Focus on Vascular Function in Female Reproduction

Inflammatory pathways in female reproductive health and disease

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Abstract

Inflammation involves alterations to vascular and immune cell function. It is well recognised that many physiological reproductive events such as ovulation, menstruation, implantation and onset of labour display hallmark signs of inflammation. These are orchestrated by specific molecular pathways involving a host of growth factors, cytokines, chemokines and lipid mediators. Resumption of normal reproductive function involves prompt and proper resolution of these inflammatory pathways. Recent literature confirms that resolution of inflammatory pathways involves specific biochemical events that are activated to re-establish homeostasis in the affected tissue. Moreover, initiation and maintenance of inflammatory pathways are the key components of many pathologies of the reproductive tract and elsewhere in the body. The onset of reproductive disorders or disease may be the result of exacerbated activation and maintenance of inflammatory pathways or their dysregulated resolution. This review will address the role of inflammatory events in normal reproductive function and its pathologies.


Introduction

The Roman encyclopaedist Aulus Cornelius Celsus (ca 25BC–ca 50AD) defined the cardinal signs of inflammation, namely rubor (redness), calor (increased heat), tumor (swelling) and dolor (pain). In 1870, Rodulph Virchow highlighted that inflammation is also associated with functio laesa or loss of function (Larhammar 1996). In response to a tissue injury or a pathogenic insult, the human body mounts a network of chemical signals that stimulate responses aimed at healing the affected tissue. These signals initiate the activation and chemotaxis of leukocytes from the general circulation to the sites of damage. Inflammatory signals also alter the function of the vasculature and the endothelium to enhance angiogenesis, vascular permeability and the extravasation of leukocytes from the blood to the inflamed tissue (Coussens & Werb 2002, Goswami et al. 2008, Serhan et al. 2008).

It is becoming increasingly accepted that many normal reproductive processes display hallmark signs of inflammation. Such processes include ovulation, menstruation, implantation and parturition (Goswami et al. 2008). All of these events are associated with upregulation in the expression of a host of inflammatory mediators, which include cytokines, growth factors and lipid mediators that influence the growth and function of the immune and vascular compartments (Coussens & Werb 2002, Goswami et al. 2008, Serhan et al. 2008). Another remarkable feature of the female reproductive tract is its capacity to resolve these inflammatory events rapidly to re-establish normal reproductive function. The resolution of inflammation involves the clearance of leukocytes and tissue debris as well as restoration of mucosal and vascular function in the affected tissue. Until recently, resolution of inflammation was considered a passive process that came about as a result of dissipation in the expression of local inflammatory mediators. However, emerging literature highlights that in response to tissue injury there are specific anti-inflammatory and pro-resolution biochemical pathways that are activated, which facilitate the re-establishment of homeostasis in the affected tissues (Serhan et al. 2008).

Little is known about the role of pro-resolution pathways in normal reproductive function. However, it is anticipated that in physiological reproductive events (such as

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Pathways regulating inflammation

In the reproductive tract, the injury and tissue remodelling caused by ovulation, menstruation and parturition trigger the inflammatory cascade. This involves a carefully orchestrated autocrine/paracrine/juxtacrine series of events to facilitate repair, remodelling and the resolution of inflammation which is regulated in a cyclical manner via the sex steroids oestradiol (E2) and progesterone. Inflammation is an active process which involves the release of inflammatory cytokines, chemokines and peptide growth factors. This establishes a gradient for the recruitment of neutrophils and macrophages to the site of injury. Injury also promotes the activation of the coagulation and fibrinolysis system, which operates in tandem to control clotting and remodelling of the vasculature. This facilitates tissue regeneration and extravasation of neutrophils at the site of injury via dilatation and oedema. Tissue remodelling also involves production of local inflammatory mediators such as kinins, histamine and eicosanoids such as prostanoids (prostaglandins (PGs), prostacyclins and thromboxanes) and leukotrienes.

Immune cells

Approximately 1% of whole human blood comprises leukocytes, more commonly referred to as white blood cells. Leukocytes are divided into two main groups: granulocytes (consisting of neutrophils, basophils and eosinophils) and agranulocytes (consisting of lymphocytes, monocytes and macrophages). Physical injury and inflammation caused by pathogens induce the release of signals such as cytokines, chemokines and growth factors to activate epithelial and endothelial cells, mast cells, macrophages, platelets and neutrophils to facilitate repair (Fig. 1). Inflammatory signals activate haematopoietic stem cells derived from bone marrow to produce monocytes (Ziegler-Heitbrock 2007). Monocytes give rise to macrophages or dendritic cells and are recruited by chemotaxis into the damaged tissue by extravasation. Once in the tissue they can phagocytose cellular debris and pathogens and stimulate lymphocytes (Ziegler-Heitbrock 2007). Neutrophils, which are a key mediator of the inflammatory response, recruit, activate and programme antigen-presenting cells to activate T cells as well as release local mediators to attract monocytes and dendritic cells (Nathan 2006). Neutrophils also generate signals to determine whether macrophages differentiate into a pro- or anti-inflammatory state and are responsible for lymphocyte expansion and lymph node drainage. In addition, neutrophils are key mediators of wound healing and microbial sterilisation as individuals with insufficient neutrophils display poor wound healing, and in severe cases this can be fatal (Nathan 2006). Mast cells are tissue-based inflammatory cells derived from CD34+ pluripotent stem cells and are recruited to the site of injury together with monocytes, macrophages and neutrophils by chemotaxis and are involved in wound healing, defence against pathogens and release of histamines to facilitate vasodilation and oedema associated with inflammation. They also produce the chemoattractant factor, interleukin (IL)16 to recruit CD4+T lymphocytes (Prussin & Metcalfe 2003). In addition to being recruited by inflammatory stimuli, immune cells also amplify and sustain the response by the release of local inflammatory mediators (cytokines, chemokines, growth factors and eicosanoids) at the site of recruitment.

