Inflammatory processes in preterm and term parturition

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Abstract

A role for the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor alpha (TNF-α) is evident in term and preterm delivery, and this is independent of the presence of infection. All uterine tissues progress through a staged transformation near the end of pregnancy that leads from relative uterine quiescence and maintenance of pregnancy to the activation of the uterus that prepares it for the work of labour and delivery. The uterus is activated by pro-inflammatory cytokines through stimulation of the expression and production of uterine activation proteins (UAPs). One of these actions is the stimulation of prostaglandin (PG) synthesis. Particularly important for labour is PGF2α and its receptor, PTGFR. In addition, pro-inflammatory cytokines are able to increase the synthesis of matrix metalloproteinases (MMPs), vascular endothelial growth factor (VEGF) and the progesterone receptor C isoform, which leads to decreased tissue progesterone responsiveness. Some of these effects are replicated by PGF2α, suggesting that it may act via its receptor to amplify the direct actions of cytokines. In turn, VEGF may enhance leukocyte recruitment to the uterus, and MMP-9 may promote activation of inactive pro-form cytokines. Pro-inflammatory cytokines also decrease the activity of 11β-hydroxysteroid dehydrogenase, which likely increases intrauterine cortisol concentrations. In turn, cortisol may drive PG synthesis. Together these feed-forward mechanisms activate the uterus, trigger the production of uterine contractile stimulants and lead to labour and delivery.

Keywords: Pro-inflammatory cytokines; Pregnancy; Birth; Uterine activation; Preterm; Prostaglandins

1. Introduction

Preterm birth (<37 weeks of gestation) is the leading cause of mortality and morbidity in newborn infants. Data from the Canadian Perinatal Surveillance Report show that 81.6% of infants born preterm have a low birth weight (LBW; <2500 g at birth) and that 60% of all neonatal deaths occur among LBW and preterm infants (Health-Canada, 1999). Further, preterm birth and LBW are associated with high neonatal and infant morbidity, including chronic respiratory illnesses, neurodevelopmental problems and long-term impairment (Berkowitz and Papiernik, 1993; Kramer, 1987).

Currently, over 60% of preterm deliveries are unexplained (Green et al., 2005), ascribable only to ‘idiopathic’ preterm labour or preterm premature rupture of fetal membranes. Some experts believe that these are associated with a (sub)clinical inflammatory response in the maternal and/or fetal tissues. Shim et al. (2004) showed that up to 70% of spontaneous

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preterm birth <30 weeks of gestation is associated with intrauterine infection, compared to only 30–40% after 30 weeks. Bacterial vaginosis alone or progression to chorioamnionitis are important risk factors for preterm birth (Leitich et al., 2003). Various sexually transmitted diseases, such as gonorrhoea and syphilis, are also associated with an increased risk of a preterm delivery (Goldenberg et al., 1997). This is true also for periodontal disease (Goepfert et al., 2004), data that strongly imply inflammatory processes in the genesis of preterm birth.

Pregnancies that display signs of infection are characterized by elevated levels of pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor alpha (TNF-α), in the amniotic fluid, myometrium, decidua, fetal membranes and maternal serum (Goldenberg et al., 2000). Pregnancies without signs of infection in the third trimester and deliveries without infection also show increased levels of IL-1β and IL-8 in the amnion, chorio-decidua and myometrium (Elliott et al., 2001). This suggests a causal role for cytokines in the process of parturition, regardless of the presence of infection.

Parturition involves five distinct yet integrated physiological events: rupture of the membranes, cervical ripening and dilatation, contractility of the myometrium, placental separation and uterine involunt (Olson, 2003). Prostaglandins (PGs), produced by the myometrium and intrauterine tissues of pregnancy, are involved in all these events. Most commonly, PGs are associated with stimulation of the myometrium. Indeed, treatment of pregnant women with PGs induces labour. Furthermore, inhibitors of PG endoperoxide H synthase (PGHS) delay the time of onset of labour (Novy et al., 1974).

