

## Cytokine control in human endometrium

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Cytokines within endometrium participate in both menstruation and implantation but also contribute to the defence mechanisms of the mucosal epithelium. Endometrium is under the control of steroid hormones, particularly progesterone and, thus, control of cytokines by this steroid is important. Although appreciable numbers of progesterone receptors are not found in endometrial leucocytes, progesterone can modulate cytokines by acting on uterine cells expressing the receptor. The NF $\kappa$ B pathway is important in the control of cytokine synthesis and can modulate production of chemokines, matrix metalloproteinases and the inducible prostaglandin synthesis enzyme COX-2. NF $\kappa$ B activity can be inhibited by progesterone by either stimulating synthesis of I $\kappa$ B, the molecule that restrains NF $\kappa$ B in the cytosol, or after binding to the nuclear receptor, competing with NF $\kappa$ B for recognition sites on the relevant gene. In this way, progesterone can limit pro-inflammatory pathways. The major palliatives for endometrial dysfunctions such as menorrhagia and dysmenorrhoea have been the non-steroidal anti-inflammatory drugs that inhibit prostaglandin synthesis. Prostaglandins have major effects on cytokine production but the direct action of prostaglandin E on leucocytes is not a pro-inflammatory response but is to stimulate interleukin 10 and inhibit interleukin 12 synthesis. The likely effect of the non-steroidal anti-inflammatory drugs is on the cells surrounding the small blood vessels, where a synergistic action between prostaglandin and chemokine will induce leucocyte entry and activation leading to lysis of connective tissue and menstruation. At the time of implantation, tight control of cytokine synthesis is required. Although leukaemia inhibitory factor is essential to implantation, the mouse knockout models show that the prostaglandin system is also essential but that there are mutually supportive pathways that compensate for the knockout of many cytokines.

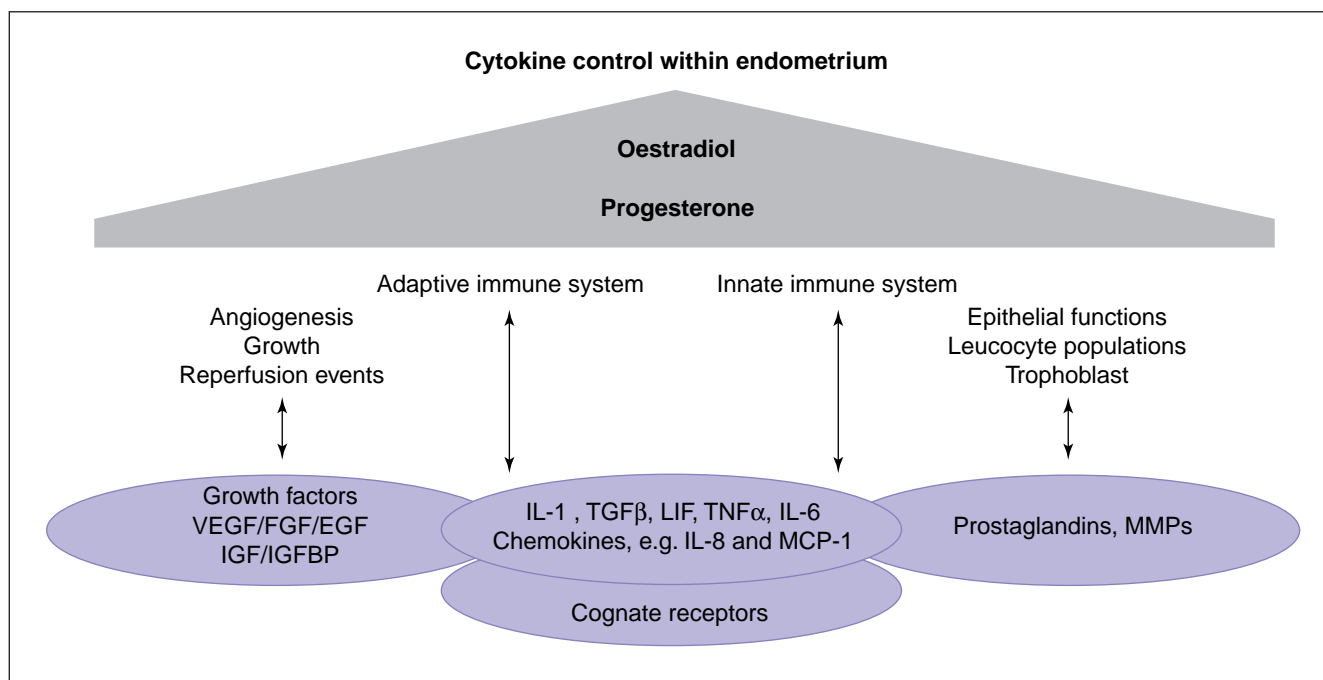
Critical reproductive events in endometrium such as menstruation and implantation have an inflammatory character (Finn, 1986). Menstruation and implantation involve both prostaglandins and cytokines and are accompanied by the ingress of leucocytes into the endometrium. Moreover, oedema is characteristic of endometrium both premenstrually and at the time of implantation. Endometrial physiology relating to these events is still poorly understood and this ignorance hinders better medical approaches to major pathologies of menstruation, such as menorrhagia and dysmenorrhoea, and failure of implantation.

One of the most revealing studies on the mechanism of menstruation was published 60 years ago by Markee (1940) who transplanted endometrium to the anterior chamber of the monkey eye, where events surrounding menstruation could be observed microscopically. The early events of menstruation involve vasoconstriction of the spiral blood vessels followed by a relaxation of the arterioles

and reperfusion of the tissue. Prolonged periods of vasoconstriction that would have induced hypoxia and the inevitable reperfusion injury were also observed. Such periods of vasoconstriction have not been confirmed in women in studies with techniques such as Doppler flow (Gannon *et al.*, 1997) and xenon clearance (Fraser *et al.*, 1987), but such techniques are unlikely to detect highly localized changes. Hypoxia would certainly be a sufficient cause of cytokine release since hypoxia affects the NF $\kappa$ B pathway (Royds *et al.*, 1998) and many genes have hypoxic response elements. Re-exposure to oxygen is likely to be accompanied by an upregulation of cytokine (Karakurum *et al.*, 1994) matrix metalloproteinase (MMP) (Fujimura *et al.*, 1999; Heo *et al.*, 1999) vascular endothelial growth factor (VEGF) (Goldberg and Schneider, 1994; Sharkey *et al.*, 2000) and prostaglandin (Kishimoto *et al.*, 1997) production. However, the events preceding vasoconstriction are less clear. The early events in menstruation are initiated by the withdrawal of the circulating progesterone as a result of the demise of the corpus luteum and are likely to involve cells close to the spiral arterioles where the constriction

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**Fig. 1.** Summary of factors affecting endometrial cytokine release. Overall control (direct or indirect) of cytokine release is exercised by the steroid hormones. The mucosal surface of endometrium can participate in both innate and adaptive immune responses and is involved in protein and fluid secretion into the lumen. In addition, growth of new tissue and accommodation of the trophoblast all involve cytokine release and control. EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; IL, interleukin; LIF, leukaemia inhibitory factor; MCP, monocyte chemotactic protein; MMP, matrix metalloproteinase; TGF, transforming growth factor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

occurs. Consequently, there are two phases: one that is initiated by decreasing progesterone and might in early stages be reversible, and a second that is inevitable after hypoxia and reperfusion. This second phase is effectively progesterone independent and involves leucocytes and epithelial cells as well as stromal cells. Most of the cells participating in this second phase will have no progesterone receptors at this point in the ovarian cycle since epithelial cells will have lost their receptors and uterine leucocytes do not possess nuclear progesterone receptors (King *et al.*, 1996).

This review examines the control of cytokines in endometrium during menstruation and in the event of conception. Both these processes involve exposure to progesterone, which is transient in the normal cycle but prolonged after implantation. Cytokines within human endometrium are controlled either directly by steroids or indirectly through cyclical changes in factors such as growth, secretion, immune defence and, after fertilization, the implanting embryo (Fig. 1). In recognition of the complexities involved, emphasis will be placed on cytokine–steroid, cytokine–prostaglandin and cytokine–cytokine interactions. Several areas such as angiogenesis and its control are reviewed elsewhere (Smith, 1998) and will not be addressed further here.

