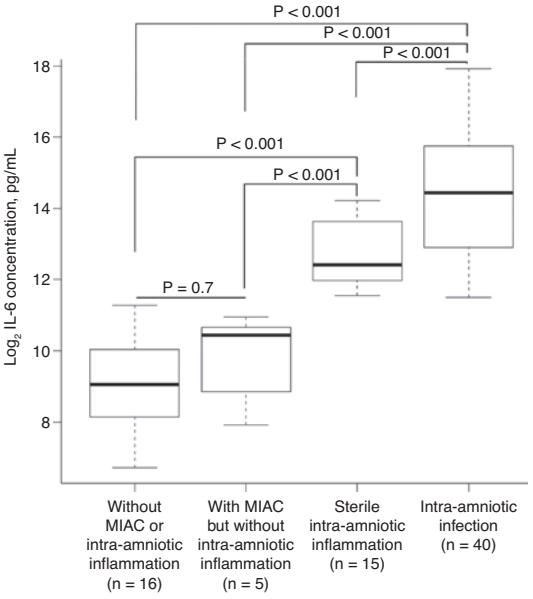

Figure 2: Amniotic fluid IL-6 concentrations in women with clinical chorioamnionitis at term.
IL-6 (pg/mL) was measured in amniotic fluid of women with clinical chorioamnionitis at term: without intra-amniotic inflammation (n = 16), with microbial invasion of the amniotic cavity (MIAC) but without intra-amniotic inflammation (n = 5), with sterile intra-amniotic inflammation (n = 15), or with intra-amniotic infection (n = 40). Data are shown as $\log_2$ concentration (pg/mL).

Women with sterile intra-amniotic inflammation had higher amniotic fluid concentrations of IL-6 than those without intra-amniotic inflammation [sterile intra-amniotic inflammation: median 5470 pg/mL (IQR 3392–14,979 pg/mL) vs. without intra-amniotic inflammation: median 531.8 pg/mL (IQR 250.6–1073 pg/mL); P < 0.001] (Figure 2). In addition, women with sterile intra-amniotic inflammation had greater amniotic fluid concentrations of IL-6 than those with MIAC but without intra-amniotic inflammation [sterile intra-amniotic inflammation: median 5470 pg/mL (IQR 3392–14,979 pg/mL) vs. MIAC without intra-amniotic inflammation: median 1386 pg/mL (IQR 350.8–1789 pg/mL); P < 0.001] (Figure 2).

We next determined whether a correlation existed between intra-amniotic inflammation (as indicated by amniotic fluid IL-6 concentrations) and the concentrations of extracellular ASC in amniotic fluid. We found that there was a significant correlation between the amniotic fluid concentrations of ASC and IL-6 in women with clinical chorioamnionitis at term (Spearman's correlation coefficient of 0.74, P < 0.001) (Figure 3).

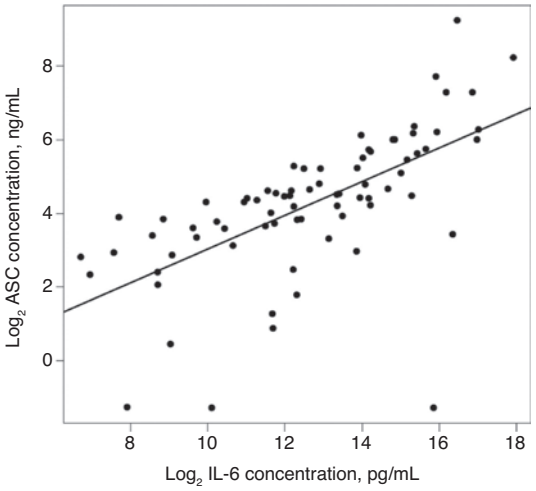

Figure 3: Correlation between ASC and IL-6 amniotic fluid concentrations in women with clinical chorioamnionitis at term. The line represents a linear fit estimating the average $\log_2$ ASC concentration as a function of $\log_2$ IL-6 concentration.

Acute inflammatory responses in the amniotic cavity and placenta of women with clinical chorioamnionitis at term

The number of WBCs in amniotic fluid was significantly higher in women with intra-amniotic infection compared to the other three study groups (Table 2). The WBC count was modestly higher in the amniotic fluid of women with sterile intra-amniotic inflammation compared to that of women without intra-amniotic inflammation, or those with MIAC but without intra-amniotic inflammation (Table 2). Women who delivered with intra-amniotic infection had reduced amniotic fluid glucose concentrations compared to the other study groups (Table 2).

