The role of Lipoxin A_4 in endometrial biology and endometriosis

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Lipoxin A_4 (LXA_4), an endogenous anti-inflammatory and immunomodulatory mediator studied in many disease states, is recently appreciated as a potentially significant player in the endometrium. This eicosanoid, synthesized from arachidonic acid via the action of lipoxygenase enzymes, is likely regulated in endometrial tissue during the menstrual cycle. Recent studies revealed that LXA_4 acts as an estrogen receptor agonist in endometrial epithelial cells, antagonizing some estrogen-mediated activities in a manner similar to the weak estrogen estriol, with which it shares structural similarity. LXA_4 may also be an anti-inflammatory molecule in the endometrium, though its precise function in various physiological and pathological scenarios remains to be determined. The expression patterns for LXA_4 and its receptor in the female reproductive tract suggest a role in pregnancy. The present review provides an oversight of its known and putative roles in the context of immuno-endocrine crosstalk. Endometriosis, a common inflammatory condition and a major cause of infertility and pain, is currently treated by surgery or anti-hormone therapies that are contraceptive and associated with undesirable side effects. LXA_4 may represent a potential therapeutic and further research to elucidate its function in endometrial tissue and the peritoneal cavity will undoubtedly provide valuable insights.

INTRODUCTION

The female reproductive tract maintains an immune surveillance system similar to other mucosal surfaces, serving as the front line against pathogens. The uterus is unique given its roles in the transport of male gametes and processing of seminal antigens. Immune crosstalk appears vital to the success of pregnancy and for tolerance of the fetal semi-allograft during implantation and throughout gestation.[1] In addition, in menstruating species such as humans and most primates, the cyclic shedding of the upper two-thirds of the endometrial surface requires rapid healing and regeneration while maintaining those defenses and minimizing inflammatory responses.[2]

Ovulation, menstruation, implantation, and parturition all represent short-term inflammatory events[3] limited by endogenous mediators that facilitate resolution of inflammation. Health is maintained by the balance between inflammation and metabolic and immune homeostasis, especially important at mucosal surfaces such as the female reproductive tract. A disequilibrium in the inflammatory response to disease underlies many immune-mediated illnesses.[4–8] Lipoxins (LXs), as well as the more recently discovered Resolvins and Protectins, are specialized pro-resolving mediators essential for the resolution of inflammation[9] In this review, we focus on a molecule likely central to this balancing act in endometrial tissue, Lipoxin A_4 (LXA_4).

LXA_4 has been implicated as an anti-inflammatory mediator in human cycling endometrium and following parturition.[10,11] The significance of LXA_4 in normal endometrial physiology is difficult to gauge given the complexities of its signaling via different receptors with varying roles in multiple cell types as well as the paucity of published data concerning its function (**Figure 1**). As an immune modulator, LXA_4 has been shown in other systems to inhibit leukocyte migration,[12] leukotriene-induced responses, including vasoconstriction and chemotactic responses,[13,14] and mitogenic signals.[15] Based on recent studies, LXA_4 and related mediators are likely to contribute to endometrial biology serving as a fulcrum between opposing forces to help maintain the balance required for tissue repair/wound healing during menstruation, tolerance toward the nascent embryonic fetal allograft, maintenance of pregnancy, and the initiation and resolution of parturition. Additionally, as attenuated LXA_4 production may contribute directly to many inflammatory conditions and chronic disease states,[16–20]

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dysregulation of LXA_4 actions may significantly impact endometrial health and reproductive function.

LIPOXIN A_4 BIOSYNTHESIS AND LIPOXYGENASE METABOLITES

In humans, the major LX biosynthetic pathways involve biosynthesis during specific cell:cell interactions and upon priming by cytokines[21,22] in the vasculature and at mucosal boundaries, such as the endometrium. LX production occurs in a transcellular manner at sites of inflammation involving two different cell types such as epithelial cells and neutrophils, for example. Three human lipoxygenase (LOX) enzymes, iron-containing enzymes that catalyze the hydroperoxidation of polyunsaturated fatty acids, have been cloned: 5-LOX, 12-LOX, and 15-LOX.[23,24] The sequential oxygenation of arachidonic acid results in LX formation. Aspirin triggers the generation of epimeric forms of LXs known as aspirin-triggered LXs, such as 15-epi-LXA_4,[25] an attribute also shared by statins.[26,27] 15-ALOX type 2, which exhibits a substrate preference for arachidonic acid converting it to 15S-hydroperoxyeicosatetraenoic acid (15S-HETE),[24] is expressed in human endometrium.[28] However, human 15-LOX isoforms exhibit allosteric product regulation,[29] and the functional significance of feedback loops *in vivo* merits further investigation.

