Molecular regulators of resolution of inflammation: potential therapeutic targets in the reproductive system

James L Hutchinson, Shalini P Rajagopal, Kurt J Sales and Henry N Jabbour

MRC Human Reproductive Sciences Unit, Queen’s Medical Research Institute, The University of Edinburgh, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK

Correspondence should be addressed to H N Jabbour; Email: h.jabbour@hrsu.mrc.ac.uk

Abstract

Inflammatory processes are central to reproductive events including ovulation, menstruation, implantation and labour, while inflammatory dysregulation is a feature of numerous reproductive pathologies. In recent years, there has been much research into the endogenous mechanisms by which inflammatory reactions are terminated and tissue homoeostasis is restored, a process termed resolution. The identification and characterisation of naturally occurring pro-resolution mediators including lipoxins and annexin A1 has prompted a shift in the field of anti-inflammation whereby resolution is now observed as an active process, triggered as part of a normal inflammatory response. This review will address the process of resolution, discuss available evidence for expression of pro-resolution factors in the reproductive tract and explore possible roles for resolution in physiological reproductive processes and associated pathologies.

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Introduction

Inflammation represents the biological response to a wide variety of tissue insults, including those mediated by pathogens, irritants, cellular dysfunction and physical trauma. When successful this results in initial containment of the insult, followed ideally by its removal, and restoration of tissue homeostasis in a process termed resolution. Inflammation itself is a highly complex process involving the coordinate action of multiple cell types, resident and recruited. It is characterised by activation of the coagulation cascade, alterations in vascular perfusion and permeability and production of local inflammatory mediators including steroids, cytokines, chemokines, growth factors and lipids (reviewed in Serhan et al. (2008) and Jabbour et al. (2009)). These physiological changes promote influx of immune cells including polymorphonuclear leukocytes (PMNs), monocytes and macrophages. Together, these cells act as both effectors and regulators of inflammation, generating and releasing antimicrobial reactive species and lytic enzymes, producing further inflammatory mediators, engulfing pathogens and debris and initiating tissue repair and remodelling (Jabbour et al. 2009).

It is perhaps to be expected that such an ancient, complex and adaptable system as inflammation should be co-opted into physiological processes. Accordingly, inflammation is widely recognised as integral to many reproductive events. Ovulation, menstruation and the onset of labour all feature local production of cytokines, chemokines and lipid mediators together with influx and activation of inflammatory immune cells including PMN and monocytes. Meanwhile, the balance between pro- and anti-inflammatory pathways is crucial to successful implantation and subsequent placentation, with immune cells playing key regulatory roles in trophoblast invasion and, later, uterine quiescence. Evidence is also mounting that inflammatory dysregulation contributes to numerous reproductive pathologies including endometriosis, menorrhagia, dysmenorrhoea, pre-eclampsia and pre-term labour (reviewed in Jabbour et al. (2009) and Maybin et al. (2010)).

Research examining the molecular pathways of inflammation in reproductive processes has largely focused either on the role of classic pro-inflammatory mediators such as prostaglandins (PG), leukotrienes (LTs) and chemokines important in the early stages of inflammation or on the cytokine balance of an established inflammatory environment in the context of the Th1/Th2 adaptive response. In recent years, it has become apparent that alongside the initial amplifying pathways of inflammation and before the establishment of adaptive immunity, further counter-regulatory biochemical and metabolic processes are activated. These serve not only to limit the extent of inflammation through suppression of classic inflammatory mediators.
(anti-inflammatory), but also to promote transition into a state of active restoration characterised by immune cell clearance and regulated repair, a feature termed ‘pro-resolution’ (Serhan et al. 2008). With this in mind it is notable that post-ovulatory and post-menstrual repair share many characteristics of wound healing in other tissues (Salamonsen 2003). Inflammatory events in the reproductive tract also involve cell types and pathways that display pro-resolution activities in other systems. Therefore, we propose that pro-resolution pathways are likely to be important in both limiting inflammation and in regulating repair and remodelling in physiological events of the female reproductive tract. These would include ovulation, menstruation, implantation, placentation and events surrounding parturition. Furthermore, dysregulation of these pathways could contribute to various reproductive pathologies, and pharmacological manipulation of these endogenous mechanisms could lead to potent and well tolerated new therapies. This review will focus on resolution mediators, outlining their synthesis and regulation with particular reference to what is known in reproductive tissues, followed by a description of their signalling and downstream anti-inflammatory/pro-resolution effector function. Finally, relevance of these pathways to inflammatory events of the reproductive tract is discussed.

Resolution effector families

The existence of mediators analogous to the pro-inflammatory PG, but which function instead to actively switch from a pro-inflammatory pathway to one restoring tissue homeostasis, was postulated as far back as 1988 (Flower 1988). The archetypal resolution mediator is the non-classical eicosanoid lipoxin A₄ (LXA₄), originally identified as one of a series of biologically active leukocyte metabolites of 15-HPETE which also includes the similar lipoxin B₄ (LXB₄; Serhan et al. 1984a, 1984b). Early experiments on lipoxins demonstrated modulatory effects on a range of activation-induced neutrophil responses with a tendency towards limiting inflammation (Lee et al. 1989, Colgan et al. 1993, Papayianni et al. 1996). Subsequently epimeric, aspirin-triggered forms of lipoxin (ATL) were discovered with similar resolving properties (Claria & Serhan 1995). Recently, a series of anti-inflammatory, pro-resolving autacoids derived from ω-3 polyunsaturated fatty acids (PUFA) have been described (Norling & Serhan 2010). These are grouped into three families termed the resolvins, protectins and maresins. These lipid mediators all appear to share certain properties/functions that could be traditionally termed anti-inflammatory, such as inhibition of leukocyte function and inflammatory cytokine secretion. Importantly, however, they also share novel pro-resolving activities such as promotion of neutrophil apoptosis, non-phlogistic macrophage phagocytosis and egress from the tissues.

