

# A role for lipoxin A<sub>4</sub> as an anti-inflammatory mediator in the human endometrium

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## Abstract

Lipoxin A<sub>4</sub> is a lipid mediator that elicits anti-inflammatory and pro-resolution actions via its receptor, formyl peptide receptor 2 (FPR2/ALX). In this study, we aimed to investigate the expression and potential role of lipoxin A<sub>4</sub> and FPR2/ALX in the regulation of inflammation associated with cyclical remodeling of the human endometrium across the menstrual cycle and during early pregnancy. Using quantitative RT-PCR analysis, we found that FPR2/ALX expression is upregulated during the menstrual phase of the cycle and in decidua tissue from the first trimester of pregnancy. We localized the site of expression of FPR2/ALX in menstrual phase endometrium and first-trimester decidua tissue to glandular epithelial cells and cells within the stromal compartment, including cells lining the blood vessels and immune cells. Measurement of serum lipoxin A<sub>4</sub> by ELISA revealed no difference in its levels across the menstrual cycle but an elevation in early pregnancy ( $P < 0.001$ ). We found that lipoxin A<sub>4</sub> was regulated by human chorionic gonadotrophin (hCG) during early pregnancy, because treatment of human decidua tissue with hCG increased lipoxin A<sub>4</sub> release ( $P < 0.01$ ). Finally, we have shown that lipoxin A<sub>4</sub> can suppress phorbol myristate acetate-induced expression of the inflammatory cytokines interleukin 6 and 8 in human endometrium and decidua tissue. These results demonstrate for the first time that lipoxin A<sub>4</sub> and its receptor FPR2/ALX can regulate inflammatory events in the human endometrium and decidua of early pregnancy.

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## Introduction

Inflammation occurs during important physiological events throughout the female reproductive tract, such as ovulation, menstruation, embryo implantation, and the initiation of labor (Jabbour *et al.* 2009). In the endometrium, inflammatory leukocytes are believed to be important in the tissue breakdown and remodeling essential for menstruation and the generation of a receptive endometrium during early pregnancy. Chronic unregulated inflammation in the endometrium has been linked with several pathologies including heavy menstrual blood loss (Smith *et al.* 2007), endometrial cancer (Wallace *et al.* 2010), endometriosis (Ryan *et al.* 1995, Arici *et al.* 1997), and recurrent miscarriage (von Wolff *et al.* 2000, Laird *et al.* 2003). Thus, the inflammation associated with menstruation, implantation, and the establishment of pregnancy must be kept under tight control in order to ensure reproductive success.

Inflammation can be controlled by the local production of endogenous anti-inflammatory mediators, such as the steroid hormone cortisol (Chapman *et al.* 2009), the cytokine interleukin 10 (IL10; Thaxton & Sharma 2010),

the protein annexin A1 (Perretti & D'Acquisto 2009), and lipid mediators including the resolvins, protectins, and lipoxins (Serhan *et al.* 2008).

The lipoxins (which include the naturally occurring lipoxin A<sub>4</sub> and lipoxin B<sub>4</sub>, and the aspirin-triggered lipoxins 15-epimeric lipoxin A<sub>4</sub> and 15-epimeric lipoxin B<sub>4</sub>) are synthesized from arachidonic acid by the action of the lipoxygenase enzymes (Chiang *et al.* 2006). Lipoxin A<sub>4</sub> has been shown to bind to and activate the G protein-coupled receptor formyl peptide receptor 2 (FPR2/ALX), a member of the FPR family (Ye *et al.* 2009). Lipoxin A<sub>4</sub> is a dual acting mediator and activates specific cellular pathways via FPR2/ALX to elicit both anti-inflammatory and pro-resolution effects (Serhan *et al.* 2008).

