The entry of fetal and amniotic fluid components into the uterine vessel circulation leads to sterile inflammatory processes during parturition

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INTRODUCTION

Dramatic advances of molecular analysis and biological profiling represent an opportunity to improve an in-depth understanding of human parturition. Parturition is characterized by the activation of innate immune and neuroendocrine mechanisms. Pregnancy is a unique immunological state in which a balance of immune tolerance and suppression may participate in the regulation of the host immune response and protection of the fetus. Oxytocin and corticotropin-releasing hormone are important neuroendocrine pathways involved in parturition (Petraglia et al., 2010). Thus, endocrine–immune interaction controls conditioning of the myometrium and plays as a prelude to the onset of labor. Furthermore, accumulating evidence suggests that ascending intrauterine infection results in premature birth and high fetal mortality (Marini and Eschenbach, 1990). There has been an increased awareness of the role of infection and inflammation at the time of parturition. Bacterial infection and sterile inflammation (a physiological process) are key mechanisms of human preterm and term labor, respectively. Several studies focused on the feedforward loop in which, near the end of pregnancy, the pro-inflammatory cytokine–prostaglandin (PG) axis activates the uterus (Golightly et al., 2011). Excessive inflammation produces uterine contractile stimuli leading to labor and delivery.

Despite considerable research and progress in the technology of reproduction, the causes of the initial events driving parturition remain obscure. We review the contemporary literature on sterile inflammation that support mechanism of parturition at term.

STUDY METHODOLOGY

The present study reviews the literature for biological studies of human parturition. Data pertaining to in vitro and in vivo studies were included. A computerized literature search was performed to identify relevant studies reported in the English language. All abstracts from Medline electronic database were reviewed to identify papers for full-text review. The web-based database were searched, combining the keywords “genome-wide,” “proteomics,” “onset,” “labor,” “term,” “myometrium,” “cervix,” “amniotic fluid,” “TLR,” “inflammation,” “immunity,” “leukocytes,” “cytokine,” “complement,” and “NF-kappaB” with “parturition.” Additionally, references in each study were searched to identify potentially missed studies. Target publications are mainly reports on human studies and animal models, as well as basic studies in gene and protein expression systems. Abstracts were not included, since they do not undergo a stringent peer review process.

PARTURIATION AFFECTED BY THE STATUS OF IMMUNITY

Alterations in maternal immunity, peripheral tolerance and fetal-maternal tolerance of uteroplacental unit have been seen during pregnancy, the so-called “immunological paradox” (Petraglia et al., 2008). In humans, there are key players in the regulation of the pathway involved in suppression of immune responses. NK
Abundant research has demonstrated complement activation in the third trimester, suggesting that the complement system is activated. C5a may be an activation of the complement cascade. Complement activation during pregnancy was characterized by an increase in complement regulatory proteins and their potential to contribute to the pathogenesis of parturition. AT TERM, the myometrium was a driver gene for inflammation-related signaling pathways. These data support the requirement of the Th1/Th2 shift for successful parturition.

In addition, one of the initial responses of this innate immunity may be an activation of the complement cascade. Complement can generate biologically active products, which trigger inflammation. Normal pregnancy was characterized by an increase in anaphylatoxin C3a in the maternal circulation in the third trimester, suggesting that the complement system is activated. C3a up-regulated pro-inflammatory and pro-labor mediators, including pro-inflammatory cytokines (IL-6 and IL-8), cyclooxygenase (COX)-2, PGD2, and PGF2α, matrix metalloproteinase (MMP)-9, and 8-isoprostane in human gestational tissues via the C5a receptor (CD88)-Mediated NF-κB activation (Lappas et al., 2012).

Abundant research has demonstrated complement activation in an innate immunity of human parturition (Gallagher et al., 1984; Benson et al., 2006; Benson, 2007; Soto et al., 2009; Gonzalez et al., 2011; Kato et al., 2012; Lappas et al., 2012), but has yet to investigate whether complement activation is the result of fetal antigen leaking into the maternal circulation.