Interleukin 13 (IL-13), is a potent inducer of 15-LOX gene expression and enzyme activity in human monocytes,[30] requiring ERK1/2 MAPK (extracellular signal–regulated kinase 1/2/ mitogen-activated protein kinase) activation.[31] IL-13 was also shown to induce the mouse homolog 12/15-LOX in monocytes while IFN-γ (interferon gamma) inhibited expression and activity of this enzyme. IL-4 also induced 12/15-LOX in mouse myeloid cells.[32] Both IL-4 and IL-13 are involved in differentiation of monocytes into M2 macrophages, which exhibit an immunosuppressive phenotype when compared with M1 macrophages.[33]

12/15-LOX is a progesterone target gene in the mouse uterus, based on the progesterone receptor knockout mouse model.[34] There is precedent for hormonal regulation of this enzyme in mucosal tissues, such as the cornea, where 17β-estradiol (E2) downregulates 15-LOX as well as LXA_4 formation.[35] In prostate cancer cells, glucocorticoids inhibit this enzyme,[36] and in vascular smooth muscle cells aldosterone stimulates 12/15-LOX expression.[37] In prostate epithelial cells, peroxisome proliferator–activated receptor-gamma (PPAR-γ) interacts with the orphan receptor ROR-α to bind the 15-LOX promoter, providing a novel negative feedback mechanism for 15-LOX and therefore LXA_4 production.[38] It remains to be seen whether such regulatory mechanisms are germane to the human endometrium.

LXA_4 levels are high at the end of the menstrual cycle and then decline after menses.[10] That is, coincidently, the time when IL-13 peaks during the normal menstrual cycle.[39] As previously noted, estrogen-mediated inhibition of this enzyme[35] would fit well with the observation that LXA_4 levels decline during the proliferative phase coincident with a rise in E2.[10] The increase in LXA_4 levels during pregnancy is likely due to human chorionic gonadotropin, which promotes LXA_4 release in the decidua of human endometrium.[10]

LOX metabolites have been implicated in reproductive function for over three decades. One study in mice provided direct evidence of the importance of LOX metabolites during implantation.[34] Using conditional PR knockout mice it was established that leukocyte and epidermal 12/15-LOX were downstream targets of PR in uterine surface epithelium. At implantation, maximal induction of both 12/15-LOX enzymes was observed, with a parallel increase in the eicosanoid metabolites 12-HETE, 15-HETE, and 13-HODE (13-(S)-hydroxyoctadecadienoic acid) in the uterus. Furthermore, leukocyte 12/15-LOX null mice exhibited impaired implantation and usage of a 12/15-LOX inhibitor confirmed these results, leading to a significant reduction in implantation sites. 12-HETE, 15-HETE, and 13-HODE activated PPAR-γ in cell-based assays, and Rosiglitazone, a PPAR-γ agonist, reversed the ability of a LOX inhibitor to inhibit implantation. This was the first demonstration that progesterone-induced synthesis of lipid mediators derived from 12/15-LOX activity activated PPAR-γ and associated signaling pathways, serving to regulate implantation in the mouse. Indeed, LOX inhibitors have been shown to indirectly reduce progesterone output in pregnancy.[40] It will be instructive to determine the relative roles of these intermediate metabolites and whether LXA_4 is also involved.

LXA_4 RECEPTORS

Of the LX family members, LXA_4 is the best characterized. LXA_4 inhibits immune cell recruitment, chemotaxis, adhesion, and transmigration, also attenuating pro-inflammatory cytokine production and promoting resolution of inflammation, thereby serving as an important brake after injury or cellular insult *in vitro* and *in vivo*. LXA_4 modulates the function of both myeloid and non-myeloid cell types.[41,42] This anti-inflammatory mediator appears promiscuous in its ability to bind and/or activate a number of both nuclear as well as membrane-bound receptors. LXA_4 directly or indirectly activates various receptors, including a subclass of peptide receptors (CysLTs (cysteinyl leukotrienes)),[15] as well as the G-protein-coupled receptor 32.[43] LXA_4 receptors also include formyl peptide receptor 2/LX A_4 receptor (FPR2/ALX), another surface membrane G-protein-coupled receptor with diverse ligands,[44,45] the aryl hydrocarbon receptor (AhR), a ligand-activated nuclear transcription factor,[46] and more recently, estrogen receptor-alpha (ERα).[28] Expression dynamics of the latter three receptors and LXA_4 itself in endometrial tissue are depicted in **Figure 2**.

FPR2/ALX, the most studied receptor, to which LXA_4 binds with high affinity,[44,45] is expressed by many different cell types, including neutrophils, monocytes, natural killer cells as well as epithelial cells, where its expression is subject to differential regulation by cytokines.[23,47] A recent study on the molecular regulation of FPR2/ALX reported that although monocytes expressed this receptor, their differentiation abrogated its expression, due to translation silencing.[48] These results suggest that FPR2/ALX is of limited relevance in tissue macrophage