The lipoxins act as anti-inflammatory mediators by inhibiting neutrophil infiltration (Takano *et al.* 1997, 1998, Hachicha *et al.* 1999) and transmigration (Colgan *et al.* 1993, Kucharzik *et al.* 2003) at sites of inflammation, in part via the induction of nitric oxide production to suppress leukocyte–endothelial interactions (Paul-Clark *et al.* 2004). Lipoxins can also inhibit the production of pro-inflammatory cytokines

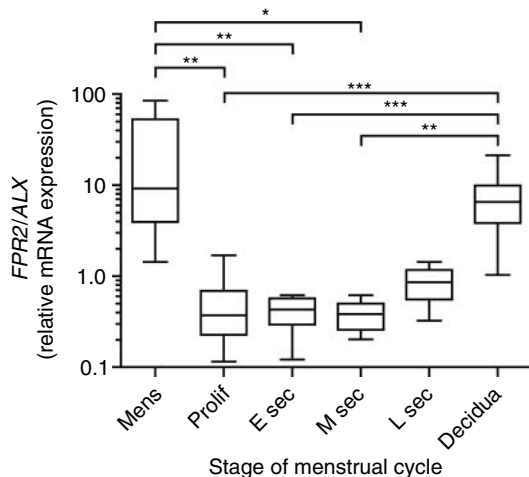
and chemokines (Wu *et al.* 2005, 2008, Bonnans *et al.* 2007), via the inhibition of transcription factors such as nuclear factor- $\kappa$ B and activator protein 1 (Gewirtz *et al.* 2002, Jozsef *et al.* 2002, Sodin-Semrl *et al.* 2004, Wu *et al.* 2008).

In this study, we aimed to determine the expression and potential role of lipoxin A<sub>4</sub> and FPR2/ALX in regulating inflammation in the human endometrium and decidua of early pregnancy. We show that FPR2/ALX is temporally regulated across the menstrual cycle and during early pregnancy and is expressed in glandular, vascular, and stromal cells. Furthermore, we show that serum lipoxin A<sub>4</sub> expression is elevated during early pregnancy and is regulated in first-trimester decidua tissue by human chorionic gonadotrophin (hCG). Finally, we show that lipoxin A<sub>4</sub> acts as an anti-inflammatory mediator in human endometrium and decidua tissue by counteracting phorbol ester-mediated induction of pro-inflammatory cytokine expression.

## Results

### Expression and cellular localization of FPR2/ALX in the human endometrium

The temporal expression pattern of FPR2/ALX mRNA expression across the menstrual cycle and during early pregnancy was investigated using quantitative RT-PCR analysis. Expression of FPR2/ALX (Fig. 1) was significantly higher in the menstrual phase of the cycle when



**Figure 1** Temporal expression of FPR2/ALX in the human endometrium and first-trimester decidua. mRNA expression levels in human endometrium across the menstrual cycle ( $n=34$ ; consisting of  $n=5$  menstrual,  $n=10$  proliferative,  $n=7$  early secretory,  $n=6$  mid-secretory, and  $n=6$  late secretory phase tissues) and first-trimester decidua tissue (7–12 weeks of gestation;  $n=27$ ) are shown for FPR2/ALX. mRNA levels are expressed relative to a standard endometrial cDNA sample. Boxes represent data lying within the 5th to the 95th percentile and whiskers represent the minimum and maximum values. \*, \*\*, and \*\*\* represent significance at  $P<0.05$ ,  $P<0.01$ , and  $P<0.001$ , respectively, as determined by ANOVA with Tukey's *post hoc* test.

compared with proliferative ( $P<0.01$ ), early- ( $P<0.01$ ), and mid-secretory ( $P<0.05$ ) phase endometrium. In first-trimester decidua tissue, FPR2/ALX expression was significantly higher than in proliferative ( $P<0.001$ ), early- ( $P<0.001$ ), and mid-secretory ( $P<0.01$ ) phase endometrium.

As the mRNA expression of FPR2/ALX was higher in menstrual phase endometrium and first-trimester decidua, we localized the site of expression of FPR2/ALX protein in these tissues by immunohistochemistry. In menstrual phase endometrium (Fig. 2A, B and C), FPR2/ALX expression was strongest in the functional layer (Fig. 2A), where it localized to glandular epithelial cells and distinct cells in the stromal compartment (Fig. 2B) along with cells lining the blood vessels and immune cells within the vasculature (Fig. 2C). Similarly, in first-trimester decidua tissue (Fig. 2D and E), FPR2/ALX expression localized to the glandular epithelium and decidualized stromal cells (Fig. 2D), cells lining the blood vessels, and immune cells within the vasculature (Fig. 2E). Tissue sections incubated with primary antibody pre-absorbed with an excess of FPR2/ALX protein were used as a negative control (Fig. 2D; inset).

