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Review

Impact of Mediators Present in Amniotic Fluid on Preterm Labour

NIKOLAOS VRACHNIS[1], STAMATIOS KARAVOLOS[2], ZOE ILIODROMITI[1], STAVROS SIFAKIS[3], CHARALAMBOS SIRISTATIDIS[4], GEORGE MASTORAKOS[1] and GEORGE CREATSAS[1]

[1]*Second Department of Obstetrics and Gynaecology, University of Athens Medical School, Aretaieio Hospital, Athens, Greece;*
[2]*Queen Elizabeth Hospital, Sheriff Hill, Gateshead, Tyne and Wear, United Kingdom;*
[3]*Department of Obstetrics and Gynecology, University Hospital of Heraklion, Heraklion, Greece;*
[4]*Third Department of Obstetrics and Gynecology, University of Athens, Athens, Greece*

Abstract. *Preterm birth continues to be one of the most important issues in current obstetric medicine, being the single largest cause of perinatal morbidity and mortality. The signals that initiate preterm and term labour remain a mystery. Intrauterine inflammation with the secretion of cytokines is one of the accepted explanations for the mechanism of initiation of preterm labour. This review discusses the current understanding of the molecular mechanisms for the initiation of preterm labour, focusing chiefly on the role of intra-amniotic fluid mediators, whether endogenous or infection-induced, in the regulation of inflammatory response pathways associated with spontaneous preterm labour. Prostaglandins (PGs) are considered to be one of the key mediators of preterm labour, with the concentration of biologically active PGs in the amniotic fluid, particularly PGE_2 and PGF_{2a}, being significantly higher in women with preterm labour. Cytokines, such as interleukins and tumour necrosis factor alpha, additionally play a dominant role in preterm labour, particularly in association with infection. Elevated amniotic fluid concentrations of extracellular matrix mediators, including metalloproteases, are also implicated in the process of foetal membrane rupture in preterm labour. Allelic variations in the main amniotic fluid mediators may be the key to understanding the disparity in the rates of preterm birth between different ethnic populations. We also discuss the role of other potential mediators such as cell-adhesion molecules, nitric oxide and novel biomarkers found in the amniotic fluid.*

Correspondence to: Nikolaos Vrachnis, MD, Second Department of Obstetrics and Gynaecology, University of Athens Medical School, Aretaieio Hospital, 124B Vasilisis Sofias Ave., 11526, Athens, Greece. Tel: +30 6974441144, e-mail: nvrachnis@med.uoa.gr

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About one in ten live births is a preterm birth. This is an enormous public health issue since many of these preterm infants survive with neurocognitive, behavioural and motor disability. To date, there is no proven accurate method of prediction, prevention or treatment of preterm birth (1-3). Thus, one of the foci of current research is to understand the underlying pathophysiology of the preterm birth process and to use this to develop better diagnostic tests and improve therapeutic strategies. It is now well recognised that labour at term resembles an inflammatory reaction, with changes in progesterone and corticotrophin realising hormone levels (4) and with an up-regulation of inflammatory cytokines and prostaglandins in the myometrium, foetal membranes, amniotic fluid and the cervix. Fluctuation in the cytokine concentrations in amniotic fluid has been shown to be indicative of various inflammatory processes and such changes may be associated with preterm labour or chorioamnionitis. Anti-inflammatory cytokines may be essential for a successful normal pregnancy, while on the other hand, increased concentrations of pro-inflammatory cytokines may be a cause of preterm labour.

Although the aetiology of preterm labour is multifactorial, in the majority of cases, a similar inflammatory reaction is seen and is thought to result from the pathologic activation of those inflammatory pathways. Infection is a pathological process for which a causal relationship with preterm labour has been established (5, 6).

Studies have demonstrated a racial disparity in spontaneous preterm birth between African-Americans and Caucasians, this probably being related to differences in patterns of

cytokine release in the amniotic fluid (7). There is increasing evidence to suggest that variation of single nucleotide polymorphisms (SNPs) in the expression of cytokine-associated genes is the cause of these ethnic differences (8).

We summarize the evidence supporting an association between certain markers of inflammation in the amniotic fluid, either endogenous or infection-induced, and spontaneous preterm labour. These intra-amniotic fluid mediators may be more representative of the foetal cytokine profile, as amniotic fluid predominantly represents foetal urinary and respiratory secretions.

Regulation of Inflammatory Response Pathways Leading to Preterm Labour

Evidence from both human and animal models suggests that changes in cytokine levels in the amniotic fluid play an important role in the pathogenesis of preterm labour (9-11). This process is believed to occur over a period of time rather than being an acute process, with the onset of myometrial contractions occurring towards the end (12). This is supported by the fact that the concentration of inflammatory mediators in the amniotic fluid has been shown to be elevated early in pregnancies complicated by preterm labour onset (9). The main mediators known to be present in the amniotic fluid, and their classification, are summarised in Table I. Amniotic fluid from women in infection-associated preterm labour contains increased concentrations of pro-inflammatory cytokines, suggesting that the underlying trigger may be a cytokine-mediated stimulation of amniotic cells that leads to prostaglandin production. There is evidence that inflammatory mediators in the amniotic fluid weaken the amniotic barrier through interference with amniotic tight junctions, thus possibly facilitating the invasion of microbes into the amniotic cavity. This is thought to be a result of a decrease in the claudin-3 and claudin-4 levels at the apical junction, and apoptosis of amniotic epithelial cells (13). Preterm labour appears to be the result of a heterogeneous group of variables known as the foetal inflammatory syndrome (14). Figure 1 provides a schematic representation of the interaction of the mediators in amniotic fluid implicated in preterm labour.