In addition to the lipid resolution mediators, a number of protein/peptide-based molecules have been identified which exhibit similar pro-resolving properties. The most prominent is the glucocorticoid effector annexin A1 (ANXA1) and its derived peptides. Similarly to lipoxins, these peptide molecules can act to inhibit leukocyte activation and function. Interestingly, a degree of unification was brought to the concept of resolution signalling with the surprising revelation that both lipoxin and resolvins family members, as well as ANXA1, are capable of signalling through a single G-protein-coupled receptor, namely formyl peptide receptor 2 (FPR2), also termed FPRL1 or ALX receptor (Perretti et al. 2002). In accordance with their extensive characterisation, the review will focus on LXA₄ and ANXA1 as exemplar lipid and protein resolution mediators respectively.

Eicosanoid and ω-3 PUFA derived resolution mediators

The various cell types involved in inflammation sense and respond to injury with an initial largely stereotypic response that is subsequently adjusted according to the nature and extent of the inflammatory insult. The dynamic balance between inflammation and resolution doubtless requires the continual integration of signals from numerous sources. Accordingly, the major pathways of lipoxin synthesis from arachidonic acid (AA), as well as those the ω-3 PUFA-derived mediators, are transeellular—that is, involving coordination of multiple cell types each expressing different subsets of enzymes (Serhan et al. 2008). These in turn show overlapping substrate specificities and are involved in synthesis of various lipid mediators including the LTs. Synthesis pathways are outlined in Fig. 1 and described in detail together with their regulation in Box 1. Briefly, the major pathways of endogenous lipoxin production from AA involve actions of successive lipoxgenases (LOX) including 5-LOX and either 12- or 15-LOX. A third pathway is described following aspirin-mediated acetylation of prostaglandin-endoperoxide synthase 2 (PTGS2; also known as cyclooxygenase-2, COX-2) which together with 5-LOX promotes formation of the ATL, contributing to the drug’s anti-inflammatory effects (Serhan 2005). The LOX enzymes and acetylated PTGS2 are also involved in synthesis of the endogenous and aspirin-triggered ω-3 PUFA-derived mediators. Although direct data on lipid resolution mediators in the reproductive tract is limited, enzyme expression patterns suggest they may be produced locally in the ovary, and in the uterus especially during pregnancy. Overexpression of 15-LOX in particular is associated with enhanced lipoxin production (Serhan et al. 2003), and interestingly 15-LOX expression is detected in myometrial smooth muscle and blood vessels (Lei & Rao 1992). Other LOX enzymes including 5- and 12-LOX have also been reported in term uteroplacental tissues.
LOX enzymes are also detected in the theca and granulosa layers of rat pre-ovulatory ovarian follicles (Kurusu et al. 2009). Direct evidence for altered resolution mediator signalling in pregnancy is provided by a recent report that LXA4 levels are significantly elevated in serum from pregnant compared with non-pregnant women (Maldonado-Pérez et al. 2010).

A change in immune cell population and enzymes expressed at an inflammatory site may alter the predominant lipid mediator produced locally in a process termed class switching. Chemical structures of the main lipoxin species are shown. AA, arachidonic acid; Ac-PTGS2, aspirin-acylated PTGS2; PTGS2, prostaglandin-endoperoxide synthase 2; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ETE, eicosatetraenoic acid; HDHA, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HpDHA, hydroperoxydocosahexaenoic acid; HpEPE, hydroperoxyeicosapentaenoic acid; LOX, lipoxygenase; LTA4, leukotriene A4; PG, prostaglandin; PMNs, polymorphonuclear leukocytes.
Box 1 Transcellular synthesis of eicosanoids
Lipoxins are so named for the multiple lipoxygenation reactions involved in their synthesis (Serhan et al. 1984a). These are mediated by the major 5-, 12- and 15-lipoxygenases (LOX), numbered according to the position at which they oxygenate arachidonic acid (Serhan 2005). Lipoxygenases, together with PTGS2, are also involved in synthesis of the resolvins, protectins and maresins from docosahexaenoic acid (Norling & Serhan 2010). Endogenous lipoxin synthesis occurs through two main pathways. Both involve 5-LOX, which is expressed in circulating neutrophils, monocytes, eosinophils and tissue macrophages. 5-LOX has a well characterised role in the generation of leukotriene (LT) A4, precursor of both LTB4 and the cysteinyl leukotrienes (CysLT; Samuelsson et al. 1987). 5-LOX works in concert with 5-LOX activating protein (FLAP) that sequesters arachidonic acid to provide substrate, markedly enhancing the selective transfer of AA to 5-LOX (Mandal et al. 2004). Decreased FLAP activity may be involved in class switching from LT to lipoxin production (Titos et al. 2005).

12-LOX is expressed by platelets and monocytes, along with blood vessel smooth muscle and endothelial cells. Three isoforms exist, each with distinct expression patterns (Yoshimoto & Takahashi 2002). 12-LOX is up-regulated by angiotensin II and VEGF (Kim et al. 1995, 2009), with organ-specific down-regulation by oestradiol (Stygar et al. 2007). A role in lipoxin synthesis was confirmed through the demonstration that purified 12-LOX converts LTA4 into LXA4 and LXB4 (Romano et al. 1993).