The most potent uterine contractile prostanoid is PGF$_{2\alpha}$, and its action is mediated by its specific receptor, PTGFR (Olson, 2005). PTGFR is a key uterine activation protein (UAP), promoting the ability of the tissues to carry out the process of parturition. Myometrial PTGFR mRNA is elevated at term and preterm birth in humans and rodents (Brodt-Eppley and Myatt, 1999; Cook et al., 2003). Also, infusion of a specific inhibitor, THG113.31, in sheep and mice delays preterm birth and prolongs gestation (Hirst et al., 2005; Peri et al., 2002). In rats, myometrial PTGFR mRNA expression rate is decreased during pregnancy, and its expression increases significantly again at term (Matsumoto et al., 1997). This suggests that PTGFR plays a central role in both pregnancy maintenance and parturition.

The purpose of this review is to describe the relationships between pro-inflammatory cytokines, PGF$_{2\alpha}$ and PTGFR and uterine activation during normal term pregnancy and at preterm birth.

2. Uterine pro-inflammatory cytokines

Interleukin-1β, IL-6 and TNF-α are pleiotropic cytokines in terms of being produced by and eliciting a response from a myriad of cells, many of which are autocrine (Lucey et al., 1996). They are also pleiotropic in eliciting a number of different cellular responses (e.g. TNF-α stimulates both survival and apoptosis) (Baker and Reddy, 1998). However, all three stimulate translocation of the transcription factor, nuclear factor kappa B (NFκB), to the nucleus and subsequent triggering of transcription of many mediators of inflammation, including each of these three cytokines (Iwamoto and Konicke, 1997; Malinin et al., 1997).

IL-6 is secreted as a 26 kDa protein that binds to the IL-6Rα (either soluble or in a complex with gp130 and the beta subunit) (Taga and Kishimoto, 1997). TNF-α is synthesized as a 26 kDa cell surface precursor (which is biologically active) (Birkland et al., 1992) that is cleaved by TNF-α converting enzyme (TACE) into a 17 kDa soluble protein that associates as an active trimer (Mohan et al., 2002). However, serine proteases are also involved in the conversion (Armstrong et al., 2006). TACE also catalyzes shedding of TNF receptors into antagonists of TNF action (Bzowska et al., 2004). IL-1β is synthesized as a 33 kDa inactive precursor that is processed by caspase-1, caspase-5 and caspase-7 into the active, secreted 17 kDa form (Martinon et al., 2002).

Macrophages are major producers of these cytokines. The decidua in late gestation has the largest concentration of leukocytes (neutrophils and macrophages) of the intrauterine tissues and it expresses the highest level of pro-inflammatory cytokine mRNA, which increases with labour (Osman et al., 2006). Further, in decidua there are a number of other cells (NK cells, decidual cells, T lymphocytes, endothelial cells, trophoblasts) implicated as producers (Dudley et al., 1996; Saito, 2000).

3. The effect of cytokines on prostaglandin synthesis and uterine activation

There is strong evidence in all species supporting an essential role for PGs in parturition; hence, their synthesis, metabolism and actions are of importance (Olson, 2003, 2005). The key regulatory steps in PG synthesis revolve around the release of the precursor, arachidonic acid, from membrane phospholipids and its conversion to an endoperoxide intermediate by PGHS. Two isoforms of the PGHS enzyme exist: PGHS-1 is expressed
in many tissues and is responsible for constitutive PG production; PGHS-2 is an inducible form of the enzyme. The increase in PG production prior to parturition is due largely to increased PGHS-2 (Hirst et al., 1995). Several cytokines, including IL-1β and TNF-α, regulate and stimulate PGHS-2 expression and PG synthesis (Kniss et al., 1997; Molnar et al., 1993). Recently, Hirsch et al. (2006) reported that mice lacking type 1 IL-1 and TNF-α receptors have significantly lower myometrial levels of PGHS-2 mRNA after Escherichia coli administration, suggesting an involvement for cytokines in myometrial PGHS-2 production.