Cytokines

Cytokines are protein and polypeptide products secreted by cells that regulate intracellular cell functions (15). They have diverse actions including from growth factor effects, chemotaxis and angiogenesis. Their actions are mediated by specific membrane receptors, which in turn activate intracellular pathways. These mediators regulate the immune response against infection and thus help maintain pregnancy; however, the inflammatory response to infection can have a detrimental effect on the pregnancy and the foetus (16). Inflammatory cytokines such as intereukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) are considered key mediators of preterm labour. The concentration of these cytokines is elevated in the amniotic fluid and foetal membranes, even in the absence of infection (17).

Inflammatory cytokines appear to have different functions in preterm birth amongst different ethnic groups. For example, elevated IL-1β and TNF-α concentrations are seen in African-American cases, whereas higher IL-6 and IL-8 levels are more commonly associated with preterm birth in Caucasian. Recent evidence from SNP analysis of genes encoding these cytokines suggests that genetic variation may be the cause of this disparity (18).

Arachidonic Acid (AA) Metabolites

Release of AA from the foetal membranes is thought to be a key step leading to the initiation of human labour. AA can be metabolised by the cyclo-oxygenase pathway to PGs and by the lipo-oxygenase pathway to hydroxyecosatetranoic acids (HETE) and leukotrienes. It is thought that AA metabolites modulate uterine contractility (19).

Tumour necrosis factor alpha. TNF-α is an inflammatory cytokine that is produced in the amniotic fluid and which is likely to play a role in the initiation of term and preterm labour. Its concentration in the amniotic fluid also significantly increases during the course of labour (20, 21). The presence of an intrauterine infection also induces the production of TNF-α in the amniotic fluid and therefore TNF-α has been implicated in the pathology of infection-associated preterm labour (22, 23). TNF-α has been shown in animal studies to be the first detectable inflammatory cytokine in the amniotic fluid after bacterial colonisation, preceding that of both IL-1 and IL-6 (24). TNF-α regulates the expression of IL-1 receptors in human amnion cells (25). It has also been shown to stimulate the production of IL-6 and IL-8 by amnion cells cultured *in vitro* (26). TNF-α is involved in the transcriptional activation of PG synthase leading to increased PGE_2 production in the amniotic fluid during infection-associated preterm labour (27). Studies have demonstrated that TNF-α concentration in the amniotic fluid increased significantly during the course of pregnancy both in women that were not in labour and also in women in term and preterm labour (28-30). This was also true in pregnancies complicated with PROM and oligohydramnios (31).

The actions of TNF-α are mediated through membrane-bound receptors 1 (TNFR1) and 2 (TNFR2). Activation of TNFR1 triggers the production of MMPs, whereas TNFR2 initiates the pro-inflammatory immune response (32, 33). Soluble forms of these receptors, sTNFR1 and sTNFR2,

Table I. *Main mediators of amniotic fluid implicated in preterm labour.*

Cytokines
Interleukins
IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17
TNF-α
Arachidonate lipoxygenase metabolites
Prostaglandins
PGE_2, $PGF_{2\alpha}$, PGD2
Leukotrienes
LTB4, LTC4, 12-HETE, 15-HETE
Extracellular matrix mediators
Metalloproteases
MMP-3, MMP-8, MMP-9
TIMP, ADAM-8
Glycosaminoglycans
Hyaluronan
Cell adhesion molecules
ICAM-1
VCAM-1
Other mediators
Hormones
Cortisol
Glycoproteins
Lactoferrin
Others
NO, RANTES, Relaxin, GCSF, ITAC, Visfatin

IL, Interleukin; TNF-α, tumour necrosis factor alpha; PG, prostaglandin; LT, leukotriene; MMP, matrix metalloprotease; TIMP, tissue inhibitors of matrix metalloproteases; ICAM-1, intercellular adhesion molecule 1; ADAM-8, A disintegrin and metalloprotease-8; NO, nitric oxide; RANTES, regulated on activation, normal T-cell expressed and secreted; GCSF, granulocyte colony-stimulating factor; ITAC, interferon-gamma-inducible T-cell alpha chemoattractant.

compete for binding to TNF-α (34, 35). Both soluble receptors have been shown to be present in amniotic fluid in term and preterm labour, but their concentration does not increase significantly in normal labour and no association between the concentration of either receptor in the amniotic fluid and preterm birth has been demonstrated (36, 37). These findings do not support the hypothesis that production of cytokine antagonists, such as sTNFR, is up-regulated preterm in order to prevent parturition. In contrast, sTNFR1 dramatically increases in infected amniotic fluid cavities, suggesting that it may have a role in down-regulating the potentially deleterious effects of TNF-α in pathologic conditions such as chorioamnionitis-complicated preterm labour (30).