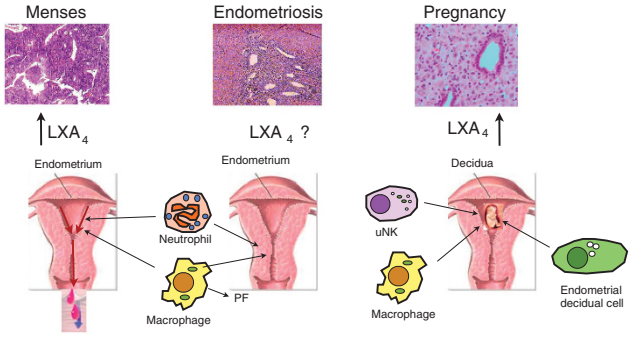

Figure 1 Lipoxin A_4 (LXA_4)-mediated actions in the endometrium at menses and in pregnancy on epithelial and stromal cells as well as on various immune cells of the innate arm. During menses, neutrophils and other immune cells are recruited just before menstruation and are normally cleared with debris, likely by macrophage-mediated efferocytosis, as a new endometrial layer forms. LXA_4 is produced via transcellular biosynthesis and 15-lipoxygenase is regulated by progesterone. In pregnancy, LXA_4 and its receptor appear to be upregulated, especially in the decidua, a putative function of LXA_4 in this environment would be to modulate macrophage activity and tissue remodeling. Elevated serum levels during gestation may fulfill immunomodulatory roles. In endometriosis (middle panel), characterized by excessive estrogen production and progesterone resistance, LXA_4 levels have not been studied. In this setting, LXA_4 biosynthesis is possibly decreased leading to a defect in the resolution of inflammation or alternatively, due to the inflammatory nature of this condition, may be overexpressed. The functional significance of LXA_4 in eutopic and ectopic endometrial tissue, as well as in the peritoneal fluid (PF), remains to be clarified. uNK, uterine natural killer cell.

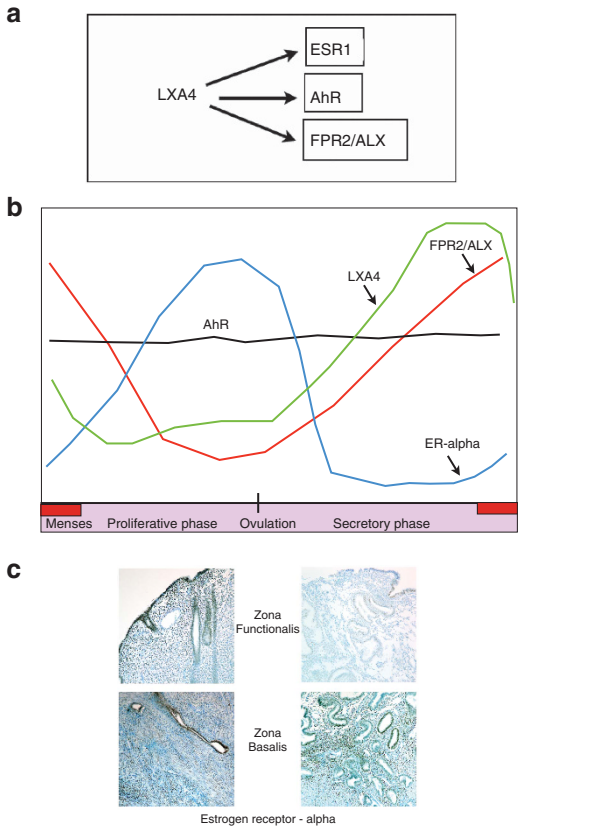

Figure 2 Serum LXA_4 levels and receptor expression dynamics in endometrial tissue. (**a**) Lipoxin A_4 (LXA_4) activates multiple receptors and has been shown to bind three present in the endometrium; formyl peptide receptor 2/lipoxin A_4 receptor (FPR2/ALX), a G-protein-coupled receptor, aryl hydrocarbon receptor (AhR), a nuclear receptor, and estrogen receptor-alpha (ESR1), which can exist in nuclear and membranous forms. (**b**) Each receptor appears to be differentially regulated, with FPR2/ALX expressed with similar temporal dynamics to LXA_4. Serum LXA_4 levels have been reported (depicted by a green line), but no data describing endometrial tissue levels have been published. LXA_4 also binds to estrogen receptor-alpha (ESR1), which increases in the proliferative phase in response to estrogen and later falls due to progesterone. AhR is constitutively expressed throughout the menstrual cycle, but its functional significance is unclear. (**c**) Immunohistochemical analyses of ESR1 expression during the secretory phase of the menstrual cycle in both the zona functionalis (left upper panel) as well as the zona basalis (left lower panel). ESR1 is downregulated in the functionalis layer (right upper panel) but maintained in the zona basalis (right lower panel). At menses, when estradiol levels are at their nadir, LXA_4 could target the nascent endometrial epithelium and stroma through ESR1 to promote early proliferation and repair. Studies on both humans and primates have provided evidence for cell division in this layer, despite the low endogenous estrogen levels. Thus, LXA_4 may serve as an early signal for endometrial regeneration and renewal.

function, but further studies are required to delineate the functional significance of this observation. Recently, expression of FPR2/ALX in human endometrium has been described.[10] MacDonald *et al.*[10] found that FPR2/ALX mRNA expression is increased during the menstrual phase compared with proliferative, early- and mid-secretory phase endometrium. As the endometrium has an upper (zona functionalis) and lower regenerative layer (zona basalis), FPR2/ALX receptor localization was found to be highest in the functionalis layer, localized to glandular epithelial and stromal cells, as well as the cells lining the vasculature and associated immune cells. We observed a smiliar expression pattern (G. Canny, unpublished data). In the first trimester of pregnancy, the decidua also exhibits high FPR2/ALX expression. Although LXA_4 levels did not change in the peripheral circulation across the menstrual cycle, serum levels were increased during early pregnancy, coincident with elevated FPR2/ALX levels.