Further, high levels of choric prostaglandin 15-hydroxy dehydrogenase (PGDH), the primary enzyme that catalyzes PGs into inactive metabolites, may prevent intact PGs crossing from the fetal into the maternal compartment or vice versa (Mitchell et al., 1993; Van Meir et al., 1997). PGDH is present at high activity in placental syncytiotrophoblast and chorionic trophoblast cells throughout gestation (Mitchell et al., 1993), although it decreases in human labour at term and preterm in the chorion (Van Meir et al., 1997). Cytokines such as IL-1β and, to a lesser extent, TNF-α are reported to decrease PGDH mRNA and activity in intact fetal membrane disks and cultured chorion and placental trophoblast cells (Pomini et al., 1999), whereas progesterone promotes PGDH expression (Patel et al., 1999).

Other evidence suggests that pro-inflammatory cytokines also stimulate PG receptor expression. Mice that are knocked out (−/−) for the IL-6 gene deliver one day later than wild-type (+/+) mice (20.7 ± 0.2 days vs. 19.7 ± 0.1 days; Robertson, unpublished data). Our preliminary observations suggest that this is the consequence of a key role for IL-6 in regulating a range of uterine activating proteins, since genetic IL-6 deficiency is associated with decreased expression of mRNA encoding PTGFR and oxytocin receptor (OTR) in the whole uterus (including myometrium, decidua and endometrium) (Robertson and Olson, unpublished data). In support of a similar regulatory pathway in human cells, the mRNA expression of PTGFR increases when human ULTR cells (a myometrium-derived cell line) are incubated with IL-6 or IL-1β (Zaragoza et al., 2006).

Interestingly, mRNA expression of vascular endothelial growth factor (VEGF) is greater in chorio-decidua from women in spontaneous preterm labour than those delivering spontaneously at term (Marvin et al., 2002). Activation of VEGF may lead to increased uterine concentrations of inflammatory cells, and this process could be stimulated by pro-inflammatory cytokines directly or indirectly via PGF2α and PTGFR. IL-1β and TNF-α each directly increase human decidual fibroblast VEGF, basic fibroblast growth factor and β-transforming growth factor protein levels (Hayashi et al., 2006). In Ishikawa cells and endometrial adenocarcinoma tissue explants, PGF2α stimulates VEGF mRNA and protein secretion via PTGFR (Sales et al., 2005). If studies should demonstrate that PGF2α increases VEGF in decidua, it might be associated with the development or maintenance of high volume arteries. VEGF is not only important for decidual growth and maintenance, but it might also stimulate extravasation of white blood cells into the decidua and therefore contribute to the inflammatory process in decidua.

Uterine activation refers to consistent changes in several genes and their proteins that prepare the uterus for labour. These uterine activation proteins, or UAPs, include the OTR, PGHS-2, connexin-43 (CX-43), inducible nitric oxide synthase (iNOS) and PTGFR (Olson, 2005). They may be key intermediates in essential processes required for labour. As a result of activation, the sensitivity and responsiveness of the uterus to contractile stimulants, such as oxytocin and PGF2α, is increased. Pro-inflammatory cytokines regulate the expression of all the UAPs, including OTR (Fang et al., 2000), PGHS-2 (Hirsch et al., 2006; Kniss et al., 1997; Molnar et al., 1993), CX-43 (Tonon and D’Andrea, 2000), iNOS (Marczin et al., 1993) and PTGFR (Zaragoza et al., 2006).

Hence, pro-inflammatory cytokines may promote term and preterm labour through stimulation of all the UAPs. As partial evidence for this, we demonstrated a few years ago that tyrosine kinase inhibitors (tyrphostins) were at least as effective as the PGHS-2 inhibitor, NS-398, in preventing LPS-induced preterm birth in mice (Mijovic et al., 2002). Tyrphostins decrease inflammatory reactions and reduce cytokine levels (Balachandra et al., 2005).

Recently, Robertson et al. (2006) utilized IL-10−/− mice to demonstrate the relationships of pro-inflammatory cytokines and preterm birth and fetal loss. They determined that it required 10-fold less LPS to stimulate a 50% preterm fetal loss in IL-10−/− mice than wild-type mice. Further, the serum, uterine and placental concentrations of TNF-α and IL-6 were considerably higher in IL-10 knockout mice than wild-type mice. Administration of recombinant IL-10 to knocked-out mice decreased the TNF-α and IL-6 responses to LPS and attenuated fetal loss.