Studies show a significant correlation of amniotic fluid levels of TNF-α (and IL-6) with PROM and chorioamnionitis (38, 39). A study which measured TNF-α concentrations in the amniotic fluid of women who had preterm deliveries showed significantly elevated concentrations in those women compared to a control group who delivered at term (40). The same study concluded that mid-trimester amniotic fluid concentrations of TNF-α (and/or IL-6) can positively identify women at risk of chorioamnionitis and subsequent preterm labour.

The association between TNF-α genetic variants and preterm labour has also been examined. One study analysed the association of SNPs in TNF-α and its receptor genes with amniotic fluid TNF-α and sTNFR (R1 and R2) concentrations in preterm labour. Significant differences between Caucasians and African-Americans were observed for both the genotype and allelic frequencies in TNF-α, TNFR1 and TNFR2 for maternal and foetal DNA. Numerous SNPs in African-Americans were associated with higher amniotic fluid TNF-α or sTNFR concentrations. In Caucasians, the association between different SNPs and cytokine concentrations was not as common (41). These allelic variations may be the key to understanding the disparity in rates of preterm birth between different ethnic populations.

Extracellular Matrix Mediators

Matrix metalloproteases. The MMPs are a family of zinc-dependent endopeptidases that are expressed in many inflammatory conditions and are capable of breakdown of connective tissue, including collagen. They play a central role in the breakdown and digestion of extracellular matrix and through this process they are implicated in foetal membrane rupture. MMPs are secreted in an inactive form, activated by local and infiltrating cells, and their action is inhibited by tissue inhibitors of matrix metalloproteases (TIMPs) which bind and inactivate these enzymes. A balance between MMP production *versus* the level of TIMPs controls tissue remodelling, although an imbalance that favours the MMPs is thought to lead to cervical ripening and foetal membrane rupture. The MMPs function is controlled by cytokines (42-45). Many studies have shown that amniotic fluid concentrations of MMPs seem to be predictive of premature delivery, as discussed below.

MMP-8: MMP-8 is a collagenase. Elevated mid-trimester concentrations of MMP-8 (and IL-6) in the amniotic fluid have been shown to be a strong predictor of intra-amniotic inflammation and spontaneous preterm delivery (44-46). Morever, increased concentrations of MMP-8 in amniotic fluid are associated with intra-amniotic infection, impending preterm delivery and adverse neonatal outcome in patients with preterm PROM (45). MMP-8 concentration in the amniotic fluid of asymptomatic women at 14-40 weeks

gestation was used as a marker of inflammation in a study assessing the role of subclinical intra-amniotic inflammation and mid-trimester shortening of the cervix on sonography. The authors concluded that 22% of women with elevated MMP-8 levels in mid-trimester amniotic fluid had a short cervix on ultrasound and a 40% risk of preterm delivery within 7 days. Currently, MMP-8 in amniotic fluid is considered a clinically useful test to predict spontaneous preterm birth in asymptomatic women (47).

MMP-9: MMP-9 is a 92 kDa type IV collagenase that is selectively expressed at the end of gestation by the amnion, trophoblasts and decidual cells. MMP-9 is the major MMP expressed during labour (48). IL-1, IL-6 and TNF-α increase expression of MMP-9 in the amniochorion. There is a correlation between MMP-9 enzyme expression and the decline of membrane tensile strength (49). *In vitro*, stimulation of the human amniochorion with either IL-1β or TNF-α results in a dose-dependent secretion of the MMP-9 pro-enzyme. IL-1β appears to be the key cytokine for induction of MMP-9 expression in the amniochorion (50). Expression of MMP-9 mRNA in a group of women with spontaneous preterm PROM was higher when compared with women in spontaneous labour at term. In the same study, expression of TIMP-2 mRNA levels in the preterm PROM group was significantly lower as compared to the group with spontaneous labour at term. This imbalance could be the cause of foetal membrane weakening and eventually preterm PROM (51).

Amniotic fluid MMP-9 is significantly elevated in women with intra-amniotic infection and is considered a sensitive predictor of chorioamnionitis (48). This increase in the MMP-9 concentration in infected amniotic fluid has also been observed in a rhesus monkey model. Injection of live bacteria (Group B Streptococcus) into the choriodecidua provokes a dose-dependent increase of TNF-α and IL-1β in the amniotic fluid, followed by augmentation of MMP-9 (and MMP-2) levels in the same compartment. In this study, 50% of animals receiving choriodecidual bacteria ended their pregnancies with spontaneous preterm labour without PROM (52).

A disintegrin and metalloprotease-8 (ADAM-8): ADAM-8 is a glycoprotein expressed in cells promoting inflammation and is implicated in a variety of biological processes involving cell-to-cell and cell-to-matrix interactions. ADAM-8 has been found to be elevated significantly in mid-trimester amniotic fluid of women later progressing to preterm as compared to term delivery (53, 54).

Interleukins. Interleukin-1 beta: IL-1 is a 17 kDa protein, 159 amino acids in length, which is mainly produced by monocytes but also by macrophages and peripheral neutrophil granulocytes. Its β form is predominant in human tissues; it acts as an endogenous pyrogen and increases the expression of cell adhesion molecules in the endothelium. IL-1β seems to have a dominant role in preterm and term labour associated with infection and can be used as a diagnostic indicator of an infective process (16, 19, 27, 55, 56).

