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Decidual cytokines and pregnancy complications: focus on spontaneous miscarriage

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Abstract

The establishment of pregnancy requires the co-ordinated implantation of the embryo into the receptive decidua, placentation, trophoblast invasion of the maternal decidua and myometrium as well as remodelling of the uterine spiral arteries. Failure of any of these steps can lead to a range of pregnancy complications including miscarriage, pre-eclampsia, fetal growth restriction, placenta accreta and pre-term birth. Cytokines are small multifunctional proteins often derived from leucocytes and have primarily been described through their immunomodulatory actions. The maternal-fetal interface is considered to be immunosuppressed to allow development of the semi-allogeneic placental-fetal unit. However, cytokine profiles of the decidua and different decidual cell types suggests that the *in vivo* situation is more complex. Data suggests that decidual derived cytokines not only play roles in immunosuppression but also in other aspects of the establishment of pregnancy including regulation of trophoblast invasion and spiral artery remodelling. This review focuses on the potential role of decidual derived cytokines in the aetiology of unexplained spontaneous miscarriage.

Keywords: decidua; cytokines; miscarriage; trophoblast invasion; spiral artery remodelling

1 *Introduction*

Several pregnancy pathologies, including pre-eclampsia (PE), fetal growth restriction (FGR) and miscarriage, are associated with deficient invasion of extravillous trophoblast cells (EVT) into the decidua and myometrium as well as incomplete remodelling of the uterine spiral arteries. EVT invasion and remodelling of the uterine spiral arteries are highly complex processes that require the dynamic interplay of many different biological signals from different cellular sources, including the decidua. Of particular note are the decidual cytokines and how their dysregulation may play roles in the aetiology of complications of pregnancy. This review will discuss the pathophysiology of spontaneous miscarriage, key processes in the establishment of pregnancy that may be causative of this pregnancy complication as well as decidual cytokines shown to be dysregulated in miscarriage, their cellular sources, known functions and how they may contribute to the pathogenesis of miscarriage.

2 *Cytokines*

Cytokines are small (5-20kDa) signalling proteins produced by a wide range of cells. They show diverse functions, including regulation of cellular invasion and both humoral and cell-based immune responses. These cell-based and humoral immune responses are mediated by different sets of cytokines that can be grouped according to their main function for example; pro- and anti-inflammatory, or associated with different T helper (Th) subsets referred to as Th1, Th2, Th17 and regulatory (Treg) cells. Major pro-inflammatory cytokines include TNF, IL-1 β , IL-6 and CXCL8/IL-8, while anti-inflammatory cytokines include IL-10 and

TGF- β . Signature cytokines of Th-associated responses are; interferon (IFN)- γ (Th1); IL-4, IL-5, IL-9 and IL-13 (Th2); IL-17A and IL-17E (Th17); IL-10 and TGF- β (Treg). Several other cytokines also exist that do not precisely fit into the described groups, like interferons, chemokines, growth factors and many others. The area is complex because of a redundancy in cytokines with antagonistic, synergistic, similar and sometimes identical effects; for instance, there are at least 37 interleukins and approximately 50 chemokines. In this review we will focus on a limited number of established and well-known cytokines in reproductive biology, but also include recent advances that confirm or challenge our understanding of this complex area of biology.

Cytokines in normal pregnancy

In the early era after the discovery of the Th1/Th2 dichotomy, Wegmann *et al.* (1993) proposed that during pregnancy a Th2 response prevails, whereas a Th1 response is detrimental to pregnancy. From a general perspective and when considering systemic changes, this view is supported by observations like the improvement of Th1-associated autoimmune disease multiple sclerosis (Confavreux *et al.*, 1998) and rheumatoid arthritis (Ostensen and Villiger, 2007) during pregnancy. However, this view is likely too simplistic as Th1 cytokines have also been shown to be essential for maintenance of pregnancy, e.g. IFN- γ is essential for successful pregnancy as it is pivotal in spiral artery remodelling and successful pregnancy outcome (Ashkar *et al.*, 2000; Croy *et al.*, 2003; Robson *et al.*, 2012). Furthermore, a study using a Th2 knockout (IL-4, IL-5, IL-9 and IL-13 ko) mouse showed normal reproductive outcome (Fallon *et al.*, 2002), although different systems may be involved in maintenance of mouse and human pregnancies. Additional cytokines, e.g. IL-15