### Serum levels of lipoxin A<sub>4</sub> across the menstrual cycle and during early pregnancy

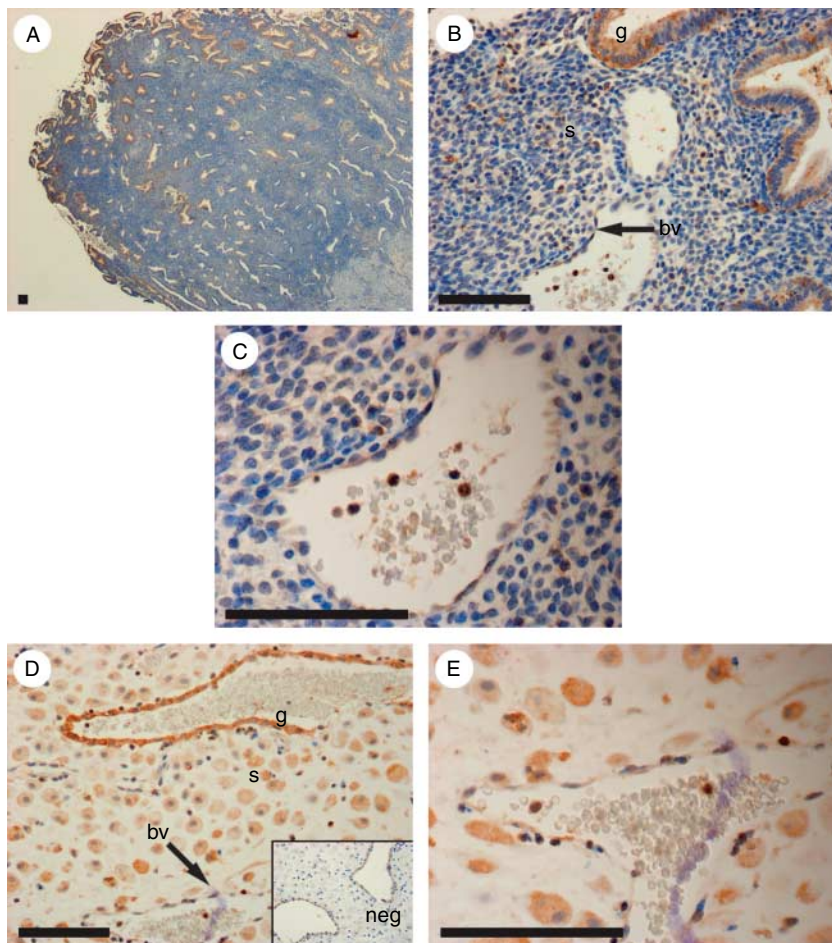
The endometrial expression pattern of FPR2/ALX suggested a role for this receptor in regulating inflammation associated with menstruation and early pregnancy. Therefore, we investigated the levels of its ligand, lipoxin A<sub>4</sub>, in the blood of women across the menstrual cycle and in early pregnancy. There was no significant variation in serum levels of lipoxin A<sub>4</sub> across the menstrual cycle (Fig. 3A). However, serum levels of lipoxin A<sub>4</sub> were significantly elevated in samples from women in the first trimester of pregnancy, when compared with levels in non-pregnant women (Fig. 3B,  $P<0.001$ ).

### Regulation of lipoxin A<sub>4</sub> release by hCG

As we observed an increase in serum levels of lipoxin A<sub>4</sub> during early pregnancy, we investigated whether lipoxin release could be regulated by hCG. Treatment of decidua tissue explants with 1 IU hCG for 8 h caused a significant increase in lipoxin A<sub>4</sub> release when compared with vehicle-treated decidua tissue ( $P<0.01$ , Fig. 3C).

