Review

Decidualization of the human endometrial stromal cell: an enigmatic transformation

Carolyn Dunn graduated from Sheffield University in 2000 with a BSc Honours degree in Biomedical Science. With an interest in human reproduction and fertility she started her PhD at the MRC Human Reproductive Sciences Unit in Edinburgh, studying the changes in cytokine release associated with human decidualization. This work has revealed that the endometrial stromal cell will release IL-15, a stimulator of uterine proliferation of natural killer cells, upon cAMP-mediated decidualisation. This finding reveals an important interaction between the human decidual cell and uterine natural killer cells.

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Abstract

Changes in human endometrium are essential to allow the establishment of pregnancy. These changes are induced *in vivo* by progesterone, and include appearance within the tissue of a specific uterine natural killer cell, characterized by an abundant expression of CD56. Changes also occur in the stromal cells, which undergo a characteristic decidualization reaction. Decidualized stromal cells are derived from the fibroblast-like cells within the endometrium, which maintain their progesterone receptors in the presence of progesterone. Prolonged exposure to progesterone induces a rounded cell characterized by release of prolactin and insulin-like growth factor binding protein-1 (IGFBP-1), and expression of tissue factor. Additional changes include the secretion of interleukin (IL)-15, vascular endothelial growth factor, and surface expression of zinc dependent metalloproteinases such as CD10 and CD13. *In vitro,* elevated intracellular cAMP as well as progesterone is necessary for decidualization. *In vivo*, these conditions may be provided by progesterone from the corpus luteum, by prostaglandin E, a stimulator of adenyl cyclase, and relaxin, which has recently been shown to be a phosphodiesterase inhibitor. Given the co-distribution of uterine natural killer cells and decidualized stromal cells, a mutual interaction might provide the correct regulatory environment for successful implantation, and penetration of the maternal blood vessels by trophoblastic cells.

Keywords: *cytokines, decidua, prolactin, prostaglandin, uterine NK cell*

Introduction

Decidualization of the endometrium is essential for successful implantation and pregnancy. This change has to be synchronized accurately to prepare for blastocyst arrival, and mainly involves the action of progesterone on oestrogen primed fibroblast-like precursor cells in the stroma. The resulting cell, the decidualized stromal cell (DSC) is critical to the development of surrounding trophoblastic, haematopoietic [e.g. uterine natural killer (uNK) cells] and mesenchymal cells (e.g. blood vessels). Considerable data have accumulated on the properties of the DSC (Brar, 2002), the majority relating to cytokines and mediators that are otherwise found in the immune system.

The decidualization reaction is initiated around the blood vessels and spreads throughout the stroma of the late luteal phase and pregnant endometrium (Bell, 1990). Because of this association with blood vessels, it is likely that decidual cells also play a key role in gating leukocyte entry and haemostasis.

This review will cover the origin and characterization of the DSC, its interaction with the leukocyte components of the stromal compartment and its participation in angiogenesis, vascular permeability and blood loss.

Cellular composition of the decidual stroma

Apart from the decidual stromal cell, the endometrium hosts a dynamic population of haematopoietic cells that play a role in both implantation, and in the absence of pregnancy, menstruation. The major leukocyte sub-types in the non-pregnant stroma are macrophages and lymphocytes. In the

latter part of the secretory phase of the cycle, macrophages and the phenotypically unique population of uterine natural killer (uNK) cells increase in number. There is a further increase in numbers of the latter cell type during the early stage of pregnancy, which in fact account for 70% of bone-marrow derived cells in the decidua of the first trimester of pregnancy. The precise role for this cell type in the endometrium is unclear, but their localization close to invading trophoblast and their surface contact with decidualized stromal cells (DSC) suggests an important interdependence (King, 2000).

Despite large numbers of uNK and monocytes, there are very few T cells and B cells (Loke and King, 1995).

Monocytes

Although the monocyte/macrophage is the second largest component of the decidual leukocyte population (25%), little is known about its phenotype. Given the data appearing on alternative differentiation of monocytes (Mantovani *et al.*, 2002), the inadequate characterization of the phenotype of the decidual monocyte/macrophage is a limiting factor in determining the function of these cells. The concentration of monocytes increases during pregnancy, and a particular influx is observed between 7 and 20 weeks of gestation (Smarason *et al.*, 1986). A high concentration of monocytes is seen around the implantation site (Kabawat *et al.*, 1985) and also in the vicinity of trophoblast cells (Bulmer *et al.*, 1988).

As in other situations, the monocyte is likely to be a source of growth factors and cytokines (Hunt, 1989), and these are likely to have autocrine as well as paracrine effects (Hunt, 1992). The decidual monocyte is certainly a major source of cytokines such as tumour necrosis factor (TNF)α (Casey *et al.*, 1989), growth factors such as colony stimulating factor (CSF)-1 (Daiter *et al.*, 1992) and prostaglandin E (PGE, Tawfik *et al.*, 1986; Lala *et al.*, 1988), an essential mediator of cAMP elevation in endometrium.

Advances in our knowledge of the decidual macrophage have been restricted by differences between human and mouse or rat decidua. In the mouse, macrophages are virtually absent from the decidua basalis but do occur in the metrial gland. However, knockout experiments have shown that CSF-1 is essential for normal macrophage differentiation in the mouse uterus (Hunt and Pollard, 1992), although in the absence of CSF-1 there is compensation by another growth factor, possible granulocyte monocyte colony stimulating factor (GMCSF) (Hunt *et al.*, 1992).

