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Estrogen Receptor α antagonists mediate changes in CCL20 and CXCL1 secretions in the murine female reproductive tract

DK Hickey, Ph.D, JV Fahey, Ph.D, and CR Wira, Ph.D

Department of Physiology, Dartmouth Medical School, USA

Abstract

Problem—Estradiol regulates chemokine secretion from uterine epithelial cells but little is known about estradiol regulation *in vivo* or the role of estrogen receptors (ERs).

Method—CCL20 and CXCL1, present in reproductive washes following treatment with selective estrogen receptor modulators (SERMs) were compared with that during estrous, and following estradiol treated ovariectomized BALB/c mice. Cellular regulation was determined using isolated vaginal and uterine epithelial/stromal cells *in vitro*.

Results—Uterine/vaginal chemokine secretion is cyclically regulated with CCL20 lowest but CXCL1 highest during high estradiol, generally mimicking estradiol effect *in vivo*. ER α but not ER β regulated CCL20/CXCL1 secretion by uterine epithelial cells *in vitro* and vaginal CCL20 *in vivo*. Estradiol/SERMs failed to alter uterine CCL20 secretion in ovariectomized mice. Diminished uterine epithelial ER α staining following ovariectomy corresponded with estradiol unresponsiveness of uterine tissue.

Conclusion—ER α regulates CCL20/CXCL1 secretion in the female reproductive tract and ER α antagonists directly oppose the regulation by estradiol. Understanding ER mediated antimicrobial chemokine expression is important to elucidate cyclic susceptibility to sexually transmitted pathogens.

Keywords

Selective Estrogen Receptor Modulators (SERMs); Estradiol; Uterus; Vagina; Estrous Cycle; Antimicrobial; Chemokine

Introduction

The innate immune system provides the initial barrier of protection against a broad range of potential pathogens that infect the female reproductive tract (FRT) (see review WIRA et al 2005) ¹. Endogenous antimicrobials are soluble factors produced by immune and epithelial cells, which inhibit pathogenic microorganism growth through direct killing and/or deactivation. Within the FRT, examples of molecules exhibiting potent antimicrobial properties include enzymes, defensins, and protease inhibitors (see review KING et al 2003) ². The structure, charge, and size of α and β defensins closely resemble that of many chemokines, therefore the study of chemokines as innate antimicrobials has emerged ³. The chemokines CCL20/MIP3 α and CXCL1/mouse KC are reported to be biologically active against a wide range of organisms. The antimicrobial capacity of CCL20 has been observed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, *Streptococcus*

pyogenes, *Enterococcus faecium*, *Staphylococcus aureus*, *Candida albicans* and recently was shown to inhibit the sexually transmitted Human Immunodeficiency Virus (HIV-1) ^{4, 5}. CXCL1 has potent antimicrobial activity against both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes*) bacteria ⁴.

Previous studies from our laboratory have shown that antimicrobial chemokines are produced by human and mouse uterine epithelial cells *in vitro* and that production is regulated by sex hormones particularly estradiol ⁶⁻⁸. When added to epithelial cells in culture, estradiol inhibits both constitutive and pathogen-associated molecular pattern (PAMP) stimulated chemokines that are secreted apically by polarized rodent and human uterine epithelial cells *in vitro* ^{6, 8}. Indirectly, estradiol treatment of uterine stromal fibroblasts results in the production of growth factors such as keratinocyte growth factor and hepatocyte growth factor, which in turn regulate the production of chemokines by uterine epithelial cells *in vitro* ^{9, 10}.

During the reproductive cycle in women, the concentration of estradiol in blood increases during the proliferative phase, peaking at ovulation, and declining during the secretory phase that is dominated by progesterone. Keller *et al*, reported a mid-cycle drop in the concentration of cytokines, chemokines, antimicrobial peptides and antibodies in human cervicovaginal lavages ¹². This includes the antimicrobial peptides, secretory leukocyte protease inhibitor (SLPI), human β -defensin 2 and human neutrophil peptides 1–3. This suppression may provide a potential period of estradiol-mediated immune vulnerability to sexually transmitted pathogens including HIV, HSV and *Chlamydia* ¹¹.