The coagulation and fibrinolysis system

During coagulation, fibrin is deposited to form a clot to limit blood loss (Fig. 1). Inflammatory cytokines such as IL6 are the main mediators of inflammation-induced coagulation (Levi & van der Poll 2005). IL6 in turn induces expression of tissue factor (TF; also known as Factor III (F3) or CD142) via signal transduction pathways such as the MAPK pathway and transcription factors such as early growth response factor 1 (Sampson & Kakkar 2002). TF then promotes thrombin generation via the activation of specific G-protein-coupled proteinase-activated receptors (PAR1–4; Sampson & Kakkar 2002, Hollenberg et al. 2008); leading to conversion of fibrinogen to fibrin (ten Cate et al. 1994, van der Poll et al. 1994, Belting et al. 2005). Thrombin also acts as a potent platelet activator to enhance fibrin deposition and
enhances TF expression by neutrophils, mononuclear cells and macrophages (Levi & van der Poll 2005).

The coagulation pathway is tightly regulated by three important anticoagulant pathways, namely the antithrombin, the protein C system and the TF pathway inhibitor (Levi & van der Poll 2005). These pathways are all impaired during inflammation to drive fibrin deposition and coagulation and are implicated in mortality and morbidity under conditions of severe or chronic inflammation (Levi & van der Poll 2005). Following tissue repair, fibrinolysis is induced as the fibrin clot is removed enzymatically by plasmin. Plasmin is produced from plasminogen following the release of plasminogen activators (tissue-type plasminogen activator (PA) and urokinase-type PA) by the cytokines IL1B and tumour necrosis factor α (TNF; Levi & van der Poll 2005). This induction of plasmin from plasminogen is counterbalanced by the induction of PA inhibitor type-(PAI)1 (Levi & van der Poll 2005). Defects in the fibrinolysis cascade due to alteration in the levels of PAI1 can contribute to thrombosis and morbidity due to inadequate fibrin removal.

Vascular changes during inflammation

During inflammation various molecules are produced which promote dilatation of blood vessels and increased vascular permeability. One example is histamine, which is produced from mast cells (Prussin & Metcalfe 2003). Histamine facilitates movement of fluid and plasma into the tissue thereby inducing oedema and swelling. This slows blood flow and allows leukocytes to marginate along the endothelium and then extravasate into the damaged tissue by chemotaxis in response to stimuli from local inflammatory mediators. In parallel, thrombin acts as a potent platelet activator to enhance fibrin deposition and remodelling of the vasculature, thereby facilitating angiogenesis.
Whereas B2 receptors are thought to be constitutively expressed, B1 receptors are upregulated during inflammation and are thought to play a role in numerous inflammatory diseases (Campos et al. 2006). Activation of both B1 and B2 receptors by kinins such as bradykinin leads to activation of diverse signal transduction pathways including activation of phospholipase Cβ, generation of inositol 1,4,5-trisphosphate and mobilisation of intracellular calcium (Calixto et al. 2001, 2004, Campos et al. 2006). Furthermore, they activate phospholipase A2 and increase arachidonic acid release giving rise to local production of inflammatory mediators (Campos et al. 2006). Activation of PARs by TF can also induce vessel sprouting and morphogenesis via the release of vascular endothelial growth factor (VEGF), which under conditions of chronic inflammation may play a role in regulating angiogenesis in disease (Boccaccio & Medico 2006). Inflammatory prostanoids such as PGE_2 and PGF_2α in addition to regulation of B2 receptor have also been shown to regulate expression of angiogenic factors such as VEGF via the activation of specific GPCRs like the E prostanoid 2 (EP2) receptor and F prostanoid receptor to promote angiogenesis, proliferation and cytoskeletal reorganisation and cell motility for tissue remodelling (Milne & Jabbour 2003, Sales & Jabbour 2004, Sales et al. 2004, Sales et al. 2008).

**Neuroimmunoendocrinology regulation**

Immune-endocrine disequilibrium attributed to stress has become a commonly cited factor when discussing unexplained reproductive failures including infertility, impaired oogenesis, miscarriages, preterm labour and impaired fetal development (as reviewed in Nepomnaschy et al. 2007). New multidisciplinary research on brain–body interactions triggered by stress in early pregnancy has shown that maternal biological responses, including localised inflammation in uterine tissue and sustained depression of progesterone production, challenge the endocrine–immune steady state during pregnancy, leading to serious consequences for the fetal environment (Arck et al. 2007). This ‘pregnancy stress syndrome’ is associated with over-activation of the hypothalamic–pituitary–adrenal (HPA) axis which triggers the release of neurohormones, and subsequently the activation of the HPA axis stimulates upregulation of key stress hormones such as corticotrophin-releasing hormone, ACTH and glucocorticoids. The elevated levels of circulating stress hormones consequently lead to altered inflammatory pathways and immune cell function affecting reproductive function (as reviewed in Nakamura et al. 2008).

**Inflammatory mediators**

**Sex steroids**

The ovarian sex steroids E_2 and progesterone are responsible for orchestrating the dynamic tissue remodelling observed in the ovary and endometrium during the normal reproductive cycle by activating gene transcription via the specific nuclear E_2 (ER) and progesterone (PR) receptors (Saunders 2005). The expression of ER and PR is under dual control of E_2 and progesterone and is spacio-temporally expressed in the endometrium (as reviewed in Critchley et al. 2001, Kelly et al. 2001 and Saunders 2005). The complex role of steroids in inflammation and their regulation of inflammatory mediators has been extensively reviewed elsewhere (Auersperg et al. 2001, Critchley et al. 2001, Richards et al. 2002, Kayisli et al. 2004, Saunders 2005, Lea & Sandra 2007, Straub 2007).

**Cytokines, chemokines and growth factors**

Cytokines are a large family of more than 100 low molecular weight proteins that function as growth and differentiation factors and immune cell modulators. The chemoattractive cytokines or chemokines are a large family grouped on the basis of the arrangement of two N-terminal cysteine residues, CXC and CC, depending on whether the first two cysteine residues have one or more amino acids between them (CX–C or are adjacent to one another (CC). Roughly 50 chemokines and 20 chemokine receptors have been identified, and they induce diverse responses such as immune cell recruitment, tissue repair and leukocyte extravasation (Thelen & Stein 2008).

