OBSTETRICS

Are amniotic fluid neutrophils in women with intraamniotic infection and/or inflammation of fetal or maternal origin?

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BACKGROUND: Neutrophils are the most abundant white blood cells found in the amniotic cavity of women with intraamniotic infection and/or inflammation. The current belief is that these neutrophils are of fetal origin. However, abundant neutrophils have been found in the amniotic fluid of women with a severe acute maternal inflammatory response but without a fetal inflammatory response in the placenta, suggesting that these innate immune cells can also be of maternal origin or a mixture of both fetal and maternal neutrophils.

OBJECTIVE: We sought to investigate the origin of amniotic fluid neutrophils from women with intraamniotic infection and/or inflammation, and to correlate these findings with acute histologic maternal and fetal inflammatory responses in the placenta.

STUDY DESIGN: Amniotic fluid was collected from 15 women with suspected intraamniotic infection and/or inflammation (positive microbiological cultures and/or interleukin-6 concentrations $\geq$2.6 ng/mL). Amniotic fluid neutrophils were purified by fluorescence-activated cell sorting, DNA was extracted, and DNA fingerprinting was performed. DNA fingerprinting was also performed in the umbilical cord and maternal blood DNA. Fluorescence in situ hybridization was assayed in women with male neonates. Blinded placental histopathological evaluations were conducted.

RESULTS: First, DNA fingerprinting revealed that 42.8% (6/14) of women who underwent a single amniocentesis had mostly fetal neutrophils in the amniotic fluid. Second, DNA fingerprinting showed that 35.7% (5/14) of the women who underwent a single amniocentesis had predominantly maternal neutrophils in the amniotic fluid. Third, DNA fingerprinting indicated that 21.4% (3/14) of the women who underwent a single amniocentesis had an evident mixture of fetal and maternal neutrophils in the amniotic fluid. Fourth, DNA fingerprinting revealed that a woman who underwent 2 amniocenteses (patient 15) had fetal neutrophils first, and as infection progressed, abundant maternal neutrophils invaded the amniotic cavity. Fifth, fluorescence in situ hybridization confirmed DNA fingerprinting results by showing that both fetal and maternal neutrophils are present in the amniotic fluid. Sixth, most of the women who had predominantly amniotic fluid neutrophils of fetal origin at the time of collection delivered extremely preterm neonates (71.5% [5/7]). Seventh, all of the women who had predominantly amniotic fluid neutrophils of maternal origin at the time of collection delivered term or late preterm neonates (100% [6/6]). Eighth, 2 of the women who had an evident mixture of fetal and maternal neutrophils in the amniotic fluid at the time of collection delivered extremely preterm neonates (66.7% [2/3]), and the third woman delivered a term neonate (33.3% [1/3]). Finally, most of the women included in this study presented acute maternal and fetal inflammatory responses in the placenta (86.7% [13/15]).

CONCLUSION: Amniotic fluid neutrophils can be either predominantly of fetal or maternal origin, or a mixture of both fetal and maternal origin, in women with intraamniotic infection and/or inflammation. The findings herein provide evidence that both fetal and maternal neutrophils can invade the amniotic cavity, suggesting that both the fetus and the mother participate in the host defense mechanisms against intraamniotic infection.

Key words: acute chorioamnionitis, clinical chorioamnionitis, cytokine, fetal inflammatory response, fever, funisitis, human, inflammation, innate immune cells, interleukin-6, intraamniotic infection, labor, microbial invasion of the amniotic cavity, parturition, phagocytosis, pregnancy, preterm birth, preterm labor, term labor

Introduction

The current belief is that the amniotic fluid is sterile under normal circumstances; yet, it contains antimicrobial properties, a low number of white blood cells (WBC) (ie, leukocytes), and proteins implicated in fetal host defense mechanisms.[1-7] In women with intraamniotic inflammation, however, leukocytes are abundant in the amniotic cavity,[8-13] and their presence is accompanied by increased concentrations of inflammatory mediators such as antimicrobial peptides,[14-18] cytokines,[19-21] and lipids.[22-33] This inflammatory process in the amniotic cavity can be initiated by microorganisms (ie, intraamniotic infection) or danger signals derived from necrosis or cellular stress (ie, sterile intraamniotic inflammation).[34-39] In both conditions, the most abundant leukocytes in the amniotic cavity are the neutrophils.[8,13]

Amniotic fluid neutrophils are a part of the innate immune host defense mechanisms that take place in the amniotic cavity of women with intraamniotic infection.[40-42] This concept is supported by evidence demonstrating that amniotic fluid neutrophils: (1) are a source of antimicrobial products[17,18,43-45] and cytokines[13]; (2) can trap and kill bacteria invading the amniotic cavity by forming neutrophil extracellular traps[46]; and (3) can phagocytize microorganisms commonly found in the lower genital tract, eg, *Streptococcus agalactiae* (also known as group B streptococcus), *Ureaplasma urealyticum*, *Gardnerella vaginalis*, and *Escherichia coli*.[47]

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Amniotic fluid neutrophils were initially thought to be of maternal origin.[48] Yet, experimental evidence showed that amniotic fluid neutrophils from women[49] or rhesus monkeys[50] undergoing preterm labor were of fetal origin. In such cases, these innate immune cells could originate from the fetal vessels of the chorionic plate.[51] However, abundant neutrophils have also been observed in the amniotic fluid of women with a severe maternal inflammatory response without a fetal inflammatory response in the placenta,[13,46] indicating that further research is required to investigate whether amniotic fluid neutrophils are of maternal origin or a mixture of both fetal and maternal neutrophils.

