Cytokines and Myometrial Signalling in Human Labour

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Introduction

Human labour is an inflammatory event, physiologically driven by an interaction between hormonal and mechanical factors and pathologically associated with infection, bleeding and excessive uterine stretch (Golightly, Jabbour, and Norman, 2011). However, the processes involved are not fully understood, especially the triggers/activators of labour. Local pro-inflammatory cytokine and chemokines have been implicated in the pathophysiology of human labour since the 1980s; with more recent data strongly linking increased intrauterine cytokine and chemokine production with both term and preterm labour (Keelan et al., 2003).

Various inflammatory mediators have been studied in reproductive tissues obtained at the time of term labour (TL) and preterm labour (PTL) showing the involvement of a range of cytokines and chemokines in the choridodecidua (Hamilton, Tower, and Jones, 2013), amnion (Gomez-Lopez et al., 2010), and placenta (Haugueldemouzon and Guerreillo, 2006). This review will be focused on recent work and current understanding of the nature and role of cytokines, chemokines and hormones and their involvement in signalling within the myometrium particularly during labour.

Myometrial inflammation

Inflammation typically involves white cell infiltration and the production of cytokines that induce changes in cell function through the modulation of gene expression. It is a highly coordinated process designed to protect the organism from infection (Meeusen, Bischof, and Lee, 2001, Martinon, Mayor, and Tschopp, 2009), but can be induced by other stimuli including chemicals and damaged cells. Generally, the inflammatory response is beneficial to the host, but when it is directed against components of the body as in joints in rheumatoid arthritis for example, or when it is excessive, such as in septic shock, inflammation can be harmful. In the myometrium, with the onset of labour at term, inflammation is thought to play a physiological role transforming the myometrium from a quiescent to a contractile state. In contrast, in preterm labour, inflammation takes on a pathological role, precipitating early delivery in response to a variety of triggers including infection, overdistension and haemorrhage.

The first reports of myometrial inflammation in association with labour appeared in the later 1980’s. Azziz et al reported the presence of inflammation in biopsies taken at the time of emergency Caesarean section and suggested that there was an underlying infective cause (Azziz, Cumming, and Naeye, 1988). Lopez-Bernal and colleagues first raised the key question of how much of the inflammatory change in the myometrium was a consequence of the labour process (Bernal et al., 1993). This question was partially addressed in a series of papers by Norman et al, in which the nature of the cellular infiltration, the changes in cytokine levels and the cells producing the cytokines were defined (Bollopragada et al., 2009). These papers established that term labour is an inflammatory event showing that the
myometrium is infiltrated by neutrophils, macrophages and T lymphocytes (Figure 1) and that these cells are the predominant source of the inflammatory cytokines (Young, 2002). Later studies have shown that the myometrial expression of chemokines and endothelial adhesion molecules are increased with the onset of labour, suggesting a potential underlying mechanism for the cellular infiltration of the myometrium (Young, 2002). The drives of the chemokine expression have also been studied and may include mechanical stretch and cytokines (see below). However, it remains unclear whether the inflammatory infiltration of the myometrium is a cause or consequence of labour. Human studies show that levels of IL-8 (Table 1) rise with established labour only (Osmers, 1995, Elliott et al., 2001, Kemp et al., 2002) In rodent pregnancies, it seems apparent that the inflammatory infiltration precedes the onset of labour (Mackler, 1999, Shynlova et al., 2012), but various groups have depleted pregnant animals of neutrophils (Timmons, 2006) or studied animals with no mast cells (Menzies et al., 2011), without delaying labour onset. Others have used chemokine knockouts, which deliver at the same time as their wild-type controls (Menzies et al., 2012). A number of animal studies have attempted to address this question using LPS, a bacterial wall polysaccharide (Fang, Wong, and Mitchell, 2000). Lye et al found that pre-treatment with a non-specific chemokine antagonist delayed labour onset in association with a reduced inflammatory infiltration (Shynlova et al., 2014), suggesting that the inflammatory infiltration is important in inflammation-induced labour onset. Indeed, macrophage depletion prevents LPS induced PTL in pregnant mice (Gonzalez et al., 2011), but neutrophil depletion had no effect (Rinaldi et al., 2014). These data suggest that macrophages but not neutrophils are important for this process.

Inflammation in reproductive tissues/compartment

The inflammatory changes may be a consequence of inflammation in other areas.

Maternal circulation:

The changes in the innate immune system during pregnancy are characterised by increased numbers of circulating monocytes and granulocytes, resulting in a higher number of total leukocytes (Tang et al., 2015). Peripheral monocyte numbers are higher; mainly due to an increase in the intermediate monocyte subset (Melgert et al., 2012). These monocytes are pro-inflammatory, producing IL-1β, IL-6 and TNF-α (Tang et al., 2015) (Table 1) and are recruited into gestational tissues, especially the decidua, during labour (Tang et al., 2015). Peripheral circulating leukocytes have also been noted to display early chemotactic responsiveness during late gestation which would aid their infiltration into uterine tissues (Gomez-Lopez et al., 2013). Recently Srikhajon et al reported that monocytes are recruited first to the myometrium by various cytokines and chemokines. Following this transmigration, activated monocytes in turn limit further chemotaxis by disrupting locally established CCL2 gradients (Table 1) (Srikhajon et al., 2014). This may serve as a negative feedback loop to control the local inflammation. On the other hand, this group also suggested that generic inhibition of chemokines limited inflammation and reduced PTB (Shynlova et al., 2014). These seeming contradictions may reflect species differences or be
determined by the stimulant. Circulating neutrophil numbers are higher in women in preterm and term labour (Yuan et al., 2009). These neutrophils are likely to be drawn into the myometrium by chemokines in particular IL-8 which is significantly higher in myometrium at term during labour than in women not in labour (Gomez-Lopez et al., 2010) and may contribute to the changes in whole blood gene expression noted in women with threatened preterm labour (Heng et al, 2014).