### Morphological components of human endometrium

The main functions of human endometrium are the provision

of a hormone-defined implantation window (Tabibzadeh *et al.*, 1998), the ability to instigate its own demise in the absence of pregnancy, and the function shared with all other mucosal surfaces of protection against invading pathogens. These different functions involve interactions between diverse constituent cell types and are both influenced by ovarian steroid hormones and controlled in a paracrine fashion within microenvironments (Tabibzadeh, 1995). Human endometrium has a mucosal epithelial surface consisting of surface and glandular epithelium, a heterologous stroma and a characteristic vascular system found only in menstruating species (old-world primates and certain bats (Rasweiler, 1991)). These components interact in the control of cytokine synthesis and release under ultimate hormonal control.

### Stroma

The stroma of human endometrium consists of fibroblasts, some macrophages and T cells but few B cells (Loke and King, 1995). A population of large granular lymphocytes (LGL) appears in the late secretory phase and numbers increase further in early pregnancy. These cells are natural killer (NK) cells, which display abundant surface expression of a specific adhesion molecule CD56 but are CD3<sup>-</sup> and CD16<sup>-</sup> negative. The function of these NK cells is not certain but it is known that they interact with the class 1 human leucocyte antigens (HLA) expressed on

extravillous trophoblast and may limit trophoblast invasion (Loke and King, 1996). In later decidua, the number of LGLs declines and these cells are sparse at term (Loke and King, 1995). Endometrial LGLs produce interferon- $\gamma$  (IFN- $\gamma$ ), particularly when interacting with macrophages or stimulated with interleukin 12 (IL-12) and IL-2 (Marzusch *et al.*, 1997). In addition, IFN- $\gamma$  is secreted by uterine neutrophil-like cells in the stroma (Yeaman *et al.*, 1998) or from lymphoid aggregates in the basalis (the lower one-third of endometrium, which is not shed at menstruation) (Tabibzadeh, 1994). The uterine lymphoid aggregates appear to be structured with T (CD8<sup>+</sup>) and B cells surrounded by a 'halo' of monocytes and macrophages (Yeaman *et al.*, 1997). These structures, which are predominant at mid-cycle and in the secretory phase, may play an important role in implantation and, as a source of IFN- $\gamma$ , may stimulate endometrial stromal cell production of monocyte chemotactic protein (MCP-1), IL-6 and granulocyte colony-stimulating factor and reduce production of IL-8 (Nasu *et al.*, 1998).

At about day 23 of the menstrual cycle, when progesterone concentrations are still high, endometrial stromal cells begin to undergo a transformation to a phenotype characteristic of cells in early decidua. The process commences in cells surrounding the spiral arterioles and gradually spreads through the endometrium (Buckley and Fox, 1989) becoming particularly evident if pregnancy ensues. The decidual cell is rounded, has myofibroblast characteristics (Oliver *et al.*, 1999) and secretes prolactin. Although progesterone is likely to be the initiating agent *in vivo*, a combination of progesterone and agents that raise intracellular cAMP is necessary for effective decidualization *in vitro* (Frank *et al.*, 1994; Tang *et al.*, 1994; Telgmann and Gellersen, 1998; Brosens *et al.*, 1999). Agents such as relaxin (Chen *et al.*, 1988) PGE (Frank *et al.*, 1994) and FSH (Tang *et al.*, 1994) all increase cAMP concentrations in endometrial stromal cells in conjunction with progestins. In human endometrial stromal cells, continuous stimulation of protein kinase A by cAMP is necessary to maintain prolactin production and this is achieved by a reduction in the availability of the negative regulatory subunit R1 $\alpha$  (Telgmann and Gellersen, 1998). The absolute necessity of progesterone has been questioned since women with very low peripheral blood progesterone concentrations can have normal pregnancies (Tang *et al.*, 1994) and decidualization can occur *in vitro* with increased cAMP alone (Telgmann and Gellersen, 1998). In addition, in rodents, progesterone alone is sufficient to induce decidualization (Paria *et al.*, 1999). These findings indicate that, in women, some other, unidentified agent influenced by progesterone and cAMP may be elaborated in secretory phase endometrium that is directly responsible for decidualization and prolactin production. Decidualization can be prevented by IL-1 (Kariya *et al.*, 1991; Frank *et al.*, 1995; Mizuno *et al.*, 1999), which reduces prolactin production as well as preventing differentiation to the decidual phenotype. However, it is not known whether IL-1 interacts with the adenylate cyclase system in endometrial cells.

## Epithelium

The epithelial surfaces of the endometrium have a dual function in both providing for implantation and for defence against infection. The role of the endometrial epithelium in implantation has been reviewed elsewhere (Aplin, 1997) and will not be addressed further here. Defence mechanisms include transepithelial passage of antibodies in the form of IgA (Kelly and Fox, 1979) as part of the adaptive immune response, but depend to a large extent on innate defences. Luminal secretions contain peptides of low molecular weight with antibacterial activity, defensins (Quayle *et al.*, 1998; Valore *et al.*, 1998) and lysosyme (Tauber *et al.*, 1993), as well as compounds such as secretory leucocyte protease inhibitor (SLPI), which have anti-viral, anti-fungal and anti-bacterial activity (Hiemstra *et al.*, 1996; Tomee *et al.*, 1997; Wiedow *et al.*, 1998). SLPI is expressed in human endometrial epithelial cells (King *et al.*, 2000) and may contribute to the luminal defences of the uterus. Other components of the innate immune defences with anti-microbial activity, such as the  $\theta$  defensins, are still being revealed (Tang *et al.*, 1999) and it now appears that defensins can also link to the adaptive immune system by attracting both dendritic and memory T cells (Yang *et al.*, 1999).

Endometrial epithelial cells are the major sources of several vasoactive substances such as prostaglandins (Lumsden *et al.*, 1984) and endothelins (Salamonsen *et al.*, 1999) that have been implicated in menstruation. However, these compounds are found in other mucosal epithelial tissues such as the gut (Egidy *et al.*, 2000) and may have a prime function as modulators of epithelial function, as well as contributing to constriction of the endometrial spiral arterioles.

## Vasculature

Cells surrounding the spiral arterioles are reported to be the origin of the decidualized stromal cells and have been shown to have myo-fibroblast characteristics. These stromal cells replicate during the secretory phase of the cycle (Abberton *et al.*, 1999), retain their progesterone receptors throughout the cycle (Critchley and Healy, 1998) and are likely to have a critical role in vascular control.

Significant health care resources are used to treat excessive blood loss at menstruation (menorrhagia) (Stirrat, 1999). It is important to consider the role cytokine control associated with endometrial blood vessels may play in this complaint. The blood supply to the superficial two-thirds of the endometrium is provided by spiral arterioles, structures found only in menstruating species. These vessels grow with increasing coiling until day 3 after ovulation (Ferenczy *et al.*, 1979). The factors governing new blood vessel growth have been reviewed elsewhere (Smith, 1998) and will not be addressed further here. The rate of proliferation of smooth muscle cells associated with the blood vessels increases after ovulation, and a deficiency in proliferation has been associated with menorrhagia (Abberton *et al.*, 1999). Studies on keratinocyte growth factor (KGF) show that this factor is progesterone dependent and contributes

to myofibroblast cell growth (Koji *et al.*, 1994). Withdrawal of steroid during the late second half of the secretory phase leads to shrinkage of the functionalis and compression of the vessels (Markee, 1940). Several vasoactive agents may be released at this time and, although compounds such as endothelins, which are predominantly synthesized in glandular tissue, may contribute (Campbell and Cameron, 1998), it is hypothesized that a subset of stromal cells plays a role in the initiation of menstruation. Mechanisms that induce the initial vasoconstriction are triggered by decreasing progesterone concentrations and, in the late secretory phase of the cycle, progesterone receptors are found in stromal perivascular cells but not in the epithelial cells of the functionalis layer (Critchley *et al.*, 1994a), placing the stromal cell in a key position. Once change is established, tissue rearrangement, involving epithelial cells as well as stromal cells, commences in an essentially irreversible process. Thus, menstruation can be considered as two seamlessly connected events.