15-LOX exists in two isoforms, termed 15-LOX-1 and 15-LOX-2. 15-LOX-1 activity is detected in resting monocytes, macrophages and dendritic cells and is up-regulated by the Th2 cytokines IL4 and IL13 (Nassar et al. 1994, Spanbroek et al. 2001) and by hypoxia (Liu et al. 2009). 15-LOX-2 expression is predominantly observed in epithelial tissues, but it is also up-regulated by macrophages in response to hypoxia (Ryderberg et al. 2004). 15-LOX metabolism of arachidonic acid generates 15S-hydroxyeicosatetraenoic acid (15S-HETE), which is rapidly converted by 5-LOX-expressing cells to lipoxins (Serhan et al. 1984a).

A third pathway for lipoxin synthesis is activated following administration of aspirin, due to its unique ability to acetylate prostaglandin-endoperoxide synthase 2 (PTGS2; also known as cyclooxygenase-2, COX-2). This modifies enzyme activity from production of prostaglandin precursors to 15R-HETE, which is subsequently converted by 5-LOX to the aspirin-triggered lipoxins, 15-epi-LXA4 and 15-epi-LXB4 (Claria & Serhan 1995). It is also possible that 15R-HETE generation by cytochrome P450 can lead to endogenous 15-epi-lipoxin production (Kalsotra et al. 2007). In addition, acetylated PTGS2 action on EHA or DHA is the first step in the generation of the E-series and 17R-aspirin-triggered resolvins D1,4 respectively (Norling & Serhan 2010).

LXA4 and LXB4 are rapidly (<30 s) inactivated by isolated monocytes through the sequential action of oxidoreductases including 15-hydroxyprostaglandin dehydrogenase (15-PGDH), but do not undergo substantial metabolism by PMNs (Maddox & Serhan 1996). Such differential control of metabolism reflects the particular resolution roles of each leukocyte type.

Glucocorticoids and ANXA1
ANXA1 was originally identified as the major effector protein of glucocorticoid-mediated anti-inflammation, discussed in Box 2. Variously termed lipocortin 1, macrocortin, lipomodulin, renocortin and calpactin 2, ANXA1 is an ~37-kDa member of the annexin family. Structurally it consists of a highly conserved core region consisting of four 70-amino acid annexin repeats containing calcium and phospholipid-binding sites, together with a unique 42-amino acid N-terminus (Weng et al. 1993). It exists in vivo in both full-length and various cleaved forms, many of which retain biological activity (Flower 1988). Calcium binding triggers a conformational change whereupon the normally buried N-terminus is displaced from the core, generating the active form of the protein (Rosengarth & Luecke 2003). ANXA1 shows a predominantly intracellular localisation under resting conditions (Spurr et al. 2010), but activation, for example by glucocorticoids, triggers mobilisation to the cell surface. There it may remain bound, in Ca2+-dependent fashion, or undergo release into the extracellular fluid (Perretti & D’Acquisto 2009).

ANXA1 expression is widespread including high levels in resting monocytes, macrophages and PMN, as well as in lymphocytes, endothelial cells, fibroblasts and in gut epithelial cells (Perretti & D’Acquisto 2009). Neutrophils in particular rapidly externalise ANXA1 following activation, e.g. by adhesion to endothelial cell monolayers or following inflammatory stimulation (Perretti et al. 1996, Gastardelo et al. 2009). Levels are notably high in reproductive tissues with levels in adult rabbit ovary and pregnant uterus higher than in immature and non-pregnant animals respectively (Tsao et al. 1995). ANXA1 is strongly expressed in human endometrial glandular epithelia as well as isolated stromal cells (Li et al. 2008). Expression is also observed in term amnionic epithelia and chorionic trophoblast with high levels evident in placental syncytiotrophoblast and placental tissue (Romisch et al. 1992, Sun et al. 1996).
Box 2  Glucocorticoids and ANXA1
The profound anti-inflammatory effects of exogenous glucocorticoids (GCs) have been known since 1949; however, it was not until 1984 that Munck et al. (1984) proposed endogenous GCs produced during the response to stress or injury functioned to protect against damage by inflammation. By this time it was clear that the majority of GC-mediated effects occurred via the ‘classical pathway’. Specifically, GC engagement of its cognate zinc finger transcription factor receptor leads to nuclear translocation, engagement of promoter GC response elements (GREs) and consequent transactivation or alternatively interaction with other transcription factors leading to transrepression activity (Flower 1988, Perretti & D’Acquisto 2009). Early research into the mechanisms of GC actions revealed that GCs act via this classical pathway to limit arachidonic acid release from cellular membranes, and hence prostaglandin production, in response to various pro-inflammatory stimuli. This led to the identification and eventual cloning of the major anti-inflammatory GC effector protein annexin A1 (ANXA1; Flower 1988).

GC responsiveness with respect to ANXA1 induction varies depending on cell type (Perretti & D’Acquisto 2009). Recent data also shows that GC induction of ANXA1 in cancer cells is correlated with the order of anti-inflammatory potency of the respective GCs (Zhang et al. 2010). GC stimulation of macrophages in vivo stimulates both release of existing stores of ANXA1 and de novo synthesis, with correspondingly biphasic kinetics (Flower 1988), while neutrophil ANXA1 levels are correlated with serum cortisol, suggesting GC regulation in vivo (Mulla et al. 2005).

Resolution receptors: one receptor to rule them all?