Amniotic fluid (AF):

Inflammatory cytokines are known to increase in AF towards term in human pregnancy and may play a role in labour by stimulating local production of prostaglandins and collagenases (Bowen et al., 2002). With the onset of TL, there are increased concentrations of IL-1β and TNF-α in AF (Romero et al., 1990, Laham et al., 1994). IL-6 has been noted to be raised in AF in women with spontaneous labour (Andrews et al., 1995) and particularly raised in PTL associated with intra-amniotic infection; and even considered a predictor for PTL before 34 weeks gestation (Chaemsaithong et al., 2015). IL-8 concentrations in AF increase progressively from early pregnancy to term and more markedly with the onset of spontaneous term labour (Romero et al., 1991, Saito et al., 1993, Laham et al., 1994). The rise in AF IL-6 precedes that of IL-8, suggesting that IL-6 has a role in the initiation of the inflammatory cascade required for the onset of labour (Kemp et al., 2002). Recent work by Romero et al have shown varying cytokine networks noted in the AF associated with PTL with intact membranes and intraamniotic inflammation (both microbial and sterile) (Romero et al., 2015). Interestingly, the chemokine CCL-20, which targets immature dendritic cells, effector/memory T-cells and B-lymphocytes increases in AF with advancing gestational age. It is further increased in the absence of infection in spontaneous TL and PTL, which suggests it has a role in the common parturition pathway (Hamill et al., 2008).

Amnion/Chorion

Inflammation has been seen in amnion and chorion with IL-1β and IL-8 increasing in concentration in the third trimester (Keelan et al., 1999, Elliott et al., 2001,). This is a key observation as it implies that the inflammatory process begins before the onset of labour. The expression of both cytokines was increased after labour with chorion producing more of each cytokine than the amnion (Elliott et al., 2001). In addition fetal membranes have exhibited selective chemotaxic activity in human labour, consequently increasing monocytes, T cells and NK cells (Gomez-Lopez et al., 2009). IL-6 and TNF-α are also increased (Young, 2002); contributing to the chemotaxis of monocytes and other immune cells into the gestational tissues, including into the myometrium and cervical stroma (Elliott et al., 2001, Golightly, Jabbour, and Norman, 2011,).

Decidua (CD)

The decidua is a highly immunologically active region of a pregnant uterus. Hamilton et al used a rat model to investigate the pre-labour changes and found a significant increase in
the numbers of macrophage infiltration of the decidua in the days prior to labour, which preceded inflammatory changes in the myometrium (Hamilton et al., 2011). This suggests that decidual inflammatory events are important in the initiation of labour (Sindram-Trujillo et al., 2004, Castillo-Castrejon et al., 2013,), supporting the hypothesis first proposed in the 1980s that decidual activation is an early event in the labour cascade (Casey and MacDonald, 1988). IL-8 is raised in CD at labour, with almost a 30 fold change in TL compared to term no labour (Hamilton, Tower, and Jones, 2013), resulting in neutrophil recruitment. These cells can release several inflammatory mediators and MMPs, which could degrade the extracellular matrix of the fetal membranes during both TL and PTL, contributing to ROM during term and preterm labour (Gomez-Lopez et al., 2010).

Choriodecidual changes are of particular interest in PTL, where it has been shown that CD56+ NK cells and T cells are increased (Hamilton, Tower, and Jones, 2013) along with an elevated expression of CCL8 which is a chemoattractant for NK and T cells (Proost, Wuyts, and Damme, 1996). These inflammatory changes implicate both the innate and adaptive immune system in the pathological process of PTL and interestingly the imbalance between these two immune systems in PTL have been demonstrated via a mouse model (Arenas-Hernandez et al., 2015).

**Placenta**

In contrast to the fetal membranes and decidua, the evidence of placental inflammation is poor (Keelan et al., 1999). The placenta is a site of peripheral monocytic activation, where monocytes encounter the villous trophoblast (Tang et al., 2015). Studies of placental cells and tissue in vitro have demonstrated their ability to respond to inflammatory stimuli such as pathogenic bacteria, LPS or IL-1 with increased production of cytokines (IL-1, IL-6, IL-10), chemokines (macrophage chemotactic protein-1[MCP-1], IL-8) and prostanoids (Denison et al., 1998, Goodwin et al., 1998, Gniesinger et al., 2001). This highlights the capacity of the placenta to play a key role in the inflammatory process associated with PTL triggered by abruption or infection.

Overall, inflammation does play a critical role in the onset and progression of labour, but where this is initiated and then propagated to is still a point of much discussion and research. It seems likely that the decidua being the maternal fetal interface is immunologically crucial, and our data (unpublished) suggests that it is the most inflammatory in PTL. Further work looking at inflammation in all compartments with comparison to peripheral blood is necessary to improve our understanding. The exact triggers for the onset of this inflammatory process is yet another uncertainty; some have suggested that the fetus releases surfactant proteins as a signal of maturity (Reinl and England, 2015), others that there is a change in maternal tolerance and still others that uterine stretch is responsible.

**Physiology (Figure 2):**
Throughout pregnancy, the uterus is dramatically remodelled to accommodate the growing pregnancy. Despite the progressive increase in size, uterine quiescence is maintained, until the onset of labour, be it at term or preterm, when the uterus transforms into an actively contractile organ, to efficiently expel the pregnancy. The growing conceptus increases intra-uterine pressure, but for the majority of pregnancy, the uterus is able to adapt and remodel to avoid any increase in wall tension. It is possible that once this adaptive mechanism is lost or overcome, the tension in the wall of the uterus rises, initiating the process, which culminates in the onset of labour. Progesterone has been suggested to play a key role in this adaptive process, particularly in animal models, where the loss of progesterone repression is associated with an increase in stretch-related pro-contraction proteins (Shynlova, Lee, et al., 2012) (connexin-43 and oxytocin receptor). In vitro stretch models of human myometrial cells (Terzidou et al., 2005) and strips (Moraitis et al., 2015) showed increased OTR expression and responsiveness respectively, while in vivo, acute uterine stretch increases prostaglandin synthesis (Manbe, Manabe, and Takahashi, 1982). Interestingly, no difference in prolabour expression was seen when comparing twin and singleton pregnancies (Lyall, 2002). Equally, excessive uterine stretch, seen in polyhydramnios, multiple pregnancy or a singleton pregnancy in a unicornuate uterus are all associated with increased rates of preterm labour (Rodriguez, 1992, Reichman, Lauffer, and Robinson, 2009, Conde-Agudelo and Romero, 2014.).