## Two phases of menstruation

### *Phase 1: initiation of vasoconstriction – cytokine changes due to steroid withdrawal*

Menstruation involves sloughing-off of all but the basal third of the endometrium and there is associated extensive tissue destruction. Lytic enzymes such as MMPs, which degrade the extracellular matrix (ECM), and proteases are clearly involved in this process and are likely to be derived from epithelial stromal and recruited leucocytes (Salamonsen and Woolley, 1999). However, menstruation is probably initiated by progesterone withdrawal and, therefore, initial events are likely to be triggered by cells that express progesterone receptors. By the latter half of the secretory phase, progesterone receptors are absent from the glandular and surface epithelium in the superficialis (Critchley *et al.*, 1994a) and have not as yet been identified in leucocytes. It is significant that progesterone receptors are expressed in stromal cells including those surrounding the blood vessels (Perrot-Aplanat *et al.*, 1994; Critchley and Healy, 1998). Although there are important paracrine interactions between stromal and epithelial cells (Tabibzadeh, 1995), a more likely location for early responses to a decrease in progesterone is the grouping of myofibroblast cells surrounding the spiral arterioles. The ECM associated with these perivascular cells will have been stabilized in the presence of progesterone and oestradiol (Lockwood *et al.*, 1998) and is therefore vulnerable to progesterone withdrawal. These perivascular cells are distinguished by a marked expression of both prostaglandins and cytokines (Cheng *et al.*, 1993a,b; Critchley *et al.*, 1994b, 1999; Jones *et al.*, 1997). If prostaglandins are modulating vascular permeability through actions on the perivascular cells, control of prostaglandins by the catabolic enzyme prostaglandin-15-dehydrogenase becomes critical. The activity of this enzyme decreases in the perivascular cells of decidua after progesterone antagonism

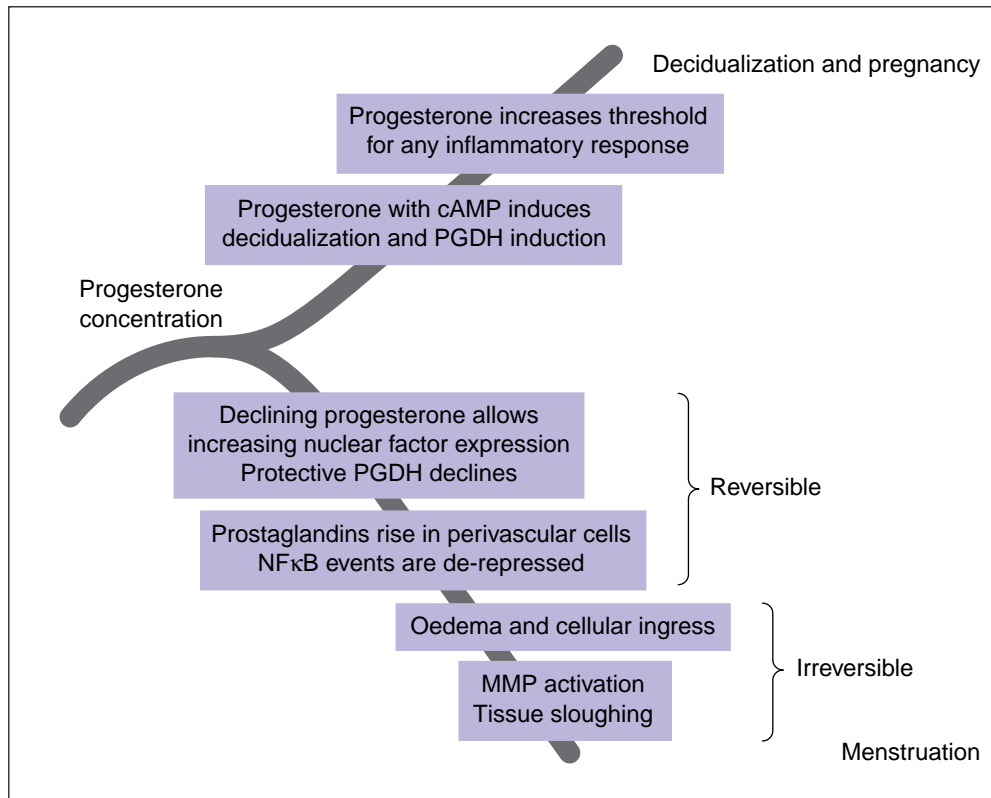
(Cheng *et al.*, 1993b) and prostaglandin dehydrogenase (PGDH) is likely to be maintained in decidual stromal cells by a combination of progesterone and increased intracellular cAMP (Greenland *et al.*, 2000). Since the perivascular cells are thought to proliferate in early pregnancy to form the population of myofibroblast-like, decidualized stromal cells, they are pivotal in their response to progesterone, initiating menstruation in response to decreasing steroid concentrations or, conversely, supporting pregnancy in response to increasing steroid concentrations (Fig. 2).

Perivascular cells in human endometrium may have a distinct mechanism for initiating prostaglandin and cytokine synthesis since they display an abundant CD40 signal on their surface (King *et al.*, in press). CD40 is a member of the tumour necrosis factor (TNF) receptor superfamily, and its ligand is a TNF- $\alpha$ -like protein CD40L or CD154. Although first recognized on B cells, the CD40–CD40L interaction has been shown to stimulate prostaglandin and IL-8 release from fibroblasts (Sempowski *et al.*, 1998; Zhang *et al.*, 1998). CD40–CD40L interactions may result in the activation of the NF $\kappa$ B pathway (Rothe *et al.*, 1995; Liu *et al.*, 1996; Takeuchi *et al.*, 1996) (Fig. 3) or, alternatively, CD40 activation may affect gene transcription through the JAK–STAT (JAK3–STAT-3 and -6) pathway (Hanissian and Geha, 1997). The source of CD40L within endometrium has not yet been identified but possible sources include lymphocyte aggregates or lymphocytes that are attracted into the tissue by chemokines. Control of the CD40–CD40L system in endometrium is unknown, but where the NF $\kappa$ B pathway is involved, an influence of progesterone is expected (Kalkhoven *et al.*, 1996). Thus progesterone withdrawal could activate such a system in a receptor-dependent process.

In the secretory phase of the menstrual cycle, the circulating concentrations of progesterone are of the order of 10–30 pmol l<sup>-1</sup> whereas, in placenta and decidua, concentrations of 20  $\mu$ mol l<sup>-1</sup> have been reported (Challis and Mitchell, 1988). The high concentrations of progesterone within the pregnant uterus are consistent with non-genomic membrane effects of progesterone and these have been implicated in both its immunosuppressive action on T cells (Ehring *et al.*, 1998) and its action on the oxytocin receptor in rats (Grazzini *et al.*, 1998). Several of these progesterone effects can be related to the blockage of potassium channels, which is not receptor-mediated (Ehring *et al.*, 1998). However, in the non-pregnant uterus, the low progesterone concentrations, the cyclical variation of progesterone receptors and the profound changes brought about by the progesterone receptor antagonist RU486 (Cameron *et al.*, 1996) indicate a classical receptor-mediated mechanism.

There are similarities between glucocorticoids and progesterone in structure, receptor sequence and response elements. Moreover, both glucocorticoids and progestins inhibit cytokine expression and the way in which they affect cytokine synthesis has now been recognized as an NF $\kappa$ B-mediated event (McKay and Cidlowski, 1999).