Early experiments examining renal function revealed a role for LXA₄ in antagonising cysteinyl leukotriene (CysLT)-mediated vasoconstriction, with competitive binding studies using [3H]leukotriene D₄ (LTD₄) indicating CysLT-mediated vasoconstriction, with competitive binding of this ligand–receptor pairing is unclear. Lastly, LXA₄ also binds the resolvin D1 (RVD1) receptor GPR32 (Schaldach et al. 2001). Following the molecular identification of the CysLT receptors it was confirmed that LXA₄ competes for binding at the LTD₄ CysLT1 receptor with equivalent sub-nanomolar affinity (Gronert et al. 1998), and further that LXA₄ can inhibit LTD₄-mediated PDGFRβ transactivation through action at CysLT2 receptor (McMahon et al. 2002). In addition to this common CysLT/LXA₄ receptor pathway, however, binding data suggested the presence of a separate specific high-affinity LXA₄ receptor on neutrophils (Fiore et al. 1993). This was subsequently cloned and identified as a GPCR related to the FPR. Later ALXR/FPR1/FPR2, as it was variously termed, was also confirmed as the major specific LXA₄ receptor on monocytes and also shown to bind ATL with equivalent nanomolar affinity (Maddox et al. 1997). Subsequent research has largely focused on the role of LXA₄ as an FPR2 ligand. In addition to these pathways, LXA₄ was shown to bind the aryl hydrocarbon receptor, inducing genes involved in xenobiotic metabolism (Schaldach et al. 1999); however, the in vivo relevance of this ligand–receptor pairing is unclear. Lastly, LXA₄ also binds the resolvin D1 (RVD1) receptor GPR32 (Krishnamoorthy et al. 2010).

In contrast to LXA₄, LXB₄ signalling is poorly understood and is apparently independent of FPR2 (Fiore et al. 1994). Limited data exists for the resolvins: RVD1 binds FPR2 with equal affinity to LXA₄ and to GPR32 with approximately fourfold higher affinity than LXA₄ (Krishnamoorthy et al. 2010), while resolvin E1 reportedly signals via both ChemR23 and the leukotriene B₄ receptor (BLT; Arita et al. 2007). Receptors for the remaining resolvins, the protectins and maresin are currently uncharacterised.

ANXA1 was shown to be responsible for glucocorticoid-mediated attenuation of inflammation-induced PG synthesis through inhibition of phospholipase A2 (PLA2) activity. This was initially assumed to be the primary mechanism of ANXA1 anti-inflammatory activity (Flower 1988). However, experiments demonstrating that exogenous ANXA1 mediated a potent and rapid inhibition of PMN infiltration in experimental models of inflammation revealed an additional mode of action (Perretti et al. 1993, Harris et al. 1995). These activities were effectively recapitulated using short peptides (Ac2-26 and Ac9-25) corresponding to the unique ANXA1 N-terminal sequence. Together with specific anti-inflammatory actions not shared with other annexin family members, this apparently precluded involvement of core domain PLA2 inhibition. It was demonstrated that the peptides signal instead through the FPR family (Walther et al. 2000). However, although the peptides can bind and activate FPR or FPR2 indiscriminately, full-length ANXA1 GPCR signalling is exclusively via FPR2 (Perretti et al. 2002, Hayhoe et al. 2006). Interestingly, short synthetic ‘antifla mmin’ peptides derived from the ANXA1 core domains, which also possess anti-inflammatory properties, reportedly bind and signal through FPR2 (Kamal et al. 2006). This would suggest that multiple protein domains cooperate to activate the receptor and potentially modulate signalling. Given the known rapid metabolism of ANXA1 by PMNs (Ernst et al. 2004), and the various cleavage products observed in vivo, this raises the possibility that regulation of ANXA1 cleavage could itself modulate signalling.
pathways activated in a spatially/temporally coordinated fashion. A new twist in the tale of ANXA1 signalling is the discovery that ANXA1 can directly associate with the p65 subunit of the ubiquitous transcription factor NF-kB, a key regulator of cellular proliferation and inflammatory gene expression (Zhang et al. 2010). This highlights alternative pathways through which ANXA1 can modulate the inflammatory process locally.

**Formyl peptide receptor 2**

As mentioned earlier, FPR2 can be activated by multiple ligands to mediate anti-inflammatory and pro-resolution activities at sites of inflammation. Human FPR2 expression is the best characterised in immune cells. It is present at high levels on the surface of resting PMNs and monocytes, with expression also observed on NK cells and at lower levels on T and B cells (Spurr et al. 2010). In addition, low levels are reported on HUVECs (Koczulla et al. 2003), and it is also observed on enterocytes under control of cytokines including interferon γ and interleukin (IL) 13 (Gronert et al. 1998), and on fibroblast-like synoviocytes (Sodin-Semrl et al. 2004). Notably, FPR2 expression is reported in endometrial stromal cells (Motohashi et al. 2005), and recently myocytes and infiltrated leukocytes in term myometrium with significant increase in labouring tissue (Maldonado-Pérez et al. 2010). Signalling through FPR2 is discussed in Box 3.

**Resolution mediator downstream effector functions**

The Fpr2−/− mouse phenotype suggests that the receptor may have either a driving or a limiting role in inflammation dependent on pathological context (see Box 3). FPR2 plays a limiting role in carrageenan-induced oedema and ischaemic-reperfusion injury (Dutton et al. 2010), whereas it contributes to Th2-driven allergic airway inflammation (Chen et al. 2010a). Meanwhile, administration of exogenous resolution mediators is protective in numerous in vivo models of pathological inflammation (Serhan et al. 2008, Perretti & D’Acquisto 2009). In vivo effects are reflected at the cellular level. In PMNs, LXA4 and ANXA1 both inhibit activation-induced L-selectin shedding and CD11b/CD18 adhesion receptor up-regulation, with corresponding inhibitory effects on PMN attachment and transendothelial/transepithelial migration, and on PMN–platelet adhesion (Strausbaugh & Rosen 2001, Filep et al. 2005, Hayhoe et al. 2006). Intriguingly, additive effects were reported for LXA4 and the glucocorticoid dexamethasone (Filep et al. 1999), suggesting possible non-redundant signalling of lipoxins and glucocorticoid-induced ANXA1. This has implications for therapeutic targeting of FPR2. High nanomolar levels of ANXA1 accelerate PMN apoptosis (Solito et al. 2003), a trait apparently not shared by lipoxins. However, the latter are able to override delay of apoptosis induced by the inflammatory protein serum amyloid A (El Kebir et al. 2007).