The uterine NK cell

Uterine NK cells are present within the human endometrium across the cycle, but rise in number around the time of implantation and remain high during the first trimester of pregnancy. They are characterized by their $CD56^{Bright}$ and $CD16^{-}$ nature (King and Loke, 1991). In contrast to the abundance of $CD56^{Bright}$ subset of NK cells in decidua, only 10% of these cells are seen in the NK fraction of peripheral blood and these cells are both smaller than their decidual counterparts and express less CD56 (Loke and King, 1991). Although the CD56 cells are in the minority in peripheral blood, their cytokine secretion pattern is very different and they can be a significant source of type-2 cytokines such as interleukin (IL)-10 (Cooper *et al.*, 2001).

The function of uNK cells has yet to be fully elucidated, although a role for uNK cells in maintaining the decidual reaction in stromal fibroblasts has been suggested by Croy *et al.* (2002).

The uNK cells do not express the genomic progesterone receptor, or the classic oestrogen receptor, ER alpha but recent data (Henderson *et al.*, 2003) describe the expression of the ER isoform, ER beta and also the glucocorticoid receptor (GR). The absence of P receptor in the uNK signifies a prominent role for the DSC in signalling responses to progesterone.

The uNK cell appears to be absent in ectopic (tubal) implantation sites (Vassiliadou and Bulmer, 1998; Marx *et al.*, 1999), although they appear in the uterine decidua in these cases. This suggests that the uNk cells may not be essential for implantation, but given the poor outcome of these pregnancies, they may have an important role in limiting trophoblast invasion.

Origin of the decidual cell

The origin of the decidual cell must still be regarded as controversial because the precursor of these cells may have developed from stem cells migrating from the bone marrow. The evidence for this is mainly confined to the rodent, and may therefore have limited relevance to humans. Chimera experiments demonstrated such an origin of the precursor cell (Kearns and Lala, 1982) by transplanting donor bone marrow to a lethally irradiated mouse. The characteristic allele of the donor bone marrow was detected in uterine decidual cells to the same extent as seen in splenic lymphocytes (Kearns and Lala, 1982).

However, two papers followed showing that in both decidual and deciduomal (induced decidualization in pseudo pregnancy) tissue the percentage of bone marrow-derived cells was small (Fowlis and Ansell, 1985; Gambel *et al.*, 1985).

In a later study, a transgenic marker was introduced into the embryonic yolk sack prenatally and the females from the litter were mated and killed at day 12 of gestation. The marker was detected by in-situ hybridization and found in decidual cells as well as macrophages and metrial gland cells (Lysiak and Lala, 1992). There is thus some dispute about the origin of these cells, but given that the precursor stromal cells possess the nuclear progesterone receptor, a feature not seen in any other cell of haematopoietic origin, a bone marrow origin of the decidual stromal cell would beg the question, how did it acquire this steroid receptor?

The cells within endometrium which decidualized are clearly fibroblast-like, and grown in culture; these are the cells that flourish to the exclusion of other cell types. However, fibroblast morphology is found in several precursor cells, in particular, recent studies on de-differentiation and re-differentiation of peripheral blood monocytes reveal a fibroblast-like intermediate in such a process (Zhao *et al.*, 2003). In those experiments, 'fibroblasts' were pluripotent stem cells that could be differentiated to epithelial, endothelial

and other cell types. The decidual precursor cells are defined by the presence of the progesterone receptor and the plain description 'fibroblast' would be misleading, and thus in this review the widely used term endometrial stromal cell (ESC) is used to denote these cells.

In vitro, the decidual transformation of the endometrial stromal cell is accomplished with progesterone, but only after prolonged culture. Alternatively, cAMP will sensitize the stromal cell to progesterone (Brosens *et al*., 1999), allowing more rapid development of the decidual phenotype (**Figure 1**). Whether in-vitro differentiation is as complete as in-vivo changes is a matter of debate (King, 2000), but it seems likely that full differentiation *in vitro* is possible with prolonged progesterone treatment, and the resulting cell has distinct processes resembling those on other dendritic cells (Montes *et al*., 1996). Prolonged culture of ESC with cAMP and progestin can induce obvious dendritic morphology (**Figure 2**). This characteristic morphology together with expression of cell surface markers CD10, CD13 and DRC-1 (the long form of CD21) (Imai *et al*., 1992; Montes *et al*., 1996) has led to the suggestion that the decidual cell closely resembles the follicular dendritic cell (FDC), a stationary cell, like the decidual cell, found in germinal centres. The main role of the FDC is to retain antigen and present it to B cells, and no parallel function can readily be seen in decidua. However, an equally important role of the FDC is to support proliferation of B cells and down-regulate their apoptotic machinery (Li and Choi, 2002). This suggests a possible similar interaction between the DSC and the uNK, with the DSC having an influence on the uNK cell, directing it towards apoptosis or survival.