### Lipoxin A<sub>4</sub> reduces the expression of inflammatory mediators in human endometrium and decidua tissue

Lipoxin A<sub>4</sub> via FPR2/ALX has been shown to mediate anti-inflammatory actions (Bonnans *et al.* 2007). To investigate whether lipoxin A<sub>4</sub> acts as an anti-inflammatory mediator in the endometrium, endometrial tissue



**Figure 2** Localization of FPR2/ALX protein in menstrual endometrium and first-trimester decidua. Localization of FPR2/ALX protein in menstrual endometrium (A, B and C;  $n=5$ ) and during early pregnancy (D and E;  $n=5$ ) is shown. Expression was detected in the glandular epithelium (g), distinct cells in the stroma (s), and cells lining the blood vessels (bv) in both tissues. FPR2/ALX was also detected in immune cells within the vasculature (C and E). Decidualized stromal cells showed expression of FPR2/ALX (D). A representative sample is shown for each tissue. Negative control (neg) is shown for decidua tissue (D, insert). Scale bar = 100  $\mu$ m.

explants were cultured with the inflammatory stimulus phorbol myristate acetate (PMA), either alone or in combination with lipoxin A<sub>4</sub>. Treatment with PMA alone increased the expression of the inflammatory cytokines *IL6* ( $P<0.001$ , Fig. 4A) and *IL8* ( $P<0.001$ , Fig. 4B). Co-treatment of tissues with PMA and lipoxin A<sub>4</sub> significantly reduced expression of *IL6* ( $P<0.01$ , Fig. 4A) and *IL8* ( $P<0.05$ , Fig. 4B). Similarly, to assess the anti-inflammatory actions of lipoxin A<sub>4</sub> during early pregnancy, decidua tissue was cultured with PMA, alone or in combination with lipoxin A<sub>4</sub>. PMA significantly increased the expression of *IL6* ( $P<0.05$ , Fig. 4C) and *IL8* ( $P<0.01$ , Fig. 4D) in decidua tissue explants. Co-treatment of decidua tissue with PMA and lipoxin A<sub>4</sub> significantly suppressed PMA-induced expression of *IL6* and *IL8* ( $P<0.05$ ).

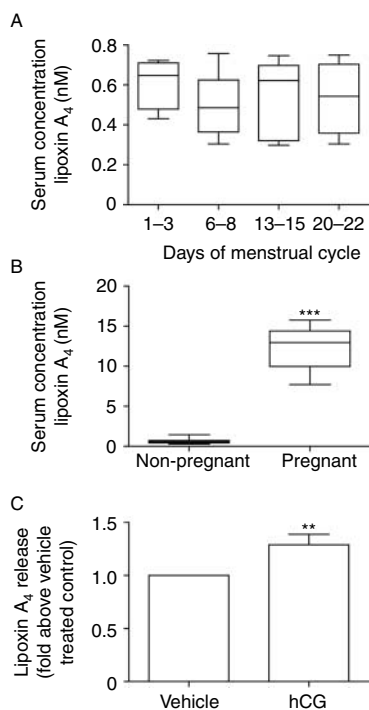
## Discussion

Endometrial events, such as menstruation and embryo implantation, are associated with inflammatory processes that must be tightly regulated to ensure reproductive success. This is achieved by a delicate balance in the production of pro- and anti-inflammatory mediators

to ensure proper tissue function and homeostasis. Lipoxin A<sub>4</sub> is a recently described anti-inflammatory and pro-resolution lipid mediator, which elicits its actions via the G protein-coupled receptor FPR2/ALX. In this study, we investigated the expression and role of the lipoxin A<sub>4</sub>-FPR2/ALX system in the human endometrium.