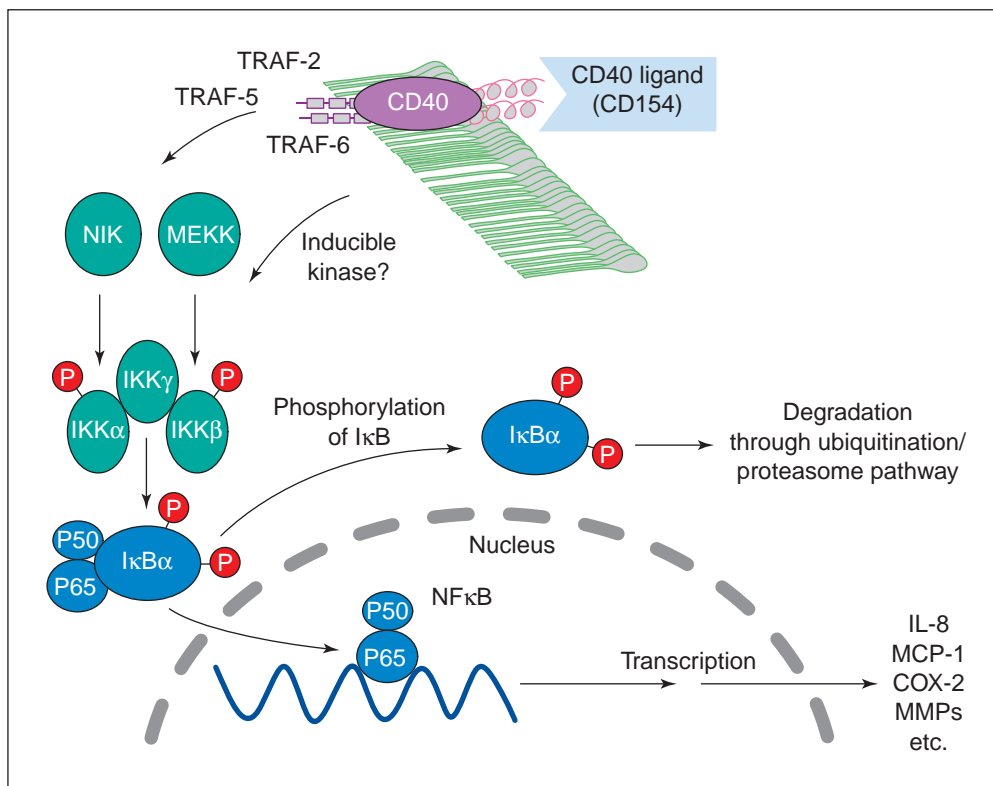


**Fig. 2.** The concentration of progesterone to which the endometrium is exposed is a major factor in governing cytokine concentrations in endometrium. If progesterone increases due to support from the corpus luteum, decidualization changes occur to support the implanting blastocyst. However, if progesterone decreases, changes are started that culminate in oedema and cellular (monocytes and neutrophils) influx, which participate in endometrial breakdown and sloughing. MMP, matrix metalloproteinase; PGDH, prostaglandin dehydrogenase.

NF $\kappa$ B is a transcription factor responsible for the upregulation of genes involved in the inflammatory response. It is sequestered in an inactive state in the cytoplasm by the endogenous inhibitor I $\kappa$ B. Most activators of NF $\kappa$ B cause the degradation of I $\kappa$ B (via a phosphorylation–ubiquitination–proteasome pathway) allowing free NF $\kappa$ B to enter the nucleus (Baldwin, 1996). The molecular mechanisms leading to the degradation of I $\kappa$ B were unclear until recently but it is now apparent that a series of protein kinases are likely to be involved in the signalling. I $\kappa$ B is phosphorylated by an I $\kappa$ B kinase (IKK) complex consisting of several proteins, including the kinases IKK $\alpha$  and IKK $\beta$  (DiDonato *et al.*, 1997) and the scaffolding protein IKK $\gamma$ . IKK $\alpha$  and IKK $\beta$  may have different functions and it appears to be IKK $\beta$  that is predominantly involved in proinflammatory signalling to NF $\kappa$ B (Delhase *et al.*, 1999; Takeda *et al.*, 1999). Upstream mitogen-activated protein kinase kinases (MAPKKK) are involved in the phosphorylation of the IKK complex. Particularly, NF $\kappa$ B-inducing kinase (NIK; Malinin *et al.*, 1997) and MAPK Erk kinase kinase 1 (MEKK1; Lee *et al.*, 1997) activate the kinase activity of the complex. These agents are activated by distinct stimuli (Nakano *et al.*, 1998) but may also act synergistically (Nemoto *et al.*, 1998). An inducible form of IKK

(IKKi) has been reported in rats (Shimada *et al.*, 1999) and, if there is a similar system in humans, an alternative control mechanism in the NF $\kappa$ B cascade will have been established.

Glucocorticoid function in controlling cytokine synthesis and release is better understood than progesterone function due, in part, to the limited expression of progesterone receptors in endometrial cell lines, which means that much of the experimental data on progesterone action is derived from studies on the breast cancer epithelial line T47D, which constitutively expresses progesterone receptors (Horwitz *et al.*, 1982). Both glucocorticoids and progesterone exert major control via effects on the NF $\kappa$ B pathway. However, not all glucocorticoid suppression of cytokines is NF $\kappa$ B-dependent (Bourke and Moynagh, 1999) and the same may yet be shown for progesterone. Glucocorticoids stimulate the production of I $\kappa$ B $\alpha$  and, in the T47D cell line, progesterone has a similar action (Wissink *et al.*, 1998) (Fig. 4). A second mechanism by which progesterone might affect NF $\kappa$ B activity is by direct competition between different transcription factors, in this case steroid receptors and NF $\kappa$ B for adjacent binding sites on the gene. This mechanism has been suggested for both the glucocorticoid receptor (Caldenhoven *et al.*, 1995) and



**Fig. 3.** Cytokine control by NFκB and CD40. CD40 is expressed in the cells surrounding the small blood vessels of human endometrium and myometrium although the nature of the ligand is not known. CD40 can alternatively act through the JAK–STAT pathway to affect cytokine transcription. IKK, IκB kinase; MEKK, mitogen-activated protein kinase Erk kinase kinase; NIK, NFκB-inducing kinase; TRAF, tumour necrosis factor receptor-associated factor.

the progesterone receptor (Kalkhoven *et al.*, 1996). A third mechanism could be the competition between NFκB and the steroid receptors for a common essential cofactor (McKay and Cidlowski, 1999).

There is experimental evidence from *in vitro* systems that progesterone suppresses the release of cytokines such as IL-8 (Ito *et al.*, 1994; Kelly *et al.*, 1994) and MCP-1 (Kelly *et al.*, 1997) that are known to be, at least in part, under the control of NFκB. In T47D cells, the production of MCP-1 is attenuated by progesterone concentrations consistent with those seen in the secretory phase of the menstrual cycle but, in these cells, no effect of glucocorticoid is seen (Kelly *et al.*, 1997).

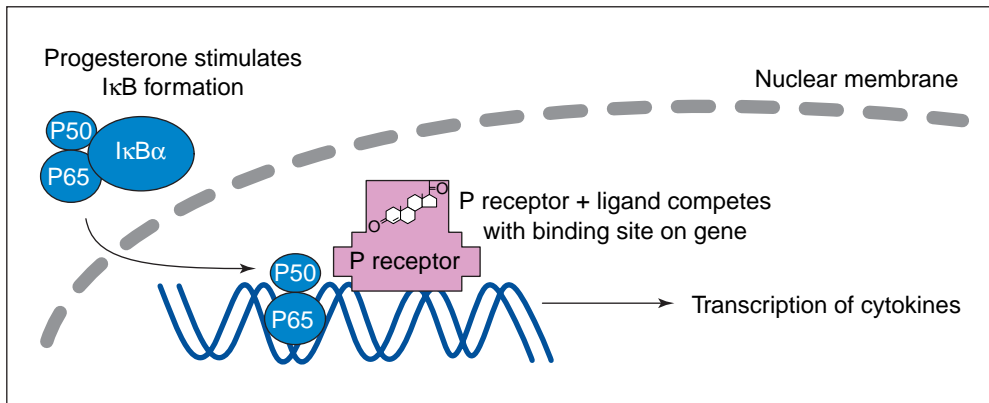
Studies *in vivo* have shown that there is reduced chemokine release from endometrium under the influence of progesterone and that progesterone withdrawal or the administration of antiprogesterin stimulates chemokine expression and release (Critchley *et al.*, 1996; Jones *et al.*, 1997).