Another physiologically relevant receptor for LXA_4 is AhR, which LXA_4 was shown to activate in murine hepatoma cells[46] and murine dendritic cells.[49] Interestingly, AhR has a role in immunity, with AhR-regulated genes being modulated by environmental toxins and pro-inflammatory cytokines.[50,51] AhR-null mice succumb significantly faster to experimental toxoplasmosis than wild-type mice and displayed greater degrees of liver damage as well as augmented serum levels of tumor necrosis factor-alpha (TNF-α), nitric oxide, and IgE but lower IL-10 production.[52] Hematopoietic defects are also observed in $AhR^{-/-}$ mice and, though classically considered important in mediating responses to environmental toxins, AhR is increasingly thought to be involved in immune responses.[53] Of note, cyclo-oxygenase 2 (COX-2), the rate limiting enzyme in prostaglandin E_2 (PGE_2) production is inducible by AhR ligands in various cell types.[50] Although AhR is present in the human endometrium, where its expression remained constant across the menstrual cycle,[54] there have so

far been no confirmatory reports demonstrating AhR activation by LXA_4 in this tissue.

LXA_4 was recently characterized as an ER ligand, stimulating expression of estrogen responsive genes, including alkaline phosphatase and PR, and inducing proliferation in human endometrial epithelial cells.[28] Interestingly, LXA_4 shares a high degree of structural similarity with the weak estrogen estriol (E3) made in large amounts by the placenta during pregnancy.[55,56] Consistent with the characteristics of a weak ERα agonist, LXA_4 also inhibited E2-mediated actions, as was previously shown for E3[57] and competed directly for ER binding with an IC_{50} of 46 nM. Arachidonic acid and 15S-HETE, the LX precursors, as well as LXB_4, an isomer, displayed minimal or no binding affinity, confirming the structural specificity. Acting in a manner similar to canonical ER signaling,[58] LXA_4 induced ERα phosphorylation and targeted this receptor for degradation by the proteasome. Further confirmatory studies in mice revealed that LXA_4 stimulated a uterotrophic response and induced estrogen-responsive genes, including PR in uterine tissue *in vivo*.

It should be noted that all the three above-mentioned receptors are promiscuous and subject to complex regulation in the different endometrial cell types and at different stages of the menstrual cycle or during gestation. Since multiple receptors may be present in the same cell, well-controlled studies are needed to fully explore potential crosstalk between these different classes of receptors and associated signaling systems, using physiologically relevant ligand concentrations and readouts.

ALTERNATIVE VIEWS ON LXA_4 AND ESTROGEN ACTION

The endometrium is a steroid hormone–dependent tissue and, in the humans, undergoes cyclic changes in response to the sequential actions of estrogen and progesterone.[59] Although the steroid hormone and gene regulation patterns of the normal menstrual cycle are beyond the scope of this review, endometrial changes have now been well characterized in both health and disease.[60–65] One of the major factors in endometrial-related diseases is the imbalance between the actions of estrogen and progesterone. Aside from the obvious example of endometrial cancer, endometriosis is a major health problem in women of reproductive age. The pathophysiology of endometriosis is intimately linked with inflammation, a resistance to progesterone, exaggerated estrogen responsiveness, and/or increased ER signaling.[66]

Estrogen action in the endometrium in both health and disease is an active area of research, and both estradiol and progesterone regulate the inflammatory events during menstruation and implantation.[62,67] It has long been known that estrogen upregulates both ERα and PR in the human endometrium[68,69] as well as in primates and other mammals.[70] Likewise, these studies have shown that progesterone downregulates both ERα and PR during the secretory phase of the cycle.[71] Early ERα immunohistochemistry data in human endometrium show that there is a marked reduction in all the cell types during the mid-secretory phase.[72–75] ER downregulated in luminal and glandular epithelium was associated with a decline in cell proliferation, except in the zona basalis.[76,77] In pregnancy, ERα is essentially absent due to its down-modulation by progesterone.[78–82] Interestingly, the timely loss of epithelial PR in the endometrium closely correlates well with the establishment of uterine receptivity in humans as well as in most mammals studied.[81,83–88] A delay in the opening of the window of implantation is associated with a correctable delay in the down-modulation of epithelial PR.[89] It has been suggested that E2 is unnecessary for normal endometrial secretory development.[90] Failure of ERα downregulation could therefore, be an indirect sign of progesterone resistance and has been reported in endometrial hyperplasia,[75] endometriosis,[91–95] and in the endometrium of women with polycystic ovary syndrome.[64] Together, these data support the association between aberrant proliferation and the dysregulation of progesterone action, leading to proliferative disorders of the endometrium and a lack of uterine receptivity.