In vivo animal models of stretch in pregnancy has been pioneered by Lye et al, who uses a unilateral pregnant rat model and compares the effect of mechanical strain imposed by the growing fetus in the gravid horn to the changes observed in empty horn. Lye et al showed that CCL-2 levels increased in the gravid uterine horn and reproduced this effect by in vitro stretch of myometrial cells (Shynlova et al., 2008). More recently Adams-Waldorf, using a non-human primate model, demonstrated the effect of stretch on the inflammatory response of the uterus by recreating uterine distension through balloon inflation. There was significant elevation of pro-inflammatory cytokines, including IL-1β, IL-6, IL-8, CCL-2 and TNF-α, which was compared to with the inflammatory response observed in human twin preterm labour (Adams Waldorf et al., 2015).

Some studies have stretched human myometrial strips and shown an increase in IL-8 levels (El Maradny et al., 1996). More recent studies revealed that prolonged stretch of human myometrial strips under high tension resulted in increased myometrial contractility (Tattersall et al., 2012). The pathway by which the myometrial contractility is enhanced has not been defined; however there is evidence the stretch stimulates the expression of a known smooth muscle stimulatory agonist, gastrin-releasing peptide. Another theory that has been postulated is that stretch of myometrium under high tension induces constitutive activation of the oxytocin receptor (Moraitis et al., 2015). This was supported by the observation that retosiban, an oxytocin receptor blocker, reduced the pro-contractile effects of stretch (Moraitis et al., 2015).

In vitro studies of human and rat myometrial cells show that mechanical stretch up-regulates pro-inflammatory factors (Shynlova et al., 2012). Our studies showed that stretch...
up-regulated IL-8 and COX-2 in a MAPK-dependent manner (Loudon, 2004, Sooranna, 2004, Sooranna et al., 2005). Later studies confirmed that stretch of myometrial cells increased the expression and release of IL-8, while showing that other chemokines and inflammatory cytokines are also increased in a predominantly NFκB-dependent manner (Hua et al., 2012). More recently, Lye et al showed that conditioned media from stretched myometrial cells induced endothelial activation and the expression of adhesion molecules, promoting the extravasation of inflammatory cells (Lee, Shynlova, and Lye, 2014).

Lee et al tested the hypothesis that the stretch enhances peripheral leukocyte extravasation into the term myometrium through the release of various soluble mediators, including cytokines and chemokines, by human uterine myocytes. Nine cytokines/chemokines were significantly increased by stretch: IL-6, IL-12p70, IL-8, CXCL1, MIF (macrophage migration inhibitory factor), G-CSF, bFGF (basic fibroblast growth factor), VEGF, and PDGF-bb (platelet-derived growth factor subunit B). The greatest effect of stretch was seen on CXCL1 and IL-8 (Lee, Shynlova, and Lye, 2014). In human myometrial cells, the stretch-induced increase in CXCL1 and IL-8 was greatest at 6 hours (Hua et al., 2012). CXCL1 and IL-8 have been widely reported to be associated with TL, when both are likely to interact with neutrophils expressing CXCR1 and CXCR2, promoting myometrial infiltration of neutrophils in the gestational tissues (Elliott et al., 2000, Bollopragada et al., 2009).

Chemokines are essential for inflammatory cell migration and also modulate immune cell activation (Griffith, Sokol, and Luster, 2014). The main chemokines implicated in the inflammatory process of labour are IL-8 and CCL-2, which act via CXCR2 and CCR-2 respectively. IL-8 is a potent chemokine for neutrophils; and its mRNA expression is increased in myometrium of women in preterm and term labour (Keelan et al., 2003). Indeed, a recent myometrial transcriptome study reported that IL-6, CXCL1 and IL-8 exhibited the greatest increase in labouring samples (Mittal et al., 2010). A more detailed study revealed that IL-8 levels increased in parallel with cervical dilation (Hebisch et al., 2001). In preterm labour, IL-8 concentrations are markedly elevated in chorioamnionitis (Yoneda et al., 2015). Interestingly, myometrial expression of CXCR2 declined with the onset of TL (Hua et al., 2012) perhaps as a result of higher IL-8 levels or the effects of increased levels of OT and PGF\(_2\alpha\), which can also repress CXCR2 expression via phospholipase C (Hua et al., 2012). Alternatively, IL-1β and TNF-α also reduce CXCR2 expression and may also be responsible for the labour-associated decline (Hua et al., 2012).

CCL-2 is a member of the CC chemokine family and is also called MCP-1 (Esplin et al., 2005, Griffith, Sokol, and Luster, 2014). It is expressed by decidual cells (Critchley et al., 1996), endometrial and myometrial cells (Arici, MacDonald, and Casey, 1995, Jones, Kelly, and Critchley, 1997,) therefore it is ideally positioned to recruit macrophages to cervix, myometrium and fetal membranes with the onset of labour. Indeed, CCL-2 is markedly upregulated in both term and preterm myometrium (Esplin et al., 2005). CCL-2 is increased in amniotic fluid from women in preterm labour particularly in the presence of infection (confirmed by histological chorioamnionitis) (Esplin et al., 2003).

