OBSTETRICS

Evidence of cardiac involvement in the fetal inflammatory response syndrome: disruption of gene networks programming cardiac development in nonhuman primates

Timothy Mitchell, MD; James W. MacDonald, MSc; Sengkeo Srinouanpranchanh, BS; Theodor K. Bammler, PhD; Sean Merillat, BS; Erica Boldenow, PhD; Michelle Coleman, PhD; Kathy Agnew, BS; Audrey Baldessari, DVM; Jennifer E. Stencel-Baerenwald, PhD; Jennifer Tisoncik-Go, PhD; Richard R. Green, PhD; Michael J. Gale Jr, PhD; Lakshmi Rajagopal, PhD; Kristina M. Adams Waldorf, MD

BACKGROUND: Most early preterm births are associated with intraamniotic infection and inflammation, which can lead to systemic inflammation in the fetus. The fetal inflammatory response syndrome describes elevations in the fetal interleukin-6 level, which is a marker for inflammation and fetal organ injury. An understanding of the effects of inflammation on fetal cardiac development may lead to insight into the fetal origins of adult cardiovascular disease.

OBJECTIVE: The purpose of this study was to determine whether the fetal inflammatory response syndrome is associated with disruptions in gene networks that program fetal cardiac development.

STUDY DESIGN: We obtained fetal cardiac tissue after necropsy from a well-described pregnant nonhuman primate model (pigtail macaque, *Macaca nemestrina*) of intrauterine infection (n=5) and controls (n=5). Cases with the fetal inflammatory response syndrome (fetal plasma interleukin-6 >11 pg/mL) were induced by either choriodecidual inoculation of a hypervirulent group B streptococcus strain (n=4) or intraamniotic inoculation of *Escherichia coli* (n=1). RNA and protein were extracted from fetal hearts and profiled by microarray and Luminex (Millipore, Billerica, MA) for cytokine analysis, respectively. Results were validated by quantitative reverse transcriptase polymerase chain reaction. Statistical and bioinformatics analyses included single gene analysis, gene set analysis, Ingenuity Pathway Analysis (Qiagen, Valencia, CA), and Wilcoxon rank sum.

RESULTS: Severe fetal inflammation developed in the context of intraamniotic infection and a disseminated bacterial infection in the fetus. Interleukin-6 and -8 in fetal cardiac tissues were elevated significantly in fetal inflammatory response syndrome cases vs controls ($P<.05$). A total of 609 probe sets were expressed differentially (>1.5-fold change, $P<.05$) in the fetal heart (analysis of variance). Altered expression of select genes was validated by quantitative reverse transcriptase polymerase chain reaction that included several with known functions in cardiac injury, morphogenesis, angiogenesis, and tissue remodeling (eg, angiotensin I converting enzyme 2, STEAP family member 4, natriuretic peptide A, and secreted frizzled-related protein 4; all $P<.05$). Multiple gene sets and pathways that are involved in cardiac morphogenesis and vasculogenesis were downregulated significantly by gene set and Ingenuity Pathway Analysis (hallmark transforming growth factor beta signaling, cellular morphogenesis during differentiation, morphology of cardiovascular system; all $P<.05$).

CONCLUSION: Disruption of gene networks for cardiac morphogenesis and vasculogenesis occurred in the preterm fetal heart of nonhuman primates with preterm labor, intraamniotic infection, and severe fetal inflammation. Inflammatory injury to the fetal heart in utero may contribute to the development of heart disease later in life. Development of preterm labor therapeutics must also target fetal inflammation to lessen organ injury and potential long-term effects on cardiac function.

Key words: cardiac, *Escherichia coli*, fetal inflammatory response syndrome, fetus, group B streptococcus, heart, morphogenesis, neonate, pigtail macaque, pregnancy, preterm birth, preterm labor, vasculogenesis

Infection, which is a leading cause of neonatal morbidity and death, is associated with most early preterm births.[1] Infection is often subclinical and thought to ascend from the lower genital tract that allows microbes to invade the placenta and amniotic fluid, which can lead to fetal bacteremia and sepsis. The fetal inflammatory response syndrome describes a condition of severe fetal inflammation that often occurs with fetal infection. The fetal inflammatory response syndrome is the counterpart to the adult condition (systemic inflammatory response syndrome) and is associated with an increased risk for multisystem fetal organ injury.[2-5] Studies have focused mainly on the relationship between the fetal inflammatory response syndrome and injury to the fetal lungs and brain, because they are often imaged and assessed postnatally. Inflammatory injury to other organs, which includes the fetal heart, has been hypothesized to occur but is more challenging to demonstrate in human neonates. Although many studies have associated prematurity, low birth weight, or fetal growth restriction with cardiovascular risk factors and heart disease later in life, the impact of perinatal infection and inflammation on fetal cardiac development is unknown.[6-16]

Accumulating evidence in humans and preterm sheep models implicates infection and fetal inflammation in altered fetal cardiac function. Fetal heart rate