and IL-18, which are not classed as Th1 or Th2 type cytokines, have been observed at the maternal-fetal interface (Chaouat *et al.*, 2002; Murphy *et al.*, 2009), and the broadening of the Th subset families to include Th17 and Treg has indeed challenged the paradigm and adding to its complexity. Although IL-17 has been mostly associated with hyper-inflammation in autoimmunity, it was recently suggested that stromal cell-derived IL-17 was present in the first trimester and had a positive role in sustaining human pregnancy by recruiting Th17 cells, and promoting trophoblast invasion and inhibiting trophoblast apoptosis (Wu *et al.*, 2014). Conversely, Th17 cells have been reported to be rare and outnumbered by an enrichment of Treg cells (Mjösberg *et al.*, 2010).

The human endometrium produces a wide variety of cytokines throughout the proliferative and secretory phase of the menstrual cycle (Tabibzadeh, 1991). These cytokines are believed to play a significant role in the modulation of the uterine environment preparing the uterus for implantation of the developing conceptus and formation of a functional placenta during the establishment of pregnancy.

Prevention of maternal rejection of the fetus requires a regulated environment, which occurs primarily at the maternal-fetal interface and the uterine tissues. Classically, naive CD4⁺ T-cells are the major producers of cytokines divided into the subsets of Th1, Th2, Th17 and Treg. However, at the maternal-fetal interface a plethora of Th-associated cytokines are also produced by trophoblast cells, stromal cells, epithelial cells, maternal T lymphocytes, macrophages, natural killer (NK) cells and other maternal leucocytes (Vince and Johnson, 2000), suggesting that maintenance and development of the fetal-placental unit is dependent on these cytokines. The presence of cytokines at the maternal-fetal interface may influence the environment by regulating processes such as implantation,

placental development, cytotrophoblast proliferation, angiogenesis, extravillous trophoblast cell (EVT) invasion, spiral artery remodelling, cellular growth and apoptosis, as well as induction of fetal tolerance (Piccinni *et al.*, 2000; Drake *et al.*, 2001; Dimitriadis *et al.*, 2005; Lash *et al.*, 2006; Murphy *et al.*, 2009). However, this is a complex area of research due to the pleiotropy and redundancy of the cytokine network.

3 *Miscarriage*

Miscarriage is the commonest gynaecological emergency (70000-90000 per year in England and Wales) (Everett, 1997) and has huge financial and personal implications. The vast majority of miscarriages occur in healthy women during the first trimester of pregnancy. Between 11 and 20% of all clinically recognised pregnancies are lost before the 20th week of gestation. Most miscarriages are sporadic, that is they are non-recurring although a sub-set of women do suffer from recurrent miscarriage defined as three or more consecutive miscarriages. It is likely that the aetiology of sporadic and recurrent miscarriage differs and therefore this article will focus on sporadic or spontaneous miscarriage.

Approximately 50% of miscarriages are associated with chromosomal abnormalities (aneuploidy) (Jauniaux and Hustin, 1992). However, the cause in the remaining 50% is unknown and the mechanisms involved in sporadic first trimester miscarriages are poorly understood (Hustin *et al.*, 1996), and it is these idiopathic cases that we will concentrate on in this review.

Approximately 70% of early miscarriages have been associated with premature and continuous intervillous blood flow, evidence arising from flow patterns using gray scale

Doppler. This was linked to oxidative stress in villous trophoblast, an effect of a thin and disrupted trophoblast shell (Jauniaux *et al.*, 1994; Jauniaux *et al.*, 2000). There is also evidence suggesting that deficient trophoblast invasion may be linked with first trimester miscarriage; using a morphological method (Hustin *et al.*, 1990) demonstrated reduced trophoblast invasion in early aneuploid miscarriages. It was this observation that led to the suggestion that trophoblast invasion and spiral artery remodelling are primarily reduced in miscarriage, although reports are inconsistent. Ball *et al.* (2006a) assessed trophoblast subpopulations and spiral artery transformation in placental bed biopsies from sporadic miscarriage cases (n=73) compared with controls (n=179), using an immunohistochemical approach. In contrast to Hustin *et al.* (1990) there was no evidence of failure of trophoblast invasion and abnormal spiral artery remodelling in the decidua or myometrium in early ($\leq 12+6$ weeks gestational age) miscarriage, suggesting that these processes do not have a pivotal role in the pathogenesis of early miscarriage. The varying results between the different studies may be due to the tissue sampled; the abnormalities in early miscarriages were detected in studies generally using aspirated gestational sacs which include cytotrophoblast cell columns and the cytotrophoblast shell (Hustin *et al.*, 1990); whereas, placental bed biopsies extend deeper into the uterine wall (Ball *et al.*, 2006a). However, reduced trophoblast invasion and spiral artery remodelling in late (≥ 13 weeks gestational age) miscarriage was observed (Ball *et al.*, 2006b).