#### Phase 2: activation of lytic mechanisms

The consequences of increased cytokine production in a perivascular location may be twofold. First, both PGE and IL-8 are synthesized and, as a result of their synergistic

action (Foster *et al.*, 1989; Rampart *et al.*, 1989; Colditz, 1990), neutrophils will be attracted into the tissue. Second, the effects of progesterone withdrawal will induce vasoconstriction–vasodilatation cycles with associated hypoxia and reperfusion which will, in turn, induce NFκB (Royds *et al.*, 1998). NFκB activation will further induce prostaglandin and cytokine release, which will affect lytic enzyme activity (Luca *et al.*, 1997). These effects are likely to be seen in all tissue affected by the intense vasoconstriction, although studies in rhesus monkeys show that such constriction is not uniform throughout the endometrium.

The exact role for infiltrating leucocytes in menstruation has yet to be clarified but they are likely to be a major source of MMPs and proteases (Salamonsen and Woolley, 1999). The neutrophil has specific granules that contain neutrophil collagenase (MMP-8) and is also a potential source of large amounts of protease (elastase). Local control of MMPs will be by cytokines since a major stimulator of MMP-1 is IL-1α derived from the glandular epithelium (Singer *et al.*, 1997). A withdrawal of progesterone dominance will allow both synthesis of IL-1 and the action of IL-1 to release MMP-1 and thus allow a geared enhancement of MMP-1 (Singer *et al.*, 1997). Although other MMPs such as MMP-9 in endometrium are stimulated by



**Fig. 4.** Two separate mechanisms for progesterone (P) control of the NFκB pathway have been described. Either the progesterone receptor acts as a competing transcription factor displacing NFκB (Kalkhoven *et al.*, 1996) or progesterone can stimulate the synthesis of the inhibitory binding protein IκBα.

cytokines, such stimulation is not sustainable, whereas MMP-1 concentrations remain high for 48 h (Singer *et al.*, 1999). It appears that TIMPs, the endogenous inhibitors of MMPs, are not increased by cytokines in parallel with the MMPs (Lockwood *et al.*, 1998; Singer *et al.*, 1999) and thus cytokine effects should result in the destruction of the supporting matrix of the endometrium and eventually lead to sloughing of tissue.

Thus, there are two pathways for activation of the lytic enzymes induced by decreasing progesterone concentrations: the induction of leucocyte entry by raising the synthesis and activity of chemotactic and vascular-active agents or a direct action on the local control of MMPs. Which of these mechanisms is triggered initially by the demise of the corpus luteum is a matter of current debate (Salamonsen and Woolley, 1999).

### Cytokine changes with implantation

If corpus luteum activity is maintained by embryo secretions, control of many of the cytokines by progesterone will continue. However, other cytokine interactions that favour survival of the conceptus will occur in local micro-environments and, inevitably, decidual cells will be affected by local production of progesterone by the trophoblast. Other cytokine changes must occur at the time of implantation to allow accommodation of the trophoblast by the maternal immune system. The trophoblast restricts expression of major histocompatibility complex (MHC) antigen but there is considerable evidence that other protective mechanisms are in place during pregnancy. There is evidence of T-cell toxicity by trophoblast (Munn *et al.*, 1998) and animal studies have indicated a predominance of Th-2 cytokines (which favour a humoral or antibody immune response) over Th-1 cytokines such as IFN-γ (which favour a cell-mediated immune response) (Wegmann, 1990). Notwithstanding this accommodation, infection can never be allowed to threaten the survival of the mother and thus,

although progesterone may raise the threshold of cytokine production, cytokine-mediated inflammatory responses are possible. During early pregnancy, before extensive changes to the vascular architecture are established, blood reaches the placenta via the capillary network (Carter, 1997) and implantation results in an increase in blood flow to the site. Prostaglandins are implicated in this phenomenon (Kennedy, 1980) but other agents such as nitric oxide (NO) may also be involved. NO is important at the time of implantation since antagonism of both NO and progesterone results in an almost complete absence of implantation sites in rats (Chwalisz *et al.*, 1999). In women, NO synthase is present in glandular epithelium stroma and myometrial blood vessels (Telfer *et al.*, 1995) and, at the time of implantation, expression of the inducible form of the synthase (iNOS) is mediated by progesterone (Buhimschi *et al.*, 1996).

The following section covers some of the complex interactions at the time of implantation.

### *Leukaemia inhibitory factor and gp130-related cytokines*

A group of cytokines that react with receptors associated with gp130 appears to play an important role in implantation. This group comprises leukaemia inhibitory factor (LIF), IL-6, oncostatin M, ciliary neurotrophic factor (CNTF), cardiotrophin (CT-1) and IL-11. Whereas many gene ablation experiments have failed to show clear effects because of redundancy of function, the homozygous LIF knockout mice are infertile since they fail to accommodate implantation (Stewart *et al.*, 1992). Similarly, ablation of the LIF receptor leads to implantation failure (Ware *et al.*, 1995). Since LIF is involved in the decidual reaction (Stewart, 1994), necessity for LIF in implantation may be restricted to those species with relatively invasive implantation. In sheep, in which implantation is synepitheliochorial, LIF may be facilitatory but not obligatory (Vogiagis and Salamonsen, 1999).

**Table 1.** Studies on gene ablation that reveal essential mediators in implantation

Gene knocked out	Observations	Reference
LIF	LIF $-/-$ animals fail to implant due to a decidual defect. Numbers of stem cells in spleen and bone marrow may be reduced.	Stewart, 1994
COX-2	The COX product essential for implantation has not been identified. Although PGE will be important, PGI <sub>2</sub> may interact through the PPAR $\delta$ receptor.	Lim <i>et al.</i> , 1997; Lim <i>et al.</i> , 1999a
IL-11 receptor	The IL-11, LIF and IL-6 receptors are all gp130 linked and all appear to play a role in implantation.	Bilinski <i>et al.</i> , 1998; Robb <i>et al.</i> , 1998
CSF-1	Early studies show that this gene is important but effects may be central since CSF affects microglial cells involved in GnRH release.	Cohen <i>et al.</i> , 1999
Hoxa 11	Hoxa 11 is essential for differentiation of uterine stromal and epithelial cells.	Gendron <i>et al.</i> , 1997
Hoxa 10	The defect is associated with a deficiency of EP3 and EP4 receptors. LIF is not affected.	Benson <i>et al.</i> , 1996; Lim <i>et al.</i> , 1999b
Cyclin D3	Implicated in implantation.	Das <i>et al.</i> , 1999
SRC-1	Decreased organ growth but still fertile.	Xu <i>et al.</i> , 1998
Prolactin receptor	There is an implantation defect in mice lacking the prolactin receptor but this defect may also be a central effect.	Ormandy <i>et al.</i> , 1997; Bole-Feysot, <i>et al.</i> , 1998
Progesterone receptor	Ablation of the progesterone receptor leads to inappropriate inflammation in the uteri of mice.	Lydon <i>et al.</i> , 1995

CSF, colony stimulating factor; EP, prostaglandin E receptor; IL, interleukin; LIF, leukaemia inhibitory factor; PG, prostaglandin; PPAR, peroxisome proliferator; SRC, steroid receptor coactivator.