MECHANICAL STRETCH OF UTERINE MYOMETRIUM AT TERM

Many investigators have analyzed genome-wide transcriptions and proteomics of the reproductive tissues at different stages of parturition. At term, uterine myometrium, including myometrial smooth muscle cells and fibroblasts, was stretched by growing fetuses. Molecular mechanism mediating stretch-induced signaling pathways has been elucidated. Cyclic mechanical stretch induced an increase in secretion of pro-inflammatory cytokines in myometrial smooth muscle cells compared to non-stretch controls (Sosnowa et al., 2004; Kendall-Wright et al., 2010; Has et al., 2012). This increase in cytokine production correlated with activation of NF-κB (Mendelson, 2009). Mechanical stretch also stimulated COX-2 expression through activation of the activated protein (AP)-1 system (Soranno et al., 2004). Thus, cyclic stretch and release in myometrial smooth muscle cells stimulated a robust activation of NF-κB and AP-1. Some of the important genes up-regulated in human myometrium during term labor were monocyte chemotactic protein-1 (MCP-1, also known as C-C chemokine motif ligand 2, CCL-2), IL-8, and TNF-α. MCP-1 was a member of a large chemokine family and displayed chemoattractive activity for monocytes/macrophages, as well as promoting macrophage activation (Explin et al., 2003). MCP-1 expression was enhanced by mechanical stretch of the uterine myometrium. IL-8 and TNF-α, whose expression was specifically restricted to myometrium after the onset of labor, were potential candidates of contraction-associated cytokines. In contrast, both IL-1β and IL-6 were present in term myometrium before and during labor, suggesting that these cytokines were involved in the preparation or conditioning for the synchronized contractions of labor (Schiringer et al., 2000). IL-1β participated in the regulation of the myometrial contractions via an increase in PGs production (Hertelendy et al., 1993). IL-1β was synthesized as an inactive precursor, pro-IL-1β, and then cleaved into the active form through cytosolic protein complex termed "inflammasomes" (Gottsch et al., 2008). The placenta expressed the inflammasomes. Cellular stress in response to inflammatory conditions accounted for activation of the inflammasomes, which occurred during labor. The previous elegant review discussed the role of the inflammasomes system and their potential to contribute to the pathogenesis of preterm birth (Abrahams, 2011). Unfortunately, we have very little understanding of their function in normal pregnancy and the onset of term labor.

These data suggest that transduction of the stretch signal in myometrial smooth muscle cells involves alteration of the gene expression signature. Activation of NF-κB and AP-1 increased expression of several genes implicated in the control of immunity and inflammation (Mendelson, 2009; Kahanati et al., 2011). MCP-1 locally mediated leukocyte migration into uterine myometrial tissues. Myometrial smooth muscle cells can play a role as immune cells and participate in the sterile inflammation at term (Khanjani et al., 2011; Shynlova et al., 2012). Taken together, mechanical stretch-induced NF-κB/AP-1 activation, which occurs prior to labor, modulates the expression of numerous inflammation-associated genes that are directly or indirectly involved in the positive feedback loop during parturition.
THE ENTRY OF FETAL AND AMNIOTIC FLUID COMPONENTS INTO THE UTERINE VESSEL CIRCULATION

Prior to labor, there were prominent changes in the myometrial fibers that increase the distance between muscle layers and promoted edema. These cells exhibited such morphology as shearing, shrinkage, and apoptosis. Endothelial cell damage in the uterine myometrium were very common at term prior to labor. The vascular lumen of endothelial cells contained fibrin and platelet thrombi, microparticles, desquamated endothelial cells, amniotic squamous cells, and mucoid material (Leong et al., 2008). The entry of amniotic fluid components into the uterine vessel circulation might be the common physiologic mechanism. Histologically, these changes were present in myometrial tissues obtained during labor at term, providing a mechanism by which fetal and amniotic fluid components may access myometrial cells (Leong et al., 2008). In addition, small amount of fetal red cells were normally detectable in peripheral blood of the mother in all pregnancies, indicating that fetal cells can enter the maternal circulation (Ahmed and Abdullatif, 2011). The presence of not only intact fetal cells but also fetal-origin nucleic acids (cell-free fetal DNA and RNA) in maternal blood has been identified. Cell-free fetal nucleic acids afford the opportunity for the promising prenatal genetic testing. Part of the fetal DNA fragments derived from the placenta. These data support that a substantial amount of fetal antigens might be transported to the uterine vasculature and maternal circulation at term prior to labor.