In a non-primate pregnant rhesus monkey model, infusion of IL-1β into the amniotic fluid stimulated the most intense contraction pattern as compared to other cytokines and induced preterm labour in all cases (57). Preterm labour induced by intra-amniotic infusion of IL-1β in a similar model was previously shown to be inhibited by the administration of dexamethasone and IL-10 intravenously, and indomethacin orally in the mother. The authors suggest that these tocolytics could be useful adjuncts in the treatment of preterm labour that is associated with inflammation or infection (58, 59). A novel study demonstrated that intra-amniotic lipopolysaccharide (LPS) induces the production of IL-1β (and IL-6, TNF-α and IL-8) expression by foetal skin cells in an ovine model of *in utero* inflammation (60). The authors propose that the foetal skin acts as an important mediator of the foetal inflammatory response and as such may contribute to preterm birth. IL-1β may also be involved in the mechanism of foetal membrane rupture. Interleukin-1β induced preterm labour and spontaneous term labour are

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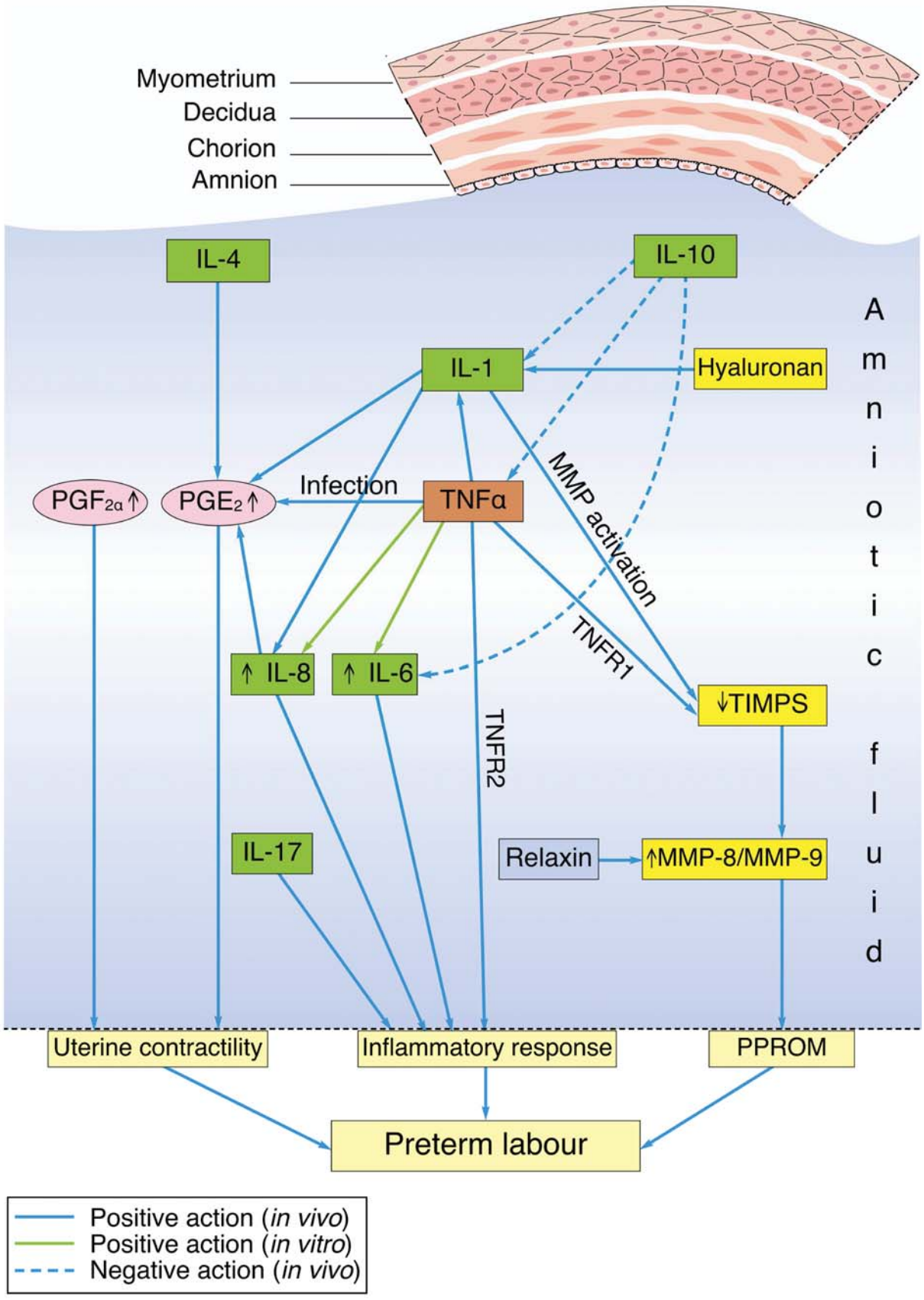
Figure 1. *Schematic representation of the interaction of the mediators in amniotic fluid implicated in preterm labour. Tumour necrosis factor-α (TNF-α) regulates the expression of interleukin-1 (IL-1) receptors in the human amnion cells. TNF-α might be implicated in the pathogenesis of preterm labour via the production of IL-6 and IL-8 as it stimulates the production of these cytokines by amnion cells cultured in vitro. TNF-α is involved in the transcriptional activation of prostaglandin (PG) synthase, leading to increased PGE_2 production in the amniotic fluid during infection-associated preterm labour. It acts via its receptor TNFR1 to trigger the production of matrix metalloproteases (MMPs) and via TNFR2 to initiate the pro-inflammatory immune response. IL-6 concentration is a marker of amniotic fluid infection. Increased levels trigger the initiation of labour via activation of an inflammatory response. IL-1 seems to have a dominant role in preterm and term labour associated with infection and this is mainly mediated via an increase in prostaglandin release. It also activates MMP production. IL-4 levels are also significantly elevated in women with preterm labour, particularly in association with infection, resulting in increased production of PGE_2 and subsequent myometrial activity. IL-10 inhibits the production of IL-1, IL-6 and TNF-α and subsequently the production of PGE_2. IL-17 produces inflammation at the foetomaternal interface. PG concentrations increase in the amniotic fluid before the onset of myometrial contractions. They induce labour by increasing myometrial contractility. MMPs, in particular MMP-8 and MMP-9, are central in the breakdown and digestion of extracellular matrix and through this process they are implicated in foetal membrane rupture. Relaxin causes increased production of MMPs. MMPs are inhibited by tissue inhibitors of matrix metalloproteases (TIMPs). An imbalance that favours the MMPs is thought to lead to foetal membrane rupture. This complex interaction between amniotic fluid mediators culminates in the onset of preterm labour through activation of an inflammatory response, preterm premature rupture of the membranes (PPROM) and increased uterine contractility.*