Although targeted disruption of the IL-6 gene leads to fertile mice, those lacking the IL-11 receptor are infertile (Bilinski *et al.*, 1998)(Table 1). Defects associated with decidual development are observed after implantation has occurred. Since LIF, IL-11 and IL-6 are all implicated in acute phase protein expression in the liver, and since some of the proteins such as  $\alpha$ 2-macroglobulin (Bell, 1979) that are expressed in early decidua are also acute phase proteins, there may be a specific role for the gp130-associated receptors in controlling protein synthesis and release. This hypothesis would certainly accord with the lack of optimum decidualization associated with both LIF (Stewart, 1994) and IL-11 (Bilinski *et al.*, 1998) deficiency, with LIF possibly acting at an earlier stage in the implantation process. Such stimulation by LIF is likely to be regulated by IL-1, TNF- $\alpha$  and transforming growth factor  $\beta$  (TGF $\beta$ ) within the decidua (Sawai *et al.*, 1995) and is consistent with early reports that favoured an effect of LIF on the blastocyst (Stewart, 1994).

A possible alternative interaction is revealed by the demonstration that LIF receptors are expressed on human trophoblast and decidual leucocytes are a major source of LIF. This finding, together with the finding that LIF stimulates chorionic gonadotrophin production by trophoblast (Sawai *et al.*, 1995), indicates that LIF is an essential mediator between maternal decidua and invading trophoblast (Sharkey *et al.*, 1999).

### *Interleukin 1 and interleukin 1-like receptors*

If one cytokine was to be singled out as having the widest impact, IL-1 would be a strong contender. IL-1 has an effect on many cell types and has crucial roles in haematopoiesis acute-phase protein expression and kidney function. It is perhaps surprising that the knockout mouse with deleted type 1 receptor (the only functional receptor) is viable and reportedly fertile (Abbondanzo *et al.*, 1996). In addition, mice deficient in IL-1 $\beta$  (Zheng *et al.*, 1995) and IL-1 $\beta$ -converting enzyme (Kuida *et al.*, 1995; Li *et al.*, 1995) are also fertile. Although redundancies can be invoked as an explanation, the survival of these mice is not fully understood at present and substitutes would have to cover a wide range of IL-1 activities within the uterus (Table 2). Compounds such as IL-18 (IFN- $\gamma$ -inducing factor) are similar to IL-1, and the IL-18 receptor was previously known as IL-1 receptor-like protein.

In non-reproductive systems, IL-1 frequently acts in a synergistic fashion with other cytokines. Synergistic action of IL-1 with TNF- $\alpha$  has been observed for IL-8 (Matsushima and Oppenheim, 1989) and PGE production (Dinarello, 1992; Fujishima *et al.*, 1993) and similar synergistic induction of COX-2 has also been reported (Bry and Hallman, 1991). IL-1 amplifies the effect of bradykinin in stimulating PGE (Angel *et al.*, 1994), a pathway that is important in pain transmission by either PGE<sub>2</sub> or PGI<sub>2</sub>.



**Table 2.** Effects of interleukin 1 (IL-1) on endometrium

	Effect	Reference
TNF- $\alpha$	TNF- $\alpha$ is stimulated by IL-1.	Laird <i>et al.</i> , 1996
IL-8	mRNA for IL-8 is stimulated by IL-1.	Arici <i>et al.</i> , 1996
LIF	TNF $\alpha$ and IL-1 induce mRNA for LIF.	Arici <i>et al.</i> , 1995; Knight <i>et al.</i> , 1999
MMP-1	IL-1 $\alpha$ from epithelium stimulates stromal fibroblast production of MMP-1.	Singer <i>et al.</i> , 1999
MMP-9	IL-1 $\beta$ raises mRNA for MMP-9 and reduces that for TIMP-1 and TIMP-3 in endometrium. IL-1 $\alpha$ raises MMP-9 activity in trophoblast.	Huang <i>et al.</i> , 1998; Meissner <i>et al.</i> , 1999
Apoptosis	Susceptibility to apoptosis in epithelial cells is blocked by IL-1 receptor antagonist.	Tanaka, <i>et al.</i> , 1998
Integrins	IL-1 receptor antagonist reduces levels of $\alpha$ -4, $\alpha$ -5 and $\beta$ -3 integrins.	Simon <i>et al.</i> , 1998
f-Fibronectin	Stimulated by IL-1 in fibroblasts (also stimulated by TGF $\beta$ ).	Meissner <i>et al.</i> , 1999
COX-2	In an endometrial epithelial cell line, IL-1 $\alpha$ stimulated PGE <sub>2</sub> and PGF <sub>2<math>\alpha</math></sub> . COX-1 remained constant.	Jacobs <i>et al.</i> , 1994; Kniss <i>et al.</i> , 1997
SIICAM-1	IL-1 $\beta$ increases soluble ICAM – a possible immunomodulatory pathway since ICAM is necessary for initial adhesion before leukocyte passage through vessel walls.	Vigano <i>et al.</i> , 1998
EP1	This prostaglandin receptor is increased in the amnion in response to IL-1 $\beta$ .	Spaziani <i>et al.</i> , 1997
IL-6	Raised in the secretory phase. Stimulated by IL-1 $\beta$ specifically in stromal cells.	Tseng <i>et al.</i> , 1996; Vandermolen and Gu, 1996
Steroids from trophoblast	Monocyte IL-1 and TNF $\alpha$ modulate trophoblast steroid synthesis.	Feinberg <i>et al.</i> , 1994

COX, cyclooxygenase; EP, prostaglandin E receptor; SIICAM, soluble intercellular adhesion molecule; IL-8, interleukin 8; LIF, leukaemia-inhibitory factor; MMP, matrix metalloproteinase; PG, prostaglandin; TGF $\beta$ , transforming growth factor  $\beta$ ; TNF- $\alpha$ , tumour necrosis factor  $\alpha$ .

(Murata *et al.*, 1997). Other subtle interactions are seen with IL-1 since macrophages primed with IFN- $\gamma$  have a reduced COX-2 expression after IL-1 treatment (Barrios-Rodiles and Chadee, 1998).

IL-1 stimulates production of MCP-1, a compound that is both chemotactic and a modulator associated with the Th-1–Th-2 dichotomy (Chensue *et al.*, 1996) and these effects are enhanced by IL-4 and IFN- $\gamma$ , although no such increase is seen with TNF- $\alpha$  stimulation (Seitz *et al.*, 1994). IL-1 has major effects on endothelial cells, affecting prostaglandins and intercellular adhesion molecule 1 (ICAM-1), IL-1, IL-6 and MHC expression. Many of the effects of IL-1 in the uterus involve endothelial cells (Table 2). Notwithstanding the fertility of mice with deletions in the IL-1 pathway, some studies indicate that intraperitoneal injection of IL-1ra prevents implantation (Simon *et al.*, 1998), although others indicate that the receptor antagonist has no effect (Abbondanzo *et al.*, 1996). Simon *et al.* (1998) reported that, where implantation is prevented by IL-1 receptor antagonist, it is due to a disturbance of the integrin expression on the epithelial cell surface (Simon *et al.*, 1998).