In the reproductive tract, coincident with the activation of the coagulation cascade, injury causes inflammatory cytokines to be released locally within tissues (Fig. 1). These cytokines act in an autocrine/paracrine manner to elicit cell-specific events, depending on the temporal nature in which they are released. For example, IL1 is a potent chemotactic cytokine, with pyrogenic and immunomodulatory actions in the reproductive tract (Amjad et al. 2006) and is an early response cytokine necessary for wound repair (Salamonsen 2003). IL6, via the IL6 receptor–GP130 complex is one of the most potent cytokines in promoting inflammatory events through expansion and activation of T cells and differentiation of B cells and the acute phase response as well as activation of the coagulation cascade as detailed above (Guazzzone et al. 2009). IL8 is known for its ability to activate macrophages and recruit neutrophils and T cells; however, it can also act as an autocrine growth factor to promote angiogenesis in endometrial vessels and proliferation of endometrial stromal cells and has been shown to enhance re-epithelialisation of skin grafts as well as to facilitate tissue remodelling (Gimbrone et al. 1989, Arici et al. 1998a, 2009).
Lipid mediators

Glycerophospholipids such as phosphatidylcholine are the main structural eukaryotic membrane lipids (Wymann & Schneiter 2008). Their release by phospholipases gives rise to arachidonic acid and lysophosphatidylcholine (Wymann & Schneiter 2008). Arachidonic acid is then enzymatically converted by cyclooxygenases (COX) to PG, prostacyclins and thromboxanes or by lipoxigenase (LOX) enzymes to leukotrienes (Rajakariar et al. 2006, Wymann & Schneiter 2008). Lysophosphatidylcholine is in turn enzymatically converted to lysophosphatic acid (LPA; Fig. 2; Mills & Mooenaar 2003, Wymann & Schneiter 2008). For many years the role of PGS in inflammation has been ascertained from studies conducted using non-steroidal anti-inflammatory drugs (NSAIDs, which function by blocking COX-catalysed synthesis of prostanoids: PGs, prostacyclins and thromboxanes (Vane & Botting 1998; COX-enzyme-specific knock-out mice (Loffin et al. 2002, Rajakariar et al. 2006)). At the onset of the inflammatory response, under the regulation of E2 and progesterone (Critchley et al. 2001), a range of growth factors, prostanooids, cytokines, chemokines and ILs promote the production of inflammatory prostanooids by inducing expression of COX enzymes. For example, mast cells and granulocytes recruited to the site of inflammation are activated to release granule contents and promote the production of PGs, thromboxanes and leukotrienes (Wymann & Schneiter 2008). As discussed earlier, bradykinin via its receptor activates phospholipase to promote prostanooid production and thrombin activation of platelets causes production of thromboxane and platelet aggregation. This is balanced by the release of prostacyclin which has a vasodilatory and anti-aggregatory effect on vascular function. Prostanoids mediate these effects in inflammation, following their binding to and activation of specific GPCRs (Fig. 2; Jabbour et al. 2006), which are present in a cell-specific manner (Coleman et al. 1994). For example, PGE2 in the CN is induced by inflammatory cytokines IL1 and TNF from activated immune cells and is responsible for regulating fever via the EP3 receptor (Murakami & Kudo 2004). Locally at the site of inflammation, COX enzymes and PGs can promote immune cell infiltration, cellular proliferation and angiogenesis to facilitate tissue remodelling (Fig. 1). LPA via its GPCRs has also been shown to play a role in inflammation in the reproductive tract via the release of cytokines such as IL6 and IL8 and modulation of urokinase-type PA (Ye 2008).

In addition to their pro-inflammatory roles, COX enzymes also play a role in producing anti-inflammatory prostanooids, including PG D2 and 15-deoxy-Δ-PG J2, which are thought to play a role in resolution of inflammation (Murakami & Kudo 2004, Rajakariar et al. 2006, Scher & Pillinger 2009). The LOX pathway is also thought to contribute towards the resolution of inflammation via the production of anti-inflammatory molecules such as lipoxins and resolvins (Serhan et al. 2008).

Inflammatory pathways in reproductive physiology

Ovulation

The hypothesis that mammalian ovulation is comparable to an inflammatory reaction was first proposed by Espey (1980) since many of the molecules responsible for inducing the inflammatory cascade including PGs, leukotrienes, bradykinin, histamine, platelet activating factor and various cytokines have been described in the ovary (Espey 1994). Ovulation is initiated by the LH surge and is controlled by the spacio-temporal expression of specific genes (as reviewed in Richards et al. 2002). The process of ovulation destroys the ovarian surface epithelium and vasculature at the site of oocyte expulsion. Inadequate resolution or remodelling and repair at the site of expulsion are thought to predispose the tissue to neoplastic transformation by the accumulation of genetic mutation following DNA damage (Fleming et al. 2006). The ovarian surface is covered by
a single layer of flat to cuboidal cells referred to as human ovarian surface epithelial cells (OSE or hOSE) or ovarian mesothelium, which is responsible for proteolytic remodelling of the ovarian surface (Auersperg et al. 2001). Although receptors for E2, progesterone and androgen are found on OSE cells, no direct effect of these steroids on OSE cellular proliferation has been observed (Auersperg et al. 2001). However, indirect effects of sex steroids on ovarian surface epithelial cell function have been observed in vitro via the upregulation of growth factors (Auersperg et al. 2001). During the mid-cycle LH surge, leukocytes migrate into the thecal layer and upon ovulation migrate into the granulosa layer coincident with rupture of the basement membrane (Bukulmez & Arici 2000). Following rupture of the ovarian surface epithelium, repair and organisation of the site is necessary to form a corpus luteum. The coagulation cascade is triggered, driven by the release of cytokines, produced locally at the site of tissue damage and by invading leukocytes as well as platelets (Fleming et al. 2006). IL1 has been shown to
upregulate pro-inflammatory genes in OSE cells, including IL6, IL8 and nuclear factor kappa b (NFKB; Rae et al. 2004b). These in turn can sustain cellular proliferation (Auersperg et al. 2001) via the activation of MAPK signalling in a positive feedback manner to facilitate rapid repair of the ovarian surface epithelium. During the repair process, infiltrating macrophages produce TNF, which also facilitates proliferation of OSE cells and produces TNF expression in OSE cells to sustain repair (Auersperg et al. 2001). Growth factors, chemokines and cytokines also induce expression of COX enzymes and promote local production of prostanoids (Rae et al. 2004b). The role of COX enzymes and PGs in the inflammation of ovulation is unclear, since inhibition of COX enzyme with NSAIDs inhibits ovulation completely. However, we can speculate on their role in initiating inflammatory pathways based on the known roles for these molecules as discussed earlier. It is feasible that COX enzymes and PGs promote immune cell recruitment, tissue remodelling and angiogenesis in the ovary post ovulation. Finally, inflammatory stimuli such as IL1A also enhance the steroidogenic environment in granulosa cells and OSE cells to increase 11β-hydroxysteroid dehydrogenase type-1. This enhances conversion of cortisone to cortisol to facilitate repair and counteract the inflammatory response (Rae et al. 2004a, Fleming et al. 2006). During this time, the TGFb family of cytokines present in exudates can exert a growth inhibitory effect on OSE cells by counteracting the proliferative effects of EGF (Auersperg et al. 2001).