Myometrium
Decidua
Chorion
Amnion
IL-4
IL-10
IL-1
Hyaluronan
Infection
TNFα
PGF2α↑
PGE2↑
MMP activation
TNFR1
↑ IL-8
↑ IL-6
↓TIMPS
TNFR2
IL-17
Relaxin
↑MMP-8/MMP-9
Amniotic fluid
Uterine contractility
Inflammatory response
PPROM
Preterm labour
Positive action (in vivo)
Positive action (in vitro)
Negative action (in vivo)

preceded and accompanied by progressive increases in amniotic fluid matrix metalloproteinase-9 (MMP-9) in rhesus monkeys (61). IL-1β (and TNF-α) has also been shown to cause significant weakening of *ex vivo* cultured foetal membranes by inducing extracellular matrix remodelling and apoptosis (62).

Interleukin-4: IL-4 is a complex glycoprotein cytokine produced by activated T-cells and mast cells. It may have a role in the pathogenesis of infection-associated preterm labour, as amniotic fluid IL-4 levels have been shown to be significantly elevated in women with preterm labour, particularly in association with infection (63). The mechanism by which this happens is thought to be through induction of cyclooxygenase-2 in amnion cells, thus resulting in increased production of prostaglandin E_2 (PGE_2) and subsequent myometrial activity (64).

Interleukin-6: IL-6 is produced by monocytes and macrophages and is one of the first cytokines to be released in response to an infectious stimulus. It is the major mediator of the acute phase response, it activates B-cells to differentiate into antibody-secreting plasma cells and mediates the differentiation of mature T-cells to cytotoxic T-cells (65). The main source of IL-6 in the amniotic fluid is the amnion (66, 67).

It is now well established that IL-6 concentrations in the amniotic fluid are elevated in women with preterm and term labour as compared to non-labouring controls. A recent systematic review showed that spontaneous preterm birth in asymptomatic women was strongly associated with elevated mid-trimester amniotic fluid levels of IL-6 (68). Evidence also indicates that IL-6 in amniotic fluid is one of the best predictors of preterm birth (69-73). Elevated amniotic fluid IL-6 concentration is a marker of chorionic and amniotic inflammation associated with amniotic fluid infection and preterm delivery (20, 74-79). Additionally, studies which measured IL-6 concentrations in the amniotic fluid of women who had preterm deliveries showed concentrations to be significantly higher in those women compared to a control group who delivered at term, even in the absence of infection (20, 40, 80-82). Levels of IL-6 (and IL-8 and INF-γ) were also shown to be elevated in the amniotic fluid of women in normal labour as compared to women undergoing an elective Caesarean section (83). These findings suggest that IL-6 plays an important role both during idiopathic preterm labour and in spontaneous labour at term in the absence of infection.

In one study, IL-6 levels were significantly higher in women with cervical incompetence and IL-6 levels appear to predict a short-latency interval between cerclage and delivery (84). Race-specific differential genetic control of amniotic fluid IL-6 levels has also been demonstrated, with IL-6 being elevated in preterm labour but not in African-Americans (82, 85).

Amniotic fluid concentrations of IL-6 also appear to be significantly higher in patients with premature rupture of membranes (PROM) pre-labour in the presence microbial invasion of amniotic fluid and histological chorioamnionitis (27, 28, 86). This is indicative of intrauterine inflammation. Elevated IL-6 level has been shown in the absence of infection, with levels much higher in preterm labour with PROM as compared to term labour with PROM (87). The amniotic fluid level of IL-6 (and IL-8 and TNF-α) is positively correlated with the duration of PROM (74, 88). This finding could be explained by different immunological mechanisms involved in the initiation of preterm and term labour. The rise in IL-6 in the amniotic fluid of cases of PROM in the presence of infection may represent an enhanced immune response to protect the foetus or a trigger for the initiation of labour, and can be a valuable test for diagnosing chorioamnionitis.