EDITORS' CHOICE

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disturbances (eg, absence of variability, arrhythmias, cardiac dysfunction) have been associated with chorioamnionitis, which is an inflammation of the placental membranes often caused by infection.[17-20] In fetuses from pregnancies with preterm premature rupture of membranes, a condition often complicated by microbial invasion of the amniotic cavity, fetal echocardiography has revealed changes in diastolic ventricular function, which may increase cardiac output.[21] In a similar cohort, strain imaging to evaluate right ventricular function found evidence for impairment of systolic and diastolic function and, in cases with funisitis (umbilical cord inflammation), dyskinesia of the right ventricle.[22] These findings are consistent with observations in preterm sheep models of intraamniotic infection (*Candida albicans*) or inflammation (lipopolysaccharide), in which fetal inflammation was associated with a reduction in mean arterial blood pressure and oxygen saturation, depressed ventricular contractility, diastolic dysfunction, and a reduction in cardiomyocyte numbers.[23-25] The mechanism that links inflammation and fetal cardiac injury is unknown and challenging to elucidate in human neonates and sheep models for ethical reasons and the lack of genomic tools, respectively.

Our objective was to identify early biologic events in the fetal heart that occur after intrauterine infection and development of the fetal inflammatory response syndrome in a nonhuman primate. We hypothesized that development of the fetal inflammatory response syndrome is associated with fetal cardiac inflammation and changes in the gene program responsible for cardiac morphogenesis, analogous to observations that we have made on the effects of intraamniotic inflammation on fetal lung development.[26,27]

Materials and Methods

Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Research Council and the Weatherall report, "The use of non-human primates in research." The protocol was approved by the Institutional Animal Care Use Committee of the University of Washington (Permit Number: 4165-01). All surgery was performed with general anesthesia, and all efforts were made to minimize discomfort.

Animals and study groups

Cases that met criteria for the fetal inflammatory response syndrome were identified retrospectively in our pregnant nonhuman primate infection program based on an elevated fetal interleukin-6 (IL-6) level (>11 pg/mL; n=5) and compared with saline controls (n=5). Fetal cardiac microarray analyses were performed on animals that were inoculated with either (1) $1-3\times10^8$ colony-forming units (CFU) of a hyperhemolytic and hypervirulent group B streptococcus (GBS) strain (GBSΔ*covR*; n=4) into the choriodecidual space,[28] (2) 5×10^4 CFU of *E coli* RS218 into the amniotic fluid (prototypic strain that causes neonatal meningitis; n=1), or (3) saline solution into the amniotic fluid and choriodecidual space (n=5);[26] citations indicate publications that describe the animal experiments and pregnancy outcomes, but fetal cardiac transcriptomics and *IL-1B*/IL-6/IL-8 were not analyzed or reported previously. Because fetal cardiac tissue from the aforementioned saline controls was not saved to allow for protein (cytokine) analysis, an additional 4 saline controls were performed to enable the comparison of cytokines from fetal cardiac tissues of saline controls with fetal inflammatory response syndrome cases.

In our model, pregnant pigtail macaques were time mated, and fetal age was determined with the use of early ultrasound scans. Temperature in the animal quarters was maintained at 72−82°F. Animals were fed a commercial monkey diet, supplemented daily with fruits and vegetable; drinking water was always available. The animal was first conditioned to a nylon jacket/tether system for several weeks before surgery, which allows free movement within the cage but protected the catheters. On days 116−125 of pregnancy (term=172 days), catheters were implanted surgically via laparotomy into the maternal femoral artery and vein, amniotic cavity, and choriodecidual interface in the lower uterine segment (between uterine muscle and fetal membranes, external to the amniotic cavity). In the *E coli* case and saline controls, an additional catheter was implanted into the fetal internal jugular vein. Fetal electrocardiography electrodes and a maternal temperature probe were also implanted. Postoperative analgesia was provided by a 25-μg fentanyl patch that was applied the day before surgery, in addition to postoperative indomethacin. After 48 hours, the animals appeared to have recovered from surgery based on a return to baseline for activity, appetite, and bowel function.

After surgery, the animal was placed in the jacket and tether with the catheters/electrodes tracked through the tether system. Cefazolin and terbutaline sulfate were administered to reduce postoperative infection risk and uterine activity. Both cefazolin and terbutaline were stopped at least 72 hours before experimental start (approximately 13 half-lives for terbutaline, 40 half-lives for cefazolin, >97% of both drugs eliminated), which represented approximately a 7−10 day period of postoperative terbutaline administration. Cefazolin (1 g) was administered intravenously each day in saline controls to minimize the possibility of a catheter-related infection. Experiments began approximately 2 weeks after catheterization surgery to allow recovery (approximately 30−31 weeks human gestation).

At our center, term gestation in the noninstrumented pigtail macaque population averages 172 days.

Intraamniotic pressure was recorded, digitized, and analyzed continuously by previously described methods. The integrated area under the intrauterine pressure curve was used as a measure of uterine activity and was reported as the hourly contraction area (millimeters of mercury•seconds per hour) over 24 hours. Preterm labor was defined as >10,000 mmHg•sec/hr associated with a change in cervical effacement or dilation.

Histology

After cesarean delivery, fetal necropsy was performed in all animals; the heart