Menstruation

The human endometrium undergoes extensive remodelling during every menstrual cycle. This process involves the disintegration of the functionalis layer of the endometrium and regeneration and differentiation of a new layer in preparation for an implanting embryo. The features of menstruation are parallel to those of an inflammatory response with the expression of inflammatory cytokines, chemokines and prostanoids. Moreover, there is an abundance of leukocytes in the endometrium prior to the onset of menstruation indicating a role for these factors and cells in the remodelling process.

The actions of PGs on the endometrium result in ischaemia, tissue necrosis and shedding of the endometrium. Endometrial PGF2α and prostacyclin (PGI2) are highest before the onset of menstruation and induce cyclic blood vessel vasoconstriction and vasodilation respectively (Baird et al. 1996). Thromboxane A2 is a potent vasoconstrictor and stimulator of platelet aggregation; whereas, PGI2 is an inhibitor of aggregation (Salamonsen et al. 1999). The level of PGs PGF2α and PGE2 during the menstrual cycle is regulated by the catabolic enzyme prostaglandin-15-dehydrogenase (PGDH) which is regulated by progesterone, following progesterone withdrawal PGDH expression declines leading to a rise in the levels of PGs peri-menstrually (Norman et al. 1991).

In the peri-menstrual period, there is a dramatic influx of inflammatory-type leukocytes: uterine natural killer (uNK) cells, neutrophils, eosinophils, macrophages and activated mast cells (Salamonsen & Lathbury 2000). The origin of uNK cells is unknown, although evidence suggests that peripheral NK cells migrate to the uterus and in the hormone-rich uterine environment they proliferate and differentiate (Moffett-King 2002, Kane et al. 2009). Regulation of the migration of leukocytes to the endometrium in response to chemotactic cytokines and chemokines (Fig. 1) is further enhanced by the action of PGE2 on the blood vessels to induce capillary leakage (Colditz 1990). Increased expression of chemotactic cytokines and chemokines prior to menstruation plays an important regulatory role in immune cell recruitment, for example IL8 and CCL2. Expression of IL8 in the endometrial epithelial cells and arterioles of the late secretory phase may regulate the recruitment of neutrophils before menstruation (Arici et al. 1998a). CCL2 has a similar expression pattern with high levels during menstruation and is a potent attractant of macrophages, T cells, NK cells, basophils and mast cells (Jolicoeur et al. 1998). The influx of leukocytes into the endometrium and their activation immediately prior to menstruation provides cellular interactions, which are critically important to matrix metalloproteinase (MMP) expression and matrix degradation. For example, eosinophils provide a wide range of secretory products, which activate mast cells. Mast cell activation results in the release of potent regulators such as TNF, histamine and mast cell-specific proteases preceding menstruation (Sivridis et al. 2001). The importance of such cellular interactions has been demonstrated by the production and activation of MMP1 and MMP3 by endometrial stromal cells during co-culture with mast cells (Zhang et al. 1998).

Finally, after the action of pro-inflammatory mediators and immune cells to induce menstruation, it is essential to regulate menstrual blood flow to allow tissue elimination without excessive bleeding. Regulation is maintained by activation of the haemostatic and fibrinolytic systems to ensure a correct balance of blood coagulation. TF is the primary initiator of haemostasis generating fibrin and leading to clot formation (Fig. 1; Lockwood et al. 1993). Fibrinolysis leading to clot degradation is regulated by the availability of plasmin by the action of PA. Progesterone increases TF expression but decreases PA expression, therefore progesterone withdrawal immediately prior to menstruation induces a haemorrhagic environment with decreased clotting and increased fibrinolysis (Casslen et al. 1995).
Implantation and placentation