Data from genetic association studies in pregnancy have demonstrated that SNPs of the IL-6 gene are associated with increased risk of preterm birth. A study has shown that the C-174G polymorphism (G allele) reduces promoter activity and thus the risk of preterm labour, whereas the G/G homozygote is associated with increased risk of preterm birth (89).

Interleukin-8: IL-8 is an 8 kDa non-glycosylated protein secreted by macrophages and monocytes following stimulation by IL-1 and TNF-α. The main action of IL-8 is recruitment of neutrophils to sites of inflammation. Elevated amniotic fluid IL-8 concentration is a marker of inflammation in the amniotic fluid and is often associated with amniotic fluid infection (90).

Interleukin-10: IL-10 is an immune-inhibitory cytokine secreted by T-lymphocytes and monocytes following LPS-induced cell activation. In the choriodecidual unit, IL-10 inhibits the production of IL-1β, IL-6 and TNF-α, and the production of PGE_2 (22). It has been shown to be effective in reducing preterm contractions mediated by inflammatory cytokines in animal model studies (91). Mid-trimester IL-10 concentration in amniotic fluid does not appear to be positively associated with preterm delivery (92).

Interleukin-17: Interleukin-17 is a key cytokine which induces inflammation and is critical to host defence. Immunohistochemical staining studies have revealed that $CD3^+$ and $CD4^+$ T-cells are the main source of IL-17 in the chorioamniotic membrane. IL-17 has been shown to produce inflammation at the foetomaternal interface in pregnancies complicated by preterm delivery (5).

Prostaglandins. PGs are considered to be one of the key mediators of the mechanisms regulating the onset of labour. They are membrane phospholipid derivatives, members of the eicosanoid family, and are produced by pregnancy tissues

and the myometrium (6). As signalling molecules, PGs act in a paracrine or autocrine manner to activate intracellular signalling and gene transcription *via* binding to G-protein receptors (17). The major site of PG synthesis and metabolism in human pregnancy is the foetal membranes. PG concentrations increase in the amniotic fluid before the onset of myometrial contractions (20). PGs have been shown to induce labour by regulating myometrial contractility as well as promoting changes in the extracellular matrix composition of the foetal membranes and by producing cervical ripening at the onset of labour (17). These changes result from alterations in the synthesis and metabolism of PGs, and expression of various PG receptors (93). Through this mechanism they are considered to play a key role in the initiation of spontaneous as well as preterm labour (93, 94).

Biologically active PGs, particularly PGE_2 and PGF2a, have well-established roles in pregnancy and labour. The concentration of these PGs in amniotic fluid is significantly higher in women with preterm labour in the presence of intra-amniotic infection rather than in women with preterm labour without infection. Similar observations have been made in patients with preterm labour and concentrations of inflammatory mediators, such as IL-6, IL-1 and TNF-α (95).

Prostaglandin E_2: PGE_2 expression by amnion cells is stimulated by inflammatory cytokines such as IL-1β, IL-6, IL-8 and TNF-α, suggesting that these cytokines may initiate labour indirectly by increasing PGE_2 production (95). Studies have shown that the increased PGE_2 biosynthesis in the amniotic fluid is mediated *via* an increase in PG endoperoxide synthase-2 mRNA expression from amnion cells. This is in turn is induced by immune mediators such as TNF-α, which are elevated in the amniotic fluid of women in preterm labour, and particularly in the presence of chorioamnionitis (96). A different study found amniotic fluid PGE_2 to be significantly higher at term rather than preterm birth. This was confirmed after taking into account factors such as race, cigarette smoking and microbial invasion of amniotic fluid (97). This finding has also been shown by another study where PG endoperoxide synthase-2 expression by amnion and chorio-decidua was higher with spontaneous labour at term as compared to preterm labour (98).

Prostaglandin F2α: The most potent uterine contractile prostaglandin is $PGF_{2\alpha}$. Its action is mediated by a specific receptor (99). Recently, $PGF_{2\alpha}$ was shown to be the only amniotic fluid eicosanoid with an amniotic fluid concentration higher at preterm birth rather than at term (97). Increased $PGF_{2\alpha}$ in amniotic fluid has been shown to be an independent predictor of preterm labour and delivery following PROM. Its concentration increases in patients with intra-amniotic inflammation, regardless of the presence of infection, and this leads to a significantly shorter preterm PROM-to-delivery interval (100).

Other PGs: PGD_2 has been described as a major product of human gestational tissues and an important component of the inflammatory reaction in both term and preterm labour (101). However, the regulation of its production and its exact role in preterm labour remains poorly defined.

HETE and leukotrienes. Preterm labour is associated with changes in the amniotic fluid concentration of HETEs. 5-HETE and 15-HETE concentrations in amniotic fluid of women in preterm labour have been shown to be elevated, particularly when associated with intra-amniotic infection (102-104).

Leukotrienes (LTs) are also implicated in the pathophysiology of preterm labour. The amniotic fluid concentrations of LTC4 and LTB4 are increased in women with preterm labour, particularly in the presence of chorioamnionitis (97, 104).