The uNK and decidual cell have a close interaction with cell-to-cell contact (King, 2000) and the DSC acts synergistically with IL-15 in stimulating growth of decidual NK cells (Verma *et al*., 2000). The IL-15 in turn can originate from the DSC (Dunn *et al*., 2002), possibly under the influence of PGE, which is derived from the decidual monocytes, representing 20% of the decidual leukocytes. The close apposition of the DSC and the decidual NK cell would enhance the interaction.

Another similar feature of the DSC and the FDC is that they both occur ectopically (Massi *et al*., 1995; van Nierop and de Groot, 2002). In the case of ectopic decidual cells, they only occur in reproductive tissue and are always associated with $CD56^{Bright}$ cells (King, 2000). This ectopic occurrence is consistent with the cells differentiating from a fibroblast-like presursor cell, although there is a real possibility that ectopic decidua arise from endometriotic areas. The progesterone receptor is obligate in the differentiation of the decidual cell, and thus appearance of isolated decidual foci is restricted to reproductive tissue or dislodged reproductive tissue such as endometriotic areas (Massi *et al*., 1995).

A dendritic morphology for the DSC is controversial, which may be because filamentous processes are difficult to see with immunohistochemistry. However, prolonged, in-vitro culture with progesterone and cAMP gives a population with clear dendritic morphology (Montes *et al*., 1996) (**Figure 2**). Clearly, the dendritic nature of the ESC would facilitate interactions with other cell types, and dendritic morphology points to such a function. Other, CD83-positive, dendritic decidual cells have been reported (Kammerer *et al*., 2000);

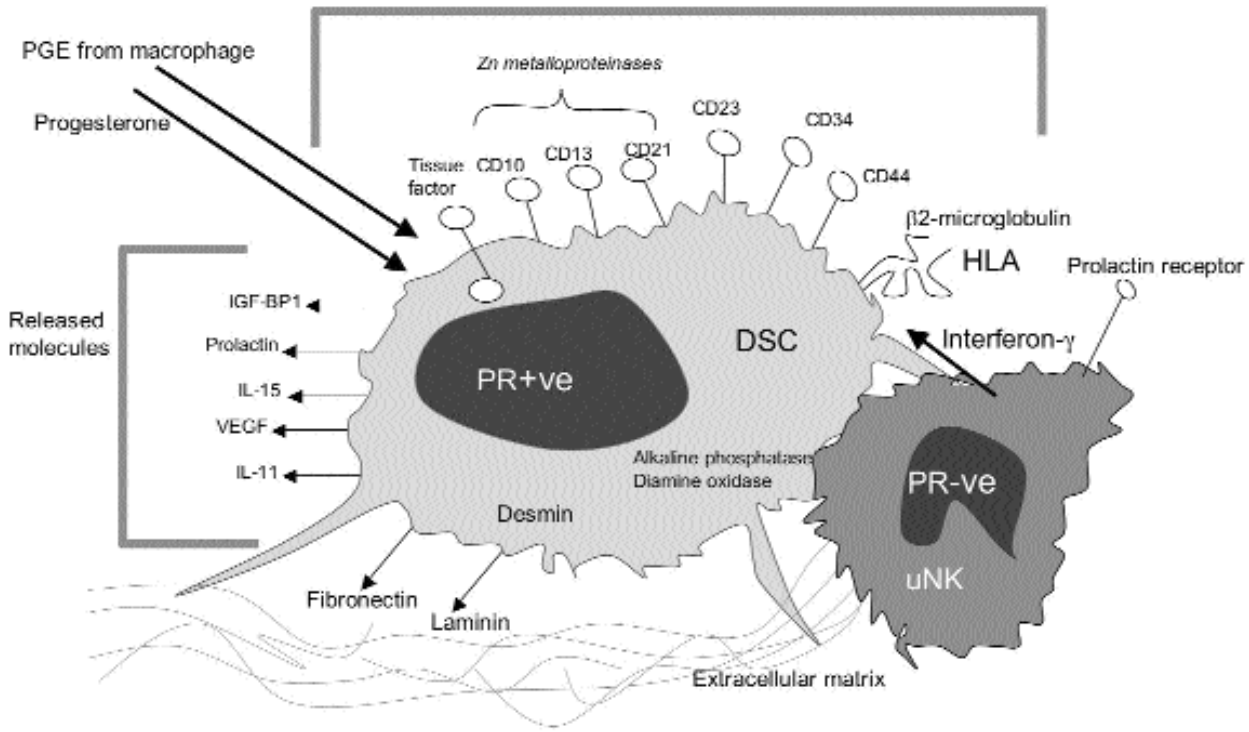

Figure 1. The human decidual stromal cell characteristically expresses and releases a distinctive set of markers and cytokines. The DSC (decidual stromal cell) is progesterone receptor positive, whereas the major leukocyte of the decidua, the uterine NK cell (uNK) is progesterone receptor negative.