Pro- and anti-inflammatory pathways are involved in the establishment of a receptive endometrium (window of implantation) and also in embryo–endometrium communication. Many cytokines produced by the embryo are pro-inflammatory, suggesting that implantation is a process in which the embryo induces inflammatory pathways in the endometrium. The endometrium responds to these embryonic signals during the window of implantation by enhancing expression of receptivity genes required for embryo adhesion and invasion (Shewin et al. 2007, Evans et al. 2009). In preparation for implantation, decidualisation in humans occurs initially during the secretory phase around the spiral arterioles and significantly throughout the endometrium if pregnancy occurs. Decidualisation, in addition to differentiation of endometrial stromal cells into decidual cells, involves initiation of inflammatory events such as infiltration of leukocytes, modification of the extracellular matrix and an increase in vascular permeability (Popovici et al. 2006, Hess et al. 2007). IL1β and TNF in particular have emerged as candidate genes responsible for the activation of the pro-inflammatory cascade at the fetal–maternal interface (Hess et al. 2007). These primary pro-inflammatory cytokines activate production of secondary mediators such as cytokines, chemokines, COX enzymes, PGs and pentraxin 3 (PTX3). PTX3 is novel mediator and plays a key role as an effector and modulator of innate resistance, inflammation and angiogenesis and is localised to perivascular and endothelial cells of first trimester decidua (Garlanda et al. 2008). Cytokines and in particular; the IL6 family members (IL11, leukaemia inhibitory factor (LIF) and IL6) play an important role in implantation. In vitro studies have shown that their receptors are expressed at the implantation site by several cell types (van Mourik et al. 2009). Gene knockout mouse models have demonstrated that both IL11 and LIF play important roles in implantation, and IL6 influences fertility and implantation efficiency (Stewart et al. 1992, Robb et al. 1998, Jasper et al. 2007). LIF is a pro-inflammatory cytokine expressed in the epithelium and decidual stromal cells and is regulated by several inflammatory mediators such as IL1, TNF, leptin, insulin-like growth factor, TGFβ (Gonzalez et al. 2004, Perrier d’Hauterive et al. 2004, Kimber 2005) and more recently prokineticins (Evans et al. 2009), suggesting intricate regulation by inflammatory pathways. Several other roles have been described for LIF including immune cell recruitment to the endometrium (Schofield & Kimber 2005) and trophoblast adhesion to extracellular matrix proteins (Tapia et al. 2008, Evans et al. 2009).

Distinct leukocyte subpopulations are present in the endometrium during implantation. Macrophages, a small number of T cells, and uNK cells predominate in the decidua especially at the sites of trophoblast invasion. Chemokines such as CCL4, CCL7 and CCL13 which recruit these types of leukocytes are upregulated in the endometrial glands during endometrial receptivity and by decidual stromal cells in early pregnancy (Jones et al. 2004). At the human implantation site, uNK cells account for 70% of the leukocytes and interact with the allogeneic placenta. By recognising paternal trophoblast ligands, uNK cells may control the extent of placental invasion. The molecular mechanism for the maternal recognition of trophoblast is via the MHC class I molecules HLA-C, HLA-E and HLA-G expressed by the trophoblast cells which are recognised by receptors (such as the killer-cell immunoglobulin-like receptors) expressed on uNK cells (Boyington et al. 2001). In humans, it is proposed that this interaction mediates the immune cell response preventing trophoblast over-invasion, but allowing placental access to the maternal blood supply (Moffett-King 2002). There is also a central role for T cell-derived cytokines in the regulation of fetal allograft survival. Changes in the production of hormones such as progesterone and relaxin play a major role in modulating T helper 1/T helper 2 (Th1/Th2)-type cytokine balance (Piccinni et al. 2000). Th1-type cytokines promote allograft rejection and compromise pregnancy. The production at the fetal–maternal interface of Th2-type cytokines such as IL4 and IL10 inhibits the Th1 responses and improves fetal survival (Piccinni et al. 2001).

Considerable evidence has accumulated indicating that PGs have an important role during implantation (Kennedy et al. 2007). PGs are elevated in areas of increased endometrial vascular permeability associated with the initiation of implantation. Further evidence comes from numerous reports that NSAIDs delay or inhibit localised increase in vascular permeability and implantation (Hamilton & Kennedy 1994), and mice with COX2 ablated have multiple reproduction abnormalities including retarded decidualisation (Cheng & Stewart 2003). The type(s) of PG and receptors involved in human embryo implantation still remains unclear and is compounded by animal studies that show species differences (Kennedy et al. 2007).

The pro-inflammatory pathways induced during implantation are regulated by anti-inflammatory mediators such as adiponectin and IL10 to prevent excessive inflammation. Adiponectin is a pleiotropic cytokine (Maeda et al. 1996) and in addition to playing an important role in regulating energy metabolism and insulin sensitivity (Yamauchi et al. 2001), it has been shown to have anti-inflammatory (Brakenhielm et al. 2004) and anti-angiogenic activities (Goldstein & Scalia 2004). Adiponectin and the two adiponectin receptors (ADIPOR1 and ADIPOR2) have been shown to be expressed in the epithelial and stromal cells of the endometrium with expression levels of the receptors peaking during the window of implantation (Takemura et al. 2006). In the endometrium, adiponectin has been demonstrated to inhibit IL1β-induced expression of IL6.
and IL8, suggesting that adiponectin signalling plays a role in regulating pro-inflammatory pathways during implantation (Takemura et al. 2006). IL10 is a well characterised anti-inflammatory and immune-modulating cytokine expressed in the endometrium and placenta (Hanna et al. 2000). IL10 has the ability to reduce inflammation by inhibiting synthesis of TNF, IL1 and other pro-inflammatory cytokines and chemokines (Moore et al. 2001). IL10 null mutant mice demonstrate IL10 as a key regulator of fetal and placental growth (White et al. 2004).

Regulation of inflammation during implantation may follow a sequential model in which pro-inflammatory is followed by anti-inflammatory or there may be a continuous balance between the pro- and anti-inflammatory environments. Despite the characterisation of several mediators of inflammation, the mechanism of inflammatory pathway regulation during implantation is unclear and warrants further investigation.

Labour

There is an emerging evidence that physiological parturition is associated with upregulation of inflammatory pathways. Labour at term is associated with a massive neutrophil and macrophage influx into the myometrium and cervix (Thomson et al. 1999, Osman et al. 2003). Myometrium, cervix and fetal membranes all release pro-inflammatory cytokines during parturition, with upregulation of pro-inflammatory cytokines being largely but not exclusively confined to invading leukocytes (Ledingham et al. 2001, Young et al. 2002, Osman et al. 2006). Normal labour is associated with upregulation of inflammatory pathways, with NFKB activation appearing to play a key role (Allport et al. 2001). More recently, genomic analysis of labouring versus non-labouring uterine and fetal tissue has confirmed that inflammatory genes are among those whose gene expression is most profoundly altered during labour (Haddad et al. 2006, Bollopragada et al. 2009). The initiating signal(s) that drives these inflammatory events is unknown, although expression of innate immune receptors that receive these signals (the Toll-like receptors) is increased towards the end of pregnancy and further increase in labour. Importantly, pro-inflammatory events are not limited to the uterus, with evidence of greater chemotactic ability of leukocytes in peripheral blood in labouring compared with non-labouring women (Yuan et al. 2009).