The interactions between eicosanoids, lipoxygenase metabolites, estrogens and nuclear receptors have not been well studied. E2 is the most potent estrogen produced in the body and like other estrogens exerts its physiological actions though binding to and translocation of their receptors. ERs belong to the nuclear hormone receptor superfamily and function as ligand-activated transcription factors.[96] Estrogen signaling is largely mediated through two receptor isoforms: ERα and ERβ, initiating both genomic and non-genomic effects.[97] Both of these ERs are abundant in reproductive tissues, ERα being the dominant receptor within the adult uterus[98] and ERβ of greater concentration in vascular cells or of interest in reproductive tissues.[74,99,100] Membrane ERs also exist, including the newly discovered G-protein-coupled receptor-30, recently studied in the endometrium.[101] Although studies using ERα-deficient mice have revealed the central role of this receptor in reproductive function at all levels of the hypothalamic–pituitary–gonadal axis,[102] ERβ-mutant mice exhibit normal puberty but reduced fecundity due to an impaired ovulation rate.[103] G-protein-coupled receptor 30 is dispensable for fertility and does not mediate estrogenic responses in mouse reproductive organs.[104]

In addition to ligand-induced activation, ERs are also subject to phosphorylation by various kinases that are themselves activated by inflammatory mediators or cytokines during endometrial remodeling.[105] TNFα was recently shown to activate ER and induce E2-regulated genes in endometrial epithelial cells,[106] indicating immunoendocrine crosstalk occurs in inflammatory endometrial conditions. The significance of membrane-initiated steroid signaling[107] in these contexts remains to be elucidated, but alternations in the inflammatory milieu could dramatically alter the actions of estrogens, their metabolites, or other ligands, such as LXA_4.

A model of LXA_4-mediated actions in endometrial tissue would depend on temporal and spatial availability of available ligands and receptors (**Figure 2**). The increased level of FPR2/ALX at menses,[10] potentially coinciding with increased local LXA_4 production due to immunocyte influx,[108] could

contribute to the early burst of endometrial healing and growth that occur at a time when endogenous E2 is relatively low. In addition, higher ERα expression noted in the basalis glands and stroma provides a potential target for LXA_4 at menses when estrogen levels are at their nadir. Whether LXA_4 initiates endometrial regeneration and healing at this time merits investigation.

The zona basalis in primates undergoes cellular proliferation in the mid-to-late secretory phase.[76,77] Given the reduced level of estrogen in the secretory phase and the virtual absence of ERα in endometrial epithelium and stroma of the zona functionalis at the time of implantation,[73,74] it is possible that LXA_4 has a role in that early proliferative activity seen in the basal glands, thought to be an important aspect of endometrial regeneration. ERβ, on the other hand, present in vascular components of the endometrium and placenta[109] or FPR2/ALX, present in the decidualized stroma, immune cells in the vasculature, and in the myometrium of human uterus,[10] may also be targets for LXA_4 exerting anti-inflammatory actions to support the ongoing pregnancy (**Figure 1**). Pregnancy is, by necessity, an immunocompromised state, whereby the growing fetus is tolerated while maintaining maternal immunity.[110] LXA_4, acting as an ERα agonist as previously observed in human endometrial epithelial cells,[28] may participate in the down-regulation of ERα observed in the late secretory phase. Loss of ERα is also essential in the pre-implantation uterus of most mammalian species.

Finally, AhR is a known receptor for LXA_4 and is present in the endometrium of secretory endometrium.[54] Considering the putative role of AhR as a suppressor of ERα signaling via several mechanisms,[111,112] it could be speculated that LXA_4 might come into play in certain circumstances. Although we showed that AhR does not seem to be involved in LXA_4 signaling in human endometrial epithelial cells,[28] actions involving this receptor in other cell types under different circumstances or *in vivo* cannot be excluded.

MACROPHAGES AS A LXA_4 TARGET

Innate immune cells, including macrophages are a major target of LXA_4-mediated bioactivities (**Figure 1**). Given that leukocytes represent 30–40% of the endometrial cell population[113] leading to LXA_4 transcellular biosynthesis, this lipid mediator likely has a role in homeostatic processes. Macrophages and uterine natural killer cells are abundant in the endometrial stroma and are found through most of the menstrual cycle. Sex hormones regulate macrophage distribution in this tissue. Macrophage numbers increase in the premenstrual endometrial stroma, coinciding with falling estrogen and progesterone levels due to the demise of the corpus luteum.[114]

Macrophages, through their ability to produce matrix metalloproteinases (MMPs), are involved in tissue remodeling and are intimately linked with both menstruation[108,113,115] and pregnancy.[116,117] It is noteworthy that LXA_4 and its analogs decrease MMP expression and activity in many different cell types[118,119] and also in peritoneal fluid cells in a mouse model of endometriosis,[120,121] of which macrophages comprise a major component.