We found that expression of FPR2/ALX is temporally regulated across the human menstrual cycle. The elevation in expression of FPR2/ALX observed in the endometrium during menstruation suggests a role for lipoxin A<sub>4</sub> in regulating the inflammation associated with endometrial remodeling and repair. Prior to the onset of menstruation, there is an influx of leukocytes into the endometrium (Salamonsen & Lathbury 2000), facilitated by the expression of pro-inflammatory chemoattractant chemokines and cytokines (Arici *et al.* 1998, Jolicoeur *et al.* 1998) and an increase in vascular permeability (Colditz 1990). These inflammatory leukocytes are thought to contribute to tissue breakdown and vascular remodeling during menstruation (Zhang *et al.* 1998, Sivridis *et al.* 2001), via the production of proteolytic enzymes such as the matrix metalloproteinases (Salamonsen & Woolley 1999). Regeneration of the





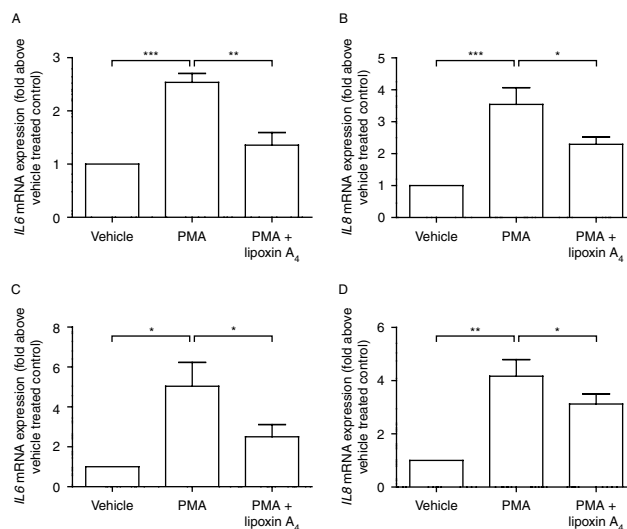
**Figure 3** Lipoxin A<sub>4</sub> serum levels across the menstrual cycle and during the first trimester of pregnancy and regulation of its release by hCG. Lipoxin A<sub>4</sub> levels in serum samples taken from a group of women on the days indicated across the menstrual cycle (A;  $n=5$  for each day of the menstrual cycle analyzed). The average level of lipoxin A<sub>4</sub> in this group of non-pregnant women (non-pregnant) was compared with serum levels measured in a group of women during the first trimester of pregnancy (pregnant;  $n=10$  from gestational ages 7–12 weeks) (B). Boxes represent data lying within the 5th to the 95th percentile and whiskers represent the minimum and maximum values. First-trimester decidua tissue ( $n=6$  from gestational ages 7–12 weeks) treated with 1 IU of hCG for 8 h showed a significant increase in lipoxin A<sub>4</sub> release into the culture medium, when compared with vehicle-treated control tissue. Data are mean  $\pm$  S.E.M. \*\* and \*\*\*Represent significance at  $P<0.01$  and  $P<0.001$ , respectively, as determined by  $t$  test.

endometrium begins soon after the onset of menstruation (Ludwig & Spornitz 1991). The mechanisms of endometrial repair have been likened to those of the wound healing process and involve angiogenesis (Jabbour *et al.* 2006) and inflammatory processes such as the removal of tissue debris by macrophages (Salamonsen & Lathbury 2000). While we observed no significant fluctuation in serum levels of lipoxin A<sub>4</sub> across the menstrual cycle, our data showing that expression of FPR2/ALX is elevated at menstruation indicates a potential role for this receptor in regulating the inflammatory events involved in endometrial tissue breakdown and regeneration at a local level. This may be achieved by local production of lipoxin A<sub>4</sub> in the endometrium, or other anti-inflammatory ligands that can activate FPR2/ALX such as annexin A1 (Perretti & D'Acquisto 2009).