Mild acute maternal (stage 1) and fetal (stage 1) inflammatory responses had a similar prevalence among the study groups; however, moderate acute maternal (stage 2) responses were significantly different (Table 2). Women with intra-amniotic infection had a higher frequency of acute chorioamnionitis and acute necrotizing chorioamnionitis than those with sterile intra-amniotic inflammation (Table 2). Similarly, severe acute fetal (stages 2 and 3) inflammatory responses were more prevalent in women who delivered with intra-amniotic infection (Table 2).

Discussion

Principal findings

In clinical chorioamnionitis at term: (1) amniotic fluid concentrations of ASC (extracellular ASC is indicative of *in vivo* inflammasome activation) and IL-6 were greater in women with intra-amniotic infection than in those without intra-amniotic inflammation, regardless of the presence of MIAC; (2) amniotic fluid concentrations of ASC and IL-6 were also higher in women with sterile intra-amniotic inflammation than in those without intra-amniotic inflammation, regardless of the presence of MIAC; (3) amniotic fluid concentrations of IL-6, but not ASC, were more elevated in women with intra-amniotic infection than in those with sterile intra-amniotic inflammation; (4) a

Table 2: White blood cell counts and glucose concentrations in amniotic fluid and the placental histopathology of the study population.

	Without intra-amniotic inflammation or infection (n=16)	With MIAC but without intra-amniotic inflammation (n=5)	Sterile intra-amniotic inflammation (n=15)	Intra-amniotic infection (n=40)	P-value
White blood cell count, cells/mm[a]	2 (0–5.5)[c]	2 (0–3)	5 (1–27.5)	560 (57.5–1373.8)	<0.001
Amniotic fluid glucose, mg/dL[a]	9 (5.8–17)	10 (9–12)	9 (8–11.5)	5 (1–9)	0.001
Acute maternal inflammatory response					
Stage 1 (acute subchorionitis)[b]	21.4 (3/14)[d]	40 (2/5)	42.9 (6/14)[c]	26.3 (10/38)[d]	0.6
Stage 2 (acute chorioamnionitis)[b]	14.3 (2/14)[d]	0 (0/5)	14.3 (2/14)[c]	47.4 (18/38)[d]	0.01
Stage 3 (acute necrotizing chorioamnionitis)[b]	0 (0/14)[d]	0 (0/5)	0 (0/14)[c]	13.2 (5/38)[d]	0.3
Acute fetal inflammatory response					
Stage 1 (acute phlebitis/ chorionic vasculitis)[b]	21.4 (3/14)[d]	40 (2/5)	28.6 (4/14)[c]	34.2 (13/38)[d]	0.5
Stage 2 (acute arteritis)[b]	7.1 (1/14)[d]	0 (0/5)	0 (0/14)[c]	23.7 (9/38)[d]	0.1
Stage 3 (necrotizing funisitis)[b]	0 (0/14)[d]	0 (0/5)	0 (0/14)[c]	7.9 (3/38)[d]	0.6

Data are given as median (interquartile range) and percentage (number/Number). [a]Kruskal-Wallis test. [b]Fisher's exact test. [c]One missing data. [d]Two missing data. MIAC, microbial invasion of the amniotic cavity.

positive and significant correlation was observed between amniotic fluid concentrations of ASC and IL-6; (5) no differences were observed in amniotic fluid ASC and IL-6 concentrations between women with and without MIAC in the absence of intra-amniotic inflammation; (6) women with intra-amniotic infection had elevated WBC counts and reduced glucose levels in amniotic fluid compared to the other three study groups; and (7) women with intra-amniotic infection presented higher frequencies of acute maternal and fetal inflammatory responses in the placenta than those with sterile intra-amniotic inflammation.

In vivo evidence of inflammasome activation in clinical chorioamnionitis at term with intra-amniotic infection