IL-10 acts as a counterbalance to cytokines that promote labour. Previous studies demonstrated that endotoxin or inflammatory mediator-induced preterm birth in rodents can be attenuated by administration of IL-10, presumably by deactivation of macrophages.
and inhibition of pro-inflammatory cytokine synthesis (Fiorentino et al., 1991).

4. Cytokines stimulate uterine activation via the NFκB system

The NFκB family of transcription factors is associated with inflammation and can be activated by pro-inflammatory cytokines. Considerable evidence accumulated since 1999 has shown that NFκB is involved with many aspects of PG synthesis and action in intrauterine tissues, especially in association with labour. This mechanism includes stimulation by TNF-α, IL-1β and LPS (Belt et al., 1999; Lappas et al., 2006). It appears that NFκB mediates IL-1β action at several levels of the PG synthesis-receptor cascade, including secretory type II PLA2 (Lappas et al., 2006), PGHS-2 (Belt et al., 1999), and PTGFR (Zaragoza et al., 2006). Our laboratory cloned and characterized the human PTGFR promoter and found it contains several response elements that bind the transcription factors NFκB, C/EBPβ and AP-1 which are associated with inflammatory cytokine action (Zaragoza et al., 2004). It contains also both repressor and enhancer regions, suggesting that PTGFR is highly regulated. The possibility exists that NFκB may also regulate specific PG synthases and PG metabolism (PGDH), but there is no evidence at present to support or refute this notion.

An inhibitor of NFκB, SN-50, was able to delay preterm birth when administered into the amniotic fluid of mice (Condon et al., 2004), and infusion of sulphasalazine, an anti-inflammatory and NFκB inhibitor, decreased uterine electromyographic activity in pregnant ewes induced to enter preterm labour with RU486, the progesterone receptor blocker (Young, personal communication). These studies suggest the participation of NFκB in preterm labour.

5. Cytokines, intrauterine cortisol and prostaglandins

Intercconversion of the active glucocorticoid cortisol and its inactive metabolite cortisone is catalyzed by 11β-hydroxysteroid dehydrogenase (11β-HSD). There are two isoforms of this enzyme (Albiston et al., 1994): 11β-HSD1, which has low affinity for cortisol, is widely distributed, operates bidirectionally and requires NAD(P)H as a co-factor; and 11β-HSD2, which is a high affinity, unidirectional oxidase and is NAD+ dependent. Human placental 11β-HSD activity is essentially entirely due to the type 2 isozyme (Stewart et al., 1995). Cortisol concentrations in pregnant women are approximately four-fold higher than fetal concentrations throughout gestation. This gradient is maintained largely through oxidation of cortisol by 11β-HSD2 in the placenta.

Recent studies suggest that excessive glucocorticoid activity in the fetus may lead to short- and long-term adverse consequences (Newnham and Moss, 2001). Therefore, we determined the effect of pro-inflammatory cytokines on human placental 11β-HSD2 expression and activity. Pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) inhibit human placental 11β-HSD2 activity through a mechanism that involves increased intracellular Ca2+ and inhibition of adenyl cyclase that could result in excessive fetal exposure to maternal cortisol (Kossintseva et al., 2006).

Also, cortisol stimulates the production of CRH in the placenta (Mancuso et al., 2004). Placental CRH secretion may affect both fetal and maternal responsiveness to stress. Increased CRH levels during pregnancy are associated with preterm birth, possibly through immuno-endocrinological mechanisms involving pro-inflammatory cytokines (Dudley, 1999).