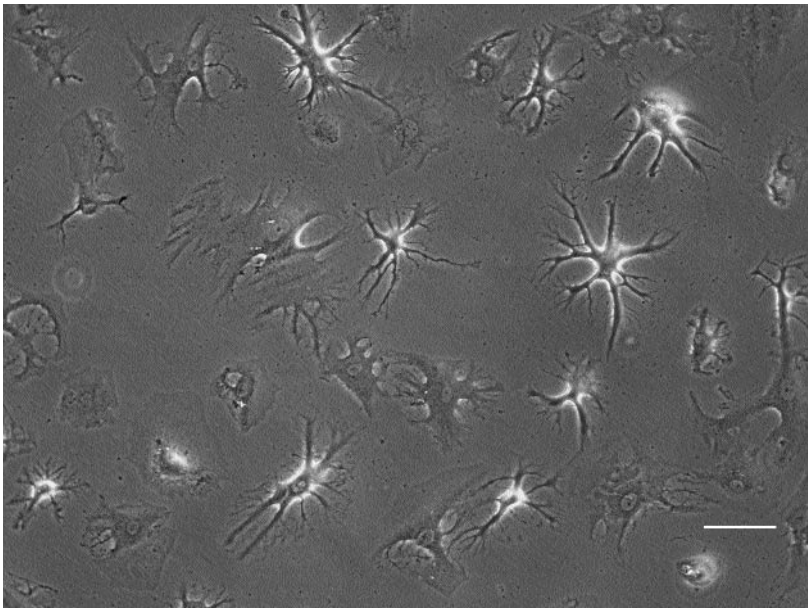

Figure 2. Human endometrial stromal cells cultured for extended periods (>14 days) in the presence of cAMP elevating agents. These cells are fully dendritic, although they do not express markers of the conventional antigen-presenting dendritic cell such as CD1a. Scale bar = 100 μm.

these are likely to be conventional antigen presenting cells, since they are CD45-positive and are associated with CD3-positive T cells.

Decidualization stimuli

Cyclic adenosine monophosphate (cAMP)

Progesterone is essential for decidualization but there is substantial evidence, largely based on in-vitro data, that intracellular concentrations of cAMP are critical. Evidence for the involvement of cAMP and its analogues in the decidualization of human endometrium has been proposed on the basis of in-vitro experiments (Tang *et al*., 1993 ; Brosens *et al*., 1996, 1999; Brar *et al*., 1997). This evidence is reinforced by findings that one initiator of decidualization (relaxin) is primarily a phosphodiesterase inhibitor (Bartsch *et al*., 2001). Intracellular second messengers allow both the transmission and amplification of signals within cells. Cyclic AMP is such a messenger, and is produced from ATP when adenylyl cyclase is activated. Activators of adenylyl cyclase result in increased cAMP concentrations, and pharmacological stimulus is provided by agents such as forskolin. Phosphodiesterases act to break down cAMP and thus counteract the effects of this second messenger. Many phosphodiesterases are known, but type IV enzymes appear to be the most important. The type IV phosphodiesterases can be specifically inhibited by compounds such as Rolipram, as well as non-specifically with agents such as theophylline. Within the endometrium, PGE_2 is an activator of adenylyl cyclase when acting through EP2 or EP4 receptors (Frank *et al*., 1994) and would therefore synergize with any natural phosphodiesterase inhibitor such as relaxin. Relaxin is released by the endometrium and the ovary at the time of decidualization (Yki-Jarvinen *et al*., 1983). The potential for PGE production is high in the secretory phase of the menstrual cycle (Maathuis and Kelly, 1978) and in first trimester decidua (Parhar *et al*., 1989), although in both situations effective local concentrations might be attenuated by progesterone-dependent PGE catabolism by prostaglandin dehydrogenase (Casey *et al*., 1980; Cheng *et al*., 1993). Arachidonic acid, the essential fatty acid from which prostaglandins are synthesized, has also been implicated in decidualization *in vitro* (Tessier-Prigent *et al*., 1999).

Prostaglandin E_2 (PGE_2)

Effective concentrations of prostaglandin within tissues are governed by both synthesis and breakdown (**Figure 3**). Phospholipase A2 and cyclooxgenase, which exists in the two isoforms COX-1 and COX-2, are the rate-limiting enzymes in PG synthesis. Catabolism and inactivation of prostaglandin is governed by prostaglandin dehydrogenase, an NAD-dependent enzyme, which in reproductive tissue is induced by progesterone (Casey *et al*., 1980; Greenland *et al*., 2000). Prostaglandins themselves have an array of roles within female reproduction. COX-1 and -2 are encoded by different genes but are both membrane-bound proteins with similar structures. COX-1 is constitutively expressed in most tissues, whereas COX-2 expression is inducible.

Animal experiments have shown that COX-2 is induced at the time of blastocyst implantation (Chakraborty *et al*., 1996; Lim *et al*., 1997) Targeted disruption of COX-2 in mice leads to problems with the key processes of ovulation, fertilization, implantation and decidualization (Lim *et al*., 1997). Decidualization can be restored in part by the prostacyclin analog carbaprostacyclin. Such inadequate decidualization poses knock-on problems for both implantation and placentation. Experiments to decipher which pathways are involved have shown that p38, but not ERK, activation is required for induced COX-2 and PPARdelta expression during decidualization (Scherle *et al*., 2000). Knockout mice for COX-1(–/–) on pregnancy day 4 demonstrate reductions in vascular permeability and PG concentration (Reese *at al*., 1999). However, these reductions were less than those predicted and further investigation revealed a compensatory response by COX-2 demonstrating interaction between the two enzymes.