Glycosaminoglycans. Hyaluronan: Hyaluronan (HA) is an immune modulator of the extracellular matrix and is released in response to infection or tissue injury. It is made of a repeating disaccharide sequence of D-glucuronic acid and N-acetyl-glucosamine. A recent study measured mid-trimester HA levels in amniotic fluid and found them to be elevated in pregnancies at risk of preterm labour (105). This study thus concluded that HA may be a component of the inflammatory response pathways involved in preterm labour.

Cell Adhesion Molecules

Cell Adhesion Molecules (CAMs) are proteins located on the cell surface that are involved with cell-to-cell or cell-to-extracellular-matrix adhesion. Intercellular adhesion molecule ICAM-1 and vascular cell adhesion molecule VCAM-1 are members of the cell-surface immunoglobulin superfamily of adhesion receptors. They are expressed on endothelial cells and induced or up-regulated by pro-inflammatory cytokines [*e.g*. IL-1 and TNF-α (106)]. Both molecules exist in transmembrane and soluble (s) forms.

Inter-cellular adhesion molecule 1. ICAM-1 is a protein continuously present in low concentrations in the membranes of leukocytes and endothelial cells. Upon cytokine stimulation, its concentration greatly increases. ICAM-1 can be induced by IL-1 and TNF-α (107). Increased circulating soluble ICAM-1 level in mid-trimester amniotic fluid is associated with a shortened length of gestation at delivery (107). Expression of ICAM-1 mRNA in amniotic membranes of women in preterm labour is significantly up-regulated as compared to women who delivered by Caesarean section at term (108). In one study, soluble ICAM-1 levels in amniotic fluid of women in preterm labour were significantly elevated (105); however, this finding has not been confirmed by others (109).

Soluble vascular cell adhesion molecule-1. A study of 13 cases of preterm delivery found that soluble VCAM-1 concentration levels in mid-trimester amniotic fluid are higher in mothers delivering preterm as compared to mothers delivering at term; however, these findings did not reach statistical significance (109).

Other Mediators

Nitric Oxide. NO is an important cell signalling molecule involved in many physiological and pathological processes. It acts through several mechanisms, including activation of soluble guanylate cyclase leading to formation of cGMP and activation of protein kinases. NO has a relaxant effect on uterine smooth muscle and is implicated in maintaining uterine quiescence during pregnancy. Nitric oxide may act as an inflammatory mediator at high concentrations.

NO metabolite concentrations are higher in amniotic fluid from women in labour than in non-labouring patients, both at term and preterm (110). Levels have also been shown to be significantly higher in patients that are in preterm labour with intra-amniotic infection compared to those without intra-amniotic infection (111, 112). These findings suggest that increased breakdown of pro-inflammatory NO in amniotic fluid may play an important role in the pathogenesis of preterm labour. Differential expression of NO synthase (NOS) isoforms in foetal membranes may also contribute to the complex regulatory roles of NO during this process (113).

RANTES. Regulated on activation, normal T-cell expressed and secreted (RANTES) protein is a potent chemoattractant of inflammatory cells that have been implicated in the mechanisms of human parturition and in the regulation of the host response to intrauterine infection. Amniotic fluid concentration of RANTES decreases with advancing gestational age (114). However, the median concentrations of RANTES in amniotic fluid increases both in preterm and term labour, with a higher concentration being found in those who delivered at term. Microbial invasion of the amniotic cavity has been shown to be associated with a significant increase in the median amniotic fluid level of RANTES in both preterm and term labour (114).

Cortisol. Glucocorticoids, including cortisol, may be important in regulating PG formation within the human foetal membranes and have been implicated in the pathogenesis of preterm labour (115). At present evidence suggests that cortisol levels in amniotic fluid do not correlate with preterm delivery as they do not change significantly in women in preterm labour as compared with women in spontaneous term labour or in women not in labour (116, 117).

Adrenomedullin. Adrenomedullin is a vasoactive peptide originally identified in pheochromocytoma and has been found to be present in amniotic fluid throughout pregnancy (118, 119). It is secreted by foetoplacental tissues and is considered to participate in uterine and placental blood flow regulation (120). Adrenomedullin is thought to be involved in the maintenance of normal pregnancy but its concentrations in amniotic fluid have not been shown to differ significantly in women with spontaneous preterm delivery and those at spontaneous labour at term (121).

Interferon-gamma-inducible T-cell alpha chemoattractant. T-cell alpha chemoattractant (ITAC) is a chemokine directing the migration of activated T-lymphocytes toward inflammatory sites (122, 123). A study has demonstrated elevated second trimester amniotic fluid concentrations of ITAC in women delivering at less than 37 weeks gestation, and this could be used as an indicator of occult infections or inflammation and therefore possibly serve as predictor of preterm delivery (123).

Granulocyte colony-stimulating factor. Granulocyte colony-stimulating factor (GCSF) is a cytokine produced by monocytes which regulates the production and maturation of neutrophil progenitor cells. Immunohistochemical staining studies have shown GCSF to be derived from endothelial cells of the foetal membranes. Its actions are mediated *via* a specific GCSF receptor (124, 125).