Changes in the recognition and adaptation to a set of foreign antigens would be a mechanism of the onset of labor. The maternal responses to an alloantigen challenge were reduced during pregnancy (Spencer et al., 2008), while, alloantigens resulted in immune-mediated fetal rejection in the term parturition. Recently, pattern recognition receptors (PRRs) responsive to unique molecules, termed pathogen-associated molecular patterns (PAMPs), have received considerable attention as possible contributors to the onset of preterm labor. Microorganisms have PAMPs that were recognized by PRRs such as TLRs. Ned-like receptors (NLRs), and the inflammasomes (Tang et al., 2012). PRRs recognized not only PAMPs, but also host-derived danger signals "alarmin" or damage-associated molecular patterns (DAMPs) derived from damaged tissue. In general, DAMPs are known to be cell-derived immunity. The fetal DNA found in the maternal circulation could act as DAMPs through PRRs such as TLR9 or AIM2 (absent in melanoma 2;Barber, 2011). The TLR9 has an ability to bind structurally highly conserved microbial molecules such as CpG motif-containing DNA and subsequently initiates the production of Th1 pro-inflammatory cytokines and chemokines (Barber, 2011). AIM2 acts as a DNA sensor in innate immunity and mediates inflammatory responses involving IL-1β. AIM2 also triggered the assembly of the inflammasomes. Cell-free fetal DNA would mediate innate immune signaling that provides an important step toward initiation of parturition.

Furthermore, hyaluronan, a component of the extracellular matrix, was a component of the DAMPs associated with NLRS. Intra-amniotic hyaluronan levels were elevated in pregnancies. Hyaluronan was released into the extracellular milieu since an amniotic fluid where it modulates immune activity. Yet, this hypothesis has not been proved.

These initial events prior to labor hint at a possible causative role. During pregnancy and prior to labor, women were tolerant of their semi-allogeneic fetal components: the maternal immune system came into contact with trophoblasts and other semi-allogeneic components, including amniotic fluid, fetal cells, and cell-free fetal DNA. The modulation of cell-mediated immunity caused by a substantial amount of DAMPs at term prior to labor may be responsible for the increased susceptibility to parturition.

INFILTRATION OF LEUKOCYTES IN UTERINE MYOMETRIUM AND CERVIX

An accumulating body of evidence has demonstrated that uterine myometrial contraction coincident with the onset of term labor was accompanied by the massive influx of leukocytes in all regions of uterine myometrium, amnion, choriodeseda, and cervix following spontaneous labor compared with non-laboring tissues (Thomson et al., 1999; Keski-Nisula et al., 2000; Osman et al., 2006). Marked myometrial inflammation was not associated with the prediction of pathological conditions such as infection (Keski-Nisula et al., 2003). The influx of fetal leukocytes into the myometrium has been implicated in the initiation of parturition in mice (Kim et al., 2006). During human labor, however, fetal macrophages from the amniotic cavity or the chorionamnios membranes did not migrate into the myometrium (Kim et al., 2006). Leukocytes in the myometrium was a maternal origin. The uterus at term was infiltrated with inflammatory cells, which was subsequently associated with advanced labor and uterine contraction, because pro-inflammatory cytokines such as TNF-α can stimulate uterine smooth muscle cell contractility (Keski-Nisula et al., 2005; Ylidon et al., 2003; Fitziiboon et al., 2009; Kanel, 2010; Lee et al., 2012). Inflammatory cells orchestrate processes required for initiation of the myometrial contraction (Thomson et al., 1999; Osman et al., 2003).

The uterine cervix must be disorganized before, during and after parturition, via release of protoxyl enzymes and followed by a tissue repair postpartum. Leukocyte density increased two to threefold between the first trimester of pregnancy and term, prior to the onset of labor (Spencer et al., 2008). A marked increase in macrophage density was observed after the onset of labor (Keski-Nisula et al., 2000; Timmons et al., 2009; Kanel, 2010). This occurred during the course of cervical softening and effacement (Ilostrom et al., 1997). In contrast, neutrophils specifically increased in the postpartum period and were involved in the postpartum tissue repair (Thomson et al., 1999; Hamilton et al., 2012).