In the normal menstrual cycle, cyclo-oxygenase expression within the endometrium has been located to both stromal and glandular compartments, with the highest concentration being seen in the latter (Lumsden *et al*., 1984). The intensity of COX-2 immuno-staining is elevated premenstrually (Jones *et al*., 1997), but potential for prostaglandin production is highest in the secretory phase (Downie *et al*., 1974; Maathuis and Kelly, 1978). This suggests COX-2 is involved in both the process of menstruation and in implantation.

Both COX-1 and COX-2 appear to be under hormonal control in the peri-implantation phase. Mid-luteal biopsies taken from women who had received the antiprogestin mifepristone 4–6 days earlier, showed that COX-1 and COX-2 expression declined in the glandular epithelium and luminal epithelium respectively, whilst no reduction in COX-2 perivascular staining occurred (Marions *et al*., 1999). Where mifepristone was administered orally on day LH + 8 and endometrial tissue obtained 36 h later, an increase in COX-2 in the glands and a general decrease in the catabolizing enzyme PGDH was observed (Hapangama *et al*., 2002). The distinct expression patterns for COX-1 and 2 within the uterus imply independent contributions to uterine PG production. Studies in decidua show that a major control of prostaglandin concentrations at this site may be due to PGDH (Cheng *et al*., 1993a), and antiprogestin administration in early pregnancy shows enhanced PGE expression especially in the four or five layers of cells surrounding the blood vessels (Cheng *et al*., 1993b).

A variety of PG synthesis inhibitors have been shown to reduce the implantation site number in mice and it has been suggested that PGs have effects on both mother and fetus (Biggers *et al*., 1981).

Relaxin

Relaxin is found in several human reproductive tissues, including the ovary, prostate and endometrium (Bryant-Greenwood and Schwabe, 1994). In endometrium, it is associated with the glandular and surface epithelium in proliferative and secretory phases, but also associated with the decidualized stromal cell in the late secretory phase and in early decidua.

Systemic administration of relaxin in mice results in laminin expression, another marker of decidualization, in ESC, further compounding a role for cAMP in the decidualization process (**Figure 3**) (Bani *et al*., 1995).

Relaxin is associated with an up-regulation of collagenase in fibroblasts (Unemori and Amento, 1990) and therefore may affect extracellular matrix rearrangements. Early studies showed that porcine relaxin increased cAMP concentrations in uterine epithelial cells (Chen *et al*., 1988) and this effect has now been explained by the demonstration that the relaxin receptor is coupled to an inhibition of the catabolic enzyme for cAMP, phosphodiesterase (Bartsch *et al*., 2001).

Secreted agents that characterize the decidual stromal cell

Prolactin

Prolactin is involved in growth and secretion, and it is particularly important in lactogenesis in mammals, in the physiology of the equivalent crop-sac of species such as the pigeon, and as a negative regulator of reproduction in seasonal

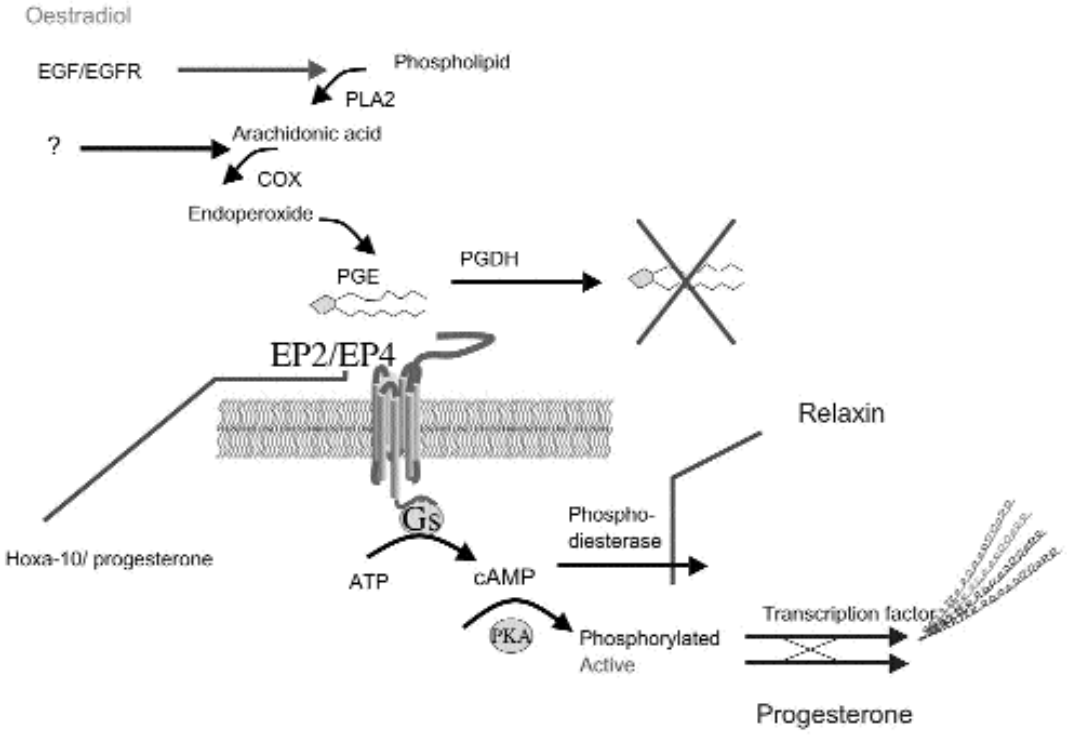

Figure 3. The mechanisms that are involved in the control of intracellular cAMP in the endometrial stromal cell. These mediators are all naturally occurring and combinations of relaxin and PGE will stimulate intracellular cAMP.