Classically, inflammation is the triad of heat, swelling and pain, and the mechanism by which these events might contribute to the process of parturition is not immediately obvious. However, further consideration suggests that release of pro-inflammatory cytokines may play a major role via stimulation of myometrial contractions. For example, myocytes pre-incubated with IL1B display greater increases in intracellular calcium (which itself is linked to smooth muscle contraction) in response to stimuli compared with myocytes incubated in control media (Tribe et al. 2003). IL1B also increases expression of COX2 (itself an initiator of myometrial contractions; Rauk & Chiao 2000) and phosphodiesterase activity in myocytes (Oger et al. 2002) again stimulating contractions. Additionally, IL1B administration stimulates preterm labour in a mouse model (Romero & Tartakovsky 1992). In the cervix, IL1B stimulates MMPs (Watari et al. 1999), which is likely involved in the process of collagen breakdown during cervical ripening which occurs before and in the early phases of parturition (Yoshida et al. 2002). Additionally, leukocytes invading the cervix release nitric oxide, which again induce cervical ripening (Thomson et al. 1997, Ledingham et al. 2000). A pathway by which inflammation induces parturition might involve initiation of inflammatory stimuli signalling via Toll-like receptors, which results in PG and MMP production in addition to leukocyte invasion into reproductive tissues, and culminating in myometrial contractility, rupture of membranes and cervical ripening (Challis et al. 2009).

Although much focus has been on the ‘pro-inflammatory’ pathways of parturition, there is some evidence that endogenous anti-inflammatory pathways are also active. For example, IL10 levels rise in amniotic fluid during labour in humans (Gotsch et al. 2008). We have recently investigated the potential role of anti-inflammatory lipid mediators such as lipoxins in parturition and shown an increase in both synthetic capacity and receptor density of these molecules in the myometrium during parturition (Maldonado-Perez et al. 2009; Fig. 3).

Inflammatory pathways in reproductive pathology

Infertility, early pregnancy loss and complications

Aberrant implantation can cause a variety of clinical problems including recurrent miscarriage, intrauterine growth retardation and preeclampsia. The cause of recurrent miscarriage is multifactorial; known causes of maternal defects include coagulation disorders, auto-immune defects and endometrial defects influencing the production of pro-inflammatory cytokines (Laird et al. 2003). Pro-inflammatory cytokines IL6, LIF and IL1B are decreased in the endometrium of women with recurrent miscarriage compared with fertile women (von Wolff et al. 2000). Impaired endovascular trophoblast invasion is the primary placental defect causing inadequate conversion of the uterine arteries and reduced utero-placental blood flow, which leads to fetal intrauterine growth restriction and the development of preeclampsia. In addition to endothelial dysfunction, there is evidence of systemic activation of maternal inflammatory cell responses in preeclampsia. There is also increased release of pro-inflammatory cytokines TNF, IL6,
soluble phospholipase A2 (a mediator of inflammatory reactions) and PTX3 into the circulation-associated preeclampsia (Redman et al. 1999, Rovere-Querini et al. 2006, Assi et al. 2007). Dysregulation of anti-inflammatory mediators has also been reported in these pathologies. IL10 levels are elevated in amniotic fluid at mid-trimester in women with intrauterine growth restriction and elevated in term placenta in women with preeclampsia (Heyborne et al. 1994, Rinehart et al. 1999). IL10 has also been reported to be aberrantly expressed in decidual T lymphocytes in women with recurrent miscarriage (Piccinni et al. 1998). A direct cause-and-effect relationship between a local defect of Th2-type cytokine expression and pregnancy loss has been reported (Piccinni et al. 2001). TGFB can inhibit Th1-type responses, which may be detrimental to pregnancy, in addition it is an important regulator of NK cells, down-regulating IFN-γ-induced activation and inflammatory cytokine production. Thus, TGFB actions during implantation are instrumental in the establishment of anti-rejection pathways to embryo survival (Jones et al. 2006). Women with high numbers of circulating NK cells have a higher risk of miscarriage as increased infiltration into the decidua of these blood-type NK cells, which express IFN-γ, are known to cause abortion. It has been shown that abortions are probably caused by IFN-γ activating the production of prothrombinase FGL2 in trophoblast and in decidua (Clark et al. 2001a). This procoagulant leads to fibrin deposition and activation of polymorphonuclear leukocytes that destroy the vascular supply to the placenta (Clark et al. 2001b).

Recent reports suggest that dysregulation of inflammatory factors play a role in endometriosis-associated reproductive failure (Gupta et al. 2008). Endometriosis is a disorder characterised by the proliferation of endometrial tissue outside the uterine cavity following retrograde menstruation of endometrial tissue into the peritoneal cavity. The concentration of inflammatory cytokines (IL1B and TNF) and PGs (PGE2 and PGF2α) produced by peritoneal macrophages (Karck et al. 1996) and pro-inflammatory chemokines for monocyte/macrophages (CCL2 and CCL5) and for granulocytes (IL8 and CXCL1) is elevated in women with endometriosis (Ryan et al. 1995, Arici et al. 1997), although it is unclear whether these pro-inflammatory changes precede or follow endometriosis. Dysregulated production of anti-inflammatory mediators has also has been reported to have an impact on female fertility (Mitchell et al. 2005) and to be decreased in the serum of women with endometriosis such as adiponectin (Takemura et al. 2005).
Menstrual disorders