**Box 3 FPR2 pleiotropy and intracellular signalling pathways: inflammation integration**

The central role of FPR2 in potentiating the anti-inflammatory effects of lipoxins and annexin A1 was confirmed in the Fpr2−/− knockout mouse, where the inhibitory effects of these ligands on IL1β- and zymosan-induced PMN migration was either abrogated or greatly reduced (Dutton et al. 2010). However, our understanding of signalling through FPR2 is complicated by its pleiotropic nature as a receptor and the promiscuity of the ligands that can activate it. FPR2 binds multiple ligands and is capable of transducing both pro- and anti-inflammatory signals. Furthermore, many studies on FPR2 signalling have used artificial ligands (MMK-1, WKYMVm and others) whose relevance to the in vivo situation is unclear. The use of assumed antagonists with overlapping specificity for FPR family members (Stenfeldt et al. 2007), and the difficulty in distinguishing true antagonists from potential anti-inflammatory/pro-resolution effectors means much early work requires cautious interpretation. As well as the resolution mediators discussed in the text, the acute phase protein serum amyloid A (SAA) and the neutrophil granule-derived antimicrobial peptide LL-37 each bind to FPR2 to elicit pro-inflammatory responses including neutrophil chemotaxis and IL8 secretion (De et al. 2000, He et al. 2003).

Lipoxin signalling in PMNs and monocytes appears to be pertussis toxin (PTX)-sensitive indicating Gi/o coupling, however, PTX-sensitive and PTX-insensitive pathways are reported following FPR2 activation by SAA (Lee et al. 2010). Reports examining intracellular Ca2+ signalling following FPR2 stimulation paint a confusing picture due to the variety of ligands, dosages and cell-types employed. A broad trend may be observed where resolution ligand stimulations at moderate but still biologically active concentrations tends towards limited or absent Ca2+ influx (Migeotte et al. 2006), with larger and more sustained responses observed with higher concentrations or with pro-inflammatory ligands (Forsman & Dahlgren 2010). Notably, certain key resolution mediator functions such as ANXA1-induced neutrophil apoptosis are Ca2+-dependent (Solito et al. 2003). Downstream intracellular effectors implicated in FPR2 signalling are appropriately diverse and include phospholipase A2, phospholipase D, ERK1/2, JNK, SAPK2/p38 and tyrosine kinases (Migeotte et al. 2006).
In contrast to their inhibitory effects on PMNs, lipoxins and ANXA1 promote monocyte adherence and chemotaxis (Maddox & Serhan 1996, Ernst et al. 2004). In addition, 15-epi-LXA₄ inhibits monocyte apoptosis triggered by serum starving (Simoes et al. 2010), suggesting that resolution mediators could regulate monocyte/macrophage function at later stages of the resolution process. Removal of extravasated neutrophils is a key requirement of resolution and accordingly lipoxins, glucocorticoids and ANXA1 all stimulate monocytes to phagocyte apoptotic neutrophils, crucially without inducing concomitant production of pro-inflammatory mediators (Liu et al. 1999, Godson et al. 2000, Scannell et al. 2007). Instead, phagocytosis is accompanied by enhanced secretion of the anti-inflammatory cytokine TGFβ, further indicating that the effect of resolution mediators on monocytes/macrophages is to promote active, non-phlogistic clearance.

Lipoxins are able to influence endothelial cell functions, through both CysLT receptor antagonism and direct FPR2 stimulation (Gronert et al. 2001). Endothelial cell modulation of leukocyte trafficking is regulated by lipoxins. Exposure of HUVEC monolayers to LXA₄ abolished subsequent LTB₄-mediated PMN hyperadhesiveness, despite LXA₄ having no effect on baseline or LPS/TNF-α-stimulated expression of the adhesion molecule ICAM-1 (Filep et al. 1999). Endothelial cells are also implicated in endothelial and inducible nitric oxide synthase-dependent increase in plasma nitric oxide following ATL treatment, which inhibited leukocyte trafficking in IL1β-stimulated murine peritonitis (Paul-Clark et al. 2004). In addition, resolution mediators are able to modulate blood flow at inflammatory sites. Lipoxins stimulate endothelial production of the vasodilator and inhibitor of platelet activation PGJ₂ (Brezinski et al. 1989), as well attenuating production and signalling of the potent pro-inflammatory vasoconstrictor endothelin-1 in a zymosan-induced murine arthritis model (Conte et al. 2010). Importantly, LXA₄ can also regulate angiogenesis by inhibiting vascular endothelial growth factor (VEGF)- and LTD₄-stimulated endothelial cell proliferation (Baker et al. 2009).