The origin of neutrophils infiltrating the amniotic cavity[49] or the chorioamniotic membranes[52] was investigated using fluorescence in situ hybridization (FISH). However, this technique is semiquantitative and does not offer the complete assessment of chromosomal complement.[53] Short tandem repeat (STR) DNA profiling, also known as DNA fingerprinting, has emerged as a state-of-the-art method with high discriminating power and sensitivity for forensic DNA analysis and paternity testing.[54]

The aims of this study were to investigate the origin of amniotic fluid neutrophils from women with intraamniotic infection and/or inflammation using DNA fingerprinting and to complement these findings with FISH, the conventional method. In addition, placental histopathological examinations were performed and correlated to the origin of amniotic fluid neutrophils.

Materials and Methods

Study population

This was a cross-sectional study of women who underwent transabdominal amniocentesis due to clinical indications or amniocentesis during cesarean delivery. Women were enrolled at Hutzel Women's Hospital of the Detroit Medical Center (April through November 2016). Amniotic fluid samples were acquired by an automatic cell counter (Cellometer Auto 2000; Nexcelom Bioscience, Lawrence, MA) to obtain the viable cell numbers, most of which are WBC or leukocytes.[13] The inclusion criteria were as follows: (1) singleton pregnancy, (2) amniotic fluid samples without blood contamination, and (3) amniotic fluid samples with a large number of viable leukocytes (including mostly neutrophils and monocytes[13]) ($>1 \times 10^6$ cells/mL) to perform fluorescence-activated cell sorting (FACS) of amniotic fluid neutrophils.

All of the women provided written informed consent to donate additional amniotic fluid for research purposes, according to protocols approved by the institutional review boards of the Detroit Medical Center (Detroit, MI), Wayne State University, and the Perinatology Research Branch, an intramural program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, US Department of Health and Human Services.

Clinical definitions

Gestational age was determined by the last menstrual period and confirmed by ultrasound examination. The gestational age derived from sonographic fetal biometry was used when the estimation was inconsistent with menstrual dating. Clinical chorioamnionitis was diagnosed by the presence of maternal fever (temperature >37.8oC) accompanied by ≥2 of the following criteria: (1) uterine tenderness, (2) malodorous vaginal discharge, (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).[20,55-58] Term delivery was defined as birth >37 weeks of gestation, whereas preterm delivery was defined as birth between 20-36 6/7 weeks of gestation.

Intraamniotic inflammation was diagnosed when the concentration of interleukin (IL)-6 in the amniotic fluid was ≥2.6 ng/mL.[59] Microbial invasion of the amniotic cavity was defined as a positive amniotic fluid culture.[60-65] Intraamniotic infection was defined as the presence of microbial invasion of the amniotic cavity with intraamniotic inflammation.[36-39,59,66-76]

Sample collection

Amniotic fluid was retrieved by transabdominal amniocentesis under antiseptic conditions using a 22-gauge needle monitored by ultrasound. Amniotic fluid was also retrieved by amniocentesis during cesarean delivery under antiseptic conditions. Amniotic fluid samples were transported to the clinical laboratory in a capped sterile syringe and were cultured for aerobic and anaerobic bacteria, as well as for genital mycoplasmas.[8,73,77-80] Shortly after collection, a WBC count was determined in each amniotic fluid sample using a hemocytometer chamber, according to methods previously described.[8] Glucose concentration was also determined[81] and a Gram stain[82] was performed in each amniotic fluid sample. Cultures, WBC count, glucose concentration, and Gram stain were not performed in all of the amniotic fluid samples collected during cesarean delivery since these samples were collected for research purposes only. However, both IL-6 concentration[13] and the presence of bacteria (bacterial live/dead staining[46,83]) were assessed in most of the amniotic fluid samples, as previously described.

FACS of amniotic fluid neutrophils

Amniotic fluid samples were passed through a sterile 15-μm filter (catalog no. 43-50015-03; pluriSelect Life Science, Leipzig, Germany) to remove epithelial cells and centrifuged at 200*g* for 5 minutes at room temperature (n = 16). The cell pellet (mostly leukocytes[46]) was washed with 1X phosphate-buffered saline (PBS) (Life Technologies, Grand Island, NY), resuspended at 1×10^6 cells in 100 μL of BD FACS stain buffer (catalog no. 554656; BD Biosciences, San Jose, CA) containing 20% human FcR blocking reagent (Miltenyi Biotec, San Diego, CA), and incubated for 10 minutes at 4°C. Next, amniotic fluid cells were incubated with the following extracellular fluorochrome-conjugated antihuman antibodies (BD Biosciences) for 30 minutes at 4°C in the dark:

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