The link between cell-free DNA, inflammation and the initiation of spontaneous labor at term

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In 1997, Denis Lo and colleagues first reported the presence of free, extracellular DNA derived from fetal sources circulating in the blood of pregnant women throughout gestation; they labeled it cell-free "fetal" DNA (cffDNA).[1] In a subsequent series of publications, these investigators reported that cffDNA makes up 3.4−6.2% of the total cell-free DNA in maternal plasma, that cffDNA is present in short fragments with only 20% >193 base pair and none >313 base pairs, that cffDNA circulating in maternal plasma has a half-life of only approximately 16 minutes, and that cffDNA levels in maternal plasma increase 11- to 12-fold from mid to late gestation.[2-4] Since that time, reports by multiple investigators have confirmed the observations by Lo et al, especially in regard to the marked increase in cffDNA levels at the end of gestation.[5-8] Of note, the presence of cffDNA in maternal plasma and its increase at term does not appear to be restricted to human pregnancies; several published reports have described similar observations in other mammals, which include subhuman primates.[9-14]

Diagnostic and physiologic utility of cell-free DNA

Although the clinical utility of cffDNA as a noninvasive tool to perform fetal genetic analyses that include gender determination, testing for Mendelian genetic disorders, and aneuploidy screening has become well-established,[15] the physiologic role for the highly regulated release of cffDNA by the placenta and fetal membranes is yet to be determined clearly.[16] In this regard, several published reports have provided data that confirm a significant relationship between elevated levels of cffDNA and the onset of parturition, both preterm and at term.[7,17-19] In addition, experimental studies have confirmed the ability of small DNA fragments (oligodeoxynucleotides) and DNA isolated from fetal tissue to stimulate a proinflammatory cascade within the pregnant mouse uterus that results in pregnancy loss (ie, preterm deliveries and/or fetal resorptions).[20-22]

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Cell-free DNA and inflammation

The influx of inflammatory cells (especially macrophages and neutrophilic leukocytes) and the increase in proinflammatory cytokines in response to DNA that was observed during the experimental studies noted earlier are similar to the inflammatory events that were observed in the human uterus, cervix, and fetal membranes at or before the spontaneous onset of labor.[23-26] The DNA-mediated stimulation of a proinflammatory response appears to be produced, at least in part, by the Toll-like receptor-9 (TLR9), a pattern recognition receptor involved in innate immunity.[20-22,27] Of note, adult vertebrate DNA is normally a poor stimulator for TLR9 because of its increased levels of cytosine methylation and because adult DNA is poorly internalized into cells where it can interact with TLR9, which is present within intracellular endosomes.[27,28] The exception to this situation appears to be DNA that is released by the placenta and fetal membranes (ie, cffDNA), which has been found to be hypomethylated and able to be internalized and results in TLR9 stimulation that leads to increased proinflammatory cytokine release.[22,27]

Cell-free DNA, methylation, and inflammation during spontaneous labor

The report in this issue of the *American Journal of Obstetrics and Gynecology* by Christina Herrera et al[29] sought to further test the hypothesis that cell-free DNA, especially when hypomethylated, is associated with increased proinflammatory cytokine levels and with spontaneous labor. Specifically, these investigators performed a prospective cohort study to assess the total cell-free DNA concentrations, the DNA methylation ratios, and interleukin-6 (IL6) levels at 26−30 and 34−36 weeks gestation and at the time of admission for delivery at term. Fifty-five women completed these studies, of which 20 women presented at term in active labor and the other 35 were not in labor on admission. The DNA in the cell-free portion of maternal plasma was extracted and measured by fluoroscopy; DNA methylation was assessed with the use of bisulfite conversion and comparative DNA sequencing, and the plasma IL6 concentrations were measured with the use of a commercial enzyme-linked immunosorbent assay. These investigators observed that the methylation ratio in total cell-free DNA that was isolated from maternal plasma was significantly higher for women who were admitted in spontaneous labor when compared with women who were not in active labor. They also observed that total cell-free DNA and IL6 levels increased significantly from 26 weeks gestation to term in plasma samples that were obtained from the pregnant women who

went into spontaneous labor, compared with those who did not. The higher methylation ratio does not support the hypothesis that spontaneous labor is associated with decreased methylation of the cell-free DNA released by the placenta and fetal membranes; however, as previously noted, the cffDNA fraction represents only approximately 5% of the total cell-free DNA circulating in the maternal blood. Therefore, the hypomethylated cffDNA levels would be overshadowed by the 20-fold high amounts of normally methylated DNA in the maternal plasma. In contrast, the increasing levels of cell-free DNA and IL6 in the maternal blood during the third trimester that was observed by these investigators do provide support for the hypothesis that spontaneous labor is associated with factors that could lead to the proinflammatory events that result in the spontaneous onset of parturition.