4 *Decidua*

Symbiotic signalling between the blastocyst and mother is necessary for successful pregnancy and requires a highly receptive endometrium (decidua) during the window of

implantation (Aplin, 2000). The decidua is comprised of luminal and glandular epithelium, stromal cells, spiral arteries, lymphatics and leucocytes. In the first trimester of pregnancy, approximately 30-40% of the cells in the decidual stroma are leucocytes, primarily uterine natural killer (uNK) cells, macrophages and T lymphocytes (Bulmer *et al.*, 1991) although other less abundant but functionally important endometrial leucocyte populations are also present including dendritic cells (Gardner and Moffett, 2003), natural killer T (NKT) (Tsuda *et al.*, 2001) cells and regulatory T cells (Mjösberg *et al.*, 2010; Ernerudh *et al.*, 2011). The decidua is a rich source of cytokines, growth factors and proteases that may contribute to regulation of EVT invasion and spiral artery remodelling.

5 *Trophoblast invasion*

EVT are naturally highly invasive although their invasion into the decidua and inner third of the myometrium is tightly regulated. In simple terms there are three general features of cellular invasion; attachment to the extracellular matrix (ECM), proteolytic breakdown of the ECM and then movement into that cleared space prior to reattachment. EVT invasiveness is associated with their phenotype, for example EVT express a unique repertoire of cell surface integrins, distinct from those expressed by villous cytotrophoblast (CTB). In particular, EVT are characterised by the expression of $\alpha 1\beta 1$ and $\alpha 5\beta 1$ integrins, while CTB express $\alpha 6\beta 4$ integrin (Damsky *et al.*, 1992). This switch in integrin expression appears to be essential for the invasive phenotype of EVT (Damsky *et al.*, 1994). Many different factors (growth factors, cytokines and chemokines) have been shown to regulate trophoblast invasion in *in vitro* models, and it is likely that *in vivo* relative local concentrations of these different compounds play roles in stimulation or inhibition of the

invasive process. Whether the natural invasive capacity of EVT is altered in miscarriage has not been investigated and studies have concentrated on alterations in decidual factors that regulate this process.

6 *Spiral artery remodelling*

Remodelling of the uterine spiral arteries is one of the most important maternal adaptations to pregnancy (Pijnenborg *et al.*, 2006). During spiral artery remodelling the blood vessels supplying the uterus undergo significant alterations that result in the decidual and superficial myometrial portions of the vessels losing their musculoelastic wall, which is replaced by fibrinoid and intramural EVT (Pijnenborg *et al.*, 2006). This remodelling process allows for maternal blood that is not under vasoactive control to be delivered to the fetal-placental unit. The underlying pathology of pre-eclampsia and fetal growth restriction is associated with reduced spiral artery remodelling, likely from reduced EVT invasion (Khong *et al.*, 1987; Pijnenborg *et al.*, 1991). Spiral artery remodelling is often described in terms of its sequential morphological features which include vascular smooth muscle cell (VSMC) separation, endothelial cell swelling, vessel dilatation, endovascular and/or interstitial EVT invasion, transient loss of endothelial cells, VSMC loss, fibrinoid deposition, presence of intramural EVT and regeneration of the endothelium (Pijnenborg *et al.*, 2006). While EVT are absolutely required for completion of successful spiral artery remodelling the initial steps occur in the absence of EVT, and may be mediated by uNK cells. However, the molecular triggers of spiral artery remodelling are not known and exactly how EVT contribute to this process is also not known.