Stretch clearly has an impact on not only pro-inflammatory mediators such as CCL-2, IL-8 and IL-6 to name a few but also on activity of oxytocin receptors and smooth muscle
agonists such as gastrin-releasing peptides. Much of the in vivo model findings have been confirmed in our in vitro work, however further work looking into the interactions between electro-mechanical signalling, hormonal interference and inflammation is necessary to understand when adaptive mechanisms that maintain uterine quiescence falter.

**Maternal tolerance**

Pregnancy has often been compared to a transplanted organ as both fetus and placenta express maternal and paternal antigens hence are like semi-allografts (Erlebacher, 2012). Breakdown in immune tolerance has been linked to rejection, which in pregnancy can have variable consequences depending on the gestation: recurrent miscarriages (Kuon et al., 2015), preterm labour (Romero, Dey, and Fisher, 2014), pre-eclampsia (Dietl, 2000) to name a few. Tolerance is maintained via factors produced at the implantation site, one such promoter of tolerance is IL-10, an anti-inflammatory cytokine (Thaxton and Sharma, 2010). IL-10 was demonstrated to be a modulator of uterine NK cell cytotoxicity; in an IL-10 depleted mice model, very low doses of LPS led to uterine NK (uNK) cell activation and fetal demise (Murphy et al., 2008). In a non-human primate model, IL-10 has been shown to inhibit IL-1β induced uterine activity (Sadowsky et al., 2003) and it seems to also have an inhibitory effect on LPS induction of matrix metalloproteinase 2 and 9 in fetal membranes (Fortunato et al., 2001).

Interferons, known for their anti-viral potential, also have an immunomodulatory role (Racicot et al., 2014). Hertelendey et al showed via human myometrial cell line cultures that cell cultures primed with IFN-γ produced significantly less prostaglandins and reduced COX-2 expression (Hertelendy and Zakár, 2004). Trophoblasts have been suggested in enabling appropriate tolerance by “educating” macrophages and adapting the cytokine profile of the local macrophages. Fest et al showed that monocytes cultured with trophoblasts (Fest et al., 2007), increased production of RANTES (which recruits T regulatory cells) and MIP-1β which both have immunosuppressive functions (Wang et al., 1999, Ramhorst et al., 2004). Dendritic cells (DC) promote cell tolerance particularly at the maternal-fetal interface, by priming T regulatory (T_{reg}) cells (Blois et al., 2007). T_{reg} cells, part of the adaptive immune system play a pivotal role in promoting fetal survival by avoiding the recognition of semi-allogenic tissues by the maternal immune system (Somerset et al., 2004, Tilburgs et al., 2009, La Rocca et al., 2014). This was seen in a mice model where depletion of CD25^{+} T_{reg} cells led to gestation failure (Aluvihare, Kallikourdis, and Betz, 2004) and a certain systemic composition of T_{reg} cells with distinct subsets have been associated with PTL (Steinborn et al., 2011).

Maternal tolerance is no doubt vital to support a pregnancy to term, and to avoid pregnancy complications such as fetal loss and pre-eclampsia. PTL without an obvious cause, commonly referred to as idiopathic PTL is presumed by many as an immunological phenomenon with various immune cells considered culprits including high uNK cells or low T_{reg} cells. Many of these conclusions have arisen from in vivo models which although highly informative, cannot take into consideration the movement, interaction and adaptability of immune cells between gestational tissue layers, between the periphery and the uterus and the mother and fetus.
Corticotropin-releasing hormone (CRH) is synthesised in the placenta and the levels of placental CRH increases as the pregnancy advances, peaking at delivery with a rapid decline postnatally (Sasak et al., 1987). CRH can induce the breakdown of mast cells, releasing histamine (Lytinas et al., 2003) and has been widely associated with cytokines especially the pro-inflammatory cytokine IL-6 (Venihaki et al., 2001). Raised maternal levels of CRH have been associated with PTL (Figure 3), suggesting a possible causative link (Vitoratos et al., 2007). Indeed, CRH can stimulate the myometrium to produce pro-inflammatory cytokines and chemokines, in particular IL-6, IL-1β, TNF-α, IL-8 and CCL2. However, this effect appears to be dependent on cAMP-PKA signalling pathway and possibly NF-κB (You et al., 2014). These cytokines can induce the chemotaxis of monocytes to the myometrium and promote inflammation, which is thought to be key for the onset of labour. For example, IL-1β and IL-6 stimulate uterine activation by increasing CX43, PGFR and OTR. In addition, CRH has been reported to have a stimulatory effect on prostaglandins (PGE2, PGF2α) (You et al., 2014).

IL-6 is a pro-inflammatory cytokine that is also recognised as a myokine. IL-6 and CRH are secreted in a pulsatile manner during active labour, with the increases in IL-6 preceding those of CRH (Papatheodorou et al., 2013). This suggests the hypothesis that IL-6 promotes the release of placental CRH and in a direct or indirect manner is associated with uterine contractility (Papatheodorou et al., 2013). IL-6 has been identified in cervico-vaginal fluid as a predictive marker of PTL in the subsequent 7 days (Jung et al., 2015). Some studies have suggested this to be secondary to sub-clinical chorioamnionitis as a majority of PTL is associated with infection (Jung et al., 2015). IL-6 concentrations, along with other cytokines do not correlate with cervical shortening (Chandiramani et al., 2012).

Aside from CRH, surfactant protein-A (SP-A) from the fetal lung can induce parturition. Surfactant is a glycerophospholipid-rich lipoprotein, produced by alveolar type II pneumocytes and is secreted into amniotic fluid with fetal breathing movements (Mendelson, 2009). In murine models, injection of SP-A into the amnion resulted in preterm delivery (Reinl and England, 2015), interestingly this was by shuttling amniotic fluid macrophages to the myometrium and increasing uterine IL-1β levels (Condon et al., 2004). SP-A deficient mice demonstrated a delay in parturition associated with suppressed myometrial inflammation and increased maternal progesterone (Reinl and England, 2015). In human models, SP-A stimulated prostaglandin synthesis (Bernal et al., 1988) and Johnston and colleagues have proposed that platelet-activating factor, a phospholipid component of fetal lung surfactant that is secreted into amniotic fluid near term, may play an important role in the activation of myometrial contractility (Toyoshima et al., 1995).