In the first trimester of pregnancy, macrophages constitute the second most predominant leukocyte population ($<30\%$) of decidual cells after decidual natural killer cells ($<70\%$)[122] and differentiate from monocytes. Decidual macrophages function in the removal of apoptotic bodies, uterine vascular remodeling, immune tolerance towards fetal antigens, immunity against external pathogens and cervical ripening and recovery[123] and elicit immunosuppressive and anti-inflammatory responses. Apoptotic body clearance results in the expression of anti-inflammatory cytokines such as IL-4, IL-6, and IL-10, with protective effects on trophoblast survival. Efferocytosis is a process whereby phagocytes engulf apoptotic cells and the latter impact macrophage phenotype. The newly discovered pro-resolving macrophage subset, Mres, appear later in the resolution program.[124] LXA_4 promotes the engulfment of apoptotic neutrophils, and tissue fragments, by macrophages, a process essential to the resolution of inflammation[125,126] and the re-establishment of tissue integrity post-menstruation.[113]

The domains of immunology and metabolism are converging, providing insights into a variety of physiological and pathological states.[127] The previously mentioned ALOX metabolites and PPAR ligands, as well as T helper type 2 (Th2) cytokines and specialized pro-resolution mediators drive temporarily distinct metabolic shifts and effector functions in macrophages.[128] Recent studies have implicated ERα in macrophage function and metabolism and demonstrated a protective role for this receptor on hematopoetic/myeloid cells in atherosclerosis-related inflammation.[129] As alluded to above, estrogens are linked with macrophage recruitment into the endometrium and are clearly implicated in immune responses. The role of ERα in LXA_4-mediated regulation of macrophage function in the endometrium and peritoneal cavity awaits elucidation.

ENDOMETRIOSIS: A COMPLEX DISEASE FOR WHICH IMPROVED TREATMENT MODALITIES ARE NECESSARY

Endometriosis, an inflammatory, estrogen-stimulated disease affects approximately 10% of women of reproductive age, and it is estimated that up to 80% of unexplained infertility is attributable to this condition.[65,130,131] First described by Daniel Schroen in 1690, several theories have been proposed but none fully explain the etiology. The most well accepted is Sampson's[132] theory of retrograde menstruation, whereby fragments of menstrual endometrium pass backward through the fallopian tubes and into the peritoneal cavity where they implant and persist. The eutopic endometrium of women with endometriosis becomes altered as shown in the baboon model,[133,134] with increased estrogen activity, cellular proliferation, and progesterone resistance.[66] The biological mechanisms linking endometriotic lesions to these endometrial alterations remains uncertain and controversial.[135] Although progesterone resistance and estrogen dominance likely contribute to the pathophysiology and survival of ectopic

lesions,[134,136,137] they probably contribute to infertility as well.[66,95,134] There is overwhelming evidence that normal immune responses, which serve to promote fertility and immunotolerance, are altered in women with endometriosis, with inflammatory changes in the intrauterine milieu, the peritoneal cavity, and systemic circulation.[65,138–140]

Cellular proliferation and inflammation are intimately linked via both hormonal and inflammatory mediators such as IL-1, TNF-α, PGE_2, or E2, which, in turn, induce growth factors, cytokines, and chemokines that promote inflammation, cellular proliferation, and angio- and lymphangiogenesis.[141,142] Estrogens directly regulate the endometrial expression of many cytokines and growth factors as well as their receptors thereby contributing to endometriotic lesion growth.[140] Conversely, TNF-α increases estrogen biosynthesis by human endometrial glandular cells and directs estrogen metabolism towards more hormonally active and carcinogenic metabolites.[143] Crosstalk between the immune and endocrine systems therefore clearly contributes to endometriosis pathology.

Dysregulations in local immune mediator concentration and/or signaling leads to increased inflammation in the peritoneal cavity and the resulting systemic changes could conceivably alter the number and profile of immune cells that traffic to the endometrium. Dysfunction in macrophage-mediated phagocytosis of cells that are transported into the peritoneal cavity by retrograde menstruation is considered an important factor in the development of endometriosis. PGE_2 diminished human macrophage-mediated phagocytosis by downregulating the scavenger receptor and lipid transporter CD36, thought to have a significant role in macrophage-mediated phagocytosis, and also increased endometriotic lesion size in mice.[144] There is some evidence that macrophages in endometriosis are M2 polarized, also known as alternatively activated macrophages, in human, mouse, and primate.[145,146] In the former study, macrophages in both inflammatory liquid and ectopic lesions were M2 polarized in endometriosis patients but not in control subjects. Adoptive transfer of alternatively activated macrophages dramatically enhanced endometriotic lesion growth in mice, and M1 polarized, inflammatory macrophages protected mice from disease establishment. It should be noted, however, that human macrophages markers are less well characterized than murine cells and extracellular marker analysis should ideally be coupled with that of pertinent effector molecules. Several different types of macrophages subsets have been described, and more are likely yet to be discovered. Macrophages display a phenotypic plasticity as a function of environmental cues, including cytokines and growth factors.[33] LXA_4 promotes M2 polarization *in vitro*.[147] Further studies are necessary to determine the precise macrophage subsets in endometrial tissue and peritoneal fluid and whether their polarization contributes to pathological responses.