7 *Homeostasis and tolerance*

The remodelling process involves the production of waste material that needs to be smoothly taken care of. Decidual macrophages and their scavenger receptors are key players in this homeostatic process (Svensson-Arvelund and Ernerudh, 2014). Along this line, decidual macrophages also take care of threatening microbes without creating hyperinflammation. Decidual macrophages are induced by M-CSF and IL-10 (Svensson *et al.*, 2011); while deficiency of IL-10 has been associated with missed abortion (Plevyak *et al.*, 2002), the role of M-CSF has not been explored in the context of spontaneous miscarriage. Decidual macrophages themselves also contribute by secretion of IL-10 (Lidström *et al.*, 2003, Heikkinen *et al.*, 2003) as well as CCL18 (Gustafsson *et al.*, 2008), and as being the most abundant antigen presenting cells (APC) at the fetal maternal interface they also interact with T cells thereby contributing to fetal tolerance. In this process, Regulatory T cells are regarded as the main contributors, with several direct and indirect effects (Ernerudh *et al.*, 2011); IL-6/TGF- β and TGF- β /IL-10 being the key cytokines in the induction and effector process, respectively.

8 *Altered decidual cytokine expression and spontaneous miscarriage*

Altered decidual cytokine expression, involved in the central processes that have been described above, has been shown to be associated with spontaneous miscarriage. However, due to the timing of sample collection it is never fully possible to conclude that this altered expression is causative of the miscarriage or a consequence of fetal and placental demise.

Some of the major cytokine candidates associated with spontaneous miscarriage are described below (Table 1).

IL-6

IL-6 is a pleiotropic cytokine that is an important mediator in acute-phase responses to injury and infection. It signals through the IL-6 receptor and the gp130 receptor subunit and the Stat pathway to activate target genes involved in cellular differentiation, proliferation, apoptosis and cell survival.

IL-6 is present in human endometrium throughout the menstrual cycle as well as in early pregnancy decidua (Tabibzadeh *et al.*, 1995). It has also been reported that EVT cells express IL-6, and it has been suggested this cytokine can influence trophoblast invasion by up-regulating MMPs (Jauniaux *et al.*, 1996; Meisser *et al.*, 1999; Salamonsen *et al.*, 2007). The role of IL-6 in regulation of EVT invasion remains unclear as different studies report different effects. Jovanovic and Vicovac (2009) reported that both endogenous IL-6 and exogenous IL-6 increased the migration and invasive potential of the trophoblast-like cell line HTR-8/SVneo due to the up-regulation of the trophoblast integrin receptors $\alpha 1$, $\alpha 5$ and $\beta 5$, receptors which bind to components within the extracellular matrix. However, Champion *et al.* (2012) reported no effect on EVT invasion from first trimester placental explants. In addition, IL-6 has been shown to induce vascular smooth muscle disorganisation in an *in vitro* model of spiral artery remodelling (Pitman *et al.*, 2013).

Studies in mouse have demonstrated that increased levels of IL-6 at the maternal-fetal interface were associated with fetal loss (Zenclussen *et al.*, 2003) and more recent data has

demonstrated increased levels of serum IL-6 in women with second trimester miscarriage compared with levels from first trimester miscarriage (Galazios *et al.*, 2011). However reduced uNK cell and decidual macrophage secreted IL-6 was reported in women with first trimester miscarriage compared with controls, while altered decidual cell expression of IL-6 and its receptors (as determined by immunohistochemistry) was predominantly shown in the placental bed of women with second trimester miscarriage (Pitman *et al.*, 2013).

CXCL8/IL-8

CXCL8, formerly known as IL-8, is classified as a pro-inflammatory multifunctional CXC chemokine, that signals through the CXCR1 and CXCR2 receptors. CXCL8 is known for its ability to recruit CXCR1 and CXCR2 expressing granulocytes, although these receptors are also expressed on monocytes, NK cells and subpopulations of CD8 cells (Takata *et al.*, 2004). In the early pregnant human decidua, uNK cells are a major producer of CXCL8 (Saito *et al.*, 1994; De Oliveira *et al.*, 2010). There are also reports describing expression of CXCL8 in the pregnant decidua and it is likely to be involved in leucocyte trafficking to the spiral arteries during the first trimester of pregnancy (Hornung *et al.*, 1997; Jones *et al.*, 2004). CXCL8 has been shown to be produced by cells of the placenta, including trophoblast, placental macrophages (Hofbauer cells) and fibroblasts, and it has been suggested that CXCL8 mediates the recruitment and accumulation of neutrophils at the maternal-fetal interface as a defence mechanism against invading bacteria (Shimoya *et al.*, 1999).