CRH and SP-A are known proteins that can increase the production of cytokines and prostaglandins, consequently triggering myometrial activity. In addition, there are likely to be other molecules released from not only the fetus and the placenta, but also from the membranes that increase myometrial inflammation. Further work to identify such molecules and its role and interactions is required.
**Progesterone and Progesterone Receptor**

The withdrawal of progesterone (P4) has long been hypothesised to be the trigger of labour, with supportive evidence from animal models, in particular sheep and goat where a fall in P4 and a concurrent increase in oestradiol precedes the onset of labour (Ravanos et al., 2015). This does not apply to humans, as there is no decline in circulating maternal P4 levels before labour. Interestingly guinea pigs are similar to humans in that they labour in presence of high maternal progesterone levels. Such model has recently shown that decreasing P4 receptors leads to a physiological mechanism of functional P4 withdrawal which is enhanced by enodogenous/exogenous prostaglandin administration (Welsh et al., 2014).

P4 maintains uterine quiescence through suppression of contraction associated proteins such as connexin 43 (Challis et al., 2000). It also exerts an anti-inflammatory action via inhibition of cytokine production and immune cell migration into the uterus and suppresses the transcription of genes that promote contractility. Interestingly in human labour, a functional impairment in P4 receptor levels have been reported near term which may reverse P4’s suppressive actions therefore promoting myometrium’s sensitivity to contract (Ravanos et al., 2015).

P4 has been suggested to maintain pregnancy primarily by inhibiting inflammation through repression of the archetypical inflammatory transcription factor NFκB (Wissink, 1996). This is mediated both via a direct interaction between the P4 receptor, PR-B, and the principle NFκB subunit, p65 and by increasing IκB levels, which binds to p65 maintaining it in an inactive state (Hardy et al., 2006). The onset of human labour is suggested to occur after P4 influence is lost by a combination of increased expression of PR-A (Mesiano et al., 2002), which inhibits PR-B, a reduction in the level of the PR co-activator, SRC1 (Condon et al., 2006) and by increased activity of NFκB, which represses PR activity via a direct interaction (Condon et al., 2003). Much of these data are based on over-expression of PR and p65, and have often been carried out in cell lines of various types. Our data suggest that P4 represses IL-1β driven COX-2 expression via the glucocorticoid receptor (GR) and not PR, despite the presence of sufficient PR to modulate the expression of the P4-responsive genes (Lei et al., 2012). Further, we show that P4 reduced IL-1β-driven COX-2 expression via the inhibition of AP-1 action rather than NFκB (Lei et al., 2015). Most work has focused on the effect of IL-1β-driven activation of NFκB on PR function, but other cytokines may also modulate PR function. Confirmation of these potential interactions awaits further study.

**Pathology (Figure 4)**

**Infection**

Infection is the leading known cause of preterm labour and unfortunately one in three preterm infants are born to mothers with an intra-amniotic infection that is largely subclinical (Romero et al., 2001). Ascending infection is seen as the main source; however...
there has been an association with periodontal disease and PTL (Manegold-Brauer et al., 2014), which suggests a possible systemic dissemination and transplacental passage.

Ascending infection is usually caused by common vaginal pathogens such as Group B Streptococcus, Mycoplasma and Ureaplasma whereas periodontal disease is commonly caused by gram negative anaerobic bacteria such as Aggregatibacter actinomycetemcomitans, Fusobacterium nucleate and Campylobacter rectus. These microorganisms and their products are typically identified by pattern recognition receptors such as toll-like receptors, which induce the production of chemokines (IL-8, IL-1, CCL-2) and cytokines (IL-1β, TNF-α) (Romero, Dey, and Fisher, 2014).

With regards to periodontitis pathogens it is likely their effect is triggered by translocation of bacterial products, such as LPS, which can trigger common parturition pathway via inflammatory mediators such as IL-6, and TNF-α (Parthiban, 2015).

PTL like TL require prostaglandins (PG). The rate limiting enzyme in prostaglandin synthesis, PGSH-2, is required to increase PG just prior to parturition (Hirst et al., 1995) and, interestingly, this is stimulated by cytokines including IL-1β and TNF-α. The key role played by these specific cytokines is shown in mice lacking receptors for both IL-1β and TNF-α, which have significantly lower levels of PGHS-2 mRNA in the myometrium following E.coli administration (Hirsch, Filipovich, and Mahendroo, 2006).

Aside from the above mentioned infections, bacterial vaginosis (BV) and STIs are recognised as a risk factor for PTL although treatment of asymptomatic women with BV does not reduce the rate of preterm births (Romero et al., 2001). One possible explanation for this association may be that BV induces the release of cytokines that trigger the onset of labour. Masson et al identified that IL-1β (in cervico-vaginal fluid) as one of most useful immunologic biomarkers that could be used to diagnose treatable discharge-causing STIs and BV (Masson et al., 2015).