Menstrual cycle disorders attributed to a dysfunctional endometrium, include dysmenorrhoea and heavy menstrual blood loss. Dysmenorrhoea is characterised by severe uterine pain during menstruation, and heavy menstrual blood loss is characterised by an abnormally heavy and/or prolonged menstrual period. Primary dysmenorrhoea and heavy menstrual blood loss are diagnosed when symptoms cannot be attributable to other underlying disease, disorder or structural abnormality in the uterus. The role of inflammatory mediators in these pathologies is not well documented except the contribution of aberrant PG production, which has been well reported. Locally produced PGs are elevated prior to menstruation and are considered primary mediators of aberrant menstruation (Sales & Jabbour 2003a, Smith et al. 2007). Increased PGE$_2$ relative to PGF$_2\alpha$ levels in endometrium and menstrual fluid have been associated with heavy menstrual blood loss, and altered PGI$_2$ and TXA$_2$ in the spiral arteries may also contribute to this condition (Lumsden et al. 1983). Dysmenorrhoea is associated with uterine hypercontractility resulting in episodes of reduced endometrial blood flow leading to ischaemia and increased pain (Rees 1989). This observation may be a consequence of increased PGF$_{2\alpha}$ production as explants from women with dysmenorrhoea produce more PGF$_{2\alpha}$ in response to arachidonic acid compared with normal endometrial explants (Lundstrom & Green 1978). Further evidence of the role of PGs comes from the administration of COX enzyme inhibitors such as ibuprofen, which have been demonstrated to reduce menstrual blood flow (Makarainen & Ylikorkala 1986) and selective COX2 inhibitors that have been used in the treatment of dysmenorrhoea and heavy menstrual blood loss (Daniels et al. 2002).

Complicated labour

The major complication of parturition is preterm labour. Preterm delivery rates in the United Kingdom are in the order of 8%, with over 70% of preterm deliveries following spontaneous preterm labour. The role of infection and inflammation within the amniotic cavity in preterm parturition has been extensively examined with good evidence that the prevalence of infection and/or inflammation is greater the earlier in gestation that preterm labour occurs (i.e. the prevalence of infection and/or inflammation is greater in preterm labour at 28 weeks compared with 34 weeks gestation; Goldenberg et al. 2000, Shim et al. 2004). Importantly, the likelihood both of preterm delivery and of poor neonatal outcome is greater in the presence of intrauterine inflammation (Shim et al. 2004). Fewer studies have examined cervical and myometrial tissues from women in preterm labour, although those that have suggested that inflammatory processes operate here also (Tornblom et al. 2004, Osman et al. 2006).

The importance of intrauterine inflammation in pre-term labour is not confined to the initiation of fetal membrane rupture, cervical ripening, myometrial contractions and preterm delivery. One of the major adverse consequences of infection/inflammation-associated pre-term delivery is neonatal brain injury, which manifests as white matter damage. A seminal study in 1997 showed that vaginal inoculation of pregnant rabbits with *Escherichia coli* induced white matter damage in the fetus within 5 days (Yoon et al. 1997b). In human pregnancy, the same group also showed that periventricular white matter injury was commoner in babies whose mothers had high cytokine levels in amniotic fluid when sampled by amniocentesis prior to delivery (Yoon et al. 1997a). A clear causal link between non-infective intrauterine inflammation and fetal brain inflammation was shown more recently by Elovitz et al. (2006), with the demonstration that intrauterine lipopolysaccharide (LPS) administration in the pregnant mouse stimulates pro-inflammatory cytokine production in the fetal brain.

A key question is whether understanding of these inflammatory pathways can be exploited therapeutically. It has been known for some time that IL1 receptor antagonists can inhibit IL1-induced preterm labour (Romero & Tartakovsky 1992). More recently, exciting data from animal studies have suggested that administration of ‘anti-inflammatory’ agents could avert not only preterm delivery, but also the risk of neonatal brain damage resulting from exposure to intrauterine inflammation. The first set of these studies focused on IL10 in a preterm labour model induced by intrauterine LPS administration. IL10 administered either on the day of LPS administration or delayed by 24 hours completely abolished preterm delivery (Terrone et al. 2001). Further studies by the same group in a rat *E. coli* preterm labour model showed that IL10 also prevented infection-induced white matter injury (Rodis-Palenik et al. 2004). More recently, Bennett et al. have shown that 15-deoxy-12,14-prostaglandin J2 (15d-PGJ2), an agent which inhibits NFkB and possibly also JNK) averts LPS-induced preterm labour in pregnant mice and LPS-induced NFkB activation in the brain of mouse pups (Pirianov et al. 2009).

Although these agents show great promise, they are at present only being trialled in animal models. Clinically, progesterone is the only agent shown to be effective in preventing preterm birth with efficacy proven for selected groups (Dodd et al. 2008). The mechanism of action of progesterone is unknown, although there is some evidence in vitro of an acute inhibitory effect on myometrial contractions (Ruddock et al. 2008). Progestogens may also act to inhibit inflammation, with inhibition of LPS-induced inflammation in human fetoplacental arteries and in myometrium (Gotkin et al. 2006) and inhibition of a
physiological rise in mouse myometrial CCL2 in vivo (Shynlova et al. 2008).

Although there has been little measurement of anti-inflammatory pathways in preterm pathological parturition, it is tempting to speculate that upregulation thereof could be a useful therapeutic strategy to prevent preterm delivery.

Reproductive tract cancers

The relationship between inflammation and cancer dates back to the 19th century, when Virchow first hypothesised that the origin of cancer was at sites of chronic inflammation (Coussens & Werb 2002). Ovarian, uterine and cervical cancers, which arise mainly from the OSE, endometrium and glandular and squamous epithelium of the cervix, are the most common gynaecological malignancies (Forman et al. 2003). The incidence of ovarian, uterine and cervical cancer as of 2005 in the United Kingdom are reported to be 17.4, 17.9 and 8.4 women per 100 000 with a mortality rate of 10.1, 3.5 and 2.4 women per 100 000.