CXCL8 has been shown to stimulate trophoblast invasion in *in vitro* models and contribute to the uNK cell stimulation of trophoblast invasion (Hanna *et al.*, 2006; De Oliveira *et al.*, 2010).

In addition, CXCL8 induces vascular smooth muscle cell disorganisation in a model of spiral artery remodelling (Pitman *et al.*, 2013).

There are few reports of CXCL8 levels in miscarriage. Madhappan *et al.* (2003) reported significantly higher levels in products of conception from women with two or more miscarriages (n=7) compared with women with normal pregnancy (n=4) or first miscarriage (n=12). Increased CXCL8 levels in uterine cervical samples have also been associated with women suffering first trimester miscarriage compared with controls (Radulovic *et al.*, 2010). In contrast, reduced levels of CXCL8 were secreted by uNK cells or decidual macrophages isolated from first trimester miscarriage compared with normal controls and expression of CXCL8 and its receptors was altered in the placental bed of women with both early and late miscarriage compared with controls (Pitman *et al.*, 2013).

TNF- α

Tumour necrosis factor (TNF) is a potent pro-inflammatory cytokine which elicits its effects via two membrane bound receptors, TNF- α RI and TNF- α RII (Haider and Knofler, 2009). TNF- α has been localized to proliferating trophoblast cells in villous tips, interstitial and endovascular EVT in the placental bed (Chen *et al.*, 1991; Pijnenborg *et al.*, 1998), and to a number of decidual cell types including T cells, uNK cells (Jokhi *et al.*, 1994), stromal cells, macrophages (Vince *et al.*, 1992) and endothelial cells (Tabibzadeh, 1991).

TNF has been reported to inhibit trophoblast invasion (Bauer *et al.*, 2004; Renaud *et al.*, 2005; Huber *et al.*, 2006; Otun *et al.*, 2011). The mechanism(s) by which TNF exerts this inhibitory effect likely include alteration of proteolytic enzymes such as the matrix

metalloproteinases (MMP's) (Bischof *et al.*, 1995) and the urokinase plasminogen activator system (Queenan *et al.*, 1987) as well as altering EVT apoptosis or proliferation (von Rango *et al.*, 2003).

Few studies have investigated TNF in human miscarriage although it has been reported to induce spontaneous abortion in rodents (Chaouat *et al.*, 1990). More recently increased decidual stromal cell secreted levels of TNF have been reported in women with spontaneous miscarriage (Sun *et al.*, 2013).

Other cytokines

Several other cytokines, such as IL-2, IL-1 β , IL-17A, have also been shown to be dysregulated in the decidua of women miscarriage (Sun *et al.*, 2013; Garzia *et al.*, 2013). However, their roles in establishment of pregnancy have not been well defined, although they do play roles in immune responses. Therefore, it is difficult to speculate whether they have a causative role in the aetiology of miscarriage or are a consequence of this devastating condition.

9 Conclusions

Reduced decidual IL-6 and CXCL8/IL-8 as well as increased TNF- α levels may negatively impact on the normal processes of pregnancy establishment by dysregulating control of trophoblast invasion as well as aspects of spiral artery remodelling and therefore contribute to the aetiology of both early and late miscarriage. However, these are very complex

processes and likely regulated by several different factors, leading to a multifactorial disease state.

Miscarriage remains one of the most prevalent obstetrical complications with little known about risk factors and aetiology. We review here the role that decidual cytokines may play in this process, but owing to the lack of known risk factors or biomarkers for this condition, there is a need for deeper insights into mechanisms before we will be able to translate this knowledge into preventative therapies.

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Table 1: Summary of the potential role of altered decidual cytokines in the pathophysiology of spontaneous miscarriage.

Cytokine	Role in early pregnancy	Levels in spontaneous miscarriage
IL-6	EVT invasion – variable results from in vitro assay systems. Spiral artery remodelling – induces VSMC disorganisation.	Serum - increased. Decidual leucocytes (uNK cells and macrophages) - decreased.
CXCL8/IL-8	EVT invasion – stimulatory. Spiral artery remodelling – induces VSMC disorganisation.	Products of conception - increased. Decidual leucocytes (uNK cells and macrophages) - decreased.
TNF- α	EVT invasion – inhibitory.	Decidual stromal levels – increased.