Chorioamnionitis (CA) is a robust inflammatory response to intra-amniotic infection, and commonly associated with an infiltration of neutrophils in response to IL-8 and CXCL-6, amongst other chemokines (Kim, Romero, et al., 2015). Damage-associated molecular pattern molecules (see below) are also able to induce such neutrophil attracting chemokine, which led to the possibility of a mutual parturition pathway. Recent work on immune cells involved in acute and chronic CA resulting in PTL has shown the importance of macrophages. It has highlighted differences in the anatomical distribution of macrophages within the fetal membranes, as well as the differing functions - both proinflammatory and immunomodulatory (Bae et al., 2016). The plasticity and flexibility of macrophages (Brown et al., 2014), enables macrophages to acquire altered phenotypes in response to different situations. This is further complicated by the uncertainty of where these macrophages originate (fetal v maternal) and the continuing conundrum of understanding the role of inflammatory signals in both TL and PTL. Indeed, variations in the onset of PTL and TL suggest that they may involve distinct inflammatory pathways, but as yet there are no definitive data on this subject.
It is important to note that sterile inflammation (defined as an inflammatory process without the presence of microorganisms) has also been associated to PTL and is more common in preterm labour with intact membranes than microbial-associated inflammation (Romero et al., 2014). The aetiology of sterile intra-amniotic inflammation is unknown; however the inflammation is understood to result from activation of the innate immune system by endogenous danger signals, derived from necrosis or cellular stress, termed damage-associated molecular pattern molecules (DAMPs), or alarmins (Gomez-Lopez et al., 2016). One such alarmin is HMGB1, which has been shown to induce PTL in a mouse model (Gomez-Lopez et al., 2016). For further detail on proposed theories on sterile inflammation please refer to Faranak Behnia’s review (Behnia, Sheller, and Menon, 2016).

Haemorrhage

Decidual haemorrhage is associated with PTL (Romero, Dey, and Fisher, 2014) and it complicates 0.5 -2% of all pregnancies (Buhimschi et al., 2010). Decidual haemorrhages were generally accepted as an acute event; however histological evaluation of the vasculopathy accompanying decidual haemorrhage provides compelling evidence that the damage is frequently chronic (Salafia et al., 1995, Elsasser et al., 2010). Placental abruption has been shown to be associated with inflammatory lesions of the placenta, in particular at preterm gestations (Nath et al., 2007) and interestingly a strong association has been noted between severe chorioamnionitis and abruption at term (Nath et al., 2007). This suggests that inflammatory pathways are common to both infection and decidual haemorrhage.

Local decidual injury leads to production of cytokines, some of which lead to drive the inflammatory labour pathway. Additionally, thrombin, which is generated from decidual-cell-expressed tissue factor (Buhimschi et al., 2010), can itself enhance the activity of cytokines such as IL-8 (Lockwood et al., 2005) and CCL-2 (Matta et al., 2007), which enhance neutrophil and macrophage infiltration, promoting inflammation. Thrombin, acting via decidual cell membrane-bound protease-activated receptors, can also induce MMPs, which enable extracellular matrix breakdown, leading to the rupture of membranes (Han, Schatz, and Lockwood, 2011). This process has been associated with preterm premature rupture of membranes (PPROM) in the absence of infection (Han, Schatz, and Lockwood, 2011) and probably explains the linkage of PPROM and placental abruption in the absence of infection (Harger et al., 1990).

Thrombin has also been shown to be a direct potent uterotonic agent in both in vitro and in vivo models (Elovitz et al., 2000). In vitro fresh whole blood stimulated myometrial contractions in a dose-dependent manner and this effect was supressed with thrombin inhibitors (Elovitz et al., 2000). In vivo thrombin increased the frequency, intensity, and tone of myometrial contractions in a dose-related fashion (Elovitz et al., 2000). Thrombin’s potential to be an enzymatic, immunological and contractile inducer defines how decidual haemorrhage can expedite labour at term and unfortunately cause PTL when occurring at an early gestation.
Senescence refers to the physiologic and biomolecular mechanisms that are normal and naturally associated with aging of a living organism (Muñoz-Espín and Serrano, 2014); however premature senescence is associated with pathology such as diabetes (Barzilai et al., 2012) and chronic inflammatory conditions (Gubbels Bupp, 2015). Senescence is also associated with a set of biomarkers that are referred to as senescence-associated secretory phenotype (SASP). SASP is recognised by production of natural compounds such as cytokines, chemokines, matrix degrading enzymes and many more (Behnia et al., 2015). Behnia et al. showed that term labour is associated with senescence of chorioamniotic membrane cells and increased pro-inflammatory SASP factors (IL-6, IL-8, GM-CSF) could function as triggers of labour (Behnia et al., 2015). Evidence of decidual senescence has been demonstrated in the basal plate of the placenta in cases with preterm labour, but not in women who delivered at term (Cha et al., 2013). Some regard senescence as an initiator of sterile inflammation, while Menon and colleagues suggest that inflammation at term, and maybe even preterm is secondary to fetal cell senescence (Behnia et al., 2015). Pathological triggers of labour include infection (systemic and localised i.e. CA), haemorrhage, and physiological deficits such as premature senescence. They all trigger pro-inflammatory markers and in general results in labour. However, it is unclear why some infections potentiate PTL and others only cause ruptured membranes and allow the pregnancy to continue to term. These variations may be due to the inflammatory marker response being stimulant (type of bacteria/antigenicity) and exposure (localised v systemic) specific and may suggest triggering distinct inflammatory pathways.

The myometrium has the ability to contract both in a non-pregnant uterus in varying phases of the menstrual cycle and also importantly, in a pregnant uterus (Pehlivanoglu, Bayrak, and Dogan, 2013). This is evidently necessary as the process of parturition can only be completed with the establishment of regular and effective contractions. The switch from uterine quiescence to the active stage of contractility is considered to be dependent on a group of proteins referred to as contraction associated proteins (CAP) (Hutchings et al., 2009) whilst the excitation-contraction coupling required for contractility is understood to occur via elevated intracellular calcium levels (Wray, 2003). For more detail please see Roger Smith’s review (Butler et al., 2013).