6. Prostaglandins and MMPs in the uterus

The matrix metalloproteinases, MMP-2 and -9, increase in decidua and other intrauterine tissues at term (Goldman et al., 2003), possibly because of a loss of progesterone activity (Goldman and Shalev, 2006). MMP-1,-2, -3 and -9 further degrade released IL-1β (but not IL-1α) into inactive forms (Ito et al., 1996). Taken together, these data indicate the complexity of responses to IL-6, TNF-α and IL-1β, especially in the decidua where proteases are activated during parturition (Tsatas et al., 1999). The potent uterine contractile prostanooid, PGF2α, produced by the decidua, may be involved in the process of decidual activation as much as direct myometrial contractile stimulation. PGF2α increases decidual expression of the genes for MMP-2 and MMP-9 and also decreases synthesis of the endogenous inhibitor of their activity, tissue inhibitor of metalloproteinase-1 (TIMP-1) (Ulug et al., 2001). Increased MMP activity in decidua, caused by PGF2α, may enhance leukocyte migration to decidua, thereby increasing cytokine release, and may also catalyze the conversion of pro-IL-1β to active IL-1β (Schonbeck et al., 1998).

7. Cytokines, prostaglandins and the progesterone receptor

Two principal progesterone receptor forms exist. The PR-A (94 kDa) is an N-terminally truncated form of PR-
B (116 kDa) (Vegeto et al., 1993). PR-A is a weaker activator of transcription than PR-B, and PR-A can act as a transrepressor of PR-B (Pieber et al., 2001). The cellular response to progesterone is dependent upon the levels and ratios of PR-A and PR-B. In co-transfection studies in amnion, increasing PR-A relative to PR-B led to an inhibition of PR-B-mediated transcription. A third isoform, PR-C, has been identified in term human myometrium (Condon et al., 2006). PR-C lacks a larger segment of the N-terminal segment than PR-A, including a major part of the DNA-binding PR domain, and therefore cannot bind to DNA. It may dimerize with PR-B in the cytosol, preventing its binding to DNA and effectively reducing its transcriptional potential.

Administration of exogenous estrogen or a progesterone receptor antagonist increases mRNA expression of PTGFR in rat myometrium, while exogenous progesterone or an estrogen receptor antagonist reduces its expression (Dong and Yallampalli, 2000). Immunohistochemical staining for the PR isoforms A, B, C and S is evident in the decidua, but the fetal membranes and placenta contain primarily PR-C (Taylor et al., 2006). Goldman et al. (2005) showed that PGF$_{2\alpha}$ reduced mRNA expression of PR-A, B and C in decidual explants, which might lead to ‘functional progesterone withdrawal’. In immortalized cells from human myometrium, PGF$_{2\alpha}$ in a dose-dependent fashion increases the relative PR-A to PR-B ratio, which has been hypothesized to decrease the effect of progesterone (Madsen et al., 2004).

Investigators have found recently that IL-1β upregulates the PR-C isoform through an action on NFκB in human myometrial and T47D breast cancer cells (Condon et al., 2006). Thus, when PR-C is increased or overexpressed, the transactivation of PR-B is decreased and progesterone action is diminished. This, in turn, would diminish the effectiveness of progesterone and facilitate the termination of pregnancy.

Interleukin-1β is known to stimulate CRH release from placental cytotrophoblast cells (Petraglia et al., 1990). Investigators reported recently that increases in IL-1β and CRH are temporally associated in human maternal peripheral serum at preterm delivery (Vitoratos et al., 2007). Interestingly, in keratinocytes, CRH probably stimulates IL-1β, IL-6 and TNF-α as it mediates the effect of lipopolysaccharide on the stimulation of pro-inflammatory cytokines (Zbytek and Slominski, 2007). If true for placental cells as well, it would suggest a positive feed-forward system involving cytokines and CRH.

8. Summary and future directions

Convincing data support a role for cytokines in mechanisms that maintain and terminate normal pregnancy. It is evident that increased levels of cytokines are associated with term parturition, that inhibition of their synthesis or action attenuates their effects and delays induced or term parturition, and preterm activation of
inflammatory cells and production of cytokines or exogenous administration of cytokines frequently leads to preterm birth. Their actions often affect processes of parturition in a feed-forward fashion, and one of these mechanisms is activation of the prostaglandin synthesis-receptor system, which has the potential to amplify the actions of cytokines (Fig. 1).

Future studies will focus on the initial increase of leukocytes in uterine tissues at term and processes that activate these cells and increase cytokine production. Also, the mechanisms of cytokines in activating the uterus will continue to be studied.

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