breeders. However, as a messenger between leukocytes, its function is enigmatic. Apart from its synthesis in reproductive tissue, it is produced by several leukocytes but particularly by activated T cells and thymocytes. Prolactin receptors are expressed in a wide range of leukocytes, including T cells, B cells, monocytes, NK cells and neutrophils (Matera *et al*., 1988; Draca, 1995). Prolactin action is particularly associated with the cytotoxic activity of the NK cell, and hyperprolactinaemia is associated with a decrease in NK-cell function (Vidaller *et al*., 1992). Interaction with steroid hormones is implicated by studies showing that the oestradiol/prolactin ratio was critical to NK-cell cytotoxicity in endometriotic lesions (Provinciali *et al*., 1995).

Progesterone treatment alone *in vitro* proved to be a weak inducer of the decidualized phenotype and prolactin production. High intracellular cAMP concentrations appear to be required to sensitize the cells to the actions of progestins providing maximal PRL expression; however, this did not appear to involve the cAMP response element (CRE) present in the prolactin gene (Telgmann *et al*., 1997).

Surprisingly, the prolactin receptor knockout mouse has no detectable immune deviation (Bouchard *et al*., 1999), suggesting that the prolactin signalling system in leukocytes can be rescued by other mediators. Despite the production of prolactin by the DSC and the demonstration of prolactin receptors in the uNK cell (Gubbay *et al*., 2002), decidualization and implantation are possible in the receptor knockout mouse but only after supplementation with progesterone to overcome the failure of corpus luteum steroid production (Reese *et al*., 2000). Thus despite its abundant release in decidua, its action in this tissue may be synergistic with other decidual secretions or with steroid hormones.

Insulin growth factor binding protein-1 (IGFBP-1)

Early studies identified placental protein 12 (PP12) as a secreted product of the secretory endometrium and early decidua (Bell, 1988) PP12 has now been identified as IGFBP-1, and concentrations in amniotic fluid peak in the second trimester but stay high in serum after reaching peak concentrations around week 15 of gestation (Bell, 1988). IGFBP-1 is found prominently in both liver and endometrium but is controlled in these two sites by different promoters. Concentrations are particularly high in decidualized stromal cells and IGFBP-1 is one of the best markers of decidualization. The exact function of this protein is uncertain, but it may be present to modulate the potent growth induction by IGF-1 an agent known to be up-regulated by oestrogen (Murphy *et al*., 1987).

The promoter for IGFBP-1 is stimulated by progesterone receptor (Gao and Tseng, 1994), but there are also multiple Sp-1 elements in the decidual promoter (Tseng *et al*., 1997). A novel form of SP-3 also exists in decidua (Gao and Tseng, 1996) and SP-3 inhibits IGFBP-1 expression. Add to this, the activation of IGFBP-1 transcription by HoxA10 in ESC but not decidual cells and the specific activation of the promoter by HoxB4 in deciduas (Gao *et al*., 2002), and it becomes clear that there is a complex and inter-related control of IGFBP-1 expression.

Tissue factor/plasminogen activator inhibitor (PAI-1)

Tissue factor (TF) is a membrane-bound inhibitor of bleeding that is seen on DSC and is likely to be important at the time the trophoblast cells invade the maternal arteries. Since the decidual cells surround the arteries they are in a good position to effect this control. In other cells, TF is found on the internal leaf of the plasma membrane and flips to the surface after membrane disturbance. This event is accompanied by increased TF mRNA expression and is accompanied by surface presentation of the better-known marker of the inner leaf – phosphatidyl serine. In view of this, it would be of interest to know the orientation of TF on the DSC membrane.

TF is stimulated in endometrial stromal cells by a combination of progesterone and ligands of the EGF receptor. TF has been demonstrated to show enhanced protein and mRNA expression in DSC of the luteal phase as well as decidua (Lockwood *et al*., 1997). Since decidualization is accompanied by an increase in EGF receptor, EGF (e.g. from glands) might play an additional role in decidualization. One such pathway would be the stimulation of cytosolic phospholipase A2 (cPLA2) by EGF, which in turn would increase prostaglandin production (**Figure 3**), as seen in other systems (Schalkwijk *et al*., 1995; Yang *et al*., 2001; Buresi *et al*., 2002). The *TF* gene is progesterone dependent (Krikun and Lockwood, 2002), but there are also multiple SP-1 sites in the promoter and activation by SP-1 has been demonstrated (Krikun *et al*., 2000).

Another progesterone dependent protein characteristic of decidualized cells is PAI-1, an agent that inhibits the fibrinolytic pathway and thus may play a role in modulating cell movement in decidua. This gene has no progesterone response element but also has SP-1 response elements in its promoter. Thus PAI-1 is indirectly controlled by progesterone and such control is likely exerted through the SP-1 system (Krikun and Lockwood, 2002).

The role of TF as a limiting factor in bleeding may be simplistic, as recent reports have suggested that TF can also be involved in angiogenesis (Abe *et al*., 1999; Rickles *et al*., 2001), in particular, the cytoplasmic tail of TF plays a role in inducing the angiogenic factor vascular endothelial growth factor (VEGF) (Abe *et al*., 1999).