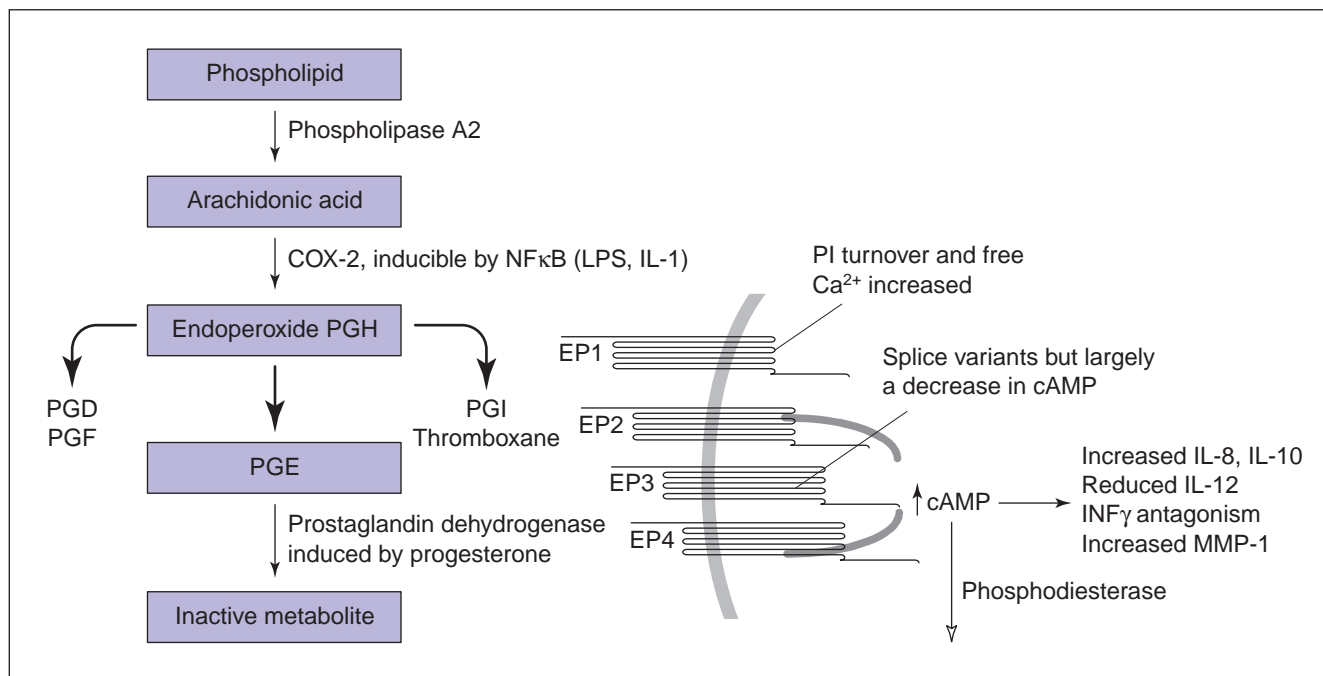
IL-1 is present in the uterus throughout the menstrual

cycle and therefore is unlikely to be directly modulated by progesterone. Such an expression pattern is consistent with a controlling function in both epithelial and endothelial cells and thus may not be directly related to implantation or the early stages of menstruation.

### Transforming growth factor $\beta$ s

TGF $\beta$  is a regulator of growth and differentiation but the role it plays in endometrial physiology is not clear, although TGF $\beta$ s 1–3 are implicated in the process of decidualization (Ando *et al.*, 1998). TGF $\beta$ s are produced and released from cells as inactive precursors that are dependent on proteolytic activation for full activity. The exact mechanisms of activation are uncertain but urokinase type plasminogen activator (uPA) and cathepsin D are competent activators (Lyons *et al.*, 1988). TGF $\beta$  is likely to be short-lived *in vivo* because of its rapid binding to  $\alpha$ 2-macroglobulin.

Four TGF $\beta$ s (1–4) have been reported in endometrium (Chegini *et al.*, 1994; Tabibzadeh *et al.*, 1998) and, although they play an immunosuppressive role in decidua (Lea *et al.*, 1992), there is some doubt about whether the



**Fig. 5.** The pathways of prostaglandin (PG) action. Control can be exercised at several stages: phospholipase, cyclo-oxygenase, prostaglandin dehydrogenase or even prostaglandin receptor expression. COX: cyclooxygenase IL, interleukin; INF, interferon. LPS: lipopolysaccharide; MMP: matrix metalloproteinase.

latent forms are converted to active forms before the decrease in progesterone at the end of the menstrual cycle allows the upregulation of proteases such as uPA (Sandberg *et al.*, 1998).

Several actions of TGFβ are associated with the control of agents affecting ECM. TGFβ stimulates both the plasminogen activator uPA (Sandberg *et al.*, 1998) and the uPA inhibitor PAI (Sandberg *et al.*, 1997). MMPs and their inhibitors (TIMPs) are also affected, and TGFβ is thought to mediate the effect of progesterone in suppressing matrilysin (MMP-7) in endometrial epithelial cells (Bruner *et al.*, 1995) although in stromal cells the evidence so far is that a similar effect is mediated by an increase in TIMPs 1 and 3 (Huang *et al.*, 1998).

Effects of TGFβ on endometrial growth have been reported, with stromal cell growth stimulated in studies *in vitro* (Marshburn *et al.*, 1994; Tang *et al.*, 1994). The effect of progesterone in stimulating TGFβ (Bruner *et al.*, 1995) is of interest since it identifies one mechanism by which stromal cells (which retain progesterone receptors in the secretory phase) can influence glandular epithelium from which progesterone receptors are lost in the second half of this phase of the menstrual cycle.

TGFβ 1, together with IL-10 and PGE<sub>2</sub>, is one of the main suppressive molecules secreted by the macrophage (Bogdan *et al.*, 1992). Many properties of PGE and TGFβ are similar and their exact interaction has yet to be established. TGFβ and PGE may both promote tolerance for trophoblast antigen in decidua and if this occurs their action

would be similar to their action in gut, where oral tolerance, the essential accommodation of food antigen, is dependent on local 'suppressive' cytokine secretion (Groux *et al.*, 1997; MacDonald, 1999; Newberry *et al.*, 1999).

### Prostaglandins

Prostaglandins have several actions relevant to cytokines and menstrual dysfunction since they are involved in both the initiation of menstruation and in pain associated with menstruation. Prostaglandins have an hyperalgesic effect, accentuating cytokine actions at nociceptors (Ferreira *et al.*, 1973). Thus, the analgesic effects of nonsteroidal anti-inflammatory drugs (NSAIDs) can be understood in the context of the two-mediator hypothesis, which impinges on many prostaglandin effects (Williams and Morley, 1973). A study in which the prostacyclin (PGI) receptor was ablated in mice showed that such animals had a higher pain threshold (Murata *et al.*, 1997) and, although little PGI is synthesized in human endometrium (Abel and Kelly, 1979), this effect might be relevant to menstrual pain originating in myometrium, where PGI is the major prostaglandin.

Prostaglandins are involved in the control of cytokine release, cell growth, differentiation and vasoactive effects. It is probably this vasoactive proinflammatory action that accounts for the anti-inflammatory action of aspirin, ibuprofen and fenamates such as mefenamic acid (Ponstan). Mefenamic acid is widely used for the

management of menstrual problems (menorrhagia and dysmenorrhoea) and although part of the action is undoubtedly analgesic, effects on blood loss have been acknowledged (Guillebaud *et al.*, 1978; Cameron *et al.*, 1987; Bonnar and Sheppard, 1996). These effects are thought to be due to the vasoactive properties of both PGE<sub>2</sub> and PGF<sub>2α</sub>. There is an increasing awareness that not all NSAID effects are mediated through the cyclo-oxygenase pathway (Shiff and Rigas, 1999; Zhang *et al.*, 1999) and therefore other possibilities, such as the action of the peroxisome proliferator (PPAR) and other nuclear receptors, ought to be considered.

Since the early studies of Pickles (1967), prostaglandins have been implicated in the mechanism of menstruation and subsequent findings have supported this tenet (Baird *et al.*, 1996). However, neither the relative contributions of COX-1 and COX-2 nor the location and the types of the prostaglandin receptors in human endometrium are known at present. Prostaglandins are also involved in implantation (for review, see Psychoyos *et al.*, 1995) and the critical role of cyclo-oxygenase has also been demonstrated by gene ablation studies (Table 2).

Prostaglandin synthesis involves the control of free substrate (arachidonic acid) concentrations by the modulation of phospholipase, control of inducible cyclo-oxygenase (COX-2) and control of the catabolic enzyme PG-15-dehydrogenase (Fig. 5). In addition, enzymes convert the common intermediate endoperoxide (PGH<sub>2</sub>) to the individual prostaglandins, although it has been suggested that synthesis of endoperoxide by COX-2 leads mainly to PGE<sub>2</sub> and PGI<sub>2</sub> (Brock *et al.*, 1999).

Prostaglandins act on heptahelical G-protein-coupled receptors, which for PGE can be of at least four types (EP1–EP4) (Fig. 5). Although prostaglandins act on cell surface receptors, they are also synthesized by COX-1 and COX-2 at the nuclear membrane and are found on both the inner and outer surfaces of this membrane, indicating a nuclear action (Spencer *et al.*, 1998). The COX enzymes are associated with cell membranes but do not have trans-membrane sequences. They attach to one face of the membrane by four short α-helices. Nuclear actions have been proposed for some prostaglandin derivatives such as those related to a dehydration form of PGD (PGJ), which interacts with the PPAR-γ receptor.