First, we showed that women with clinical chorioamnionitis at term and intra-amniotic infection had the highest amniotic fluid concentrations of extracellular ASC, which coincides with the most elevated concentrations of IL-6 (i.e. intra-amniotic inflammation). These results are in line with a previous report indicating that women with clinical chorioamnionitis at term and intra-amniotic infection have higher amniotic fluid concentrations of IL-1β – the main product of inflammasome activation [97] – than those without intra-amniotic inflammation [46]. Recently, it was reported that the chorioamniotic membranes from women who underwent labor at term with acute histologic chorioamnionitis, a placental lesion strongly associated with intra-amniotic infection [21, 93, 94, 98–107] and clinical chorioamnionitis at term [85] had (1) elevated mRNA and protein levels of NLRP3 and NLRC4 (i.e. inflammasome sensor molecules), (2) increased expression and amounts of active caspase-1, (3) high concentrations of mature IL-1β, and (4) enhanced inflammasome assembly (i.e. ASC/caspase-1 complexes), compared to those without this placental lesion [53, 54]. Furthermore, genital mycoplasmas, the most common microorganisms found in women with clinical chorioamnionitis at term and intra-amniotic infection [40, 85, 108], are capable of activating the inflammasome pathway in murine macrophages [109]. Together, these findings indicate that the inflammasome is involved in the mechanisms that lead to microbial-associated intra-amniotic inflammation in women with clinical chorioamnionitis at term.

Women with clinical chorioamnionitis at term and intra-amniotic infection have high numbers of amniotic fluid immune cells [108], which are more likely to be of maternal origin or a mixture of both fetal and maternal neutrophils [110]. These neutrophils actively participate in the mechanisms of host defense in the amniotic cavity by releasing cytokines [108] and anti-microbial molecules [111–113], performing phagocytosis [114], and forming neutrophil extracellular traps [115, 116]. In addition, women with clinical chorioamnionitis at term and intra-amniotic infection present severe acute inflammatory lesions in the placenta [40, 46, 85, 117], suggesting that both amniotic fluid neutrophils and the chorioamniotic membranes are a source of extracellular ASC in the amniotic cavity.

In vivo evidence of inflammasome activation in clinical chorioamnionitis at term with sterile intra-amniotic inflammation

In the current study, we reported that women with clinical chorioamnionitis at term and sterile intra-amniotic inflammation had higher amniotic fluid concentrations of extracellular ASC than those without intra-amniotic inflammation. These results are consistent with a previous study demonstrating that women with clinical chorioamnionitis and sterile intra-amniotic inflammation had higher amniotic fluid concentrations of IL-1β than those without intra-amniotic inflammation [46]. In addition, women with clinical chorioamnionitis at term had elevated concentrations of high mobility group box (HMGB) 1 [118], a prototypical alarmin [119, 120], whose intra-amniotic administration leads to preterm labor and birth in mice [121]. Importantly, the inoculation of the chorioamniotic membranes with this alarmin induces inflammasome activation [122]. Therefore, we suggested that, in the context of sterile intra-amniotic inflammation, the mechanisms leading to term and premature labor involve the inflammasome [52–57, 123]. Herein, we provided further evidence supporting our hypothesis by showing that there is inflammasome activation in the setting of sterile intra-amniotic inflammation in women with clinical chorioamnionitis at term.

Intra-amniotic infection vs. sterile intra-amniotic inflammation in clinical chorioamnionitis at term

Amniotic fluid ASC concentrations were comparable between women with intra-amniotic infection and sterile intra-amniotic inflammation, confirming that both inflammatory responses are similar in nature [46]. This is consistent with previous studies showing that the inflammasome can be activated by both microbes [59, 124–134] and alarmins [135–143]. However, sterile signals generate weaker and delayed inflammasome-dependent inflammatory responses compared to those triggered by microbial

signals [144]. The latter findings provide an explanation as to why, herein, women with clinical chorioamnionitis at term and sterile intra-amniotic inflammation had lower amniotic fluid IL-6 concentrations and a reduced number of amniotic fluid leukocytes compared to those with intra-amniotic infection. Consistently, women with clinical chorioamnionitis at term and sterile intra-amniotic inflammation have reduced pro-inflammatory lipid mediators of the 5-lipoxygenase pathway in amniotic fluid compared to those with intra-amniotic infection [145]. Nonetheless, women with sterile intra-amniotic inflammation presented mild/moderate acute inflammatory lesions in the placenta, suggesting that alarmins could cause placental and fetal damage. Collectively, these data suggest that the intra-amniotic inflammatory process initiated by alarmins is milder than that triggered by microbes in women with clinical chorioamnionitis at term; yet, it can be deleterious to the fetus.

Conclusion

The data presented herein showed that the intra-amniotic inflammatory response, either induced by alarmins or microbes, is characterized by the activation of the inflammasome – as evidenced by elevated amniotic fluid concentrations of extracellular ASC – in women with clinical chorioamnionitis at term. Such a process is similarly observed in both women with intra-amniotic infection and sterile intra-amniotic inflammation, suggesting that both inflammatory processes could cause placental and fetal damage.

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