In endometriosis, establishment of an immunotolerant (Th2/Treg (regulatory T cell)) environment[139,148,149] appears to be replaced by an inflammatory Th1/Th17 immune response.[1,150] Retinoids, vitamin A derivatives that mediate diverse physiological functions, exert their pleiotropic effects through the interaction with nuclear receptors, defined as retinoic acid receptors and retinoid X receptors (RXRs).[151] Retinoic acid (RA) as an important intermediary down-stream effector of progesterone action is also involved in immune cell programming.[152] Retinol-binding proteins are under the regulation of progesterone in primates[153] and human endometrium. Among six retinoid receptors examined, RXRγ immunoreactivity was exclusively detected in the epithelial cells of the secretory phase endometrium but not of the proliferative phase.[154] These data indicate that its expression is induced by progesterone. It is noteworthy that nuclear receptors such as RXR and PPAR-γ heterodimerize and it seems that these dimers provide cells such as macrophages with a coordinated and inter-related network of transcriptional regulators for interpreting local metabolic changes resulting in subtype differentiation.[152] T-cell differentiation is altered toward an inflammatory phenotype in endometriosis, RA and PPAR-γ being essential for Treg differentiation.[155,156] Inflammatory changes, including augmented IL-6 and IL-23, promotes conversion of Tregs to Th17 cells.[157] Of note, PPAR-γ selectively inhibits Th17 differentiation of CD4+ T-cells.[156] As such, several nuclear receptors likely impact physiological and pathological processes in endometrial tissue, conceivably involving LXA_4.

LXA_4 inhibits endometriosis progression in the mouse preclinical model.[121,158] As shown for LXA_4 or a stable analog,[121,159] pretreatment with a combination of progesterone, RA, and TGFβ greatly attenuates MMPs expression and reduces endometriotic lesion growth.[160] Interestingly, LXA_4 also suppresses phorbol myristate acetate–induced expression of the inflammatory cytokines IL-6 and IL-8 in human decidua tissue[10] and attenuates CCL2, IL-6 and inhibit nuclear factor (NF)-κB and Akt pathways in other animal models of inflammation.[161,162] These observations may constitute mechanisms underlying its beneficial effects in preclinical endometriosis models but also raise the question whether LXA_4 is a down-stream effector of progesterone and RA actions. Interestingly, a recent paper demonstrated the protective effect of fish oil in endometriosis in a chimeric model where human endometrial tissue was injected into the peritoneum of nude mice.[163] Mice administered fish oil exhibited fewer leukocytes within lesions and less collagen deposition at adhesions indicating that dietary intervention may prevent postsurgical adhesion development. These effects may well be due to the formation of pro-resolving lipid mediators such as resolvins and protectins, metabolized from omega 3 fatty acids.[9]

Hormone production, signaling, and metabolism are significantly perturbed and progesterone resistance comprises part of the pathology.[66,164] Treatment of endometrial epithelial cells with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent AhR ligand, altered the expression of PR-B[165] as a possible mechanism of progesterone resistance, this environmental toxin having been implicated in endometriosis development. PR-B in the uterus can exert an anti-inflammatory action, opposing PR-A.[166] PR-A induced inflammatory cytokines

IL-8-, IL-1β-, and NF-κB-regulated genes, while PR-B induced FKBP52 and NFKB1A, an inhibitor of the NF-κB pathway. FKBP52 has been shown to be decreased in endometriosis.[167,168] This PR chaperone protein is required for proper progesterone-mediated actions and its reduction in this disease may be a major determinant in progesterone resistance.[136]

Progesterone resistance in endometriosis is associated with a decrease in RA activity;[169] RA uptake protein STRA6 and cellular RA-binding protein 2 (both progesterone-regulated genes) are reduced in eutopic endometrium in endometriosis. Women with unexplained pregnancy loss also have reduced RA-binding protein 2.[170] A recent study showed that RA treatment reduced endometriosis lesion size in the mouse model, with decreased IL-6 and MCP-1,[171] actions similar to those elicited by LXA_4. RA acts in concert with progesterone and PPAR-γ to limit inflammation; specifically inhibiting the expression of the pleiotrophic cytokine IL-6 at the promoter, limiting its production[172] along with IFN-γ, while reduced RA action results in augmented IL-6 expression.[169] In endometriosis, stromal cells produce increased IL-6 in response to IL-1β.[173]

Estrogen's ability to inhibit LXA_4 production in other mucosal tissues[35] has important potential implications for endometriosis. As progesterone opposes estrogen action, progesterone resistance leads to increased estrogenicity in eutopic and ectopic endometrial tissue, perhaps also in the peritoneal compartment. ERα itself is one of the best recognized markers of progesterone resistance. In endometriosis, ERα is more abundant and displays a failure of downregulation in eutopic[95] and ectopic tissue.[174,175] The underlying reasons are unknown but could include aberrant modification and/or targeting to the proteasome, as part of abnormal responses to this hormone, or could be linked to progesterone resistance. ERα is more important than ERβ in endometriosis lesion development in mice, as recently demonstrated in an immunocompetent model.[176] In this study, ERα-null lesions were associated with increased inflammation, it is therefore tempting to speculate that endogenous ERα ligands mediate this effect.