It is now well established that infection of the cervix with human papillomavirus (HPV) is the main cause of cervical cancer in women (zur Hausen 2009). However, the aetiology of ovarian and endometrial cancers is multifactorial and less well-defined. In the case of ovarian cancer, it is considered that the inflammatory environment caused by repetitive ovulation over the lifetime of a women increases the risk of genetic error and mutation during the repair process (Fleming et al. 2006), leaving the OSE susceptible to neoplastic transformation in subsequent ovulation and repair cycles. Epidemiological and experimental observations have implicated hormonal fluctuations in sex steroids, and androgen exposure in particular, in the pathogenesis of ovarian cancer; however, the mechanism of actions of steroid hormones in the regulation of ovarian cancer remains unclear (Auersperg et al. 2001). Genetic mutations in the BRCA1 and BRCA2 genes have also been implicated in the pathogenesis of the disease as they have been associated with a genetically inherited incidence of ovarian cancer (Fleming et al. 2006). Endometrial adenocarcinomas on the other hand are associated mainly with post-menopausal women and are thought to arise from excessive E2 exposure in the setting of endometrial hyperplasia (Persson 2000), although other factors including poly cystic ovarian syndrome and obesity are also thought to play a role.

The interplay between inflammation and cancer has been extensively reviewed (Sporn & Roberts 1986, Coussens & Werb 2002, Modugno et al. 2005, Goswami et al. 2008). In the context of the gynaecological malignancies, inflammation can contribute to the initiation and progression of disease via the release of local mediators, ILs, growth factors and cytokines, to facilitate immune cell recruitment, cell proliferation and angiogenesis and sustain tumour growth. Tissue damage, be it is post-ovulatory damage or damage by chemical carcinogens or viral infection agents can cause activation of the coagulation cascade, as described earlier, and there is now much evidence in support of the coagulation cascade in mediating tumour cell adhesive spreading. For example, TF is increased in cell lines containing inactivating mutations of p53 and PTEN (Boccaccio & Medico 2006) and is thought to enhance tumour metastasis directly by enhancing cell motility as its extracellular domain interacts with several integrins (Beling et al. 2005). TF activation of PAR1/integrin αvβ5 signalling via thrombin can enhance cell motility and metastasis (Beling et al. 2005). Furthermore, the TF cytoplasmic domain has been shown to negatively regulate integrin α3β1, which mediates metastatic arrest (Beling et al. 2005). Dysregulation of TF phosphorylation and upregulation of PAR1 is thought to contribute to the aggressive behaviour of some cancer cells (Beling et al. 2005). The kallikrein–kinin system of proteinase-mediated signalling has also been implicated in cancer. For example, a link has been made between PAR1 receptor expression and mammary tumour cell metastasis and invasion (Hollenberg et al. 2008); however the role of these pathways in ovarian, endometrial and cervical cancers needs to be investigated.

We and others have shown that the inflammatory COX–PG axis is elevated in ovarian, endometrial and cervical cancers (Ryu et al. 2000, Tong et al. 2000, Jabbour et al. 2001, Sales et al. 2001, 2002, Gupta et al. 2003, Sales & Jabbour 2003a, 2003b, Daikoku et al. 2005, Munkarah & Ali-Fehmi 2005, Khunamormpong et al. 2009). This pro-inflammatory pathway can be induced by a variety of stimuli, including LPS, cytokines, growth factors and tumour-promoting chemical carcinogens (Modugno et al. 2005, Goswami et al. 2008). In cervical cancers, a recent study has shown that HPV infection induces expression of COX2 (Subbaramaiah & Dannenberg 2007). Furthermore, PGE2 has been shown to regulate hormone-dependent diseases of the endometrium by upregulating aromatase expression and local E2 production, which can in turn upregulate COX enzyme expression (Bulun et al. 2000). Elevated PG biosynthesis, as a consequence of elevated COX enzyme expression in ovarian, endometrial and cervical epithelial cells, can promote and sustain tumourigenesis via the activation of specific prostanoid GPCRs and second messenger systems to enhance the expression and delivery of potent growth factors, cytokines and chemokines (Fig. 2; Sales & Jabbour 2003a, 2003b, Jabbour & Sales 2004, Jabbour et al. 2006, Goswami et al. 2008). These local mediators enhance the recruitment of immune cells, inhibit apoptosis and enhance cell proliferation, tumour angiogenesis and promote cell migration and metastasis (Fig. 2).

This has led us and other investigators to speculate that inhibition of the inflammatory COX–PG axis could be of...
therapeutic relevance for women with gynaecological malignancies. Indeed, the risk of cancer and epithelial ovarian cancer in particular in women on NSAID treatment for at least 6 months is reduced (Fleming et al. 2006). This is due to the inhibition of COX enzyme activity and suppression of transcriptional transactivators such as NFKB, which leads to a reduction in the expression of local mediators, such as pro-inflammatory cytokines, growth factors and ILs (Fleming et al. 2006, Goswami et al. 2008). LPA, which is present in follicular fluid and elevated in ascites from patients with ovarian, endometrial and cervical cancer, is another bioactive lipid that has recently been shown to play a role in reproductive tract pathology. LPA acting via its specific GPCRs has been shown to promote cellular proliferation, growth, migration and survival of ovarian, endometrial and cervical cancer cells by inducing local expression of growth factors and cytokines such as IL6 and IL8 (Fig. 2; Ye 2008). It is anticipated that the activation of the LPA system in parallel to the COX–PG system could enhance the inflammation in reproductivepathologies associated with aberrant expression of LPA or PG receptors (Fig. 2).

Little is known about the role of anti-inflammatory cytokines and lipids in reproductive tract cancer. It would be tempting to speculate that their expression, synthesis and function are suppressed to maintain an exacerbated inflammatory environment conducivefor growth and metastases of these cancers. Future work is warranted to address the role for such molecules in female reproductive cancers and their potential exploitation for therapeutic intervention.

Conclusions

It is well accepted now that reproductive processes are regulated by inflammatory events. Tight control of the onset and resolution of these inflammatory events ensures normal reproductive function. Exacerbated or premature activation of inflammation can contribute to disease. Understanding the molecular control of inflammation and its resolution in the reproductive tract may give us insight into how these may be corrected therapeutically in disease.

Declaration of interest

H N Jabbour is a named inventor on several patents for the treatment of endometrial pathologies or preterm labour. J E Norman is a named inventor on a patent for the treatment of preterm labour.

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Placenta
Inflammation in reproductive processes

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