Cytokines

IL-11

The human endometrium produces many cytokines, some of which are important in the induction and maintenance of the DSC, Three cytokines (leukaemia inhibitory factor [LIF], IL-6 and IL-11) which all use gp130 for signal transduction appear to play critical roles at implantation but only IL-11 is directly associated with the function of the DSC. IL-11, a 23 kDa non-glycosylated protein with no cysteines, is produced by decidualized stromal cells, glandular epithelial cells and by endothelial cells within the human uterus where concentrations are greatest in the secretory phase (Dimitriadis *et al*., 2000). In mice, it has been shown to be essential for decidualization, as shown by studies with knockout mice for

the IL-11 receptor alpha (Bilinski *et al*., 1998; Robb *et al*., 1998). Using human ESC cultures, IL-11 has been identified as being both a product of these cells during decidualization and also a key factor in promoting the decidual transformation of these cells (Dimitriadis *et al*., 2002). They showed that neutralization of endogenous IL-11 via addition of an anti-human IL-11 antibody reduced prolactin and IGFBP-1 expression by these cells, both markers of decidualization. Links with LIF and prolactin in producing this decidual transformation have also been implied (Dimitriadis *et al*., 2002).

IL-1

The decidualization of ESC may need to be modulated and for this to be achieved, there must be cytokines present that are capable of limiting the decidual process. One such cytokine is IL-1, which has been shown to prevent prolactin production and decidual-associated morphological changes (Kariya *et al*., 1991). Specifically IL-1 beta inhibits in-vitro decidualization of ESC (Frank *et al*., 1995). Notwithstanding this inhibitory effect, appropriate expression of IL-1 may be essential for implantation, since administration of the receptor antagonist blocks implantation in a mouse model (Simón *et al*., 1994). The role of IL-1 in the epithelial layer is further emphasized by studies of IL-1 receptor antagonist (IL-1ra) in human endometrium (Simón *et al*., 1995). The predominant (intracellular) form of the antagonist fades from the glands in the secretory phase of the cycle, thus allowing a potential role for IL-1 in the implantation window (Simón *et al*., 1995).

Tanaka *et al*. (2000) demonstrated that the inhibition by IL-1 beta in ESC can be antagonized by IL-1ra administration. This fits with the in-vivo situation, since IL-1ra concentrations are 10- to 30-fold greater than those of IL-1 beta. In addition, IL-1ra is produced by first trimester trophoblast (Kelly *et al*., 1995). Although IL-1 inhibits decidualization induced by cAMP, in the absence of cAMP, IL-1 can induce decidualization, possibly by inducing PGE synthesis (Strakova *et al*., 2000, 2002).

IL-15

The cytokine IL-2 is an effective stimulator of uterine $CD56^{Bright}$ NK cell proliferation (Ferry *et al*., 1990; King *et al*., 1992). The cytokines IL-2 and IL-15 share common (β and γ) receptor elements and have been demonstrated to stimulate the proliferation of uNK cells *in vitro* (Verma *et al*., 2000). Moreover, a specific role for IL-15 in NK cell development is seen in the IL-15 γ-receptor knockout mouse, which is devoid of NK cells (Miyazaki *et al*., 2002). IL-2 is absent from human endometrium, whereas IL-15 is present in both the stroma and the glands (Kitaya *et al*., 2000) and is therefore a prime candidate for stimulating uNK cell proliferation *in vivo*. It remains a contentious issue as to whether uNK cells actually proliferate within the endometrium itself or whether they proliferate before they infiltrate the stroma. The topic has been discussed in Pollack and Linnemeyer (1996) in the context of the murine situation.

Using in-vitro culture systems of human ESC, it has been possible to show that production of IL-15 by these cells increases under the influence of decidualizing stimuli (Dunn *et al*., 2002). It could therefore be proposed that DSCs secrete IL-15, which maintains the uNK cell population via stimulating proliferation. In turn, the uNK cells may secrete factors beneficial to DSCs. Furthermore, decidualized ESC are a source of prolactin and uNK cells have been shown to be a novel target for this protein (Gubbay *et al*., 2002), thus offering further support to this proposed cell–cell interaction.

Cell surface expression and adhesion molecules

The DSC interacts with the other major cell types in the decidua, the endothelial, epithelial and leukocyte population as well as the trophoblast. Such interactions will involve cell–cell contact. In-vitro determination of surface antigens can be affected by cell culture conditions, for example, CD34, a marker of haematopoietic precursor cells, is not seen on the surface of DSC grown in the presence of high concentrations of fetal calf serum (FCS; Garcia-Pacheco *et al*., 2001). When grown with low FCS, decidual cell express at least two cell-surface endopeptidases (CD10, CD13) (**Figure 1**), two markers normally associated with B cell function (CD21, CD23), and a marker of an immature haematopoietic cell, (CD34). Two costimulatory molecules (CD80 and CD86) are also expressed to some degree (CD86 is expressed in 17–44% of cells and CD80 in 10–13%) (Garcia-Pacheco *et al*., 2001). HLA class I (Komatsu *et al*., 1998) and HLA-DR but not CD14 are seen (Garcia-Pacheco *et al*., 2001).