However, data from human studies showed that ERβ is overexpressed in endometriotic lesions and may have a role in the pathophysiology. A high ERβ-to-ERα ratio in endometriotic stromal cells is associated with suppressed PR and augmented COX-2 levels contributing to progesterone resistance and inflammation.[100] High levels of ERβ result in suppressed ERα expression and estradiol responses in endometrial and endometriotic stromal cells.[174] We have recently demonstrated increased ERα and ERβ expression in ectopic and peritoneal tissue of patients with peritoneal endometriosis compared with control subjects, with a particularly marked elevation in ERβ observed,[175] as has also been reported for ovarian endometriosis.[164] The respective role of stromal and epithelial cells within lesions as well as the myeloid cells in the peritoneal cavity, and their immune and metabolic products, remains unclear. ER crosstalk and regulation is likely to be crucial. This complex area of biology necessitates further research and differences between murine models and the human pathology likely exist. Advances in understanding of the latter will necessitate standardized, well-designed studies and facilitate the discovery of relevant biomarker panels, which will hopefully serve to decrease the long delay before diagnosis.[177–179]

Estrogen production is also increased in endometriosis,[180] likely influenced by the inflammatory environment.[181] Here, the COX-2-derived eicosanoid PGE_2, as a potent inducer of aromatase and Steroidogenic acute regulatory protein expression, has a pivotal role.[182] Furthermore, estrogen degradation is regulated by progesterone and defective in progesterone resistance.[183] Estrogen may be pro-inflammatory in endometriosis[184] and contributes to the pathophysiology of the disease as a mitogen causing aberrant proliferation[185] and inhibition of apoptosis,[186] decreasing the tumor-suppressor phosphatase and tensin homolog deleted on chromosome 10 via NF-κB dependent pathways.[187]

Defective immunity and decreased production or activity of anti-inflammatory mediators such as LXA_4 could account for the development of sustained inflammation seen in the reproductive tract and peritoneal fluid[188] of endometriosis patients. Alterations in endometrial stroma have long been noted,[173,189] including an exaggerated response to IL-1β and TNF-α, resulting in excessive production of the inflammatory cytokines ENA-78 (epithelial neutrophil–activating peptide-78), IL-6, and IL-8.[190] Other inflammatory cytokines, including IL-17, increase IL-8 secretion and expression of COX-2[191] and aromatase,[192,193] making this pathway an attractive therapeutic target.[194] LXA_4 production is induced by IL-13 in monocytes,[30] and IL-13 is regulated during the menstrual cycle, with expression induced by ovarian steroid hormones and cytokines.[39,195] Studies on micro(mi)RNA expression[196,197] have revealed a wide variety of changes in this disease. Let-7 miRNA was one of those most upregulated in the endometrium of women with endometriosis[196] and intruigingly, a let-7 miRNA polymorphism has been associated with endometriosis.[198] Let-7 miRNAs inhibit IL-13 expression.[199]

Current treatments are directed toward surgical excision of ectopic endometrial tissue, and symptom alleviation, usually by targeting hormones or their receptors. Upon cessation of treatment, endometriosis frequently recurs. Furthermore, surgery can result in adhesion development, which can also lead to chronic pelvic pain and infertility.[200] Novel approaches are therefore necessary as traditional therapies, the majority of which target hormones or their receptors, have been hampered by poor bioavailability, undesirable side effects, and a negative impact on fertility.[201,202] As inflammation underlies the major endometriosis-associated symptoms, notably infertility and pain, a well-tolerated immunomodulatory therapy targeting inflammatory changes associated with this disease could improve symptoms without the untoward side-effects of existing treatments. As detailed above, LXA_4 exerts a protective effect in preclinical endometriosis models, through anti-inflammatory and anti-angiogenic mechanisms.[120,121,159]

Importantly, cycling remained unchanged, indicating that LXA_4 does not alter ovarian function.

In conclusion, complex and interrelated pathways link the immune response to steroid hormone actions. Studies on endometrial function and endometriosis confirm the importance of the balance between inflammation and its resolution. Pregnancy itself is dependent on this compromise between self-defense and beneficence.[110] Future research efforts will be needed to clarify the functional ramifications of LXA_4 as an anti-inflammatory modulator and ER agonist. As a molecule with multiple modes of action and the ability to mitigate some E2-mediated responses,[28] as well as potentially divergent actions on myeloid and non-myeloid cell types, revelations on its role in endometrial physiology and metabolism will likely identify therapeutic opportunities. Indeed, research in the emerging domain of immuno-metabolism is predicted to generate key insights into reproductive biology and disease.

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DISCLOSURE

The authors declare no conflict of interest.

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