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.

Reproduction (2009) **138** 903–919

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, Rodolph 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

menstruation, ovulation, implantation and the onset of labour), their expression may be temporally regulated and important in the maintenance of proper reproductive function.

Furthermore, it is well recognised that inflammation-mediated alterations in immune cell and vascular function are important components of many pathologies that include cancer, chronic inflammatory diseases, allergy, asthma, atherosclerosis, autoimmunity, transplant rejection and metabolic and degenerative diseases. Moreover, alterations or disruption in the onset of the pro-resolution pathways may lead to uncontrolled inflammation and the onset of disease, and there is mounting evidence that in animal models of disease the administration of pro-resolution mediators can help control and resolve inflammation (Serhan *et al.* 2008). However, to date the role of pro-resolution pathways in reproductive disorders or their pathologies known to be associated with excessive or premature onset of inflammatory pathways (for example menstrual disorders, infertility, premature onset of labour, cancers of the female reproductive tract) remains to be elucidated.

This review will discuss the role of inflammatory pathways in female reproductive health and will address how alterations in these pathways may contribute to the pathologies of female reproductive function.

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 (E_2) 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 (PARs1–4; 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

Figure 1 Schematic diagram highlighting the pathways involved in inflammation and tissue repair. Damaged tissue releases a host of local inflammatory mediators including cytokines, interleukins, growth factors and prostaglandins. These mediators activate numerous pathways, which act synergistically to control tissue remodelling and repair. For example, inflammatory mediators are released to facilitate cellular proliferation and repair the damaged tissue and act together with histamine, released from mast cells, to alter vascular tone and facilitate movement of fluid and plasma into the tissue to promote 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.

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 anti-thrombin, 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 counter-balanced 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 tissue (Fig. 1). In addition, thrombin produced locally within the vasculature is known to promote vasorelaxation in endothelium-containing vessels via the activation of PAR1 and 2 receptors to enhance blood flow to the site (Hollenberg *et al.* 2008). Members of the kallikrein-kinin system of blood proteins, such as bradykinin, are also produced at the site of inflammation by the proteolytic cleavage of a kininogen precursor, high molecular weight kininogen (HMWK or HK). Kinins exert their effects by binding to two classes of G protein-coupled receptors (GPCRs), B1 and B2 (Campos *et al.* 2006).

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 C β , 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 prostanoids such as PGE₂ (Calixto *et al.* 2001, Campos *et al.* 2006), which in turn can induce expression of B2 receptor. 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 PGE₂ and PGF_{2 α} 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 2003a, Jabbour & Sales 2004, Sales *et al.* 2004, 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 E₂ 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 E₂ (ER) and progesterone (PR) receptors (Saunders 2005). The expression of ER and PR is under dual control of E₂ 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 (Guazzone *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,

1998b, Kayisli *et al.* 2002, Salamonsen 2003, Ulukus *et al.* 2005). Furthermore, activation of the coagulation cascade directly promotes the production of inflammatory cytokines and chemokines. For example, fibrin can stimulate mononuclear cells and endothelial cells to produce IL6 or IL8 (van der Poll 2001, van der Poll *et al.* 2001), while thrombin can act on endothelial cells to enhance production of IL8, monocyte chemoattractant protein 1 and E-selectin (van der Poll 2001). These ligands can act via their specific receptors on cells within the microenvironment to activate diverse signal transduction cascades, target genes and adhesion molecules and are potential targets for therapeutic intervention for numerous inflammatory diseases (Feldmann 2008).

In addition to cytokines and chemokines, peptide growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and transforming growth factor (TGF) have been identified as paracrine mediators in platelets, macrophages and lymphocytes, where they play a multifactorial role (Auersperg *et al.* 2001). For example, TGF β (TGF β) and PDGF are potent leukocyte chemoattractants. PDGF released from platelets is a chemoattractant for fibroblasts and stimulates the production of collagenase by fibroblasts to promote remodelling of the extracellular matrix (Sporn & Roberts 1986, Wahl *et al.* 1989). TGF β released from platelets, T lymphocytes and monocytes can induce PDGF, fibroblast growth factor, IL1 and TNF in a host of cell types to regulate tissue repair by controlling the recruitment of new cells and the formation of new matrix and blood vessels necessary for the repair process (Sporn & Roberts 1986, Wahl *et al.* 1989).

Lipid mediators

Glycerophospholipids such as phosphatidylcholine are the main structural eukaryotic membrane lipids (Wymann & Schneider 2008). Their release by phospholipases gives rise to arachidonic acid and lysophosphatidylcholine (Wymann & Schneider 2008). Arachidonic acid is then enzymatically converted by cyclooxygenases (COX) to PG, prostacyclins and thromboxanes or by lipoxygenase (LOX) enzymes to leukotrienes (Rajakariar *et al.* 2006, Wymann & Schneider 2008). Lysophosphatidylcholine is in turn enzymatically converted to lysophosphatidic acid (LPA; Fig. 2; Mills & Moolenaar 2003, Wymann & Schneider 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 (Loftin *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, prostanoids, cytokines, chemokines and ILs

promote the production of inflammatory prostanoids 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 & Schneider 2008). As discussed earlier, bradykinin via its receptor activates phospholipase to promote prostanoid 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 prostanoids, 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