Lipoxin A<sub>4</sub> has previously been demonstrated to elicit anti-inflammatory actions in a variety of cell types, including epithelial cells (Bonnans *et al.* 2007),

endothelial cells (Wu *et al.* 2008), and myometrial tissue explants (Maldonado-Perez *et al.* 2011), where it facilitates tissue remodeling events by reducing the expression of inflammatory chemokines and cytokines. In order to confirm that lipoxin A<sub>4</sub> could regulate inflammatory events in the endometrium, we treated endometrial tissue with the general inflammatory stimulus PMA. This elevated the expression of the pro-inflammatory cytokines *IL6* and *IL8*. Co-treatment of endometrial tissue with PMA and lipoxin A<sub>4</sub> suppressed PMA-mediated induction of *IL6* and *IL8* expression. These data confirm that lipoxin A<sub>4</sub> has the capacity to mediate anti-inflammatory actions in the endometrium.

In addition to its anti-inflammatory effects, the lipoxin A<sub>4</sub>-FPR2/ALX system can also elicit pro-resolution actions in order to regulate tissue homeostasis (Serhan *et al.* 2008). The resolution of inflammation involves the removal of leukocytes and debris from the site of inflammation and was once thought to be a passive process caused by the dispersal of chemotactic gradients (Serhan 2004). However, it is now recognized that the resolution of inflammation is an active process involving the activation of distinct biochemical pathways that can be mediated by the lipoxins (Serhan *et al.* 2008). To promote resolution, the lipoxins accelerate the clearance of neutrophils from inflamed tissues via the promotion of



**Figure 4** Anti-inflammatory action of lipoxin A<sub>4</sub> in endometrium and decidua tissue. Cultured endometrial tissue consisting of four individual tissues taken across the menstrual cycle (one proliferative, two early secretory, and one late secretory samples) stimulated with the inflammatory stimulus PMA showed a significant increase in the expression of the inflammatory cytokines *IL6* (A) and *IL8* (B). The expression of these markers was significantly reduced when tissue was cultured with PMA in combination with lipoxin A<sub>4</sub>. PMA also stimulated an increase in the expression of *IL6* (C) and *IL8* (D) in cultured decidua tissue ( $n=6$  from gestational ages 7–12 weeks), which was significantly attenuated by the presence of lipoxin A<sub>4</sub>. Data are mean  $\pm$  S.E.M. \*, \*\*, and \*\*\*Represent significance at  $P<0.05$ ,  $P<0.01$ , and  $P<0.001$ , respectively, as determined by  $t$  test.

neutrophil apoptosis (El Kebir *et al.* 2007), monocyte migration (Maddox & Serhan 1996), and the non-phagocytosis of apoptotic neutrophils by monocyte-derived macrophages (Godson *et al.* 2000). Our observation that FPR2/ALX is expressed on glandular epithelial, stromal, vascular, and immune cells in the endometrium during menstruation suggests that pro-resolution lipoxin A<sub>4</sub>-FPR2/ALX signaling may contribute to controlling the removal of tissue debris after menstruation. This is essential for wound healing and tissue regeneration in the endometrium.

Inflammatory processes are also important during early pregnancy, for both the establishment of a receptive endometrium and in embryo–endometrial cross talk. Decidualization of the stroma occurs in the human endometrium in preparation for embryo implantation and trophoblast invasion. As well as differentiation of the stromal cells, this involves inflammatory events such as infiltration of leukocytes, modification of the extra-cellular matrix, and an increase in vascular permeability (Hess *et al.* 2007). The developing embryo also releases many pro-inflammatory cytokines, and it is thought that this induces inflammatory pathways in the endometrium to further enhance its receptivity (Dimitriadis *et al.* 2005). Dysregulated inflammatory responses have been associated with recurrent miscarriage (von Wolff *et al.* 2000, Laird *et al.* 2003); therefore, these inflammatory pathways need to be tightly regulated to ensure reproductive competence.