In addition to the above evidence of the action of prostaglandin inhibitors, there are other factors implicating prostaglandins in menstruation. In anovular women, whose endometrium is influenced by oestradiol but not progesterone, the production of prostaglandin is negligible in biopsies but synthesis is seen when the substrate arachidonic acid is made available (Smith *et al.*, 1982). This finding indicates that the ability to provide precursor is progesterone dependent. However, some actions of prostaglandin may not be possible under the influence of progesterone since release in culture is attenuated (Abel and Baird, 1980). Prostaglandin dehydrogenase (PGDH) provides strong catabolizing activity (Casey *et al.*, 1980),

which may account for some of the reduction. Greenland *et al.* (2000) have shown that PGDH is progesterone-dependent in reproductive tissues. Progesterone may also inhibit the inducible synthesis of COX-2 in endometrium (Bracken *et al.*, 1997; Critchley *et al.*, 1999) and decidua (Ishihara *et al.*, 1995). Since COX-2 can be induced via the NFκB pathway (Fig. 3), this may be a major point of action of progesterone (Kalkhoven *et al.*, 1996).

Thus, progesterone withdrawal is a stimulus to prostaglandin production in certain microenvironments, for example, in early decidua, where prostaglandin dehydrogenase becomes undetectable and PGE is clearly evident in the cells surrounding the small blood vessels (Cheng *et al.*, 1993a,b). Towards the end of the menstrual cycle, there is a physiological withdrawal of progesterone and again COX-2 expression is increased at this location (Jones *et al.*, 1997).

The COX-2-deficient mouse has defective decidualization and implantation (Lim *et al.*, 1997) and other knockout mice, such as HOX-10-deficient knockout mice, have a deficiency in the progesterone control of prostaglandin receptors (Lim *et al.*, 1999b). These findings indicate an important role for prostaglandins in implantation as well as in decidualization. Decidualization is a cAMP-dependent process and, in experiments *in vitro*, PGE enhances decidualization (Frank *et al.*, 1994). The process is mediated by COX-2 *in vivo* (Han *et al.*, 1996) but progesterone is also essential and combinations of progesterone and cAMP-increasing mediators are effective in inducing decidualization *in vitro* (Brosens *et al.*, 1999). The cAMP would by-pass any PGE effect since PGE interacts with EP2 or EP4 receptors to give an increased intracellular cAMP concentration. However, for implantation, the evidence is strongest for a role in implantation for COX-2 rather than for PGE and it has been suggested that prostacyclin is the key prostaglandin interacting with nuclear PPARδ (Lim *et al.*, 1999b) although, in NIH 3T3 cells at least, the main product from activation of nuclear COX-2 is PGE (Spencer *et al.*, 1998). In women, the synthesis of PGI<sub>2</sub> and therefore its involvement in implantation is less likely since human endometrium produces very little of this prostaglandin (Abel and Kelly, 1979).

Cytokines such as IL-1 and TNF-α stimulate prostaglandin production, and PGE is a major inhibitor of lipopolysaccharide-induced IL-1 production by monocytes (Kunkel *et al.*, 1986) and thus a negative feedback regulation of immune responses in such cells is apparent. PGE is involved in stimulating MMP production (Lindsey *et al.*, 1996; Zeng *et al.*, 1996; Shankavaram *et al.*, 1997), stimulation of IL-8 and IL-10 production (Strassmann *et al.*, 1994; Agro *et al.*, 1996; Denison *et al.*, 1999), antibody class switching in B cells (Phipps *et al.*, 1991; Roper *et al.*, 1990) and inhibition of IL-12 synthesis from activated monocytes (Kraan *et al.*, 1995). All of these properties of PGE are consistent with the damping of any Th-1 (cell-mediated) response within decidua, where a major source of PGE would be the trophoblast (Kelly *et al.*, 1995). The

suppression of IL-12 is important since it has been shown to activate maternal lymphocytes (both peripheral and decidual) to attack trophoblast (Hayakawa *et al.*, 1999).

### Chemokines

Chemokines are chemotactic cytokines of 8–10 kDa that are classified by their distribution of cysteines. These compounds have similarities in gene sequence, protein sequence and tertiary structure. There are two critical cysteine bonds with either one ( $\alpha$ - or CXC (cystein-any amino acid-cysteine) chemokines) or no ( $\beta$ - or CC (cysteine-cysteine) chemokines) amino acids separating the N-terminal cysteines. A further group (fraktalkines) has three amino acids (CX3C) separating the N-terminal cysteines. The individual classes can be further subdivided: in the  $\alpha$ -group, those with a ELR (Glu-Leu-Arg) motif next to the cysteine nearest the N-terminus, primarily attract neutrophils. Chemokines not only attract cells but also activate them and contribute to angiogenesis and haematopoiesis. Activation may depend on cell type and  $\beta$ -chemokines such as MCP-1 skew T-cell populations into a Th-2 (humoral response pattern of cytokine release) as opposed to a Th-1 (cell-mediated) response (Chensue *et al.*, 1996). The MCP-1  $-/-$  mouse has been used to demonstrate an absolute necessity for MCP-1 in mounting a Th2 response (Gu *et al.*, 2000). Some chemokines can selectively attract haematopoietic progenitor cells out of bone marrow, for example, during inflammatory events and thus locally derived decidual signals may attract leucocytes to decidua.

The presence of the  $\alpha$ -chemokine IL-8 and the  $\beta$ -chemokine MCP-1 have been demonstrated in perivascular cells (Critchley *et al.*, 1994b, 1999; Jones *et al.*, 1997) and the mRNA and protein for these chemokines increase perimenstrually, indicating a role in the early stages of menstruation (Critchley *et al.*, 1999; Milne *et al.*, 1999). Eotaxin, an eosinophil chemotactic agent, has also been identified in a perivascular location in the late secretory phase of the cycle (Zhang *et al.*, 2000). In human endometrium, chemokines may also be responsible for the increasing number of monocytes in the second half of the menstrual cycle (Kamat and Isaacson, 1987).

Epithelial cells act as both a physical barrier to and a target for infection in endometrium. Thus, these cells must possess a competent response to infection, and the uterine epithelium is a source of several chemokines such as MCP-1 (Jolicœur *et al.*, 1998), macrophage inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ ) (Akiyama *et al.*, 1999) regulated-upon-activation, normal T cell expressed and presumably secreted (RANTES) (Altman *et al.*, 1999) and eotaxin (Zhang *et al.*, 2000), although here their primary role may be to participate in any immune defences raised against infection. Expression of MCP-1 in glandular cells is particularly noticeable in endometriotic tissue (Jolicœur *et al.*, 1998) or in tissue that is otherwise stimulated. RANTES is also produced by endometrial stromal cells and its synthesis is enhanced by lipopolysaccharide, TNF and IL-1

(Arima *et al.*, 2000). This effect of lipopolysaccharide indicates that these cells also can recognize infectious agents.

### Conclusions

Menstruation has been shown to involve cytokines and MMPs and is initiated by the decrease in the circulating concentrations of ovarian progesterone. However, the first stages in menstruation are not clear and the cells that first respond to the decrease in progesterone have not been identified. Many of the vasoconstricting agents that may affect blood loss during menstruation (for example, endothelins and prostaglandins) are produced mainly in the glandular epithelium, a site not obviously relevant to the blood vessels. Attention may now have to be directed to the cells immediately surrounding the spiral arterioles. These cells have been shown to respond to progesterone and are potent sources of cytokines and prostaglandin. Moreover, these cells appear to possess components of the CD40 signalling system, which exerts control over cytokine production in cells as diverse as B cells and fibroblasts. Concentration on such signalling pathways over the next decade may result in a new approach to the control of endometrial function that will allow better medical intervention in distressing complaints such as dysmenorrhoea and menorrhagia.

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