Expression of pro-inflammatory mediators is also down-regulated by resolution mediators. Data from the Anxa1⁻/⁻ mouse reveal an inhibitory role for ANXA1 in inflammatory IL1β production (Hannon et al. 2003), although synovial TNF-α is reduced by ANXA1 immunoablation in rat adjuvant-induced arthritis (Yang et al. 1999). LXA₄ analogues have been shown to reduce PMN peroxynitrite-induced NF-κB- and AP-mediated gene expression, including that of the key chemokine IL8 (El Kebir et al. 2007). LXA₄ inhibited ~60 of 125 genes up-regulated by Salmonella typhimurium in model epithelia including a subset regulated via NF-κB. However, no effect was observed upon exposure to LXA₄ in the absence of inflammatory stimulation, suggesting resolution mediator action on epithelial cells is predominantly through inhibition of pro-inflammatory pathways (Gewirtz et al. 2002). Resolution mediators also promote clearance of existing pro-inflammatory ligands. LXA₄, as well as resolvin E1 and protectin D1, enhance CCR5 expression on apoptotic neutrophils leading to sequestration of the chemokine ligands CCL3 and CCL5 (Ariel et al. 2006).

Resolution mediators can also influence tissue remodelling. In addition to the anti-angiogenic effects discussed, they have been shown to regulate the production and actions of matrix metalloproteinases (MMPs). LXA₄ inhibited IL1β-induced MMP3 synthesis in synovial fibroblasts with concurrent up-regulation of the counter-regulatory molecules tissue inhibitor of metalloproteinase-1 and -2 (Sodin-Semrl et al. 2000). However, ANXA1 was shown to stimulate secretion of MMP1 in the same cell type suggesting that individual mediators might have functionally distinct roles in tissue remodelling (Tagoe et al. 2008).

Comparatively little is known about effector functions of resolution mediators other than LXA₄ and ANXA1. However, similar modulatory actions on PMN and monocyte migration and adhesion to those described have been noted for LXB₄ (Maddox et al. 1998), as well as for the resolvins, protectins and maresins (Norling & Serhan 2010). ω-3 PUFAs-derived eicosanoids are also observed to inhibit pro-inflammatory cytokine production, while protectins in particular may be involved in establishing the T cell Th2 phenotype (Ariel & Serhan 2007).

Resolution pathways in the ovary

It is well established that ovulation, subsequent ovarian repair and remodelling associated with corpus luteum formation resemble an acute inflammatory response (Jabbour et al. 2009). Pathways activated display extensive overlap with those regulated by resolution mediators including vascular changes, cytokine production, immune cell migration, apoptosis and ECM breakdown and repair. Both cyclooxygenase and LOX inhibition have been shown to suppress ovulation in the rat via inhibition of the LXA₄ inducer PGE₂, and LOX expression in rat ovaries is enhanced by hCG induction of ovulation (Gaytan et al. 2006, Kuruś et al. 2009). More direct evidence for ovulation-associated resolution mediator production is provided by a peak in ovarian levels of the lipoxin precursor 15-HETE (Tanaka et al. 1989). However, rather than a role in ovulation per se it is more probable that mediators are involved in post-ovulatory healing and tissue remodelling. Elevated glucocorticoid concentrations observed in pre-ovulatory follicular fluid have been proposed to contribute to these processes (Andersen 2002). This is probably to be mediated at least in part by the highly expressed glucocorticoid effector ANXA1. Resolution signalling could act to terminate ovulation-associated extracellular
matrix breakdown by MMPs, levels of which decrease following a peak at ovulation (Curry et al. 1992). Resolution mediators may also contribute to the unusual scarless healing observed in post-ovulatory surface epithelia (as well as post-menstrual endometrium). The resolution mediator target VEGF contributes to fibrosis while LXA₄ has been directly shown to inhibit profibrotic actions of PDGF (Rodgers et al. 2005).

Intriguingly, temporal regulation of leukocyte subset migration into the ovary recalls the selective properties of resolution mediators in inflammatory responses. Initial influx of neutrophils at ovulation is followed by a delayed monocyte chemotaxis, while a further selective increase in monocyte/macrophage number is observed at luteolysis (Brannstrom et al. 1994). In addition, luteal macrophages display a pro-resolution M2-type phenotype characterised by phagocytic activity and Th2 cytokine production (Hashii et al. 1998). Macrophages have been proposed as causal in the demise of the corpus luteum, being capable of producing many pro-apoptotic factors including TNF-α. Indeed, macrophage ablation by splenectomy in pseudopregnant rabbits delays luteolysis. However, this model also reveals a role for macrophages in stimulating luteal progesterone production (Endo & Kanayama 1998). The potential for ANXA1 and LXA₄ to influence these processes is illustrated by the abilities of dexamethasone and PGE₂ to delay luteal regression in various animal models (Wang et al. 1993, Vanderwall et al. 1994). Thus, the balance between inflammation and resolution may be central to macrophage regulation of luteal cell survival and function. It may also ensure rapid clearance of apoptotic cells at luteolysis to prevent excessive inflammation due to secondary necrosis, which could otherwise damage surrounding ovarian tissue.

Resolution pathways in the uterus

**Menstrual function**

As in the ovary, inflammatory regulation appears to be central to uterine physiological reproductive events. Proliferation, subsequent differentiation and maintenance of the endometrial functionalis are under sequential control of oestrogen and progesterone, combining to create a ‘receptivity window’ for implantation following ovulation. In the absence of fertilisation, withdrawal of hormonal support following demise of the corpus luteum triggers menstruation. Characteristic features include local cytokine and chemokine up-regulation, inflammatory prostanoid production, leukocyte recruitment and activation, vascular changes and MMP production which together promote disintegration and shedding of the functional layer with associated blood loss (reviewed in Maybin et al. (2010)). Resolution mediators have the potential to regulate these events and promote the transition to endometrial repair. LOX metabolites are among the most abundant AA derivatives present in menstrual blood and include 15-HETE (Hofer et al. 1993). Importantly, ANXA1 secretion is detected from cultured human endometrial samples and can be inhibited by progesterone (Gurpide et al. 1986). Hence, hormonal withdrawal at menses could trigger ANXA1 release together with LXA₄ production, associated with enhanced LOX activity. The role of resolution mediators in restoration of endometrial tissue homoeostasis leading to scarless healing following menstruation is currently unknown. However, post-menstrual repair is delayed following neutrophil depletion in a mouse model revealing a role for these cells in resolution potentially via LOX enzyme expression (Kaitu’u-Lino et al. 2007).