We found elevated expression of FPR2/ALX in the decidua of early pregnancy compared with non-pregnant endometrium across the menstrual cycle. In addition, serum lipoxin A<sub>4</sub> levels were also significantly increased during the first trimester of pregnancy compared with levels in non-pregnant patients, indicating potential fetal–maternal cross talk in the regulation of the inflammatory response during early pregnancy. This led us to investigate the potential of hCG to regulate lipoxin A<sub>4</sub> release. hCG is released from the developing blastocyst during early pregnancy and maintains progesterone production from the corpus luteum and directly regulates gene expression in the endometrium to facilitate the establishment and maintenance of pregnancy (Srisuparp *et al.* 2001, Licht *et al.* 2007). We observed an increase in lipoxin A<sub>4</sub> release from cultured decidua tissue treated with hCG. Whether circulating levels of lipoxin A<sub>4</sub> correlate with higher levels seen at the maternal–fetal interface during pregnancy is unclear. However, our data strongly suggest that decidual tissue, in response to embryonic hCG, is capable of secreting lipoxin A<sub>4</sub> which in turn may contribute to the higher levels seen in early pregnancy.

To confirm that lipoxin A<sub>4</sub> could regulate inflammatory events in the decidua of early pregnancy; we treated decidua tissue from pregnant women with PMA. Similar to our observations for treatment of non-pregnant endometrium, we found that PMA enhanced the

expression of *IL6* and *IL8* in decidua tissue explants. This was suppressed by co-treatment of decidua tissue with PMA and lipoxin A<sub>4</sub>. Our data showing that lipoxin A<sub>4</sub> can moderate the expression of *IL6* and *IL8* in the decidua suggest a mechanism whereby lipoxin A<sub>4</sub> may regulate physiological inflammation during early pregnancy, by controlling the inflammatory processes required for decidualization and the generation of a receptive endometrium.

In summary, we demonstrate for the first time expression of the lipoxin A<sub>4</sub> receptor FPR2/ALX in the human endometrium and decidua from the first trimester of pregnancy and show its temporal regulation across the menstrual cycle. We highlight that lipoxin A<sub>4</sub> serum levels are elevated during early pregnancy and that its release from the decidua can be regulated by hCG. Furthermore, we show that lipoxin A<sub>4</sub> has an anti-inflammatory action in human endometrium and decidua tissue. We believe these data demonstrate that lipoxin A<sub>4</sub> signaling via FPR2/ALX represents a novel mechanism to control the inflammation associated with menstruation and the establishment of pregnancy.

## Materials and Methods

### Reagents

DMEM/F-12 GlutaMAX culture medium was obtained from Invitrogen. Lipoxin A<sub>4</sub> (used at a final concentration of 500 nM) was purchased from Calbiochem (Nottingham, UK). PMA; used at a final concentration of 100 nM and recombinant (hCG; used at a final concentration of 1 IU) were purchased from Sigma. FPR2/ALX polyclonal antibody and blocking peptide were obtained from MBL International (Woburn, MA, USA).

### Tissue and serum collection

Endometrial tissue was collected from 34 women aged 21–39 years (consisting of *n*=5 menstrual, *n*=10 proliferative, *n*=7 early secretory, *n*=6 mid secretory and *n*=6 late secretory phase tissues) with no underlying endometrial pathology and regular menstrual cycles (25–35 days) who had not received any hormonal preparation for 3 months preceding biopsy collection. The phase of the menstrual cycle for each tissue was confirmed by histological assessment by a pathologist. Biopsies were dated according to stated last menstrual period and confirmed with hormone analysis for circulating estradiol and progesterone levels; they were consistent with both the stated last menstrual period and the histological assessment of the stage of the menstrual cycle. First-trimester decidua tissue (7–12 weeks gestation; *n*=27) was collected from women undergoing elective surgical termination of pregnancy, with gestation confirmed by ultra-sound scan. Blood samples were obtained from two groups of women. The first group included healthy non-pregnant women who attended four visits during a single menstrual cycle (days 1–3 (menstrual), days 6–8 (follicular), days 13–15 (peri-ovulatory), and days 20–22 (luteal)). The second group included women undergoing

elective surgical termination during the first trimester of pregnancy (7–12 weeks gestation). Blood samples were taken from the antecubital fossa and transported to the laboratory on ice, where serum was separated by centrifugation and stored at  $-20^{\circ}\text{C}$ . Ethical approval was obtained from Lothian Ethics Research Committee, and written informed consent was obtained before tissue or blood collection.