GCSF is significantly elevated in the amniotic fluid, in both term and preterm labour (125, 126). Since this is also true in association with preterm labour complicated by intra-amniotic infection, amniotic fluid levels of GCSF have been found to be a reliable predictor of chorioamnionitis. This suggests that GCSF may be an early regulator of infection-induced preterm labour (125). However, another study failed to demonstrate a correlation between GCSF concentrations in amniotic fluid and term or preterm labour (126).

Lactoferrin. Lactoferrin (Lf) is an iron-binding glycoprotein with antimicrobial properties, which is released from secondary granules of activated neutrophils in response to inflammation. It is found in many biological fluids, including amniotic fluid. Its concentration increases with advancing gestation (127-129).

The biological significance of Lf concentration changes in amniotic fluid has been demonstrated by the fact that such concentrations in cases of chorioamnionitis are significantly higher than in non-infected cases (129, 130). The same authors also demonstrated that IL-6 production by cultured amnion cells was significantly suppressed by Lf, suggesting that Lf may act as a self-defence mechanism against intrauterine infection.

Similar results demonstrated that intra-amniotic infection is associated with a significant increase in amniotic fluid Lf concentration in patients with preterm labour, term labour and preterm PROM (127). Spontaneous term labour was associated with a reduction in the Lf concentration in the amniotic fluid, again suggesting that Lf acts as part of the host defence mechanism against intra-amniotic infection. It has been suggested that high amniotic fluid levels of Lf in patients at less than 32 weeks gestation are highly suggestive of intra-amniotic infection, and therefore the authors suggest that such patients may benefit from antibiotic treatment (131).

Visfatin. Visfatin, a novel adipokine with diabetogenic and immunoregulatory properties, was originally identified as a pre-B-cell colony-enhancing factor. Its specific physiological role has not been completely elucidated, but there is evidence that it is implicated in the pathophysiology of insulin resistance as well as in various acute and chronic inflammatory disorders (132). Visfatin is expressed by amniotic membranes, cytotrophoblast and decidua and in particular when foetal membranes are exposed to mechanical stress or pro-inflammatory stimuli. It is a physiological constituent of amniotic fluid and its concentration is increasing with advancing gestational age.

A study demonstrated that among women with preterm labour with or without PROM who delivered preterm, the median visfatin concentration in amniotic fluid was significantly higher in the presence of infection regardless of the membrane status. The authors therefore concluded that visfatin may have an important regulatory role in the inflammatory response in acute infection (133).

Relaxin. Relaxin is a peptide hormone with structural similarity to insulin (134). In human pregnancy, relaxin is both a systemic hormone that is secreted from the corpus luteum and a paracrine hormone at the maternal-foetal interface formed by the decidua, placenta and foetal membranes. Relaxin causes increased production of the MMPs (135). The important role of relaxin in the initiation of preterm labour is supported by the fact that expression of the relaxin gene and protein in the decidua and the placenta is increased during preterm as compared to term labour (136). In the absence of infection, decidual relaxin expression is increased in patients with preterm PROM. An *in vivo* animal model has demonstrated that this increase in foetal membrane expression of relaxin is independent of an infection-mediated cytokine response, but in the absence of infection, it causes increased intra-amniotic concentrations of IL-6 and IL-8 from the foetal membranes (134). This may induce a local sterile inflammatory process which potentially contributes to extracellular matrix degradation and weakening of the foetal membranes, thus leading to PROM and preterm labour (136-139).

Conclusion

Induction of pro-inflammatory cytokines and chemokines plays an important role in the activation of the cascade of events resulting in preterm labour. Considerable evidence from human and animal studies suggests that changes in cytokine levels in the amniotic fluid are important in this process. Cytokines and arachidonate/lipooxygenase metabolites play a central role in the pathogenesis of preterm birth, particularly in association with infection. This is based on the observation that increased concentrations of these mediators are found in patients with intra-amniotic infection and preterm labour. *In vitro* studies have also shown that bacterial products stimulate the production of pro-inflammatory cytokines by human decidua.

The precise mechanism of foetal membrane breakdown remains to be elucidated; however, the fact that extracellular matrix-degrading enzymes, such as MMPs, are implicated is well established. The concentration of these enzymes in amniotic fluid is increased in women with preterm labour and this increase is thought to contribute to premature rupture of the membranes.

The identification of differences in the expression of amniotic fluid mediators will enable assessment of susceptibility to preterm birth. Recent evidence from SNP analysis of genes encoding amniotic fluid cytokines suggests that genetic variation may be the cause of this disparity in cytokine concentrations. Allelic variations may, thus, be key to understanding the different rates of preterm birth among various ethnic populations.

Clinical predictors of preterm labour are reliable only in the later stages of the pathological process, at which point, measures to prevent preterm labour may be ineffective as this process is difficult to reverse. By developing tests that detect the molecular changes early on, prior to the onset of clinical symptoms, it might be possible to initiate effective preventative treatments. Human and animal models can be used to describe the pathophysiological events associated with mediator changes in the amniotic fluid and suggest methods for preventing preterm birth. The exact physiological and pathophysiological role of these mediators, however, has yet to be elucidated and further research is required. The current challenge remains to fully understand mediators in amniotic fluid and their pathways of action in order to help both prevent and treat preterm labour.

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