Future investigation regarding cell-free DNA

As mentioned previously, adult vertebrate DNA is a poor stimulant for a proinflammatory response; therefore, one could hypothesize that the high levels of methylated cell-free DNA that was found in the maternal systemic circulation by Herrera et al is a protective mechanism that prevents the potentially detrimental effects that would occur with a robust systemic inflammatory response if hypomethylated cffDNA was not overshadowed. In contrast, the proinflammatory events that potentially stimulate parturition occur within the pregnant uterus, where high levels of hypomethylated DNA that are released by the placenta and fetal membranes would be in position to stimulate local inflammatory and decidual cells directly. To better understand these relationships, future research by Herrera et al must assess the methylation status of the cell-free DNA fraction that is released by the gestational tissues (ie, the placenta and fetal membranes) and the effect of this hypomethylated DNA on the innate immune response that occurs locally within the pregnant uterus. ■

REFERENCES

1. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485-7.
2. Lo YM, Tein MS, Lau TK, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet 1998;62:768-75.
3. Chan KC, Zhang J, Hui AB, et al. Size distributions of maternal and fetal DNA in maternal plasma. Clin Chem 2004;50:88-92.
4. Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. Am J Hum Genet 1999;64:218-24.
5. Ariga H, Ohto H, Busch MP, et al. Kinetics of fetal cellular and cell-free DNA in the maternal circulation during and after pregnancy: implications for noninvasive prenatal diagnosis. Transfusion 2001;41:1524-30.
6. Birch L, English CA, O'Donoghue K, Barigye O, Fisk NM, Keer JT. Accurate and robust quantification of circulating fetal and total DNA in maternal plasma from 5 to 41 weeks of gestation. Clin Chem 2005;51:312-20.
7. Majer S, Bauer M, Magnet E, et al. Maternal urine for prenatal diagnosis: an analysis of cell-free fetal DNA in maternal urine and plasma in the third trimester. Prenat Diagn 2007;27:1219-23.
8. Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. Prenat Diagn 2013;33:662-6.
9. Khosrotehrani K, Wataganara T, Bianchi DW, Johnson KL. Fetal cell-free DNA circulates in the plasma of pregnant mice: relevance for animal models of fetomaternal trafficking. Hum Reprod 2004;19:2460-4.
10. Wang G, Cui Q, Cheng K, Zhang X, Xing G, Wu S. Prediction of fetal sex by amplification of fetal DNA present in cow plasma. J Reprod Dev 2010;56:639-42.
11. De Leon PM, Campos VF, Dellagostin OA, Deschamps JC, Seixas FK, Collares T. Equine fetal sex determination using circulating cell-free fetal DNA (ccffDNA). Theriogenology 2012;77:694-8.
12. Kadivar A, Hassanpour H, Mirshokraei P, Azari M, Gholamhosseini K, Karami A. Detection and quantification of cell-free fetal DNA in ovine maternal plasma: use it to predict fetal sex. Theriogenology 2013;79:995-1000.
13. Jimenez DF, Tarantal AF. Quantitative analysis of male fetal DNA in maternal serum of gravid rhesus monkeys (Macaca mulatta). Pediatr Res 2003;53:18-23.
14. Mitsunaga F, Ueiwa M, Kamanaka Y, Morimoto M, Nakamura S. Fetal sex determination of macaque monkeys by a nested PCR using maternal plasma. Exp Anim 2010;59:255-60.
15. Wright CF, Burton H. The use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis. Hum Reprod Update 2009;15:139-51.
16. Phillippe M. Cell-free fetal DNA: a trigger for parturition. N Engl J Med 2014;370:2534-6.
17. Farina A, LeShane ES, Romero R, et al. High levels of fetal cell-free DNA in maternal serum: a risk factor for spontaneous preterm delivery. Am J Obstet Gynecol 2005;193:421-5.
18. Jakobsen TR, Clausen FB, Rode L, Dziegiel MH, Tabor A. High levels of fetal DNA are associated with increased risk of spontaneous preterm delivery. Prenat Diagn 2012;32:840-5.
19. Dugoff L, Barberio A, Whittaker PG, Schwartz N, Sehdev H, Bastek JA. Cell-free DNA fetal fraction and preterm birth. Am J Obstet Gynecol 2016;215:231.e1-7.
20. Thaxton JE, Romero R, Sharma S. TLR9 activation coupled to IL-10 deficiency induces adverse pregnancy outcomes. J Immunol 2009;183:1144-54.
21. Sun Y, Qin X, Shan B, et al. Differential effects of the CpG-Toll-like receptor 9 axis on pregnancy outcome in nonobese diabetic mice and wild-type controls. Fertil Steril 2013;99:1759-67.
22. Scharfe-Nugent A, Corr SC, Carpenter SB, et al. TLR9 provokes inflammation in response to fetal DNA: mechanism for fetal loss in preterm birth and preeclampsia. J Immunol 2012;188:5706-12.
23. Thomson AJ, Telfer JF, Young A, Campbell S, Stewart CJ, Cameron IT, et al. Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. Hum Reprod 1999;14:229-36.
24. Osman I, Young A, Ledingham MA, et al. Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. Mol Hum Reprod 2003;9:41-5.
25. Haddad R, Tromp G, Kuivaniemi H, et al. Human spontaneous labor without histologic chorioamnionitis is characterized by an acute inflammation gene expression signature. Am J Obstet Gynecol 2006;195:394.e1-24.
26. Unal ER, Cierny JT, Roedner C, Newman R, Goetzl L. Maternal inflammation in spontaneous term labor. Am J Obstet Gynecol 2011;204:223.e1-5.
27. Goldfarb I, Adeli S, Berk T, Phillippe M. Fetal and placental DNA stimulation of TLR9: a mechanism possibly contributing to the proinflammatory events during parturition. Reprod Sc 2017 Jan 1: 1933719117728798. https://doi.org/10.1177/1933719117728798. [Epub ahead of print].
28. Phillippe M. Cell-free fetal DNA, telomeres, and the spontaneous onset of parturition. Reprod Sci 2015;22:1186-201.
29. Herrera CA, Stoerker J, Carlquist J, et al. Cell-free DNA, inflammation, and the initiation of spontaneous term labor. Am J Obstet Gynecol 2017;217:583.e1-8.