### Tissue culture and treatment

Non-pregnant endometrial tissue and decidua tissue for explant studies were chopped finely and maintained in serum-free DMEM/F-12 medium. For each individual patient sample, tissue was divided into six roughly equal portions that were then treated with the three treatments each in duplicate. Tissue was incubated overnight in serum-free DMEM/F-12 culture medium supplemented with 100 IU penicillin and 100  $\mu\text{g}$  streptomycin, before treatment with 1 IU hCG, 100 nM PMA, or 100 nM PMA + 500 nM lipoxin  $\text{A}_4$ . Tissue explants were incubated for up to 8 h at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$  before tissue was collected for RNA extraction and RT-PCR analysis, or conditioned medium was collected for ELISA.

### Taqman quantitative PCR

Total RNA was extracted from tissue using the RNeasy mini kit and RNase-free DNase set from Qiagen, according to the manufacturer's guidelines. RNA samples were quantified and reverse transcribed using the SuperScript VILO cDNA synthesis kit from Invitrogen. PCR reactions were carried out in duplicate using an Applied Biosystems ABI 7500 system, UK. Primer and FAM-labeled probe sequences were as follows (5'–3'): *FPR2/ALX*, forward GCCATCTGCTATGGGCTCAT and reverse CGTAAGGGACGGCTGGATT; probe, CAGCCAAGATCCACAAAAGGGCATG; *IL6*, forward GCCGCCCCACACAGACA and reverse CCGTCGAGGATGTACCGAAT; probe, CCACTCACCTCTTCAGAACGAATTCACAAAC; *IL8*, forward CTGGCCGTGGCTCTCTT and reverse TAGCACTCCTTGGCAAAGT; probe, CCTTCCTGATTTCTGCAGCTCTGTGTGAA. The expression of analyzed genes was normalized for RNA loading using 18S ribosomal RNA primers and probe (Applied Biosystems, Warrington, UK). Results were calculated relative to a standard included in all reactions (endometrial tissue cDNA). Tissue expression levels are shown as relative to the endometrial tissue cDNA standard, and experimental data are expressed as fold change compared with control.

### Immunohistochemistry

Localization of FPR2/ALX was performed by immunohistochemistry in 5  $\mu\text{m}$  paraffin-embedded sections using the Vision Biosystems Bond Immunostaining Robot under normal operating conditions (Leica Microsystems, Wetzlar, Germany). Immunostaining was performed across the menstrual cycle/decidua on tissue sections from five different patient samples per stage of the cycle/decidua following antigen retrieval in 0.01 M sodium citrate buffer (pH 6), using primary antibody specific for FPR2/ALX (1:200; 5  $\mu\text{g}/\text{ml}$ ). Control tissue was

incubated with primary antibody pre-absorbed with a ten times excess FPR2/ALX protein (50  $\mu\text{g}/\text{ml}$ ) overnight at  $4^{\circ}\text{C}$ .

### Lipoxin $\text{A}_4$ ELISA

Lipoxin  $\text{A}_4$  levels were measured in longitudinal serum samples taken at four points across a single menstrual cycle from the same group of five women, a second group of ten patients during the first trimester of pregnancy, and in conditioned medium from decidua tissue ( $n=6$  different patients from gestational ages 7–12 weeks) stimulated with 1 IU hCG, using an ELISA kit according to the manufacturer's instructions (Neogen Corporation, Lexington, KY, USA). Lipoxin  $\text{A}_4$  was extracted from serum samples using  $\text{C}_{18}$  Sep-Pak columns (Waters, Elstree, UK) before analysis. Each sample was assayed in duplicate.

### Statistical analysis

Data are shown as mean  $\pm$  s.e.m. and were analyzed by *t* test or one-way ANOVA with Tukey's *post hoc* test as described in the figure legend (GraphPad Prism, San Diego, CA, USA). All tissue samples were treated in duplicate at the same time to provide two pools for each treatment condition. These were then averaged to a mean value prior to statistical analysis between and among treatment groups. Statistical analysis was performed on the untransformed data and presented as fold increase above vehicle control. All samples within an experiment or set of experiments were subject to the same pattern of analysis.

### 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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