Figure 2 Schematic diagram outlining the pathways regulating lipid signalling. Glycerophospholipids are the main eukaryotic membrane lipids. Their diacylglycerol (DAG) backbone carries a phosphate (phosphatidic acid) esterified to either choline, ethanolamine, serine or inositol. Hydrolysis of membrane lipids by cytosolic phospholipase A2 (cPLA2) gives rise to arachidonic acid and lysophosphatidylcholine. Arachidonic acid is then stereospecifically oxygenated through the cyclooxygenase (COX) pathway, thereby producing prostaglandins (PG). Although the metabolic pathways responsible for LPA biosynthesis are poorly delineated, LPA can be produced by hydrolysis of phosphatidic acid, following its release from glycerophospholipids by phospholipase D (PLD), by soluble phospholipase A2 (sPLA2) or phospholipase A1 (PLA1) or by the cleavage of lysophosphatidyl choline by ATX/lysoPLD. In addition, the phospholipase C (PLC) breakdown products of membrane lipids, DAG and inositol 1,4,5 trisphosphate (IP3) serve as lipid second messengers to promote cell signalling. Once biosynthesised, PG and LPA are released from the cell and exert their biological function via specific G-protein-coupled receptors. Ligand–receptor activation of either LPA or PG pathways can regulate their own biosynthesis and that of each other in a positive feedback manner to regulate inflammatory signalling in normal reproductive physiology as well as disease by altering vascular function, immune cell recruitment, tissue remodelling and cellular function.

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 E_2 , 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 by growth factors present in the follicular fluid (Fig. 1; Yamada & Gentry 1995, Bukulmez & Arici 2000). At this time, EGF and PDGF are released from platelets and together with the pro-inflammatory cytokines and induction of COX enzymes and prostanoids stimulate OSE growth (Auersperg *et al.* 2001). Invading leukocytes release nitric oxide to facilitate vasodilatation and secretion of IL1 for tissue remodelling (Fleming *et al.* 2006). IL1 has been shown to

upregulate pro-inflammatory genes in OSE cells, including IL6, IL8 and nuclear factor kappa β (NF κ B; [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 11 β -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 TGF β 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 PGF $_{2\alpha}$ and prostacyclin (PGI $_2$) are highest before the onset of menstruation and induce cyclic blood vessel vasoconstriction and vasodilation respectively ([Baird et al. 1996](#)). Thromboxane A $_2$ is a potent vasoconstrictor and stimulator of platelet aggregation; whereas, PGI $_2$ is an inhibitor of aggregation ([Salamonsen et al. 1999](#)). The level of PGs PGF $_{2\alpha}$ and PGE $_2$ 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 chemoattractant cytokines and chemokines ([Fig. 1](#)) is further enhanced by the action of PGE $_2$ 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 ([Jolicœur 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 (Sherwin *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). IL1B 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, TGF β (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 IL1B-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-inflammation is followed by anti-inflammation 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 NF κ B 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,

Figure 3 Schematic diagram of the pro- and anti-inflammatory pathways operating in labour. Pro-inflammatory pathways are shown on the right hand side of the diagram. Parturition is associated with the influx of leukocytes (principally neutrophils and macrophages) into the myometrium and cervix during parturition. These cells release cytokines such as interleukin (IL)1, IL6 and IL8. These cytokines upregulate matrix metalloproteinase (MMP) expression and activity in the cervix, causing collagen breakdown and cervical ripening. In the myometrium, IL1 and possibly the other cytokines increase the expression of cyclooxygenase (COX)2, leading to increased prostaglandin production. This, together with IL1's activity in increasing calcium entry into myometrial cells will stimulate myometrial contraction. In parallel with these pro-inflammatory events, anti-inflammatory events are also upregulated in labour. Specifically, amniotic fluid IL10 production is increased. In the myometrium, synthetic capacity for the lipoxin pathway and its receptor (ALX) is also upregulated during labour. The upregulation of the anti-inflammatory pathway likely acts as a counterbalance to the pro-inflammatory pathway during the process of parturition. Inappropriate early activation of the pro-inflammatory pathway may initiate preterm labour, and thus strategies to upregulate the anti-inflammatory pathway are of interest in prevention or treatment of preterm birth.

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 PGF_{2 α}) 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 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 PGE₂ relative to PGF_{2α} levels in endometrium and menstrual fluid have been associated with heavy menstrual blood loss, and altered PGI₂ and TXA₂ 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_{2α} production as explants from women with dysmenorrhoea produce more PGF_{2α} 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 preterm 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 preterm 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 (Rodts-Palenik *et al.* 2004). More recently, Bennett *et al.* have shown that 15-deoxy-12,14-prostaglandin J2 (15d-PGJ2, an agent which inhibits NFκB and possibly also JNK) averts LPS-induced preterm labour in pregnant mice and LPS-induced NFκB activation in the brain of mouse pups (Piryanov *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 life time 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 E_2 exposure in the setting of endometrial hyperplasia (Persson 2000), although other factors including polycystic 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 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 (Belting *et al.* 2005). TF activation of PAR1/integrin $\alpha v \beta 5$ signalling via thrombin can enhance cell motility and metastasis (Belting *et al.* 2005). Furthermore, the TF cytoplasmic domain has been shown to negatively regulate integrin $\alpha 3 \beta 1$, which mediates metastatic arrest (Belting *et al.* 2005). Dysregulation of TF phosphorylation and upregulation of PAR1 is thought to contribute to the aggressive behaviour of some cancer cells (Belting *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, Khunamornpong *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, PGE_2 has been shown to regulate hormone-dependent diseases of the endometrium by upregulating aromatase expression and local E_2 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 reproductive pathologies 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 conducive for 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.

Funding

This research has been supported by the Medical Research Council (U.1276.00.004.00002.01) and core funding for the Edinburgh Tommy's Centre for Maternal and Fetal Health, PiggyBank Kids and Action Medical Research (Grant no. SP4275).

Acknowledgements

We would like to thank Ronnie Grant for assistance with graphics.

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Received 16 June 2009

First decision 17 August 2009

Revised manuscript received 23 September 2009

Accepted 30 September 2009