The up-regulation of proinflammatory cytokines within labouring myometrium stimulates and potentiates uterine contractions (Voltolini et al., 2015). IL-1β enhance myometrial contractility via different pathways, promoting basal and store-operated calcium entry (Tribe, 2002), upregulating TrpC expression (calcium entry channels; Dalrymple et al., 2004) and increasing the expression of selected phosphodiesterases, enzymes involved in the control of intracellular levels of cyclic nucleotides (Oger et al., 2002). TNF alpha reduces the
expression of Galphas, the component of the G-protein receptor complex that links to adenyl cyclase and which increases intracellular cAMP levels promoting myometrial relaxation (Chapman et al., 2005). Interestingly, LPS increased the contraction of an isolated mouse uterine horn preparation (Mackler, 2003) and uterine myocytes in vitro through the Rho/ROCK signaling pathways (Hutchinson et al., 2013) and co-culture of uterine myocytes and monocytes enhances cytokine production and contraction (Rajagopal et al., 2015). Myometrial cells are able to produce cytokines such as IL-1β, IL-6, IL-8, TNF-α, which is enhanced by infiltrating immune cells (Young, 2002) such as macrophages, promoting a positive feedback loop to sustain the myometrial contractility. It is important to recognise that the effect of both cytokines and pro-inflammatory agents such as LPS are dose-dependent based on in vitro data; this is unlikely to reflect the reality of an in vivo system as other confounders may modify the effect. Such confounders may be innate control agents, which limit the severity of inflammation such as the production of IL-10 in response to IL-1β (Sadowsky et al., 2003). The production/release of such immunomodulatory cytokines may be derived from other tissues e.g. decidua; this is difficult to factor into in vitro models and does limit interpretation of such data. However some models have attempted to address this crosstalk by co-culturing with agents such as progesterone and IL-10 (Rajagopal et al., 2015).

The Indirect Effects of Inflammation

Inflammation drives the expression of CAPs include the oxytocin receptor, prostaglandin receptors (Figure 3) and the gap junction protein connexin 43 (Hutchings et al., 2009).

Myometrial oxytocin system

The oxytocin receptor (OTR) mediates the effects of oxytocin (OT) on the myometrium. It is a key regulator of myometrial function. Its expression increases with advancing gestation (Fuchs et al., 1991), peaking in early labour (Rivera et al., 1990), corresponding to the clinical observation of increased uterine sensitivity to OT (Kimura et al., 1996). OT increases myometrial contractility via increases in intracellular calcium, mediated through its G-protein coupled receptor, OTR. How inflammatory cytokines affect OTR expression is debated. Some authors show that IL-1β down-regulates myometrial OTR expression (Rauk and Frievbe-Hoffmann 2000, Schmid, Wong, and Mitchell, 2001, Helmer, 2002), while others have shown that it increases OTR expression (Terzidou et al., 2006). The effect is certainly time dependent and may explain some of the conflicting data (Terzidou et al., 2006). Myometrial and decidual synthesis and release of OT was increased by IL-6 and IL-1β (Friebe-Hoffmann et al., 2007), suggesting that the acute effects of inflammation would be to increase the activity of the myometrial OT system, consistent with the observation that acute exposure to IL-1β increases OT-induced contractility, but chronic exposure reduces it (Molnár, Romero, and Hertelendy, 1993, Rauk, 2000). Intriguingly, OT has been shown to activate the NF-κB pathway, increasing the expression of several key inflammatory labour-associated genes in both myocytes and amnion cells including IL-8, IL-6, CCL-5 and COX-2.
Prostaglandin/Prostaglandin receptors and Cytokines

Prostaglandins (PG) are known to initiate labour and enable contractions via cervical ripening, membrane rupture and uterine contractility. Phospholipase A2 releases arachidonic acid, which is converted into PGH$_2$ by cyclooxygenase 1 and 2 (Simmons, 2004). PGH$_2$ can be converted into the four main PGs: PGE$_2$, PGF$_{2\alpha}$, PGD$_2$ and prostacyclin (PGI$_2$) (Sykes et al., 2014), of which PGE$_2$ and PGF$_{2\alpha}$ are known be potent inducers of uterine contractility in spontaneous labour (Crankshaw and Dyal, 1994). Inflammatory cytokines have long been recognised to drive PG synthesis in human myometrial cells (HertelendyM et al., 1993, Molnár, Romero, and Hertelendy, 1993, Pollard and Mitchell, 1996) via the activation of NFkB and MAPK, p38 (Belt et al., 1999, Bartlett, Sawdy, and Mann, 1999). PGs are recognised to be pro-inflammatory and contribute to inflammatory conditions throughout the body such as in asthma (Claar, Hartert, and Peebles, 2014) and cancer (Rose, Gracheck, and Vona-Davis, 2015). PGs can act as cytokine amplifiers and in particular increases activity of IL-1β (Aoki and Narumiya, 2012), which as mentioned plays a substantial role in initiating labour and contractility. PGs contribute to the physiological inflammatory reaction seen in labour; for example PGE$_2$ enhances migration of leukocytes towards the cervix, which in turn leads to an increased production of IL-8 (Hertelendy and Zakár, 2004). PGF$_{2\alpha}$ indirectly can activate IL-1β in the decidua and consequently increase production of MMP-9 (Christiaens et al., 2008) which is known to participate in breakdown of the extracellular matrix leading to ruptured fetal membranes (Vadillo-Ortega and Estrada-Gutiérrez, 2005). Additionally, PGE$_2$ interacts with LPS to induce IL-6, COX-2 and IL-1β via EP$_4$ on macrophages (Aoki and Narumiya, 2012) indicating PGs’ role in infection associated preterm labour.

Connexin 43 and Cytokines

Connexins are a family of homologous proteins (21 in humans), each of which is the product of a distinct gene (Söhl and Willecke, 2003). Connexins differ greatly in size, providing a convenient method of distinguishing them: connexin 43 (Cx43) is 43kD. Their best known function is to form the intercellular membrane channels of gap junctions, which allow direct sharing of small molecules between cells in a process known as gap junctional intercellular communication (Winterhager and Kidder, 2015). Cx43 is a recognised as one of the contraction associated proteins (Hutchings et al., 2009).