At the time of decidualization, considerable changes occur in the structure and function of extracellular matrix in the endometrium. These changes provide anchorage for cells, but in addition, the new matrix can affect macrophage phenotype (McKay *et al*., 1992). The specific composition of the matrix may play a part in assisting trophoblast migration (Loke *et al*., 1989). Loke has suggested that the distribution of laminin, which is localized close to the decidual cell and therefore absent in myometrium, is a controlling factor in trophoblast invasion. Fibronectin also remains localized around the decidual cell (Aplin *et al*., 1988) and since it contains the arginine–glycine–aspartic acid (RGD) motif, it may play an anchoring role. One of the main markers of decidualization, IGFBP-1, also has an RGD sequence, but the significance of this is not clear. CD44, which binds hyaluronic acid, is strongly expressed on decidualized stromal cells (Behzad *et al*., 1994) and may play a role in leukocyte adhesion. However, this molecule might also account for the oedema seen at the implantation site (Okada *et al*., 2001), since hyaluronic acid bound to surface-expressed CD44 will attract water and thus cause swelling.

The increase in matrix synthesis in the decidualizing cell is amplified because it is accompanied by a progesterone controlled decrease in lytic enzymes such as matrix metalloproteinase (MMP)-1 and MMP-3, as well as a fall in the activity of the 55 kDa urokinase-type plasminogen activator (uPA) and the 67 kDa tissue-type PA (tPA) due to increased PAI-1 concentrations (Schatz *et al*., 1995).

One additional cellular interaction of the DSC is through expression of MHC class I (Komatsu *et al*., 1998). The expression of these molecules increases upon decidualization and they are likely to protect the DSC against the uNK in the classical manner (King, 2000). Gk beta microglobulin, which

is the invariant (β) chain of the HLA class I complex (and is essential for class 1 expression), is also induced upon decidualization (Komatsu *et al*., 1998). The likely receptor for HLA class-I on the uNK is CD94/NKG2A, which is present in higher amounts on the uterine NK cell than on the peripheral NK cells (King, 2000; Loke and King, 2000). This interaction of the uNK with class I molecules will give the NK cell a do-not-kill message, which will ensure the survival of DSC in close contact with the uNK cell.

Angiogenic or permeability signals

Changes in vascular permeability that accompany decidualization will promote leukocyte invasion. Excessive neutrophil entry into the uterus is restricted by progesterone (Staples *et al*., 1983) and progesterone antagonism *in vivo* results in PGE appearance in the cells surrounding the blood vessels (Cheng *et al*., 1993a,b) and accompanying increase in chemotactic agents (Critchley *et al*., 1996, 1999) and leukocyte numbers (Critchley *et al*., 1996, 1999). Since progesterone receptors in stroma are confined to the non-leukocyte population, these DSC exercise an important control on leukocyte invasion.

The local population of uterine NK cells has been reported to express angiogenic growth factors, including vascular endothelial growth factor (VEGF)-C and placenta growth factor mRNA in the secretory phase and angiopoietin-2 mRNA in the late secretory phase (Li *et al*., 2001). Since IL-2 and IL-15 can up-regulate VEGF-C in isolated uNK cells, these are lines of evidence to implicate a role normal and abnormal uterine angiogenesis and regeneration.

VEGF is also a permeability factor and is responsible for increased permeability of the endometrial epithelium (Hastings *et al*., 2003). It is likely (though not proven) that VEGF, which can be induced by PGE (Ben-Av *et al*., 1995; Cheng *et al*., 1996), plays a role in increased permeability at the implantation site in mammals.

Notwithstanding the uNK cell contribution of VEGF, the DSC itself can synthesize VEGF (Sugino *et al*., 2002) and the factors controlling VEGF in decidua must be the subject of further research.

The role of decidualization in vascular remodelling has been highlighted by the finding that uterine vascular changes occur in an ectopic pregnancy (Craven *et al*., 1998). However, the extent of any such changes in the uterus at the time of a tubal pregnancy are limited, and do not reflect the extensive change associated with trophoblast invasion (King and Loke, 1997).

Hox10$^{-/-}$ mice exhibit defective vascular development as well as poor decidualization (Benson *et al*., 1996), and both of these may derive from the impaired progesterone–PGE interaction which is present in the uteri of these mice (Lim *et al*., 1997). In particular, despite the normal expression of many progesterone-associated genes, the message for the prostaglandin receptors EP3 and EP4 is both significantly reduced and aberrantly regulated by progesterone.

Conclusions

In the context of cellular secretions and surface markers (if not of morphology), the decidualized stromal cell of the endometrium is a well defined cell with a poorly defined function. Both cell surface markers and secreted proteins point to a modulating action on local leukocytes, among which the uNK cell must be the main candidate, but the exact communication between these cells is uncertain.

Most of the studies of the physiology of the DSC cell have been performed *in vitro* and these have uncovered the role of cAMP in the decidualization process. Many of the agents known to induce decidualization (particularly relaxin and PGE) turn out to be eventual modulators of cAMP.

Many questions remain unanswered: does the decidual cell play a role in menstruation (Bell, 1990)? Does the decidual cell promote the growth and differentiation of the uNK cell? Is the decidual cell the main source of extracellular matrix deposition and what is the role of the DSC in promoting angiogenesis?

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