Taken together, leukocyte infiltration is a complex process involving at least two steps, including the first step, mechanical stretch of uterine myometrium without involvement of resident macrophages, and then the second step, the entry of fetal and amniotic fluid alloantigens into the maternal circulation. These steps might drive myometrial chemokine expression primarily via activation of NF-κB, which in turn results in a prominent leukocyte
infiltration into the uterus. DAMPs such as fetal antigens activated infiltrated macrophages via the TLR/AIM2/NF-κB pathway. The infiltration of myometrium (before the onset of labor) and cervix (in the postpartum) with activated leukocytes has been associated with the initiation of parturition and a rapid repair postpartum, respectively.

**INFLAMMATORY GENE REGULATORY NETWORKS IN PARTURITION**

Several investigators used a network mining algorithm to identify tightly connected gene expression pathways that were frequently present in microarray data set samples. Global gene expression analyses and single gene approaches revealed that human labor involved the infiltration of specific leukocyte subsets and the secretion of autocrine and paracrine mediators, including NF-κB, pro-inflammatory cytokines (IL-1, IL-6, TNF-α), chemokines (IL-8/CXCL-8, MCP-1/CCL-2), IL-1 receptor accessory protein (IL-1RAP), TLRs, COX-2, contraction-associated proteins (CAPS), oxytocin receptor (OXTR), connexin-43 (CX-43), the prostaglandin F receptor (FP), hypoxia-inducible factor (HIF), thrombospondin 1 (TSP-1), MMP-2, and MMP-9 (Vora et al., 2010; Li et al., 2011; Lim et al., 2012). The majority of these genes were downstream targets of NF-κB and PRRs such as TLRs. Spontaneous term labor group is associated with increasing activation of the NF-κB signaling network relative to the term no labor cohort (Vora et al., 2010). Therefore, sterile inflammation has underlied parturition at term and the TLR/NF-κB axis in macrophages is an essential pathway.

**ENDOCRINE AND PROSTANOID PATHWAYS**

Myometrial contractility has been the predominant focus for the mechanism that contributes to regulation of development of parturition and initiates labor. The endocrine status affects the process of parturition. The potential factors included PGs, oxytocin,
nitric oxide, COX-2, cytokines, as well as endocrine mediators such as estrogen, progesterone, corticotrophin releasing hormone, and cortisol. Activation of contractile proteins (e.g., COX-2, OXTR) was directly promoted by transcription factors NF-κB and AP-1. Such changes in prostanooids and COX-2 pathways seem to be inflammation-mediated physiological responses at the later stages of parturition. It is likely that all of these factors were involved in the feedforward loop during parturition (Christians et al., 2008; Golightly et al., 2012).

SUMMARY
This review focuses on the contribution of biological, biochemical, and genetic changes during each phase of activation in the process of parturition. This process consists of four steps (Figure 1). The first step is mechanical stretch of myometrium which can promote the entry of amniotic fluid components into the uterine vessel circulation at term prior to labor. The constituents of amniotic fluid include fetal and amniotic fluid-derived DAMPs such as cell-free fetal DNA and RNA. The second step consists of immune cells trafficking and activation which might be induced by fetal and amniotic fluid-derived DAMPs such as cell-free DNA in the course of sterile inflammation by infiltrating leukocytes and subsequent DAMP activation.

ACKNOWLEDGMENTS
Grant support: Supported by Grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan to the Department of Obstetrics and Gynecology, Nara Medical University (Hiroshi Kobayashi).

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In conclusion, there are at least two phases of human parturition: the initial wave of the entry of amniotic fluid components into uterine vasculatures, which would be followed by the second big wave of sterile inflammation by infiltrating leukocytes and subsequent DAMP activation.
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Molecular basis of human parturition

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any com- mercial or financial relationships that could be construed as a potential con- flict of interest.

Received: 08 August 2012; accepted: 09 October 2012; published online: 23 October 2012.

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