Dysregulation of resolution pathways could contribute to menstrual pathologies. Cortisol inhibits angiogenesis in endometrial explants, and levels of the cortisol-inactivating enzyme 11β-hydroxysteroid dehydrogenase-2 are elevated in women with menorrhagia (Rae et al. 2009). This suggests perimenstrual ANXA1 levels may also be decreased in this disorder and contribute to aberrant angiogenesis. ANXA1 regulation of PTGS2 activity could also influence elevated endometrial PG levels associated with menorrhagia as well as primary dysmenorrhoea (Maybin et al. 2010). PGE₂-induced LXA₄ production could plausibly contribute to the reduction in VEGF observed in menorrhagia patients, which alongside the beneficial anti-angiogenic effects of cortisol suggests that precise temporal regulation of tissue repair factors by resolution mediators could be critical to an ordered transition from menstruation to the endometrial proliferative phase.

There is also evidence implicating resolution mediator dysregulation in the pathogenesis of endometriosis. This disorder is characterised by extraterrine proliferation of endometrial tissue, and though the causes remain unclear numerous inflammatory mediators and pathways show altered expression patterns. Proteomics studies reveal ANXA1 overexpression in glandular epithelia of eutopic endometrium from endometriosis patients versus healthy controls (Li et al. 2008), and further in ectopic versus eutopic endometriosis samples (Meola et al. 2010). Full-length ANXA1 protein was also detected in peritoneal fluid samples from endometriosis patients, while the receptor FPR2 was also up-regulated in ectopic endometriosis samples (Motohashi et al. 2005). Direct evidence for the therapeutic potential of resolution mediators is provided by the demonstration that endometriosis progression in a mouse model was inhibited by exogenous ATL administration (Chen et al. 2009). This was accompanied by decreased pro-inflammatory cytokine formation as well as reduced lesion production of MMP2 and MMP9 (Chen et al. 2010b). The effects of lipoxins on endothelial cell function may also be relevant, since angiogenesis is a known feature of endometriosis.
Together, these data on lipoxins and ANXA1 in endometriosis reveal resolution mediator signalling capability in this pathology.

**Establishment of pregnancy**

Establishment and maintenance of pregnancy may also involve pro-resolution pathways. Successful implantation requires carefully regulated local expression of numerous pro- and anti-inflammatory cytokines, chemokines, leukocyte adhesion molecules and angiogenic factors. Together, these mediate blastocyst attachment, endometrial decidualisation and placentation including vascular remodelling (reviewed in van Mourik et al. 2009). Numerous resolution pathway targets are involved in these processes including VEGF, IL1, IL6, IL10, TGFβ, TNF-α, MMPs and others. Lipoxins are implicated in a mouse model of implantation. Uterine 12- and 15-HETE levels in wild-type animals showed a sharp peak coincident with implantation, with LOX activity, particularly 12/15-LOX isoforms, crucial to this process (Li et al. 2004). LOX metabolites including 15-HETE were demonstrated to act via the NF-κB inhibitor PPAR-γ, a known LXA₄ target whose neuroprotective effects upon agonist stimulation are themselves mediated by LXA₄ (Weinberger et al. 2008, Sobrado et al. 2009).

Leukocytes are central regulators of pregnancy establishment and maintenance, uNKs in particular may be critical determinants of menstruation versus decidualisation. As well as regulating endometrial angiogenesis, uNKs are thought to modulate trophoblast invasion and immune response to the foetal allograft. LXA₄ inhibits peripheral NK cell polarisation and cytotoxicity (Ramstedt et al. 1987), but whether uNKs like their peripheral counterparts express FPR2 is unknown. Following placentation uNK cell numbers decline from ~70% of total decidual leukocytes during the first trimester to only 3% at term, while macrophage numbers remain relatively constant (at least 10%) throughout pregnancy until labour. These cells are thought to play a role in the systemic shift towards immunosuppression and tolerance observed during pregnancy, which may be an epiphenomenon reflecting local uterine cytokine signalling. In this context, the elevated LXA₄ levels described in pregnant serum may well result from significant local uterine production (Maldonado-Pérez et al. 2010). IL4, IL13 and IL10 expression at the maternal–foetal interface reflects the M2 gene expression profile of first trimester decidual macrophages (Gustafsson et al. 2008). These cells also produce the LXA₄ inducer PGE₂, that acts to inhibit leukocyte activation (Parhar et al. 1989). ANXA1 levels also increase in uterine tissue with pregnancy (Romisch et al. 1992, Sun et al. 1996). However, the functional role of ANXA1 in pregnancy remains to be elucidated. It is tempting to speculate that this contributes to the macrophage M2 phenotype, perhaps via a functional role in apoptosis induction and clearance in decidual tissue that is required to accommodate the developing foetus. This hypothesis is supported by the demonstration that glucocorticoids induce apoptosis in third trimester decidual explants, including cortisol via conversion to active cortisol by up-regulated decidual 11β-HSD1 (Chan et al. 2007).