Cx43 gap junctions are scarce in the myometrium of the non-pregnant uterus but increase in size and abundance with parturition in both humans and animals (Chow and Lye, 1994, Orsino, 1996). Doring et al has shown in a mouse model that ablation of Cx43 delays parturition. This was shown both in vitro and in vivo (Doring, 2006). Cx43 is impacted by inflammation. In an in vitro model, monocytes in presence of TNF-α and IFN-γ increased protein and mRNA levels of Cx43 (Eugenin et al., 2003). This would increase the contractility
potential of the myometrium. It is also raised in response to LPS (Chang et al., 2012) and is raised in association with preterm labour (Balducci et al., 1993).

\text{PGF}_2\alpha \text{ has also been shown to increase Cx43 and PTGS2 expression in myocytes, the effect of which is enhanced by IL1\beta (Xu et al., 2013).}

In summary, the three CAPs have been shown to be stimulated by cytokines, in particular IL-1\beta, but as noted with OT, exposure duration may have variable effect on the CAPs (this has not been studied with regards to PG and Cx43). Interactions between CAPS and cytokine/chemokines draw a variety of immune cells, however the particular role of these cells are unclear, as they may be acting in an immunomodulatory capacity as opposed to the presumed inflammatory role.

**Future research**

Labour at term is clearly associated with inflammation. Inappropriate initiators of this inflammation seem to trigger PTL as described above. It is evident from this review that there is a multitude of factors that enable and promote the myometrium to contract (Figure 2 and 4). In fact there is a growing body of evidence to suggest that the beginning of labour may be initiated in other gestational tissues before the myometrium is involved.

Cytokines play a significant role in establishing the inflammatory environment that is associated with labour, however there is much more to understand. Certain cytokines are repeatedly implicated in the various steps of labour; however the exact role of each cytokine is unclear. It is understood that they are chemotactic to leukocytes, but there is little understanding of the leukocytes’ exact function. Further work to identify leukocyte phenotype and function needs to be considered.

Future work needs to focus on the trigger of labour as this seems to be the one question that we are unable to truly answer. By unravelling this mystery it could be possible to identify effective therapeutic targets for those at risk of PTL. Longitudinal studies will be necessary to understand the molecular and immunological changes in normal pregnancy as this may enable identification of biomarkers and improve risk assessment. Newer high-throughput techniques such as metabolomics and proteomics could complement our current methods, and enhance our understanding of labour, which is the ultimate key in tackling PTL.


Table 1 Summary of nature and role of key soluble mediators in myometrium

**Figure 1** – Leukocytes infiltrating the myometrium during parturition. From Thomson A et al., Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process, Human Reproduction, 1999, volume 14, issue 1, pages 229–236, by permission of Oxford University Press.

**Figure 2** - The effect of physiology (including stretch, surfactant protein-A and Corticotropin-releasing hormone) on cytokines and myometrial contractility

**Figure 3** - Pathology and hormones that promote myometrial contractility (adapted from Romero R et al, Preterm Labour, one syndrome, many causes. Science, Aug 2014)

**Figure 4** - The effect of pathology (including haemorrhage, infection and premature senescence) on cytokines and myometrial contractility
Table 1 Summary of nature and role of soluble mediators in myometrium

<table>
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<tr>
<th>Cytokine/Chemokine</th>
<th>Role in myometrium</th>
<th>Evidence</th>
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| IL-1β              | • Pro-inflammatory IL-1 cytokine superfamily  
• Source – monocytes, macrophages mainly  
• Stimulates arachidonic acid release, activate phospholipid metabolism and increase the production of prostaglandins by the myometrium  
• IL-1β activates a signal transduction system involving NF-κB to increase the expression of COX-2 which is increased in the myometrium during labour and stimulate the production of PGE2 by myometrial cells | Peltier, 2003, Krishnan et al., 2014                                                        |
| IL-6               | • Pro-inflammatory cytokine and anti-inflammatory myokine  
• Source – monocytes, macrophages, endothelial cells  
• IL-6 has no effect on prostaglandin production by myometrial cells and is unable to stimulate myometrial contractions  
• This cytokine may play a role in labour by increasing the expression of oxytocin receptors on myometrial cells to increase their responsiveness to oxytocin  
• IL-6 can also increase oxytocin secretion by myometrial cells | Peltier, 2003                                                                                   |
| IL-8               | • Chemotactic and pro-inflammatory cytokine  
• Source – macrophages, endothelial cells  
• IL-8 is chemotactic to neutrophils  
• Increased in myometrium in term labour compared to preterm labour; may work by increasing PGE2  
• Progesterone and dexamethasone have been shown in vitro to inhibit IL-8 | Baggiolini, Loetscher, and Moser, 1995, Keelan et al., 2003, Terzidou et al., 2006.               |
| TNF-α              | • Pro-inflammatory cytokine  
• Source – macrophages, monocytes  
• Stimulates arachidonic acid release, activate phospholipid metabolism and increase the production of prostaglandins by the myometrium | Peltier, 2003, Idriss and Naismith, 2000.                                                        |
| CCL2               | • Pro-inflammatory soluble chemoattractant cytokine  
• Source – monocytes, lymphocytes, endothelial cells, fibroblasts  
• Chemotactic to monocytes, NK cells, CD4⁺ T cells  
• Uterine smooth muscle cells can secrete CCL2 which can lead to inflammation by promoting | Shynlova et al., 2008.                                                                          |
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<th>Recruitment of monocytes to myometrium</th>
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<td>• Mechanical stretch of the myometrium increases expression of CCL2</td>
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(A) Granulocytes are sparse in myometrium collected from women not in labour (arrow) and (B) abundant in myometrium obtained from women during labour

Identification of macrophages in lower segment myometrium obtained (A) before and (B) during labour

Identification of neutrophils in lower segment myometrium obtained (A) before and (B) during labour

Figure 1 – Leukocytes infiltrating the myometrium during parturition. (Thomson A et al, Human Reproduction, 1999)