Deficiencies in spiral artery remodelling during placentation may contribute to development of pre-eclampsia, a disorder associated with inflammatory activation and cardiovascular dysfunction. In particular, pre-eclampsia is associated with a maternal Th1/M1 phenotype, with decreased placental and serum IL10 and increased monocyte production of pro-inflammatory cytokines including IL1β, IL6 and IL8 compared with normal pregnancies (Laresgoiti-Servitje et al. 2010). It is plausible that dysregulated production/action of resolution mediators can contribute to pre-eclampsia development and progression. This is highlighted by a recent study demonstrating that enhanced IL1β production by monocytes from pre-eclamptic patients can be inhibited in vitro by LXA₄ treatment (Wang et al. 2010).

**Labour**

Unlike the anti-inflammatory uterine environment observed through most of pregnancy, labour is accompanied by marked influx of PMNs and macrophages into the cervix, myometrium, decidua, placenta and foetal membranes. This corresponds with widespread up-regulation of pro-inflammatory cytokines, PG, vascular adhesion molecules, proteases and smooth muscle activity leading to cervical ripening, membrane rupture and myometrial contraction. Platelet P-selectin expression and adherence to peripheral monocytes are increased during the third trimester of pregnancy (Robb et al. 2010). Circulating leukocytes also increase their expression of adhesion and activation markers including CD11b and L-selectin during labour itself (Yuan et al. 2009), with infiltrating cells showing a similar activated phenotype. Thus, it appears that central to the process of labour is an immune switch, from anti-inflammatory pathways actively maintaining uterine quiescence towards local and systemic pro-inflammatory activation. A further increase in serum LXA₄ levels observed as term approaches (Maldonado-Pérez et al. 2010) may therefore represent a homoeostatic adjustment maintaining uterine quiescence in the face of increased inflammatory activation. This would ultimately be overwhelmed by the inflammatory cascade, ensuring rapid and complete parturition following eventual, irreversible onset of labour. Pro-resolution pathways activated as part of the inflammatory response during labour are also highly likely to contribute to post partum recovery through
roles in immune cell down-regulation and tissue clearance.

Inflammation, either idiopathic or infection induced, may act to trigger the premature onset of labour and this may in part result from dysregulated action of resolution molecules (Jabbour et al. 2009). LXA₄ treatment inhibits pro-inflammatory cytokine production by term myometrial explants in response to LPS (Maldonado-Pérez et al. 2010). Levels of resolution mediators may themselves be reduced during labour. A decrease in 15-LOX myometrial smooth muscle expression is observed at labour (Lei & Rao 1992), concomitant with elevated 5-LOX/ decreased 5-LOX activating protein (FLAP; Brown et al. 1999). Such alterations in LOX activity suggest possible class switching from LXA₄ to LT production. Interestingly, comparison between term Caesarian and labouring samples reveals a decrease in ANXA1 expression in amnion and placenta at parturition (Lynch-Salamon et al. 1992, Myatt et al. 1992, Bennett et al. 1994). Together, these data are supportive of a decrease in resolution signalling associated with the onset of labour.

Resolution pathways as therapeutic targets in the female reproductive tract

The rationale behind many non-steroidal anti-inflammatory therapies is based on our understanding of the early, amplifying events of inflammation. Consequently, these therapies have focused on disrupting the machinery of pro-inflammation. Recent advances in the field of resolution challenge this approach, revealing a network of endogenous pathways that actively terminate the inflammation programme and restore tissue homoeostasis. Inflammatory processes affected by these specialised pro-resolution mediators are known to be important in reproductive events and pathologies, namely acute vascular changes, leukocyte infiltration and activation, production of cytokines, chemokines and lipid mediators and tissue remodelling events.

Therapeutic targeting of resolution mechanisms has focused on developing drugs that mimic resolution mediator actions (Burli et al. 2006). This is based on the assumption that direct activation of resolution processes will avoid side effects associated with glucocorticoids and aspirin, and give enhanced potency compared with conventional NSAIDs which may themselves disrupt pro-resolution pathways (Perretti & Dalli 2009). Accordingly, stable LXA₄ analogues, peptide FPR2 ligands and cleavage-resistant ANXA1 mutants have been developed (Duffy & Guiry 2010, Pederzoli-Ribeil et al. 2010). Multiple overlapping yet distinct pathways with respect to ligand-receptor recognition and signalling among resolution mediators suggest tissue specificity and therapeutic function could be tailored. For example, ANXA1 mimetics can be generated that selectively activate FPR2 or directly inhibit NF-κB activity. This may in turn allow for actions relating to anti-inflammation or resolution to be selectively targeted. The potential for inter-regulation between different resolution mediators is also relevant, for example corticosteroids are able to reduce LXA₄ production (Bhavsar et al. 2010). PLA2 inhibition by ANXA1 could itself influence generation of pro-resolution eicosanoids.

Key questions relevant to female reproductive functions concern the significance of resolution pathways in the cyclical scarless tissue repair observed in the female reproductive tract. In addition, female reproduction is regulated by steroid hormones and the influence of these on resolution molecule production and action needs to be clarified. Progesterone in particular displays anti-inflammatory and immunomodulatory properties reminiscent of known resolution mediator functions (Jabbour et al. 2009). Better characterisation of resolution mediator production, metabolism and receptor expression within the reproductive tract is required to pinpoint possible opportunities for intervention in conditions including menstrual disorders, endometriosis, recurrent miscarriage, pre-eclampsia and pre-term labour.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References


Strausbaugh HJ & Rosen SD 2001 A potential role for annexin 1 as a physiologic mediator of glucocorticoid-induced L-selectin shedding from myeloid cells. Journal of Immunology 166 6294–6300.


Walther A, Riehemann K & Gerke V 2000 A novel ligand of the formyl peptide receptor: annexin 1 regulates neutrophil extravasation by interacting with the FPR. Molecular Cell 5 831–840. (doi:10.1016/S1097-2765(00)08023-8)


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