Estrogenic responses are mediated by two estrogen receptors subtypes ER α and ER β , which are members of the nuclear receptor superfamily of ligand-dependent transcription factors. In addition to estradiol, estrogen receptors bind a number of other pharmacological compounds that regulate ER-mediated responses termed selective estrogen receptor modulators (SERMs) ^{13, 14}. SERMs induce different profiles of estrogenic/antiestrogenic actions that are cell-type specific in which agonist or antagonist effects vary depending on the cellular complement of co-activators or co-repressors ¹³. SERMs interact with differing affinities to ER α and/or ER β to regulate gene transcription ¹⁵. To understand the role of ER subtypes in regulating *in vivo* chemokine production in the FRT, we investigated three SERMs, each with differing affinity to ER α and ER β . ICI 182,780 (Faslodex) is an ER antagonist with a high affinity to both estrogen receptor subtypes ^{16, 17}. Y134 is highly selective for ER α over ER β , and has shown to act as an antagonist in reproductive tissue ^{18, 19}. PHTPP specifically interacts with ER β , and has shown to act as a full antagonist in human endometrial cancer cells ²⁰. In contrast, estradiol acts as a direct agonist for both receptor subtypes.

Using female BALB/c mice we evaluated the effects of estradiol and SERMS in mediating the secretion of CCL20 and CXCL1 *in vivo*. We observed a relationship between the stage of the reproductive cycle, and the luminal secretion of CCL20 and CXCL1. Using specific estrogen-receptor antagonists we show that estradiol regulation of CCL20 and CXCL1 secretion is mediated by ER α and not the ER β subtype. Understanding the specific roles of estrogen receptor subtype in mediating the production of antimicrobial peptides in the FRT may provide new therapeutic targets to promote the local secretion of specific protective innate immune factors, thus protecting the reproductive tract from sexually transmitted pathogens.

Material and Methods

Mice

Sexually mature, BALB/c female mice were obtained from the National Cancer Institute colony at Charles River Laboratories (Wilmington, Massachusetts). Mice were maintained in a constant-temperature room with fixed light/dark intervals (12 hr) and allowed food and water *ad libitum*. All procedures involving animals were conducted after approval of the Dartmouth College Institutional Animal Care and Use Committee. Stages of the estrous cycle (proestrus, estrus or diestrus) of BALB/c mice were determined by examining the proportion and morphology of leukocytes and epithelial cells present in daily vaginal smears as previously described ²¹.

Collection of vaginal and uterine secretions

Vaginal secretions were collected by gently flushing the vaginal vault three times with 50µl of sterile PBS using a 200µl pipette tip inserted 5mm in vagina. For uterine fluid collection, upon sacrifice, the entire upper FRT was removed and each uterine horn was flushed with a total of 100µl of PBS. Uterine and vaginal lavage fluid were centrifuged at 10,000g for 5 min to remove cellular components prior to supernatants being frozen at -80°C until assayed via ELISA.

Preparation of 17β-Estradiol and SERMs

For *in vitro* and *in vivo* studies 17β-estradiol, ICI 182,780 (7a,17b-[9-[(4,4,5,5,5-Pentafluoropentyl)sulfinyl]nonyl]estra-1,3,5(10)-triene-3,17-diol); Y134 ([6-Hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3-yl]-[4-[4-(1-methylethyl)-1-piperazinyl]phenyl]methanone); and PHTPP (4-[2-Phenyl-5,7-bis(trifluoromethyl)pyrazolo [1,5-a]pyrimidin-3-yl]phenol) were all purchased from Tocris, (Minneapolis, Missouri). Treatments were prepared in 100% ethanol (Sigma-Aldrich, St Louis, Missouri) at a concentration of 1mg/ml. The ethanol was evaporated and each treatment was resuspended to the appropriate concentration in complete Cellgro® (Invitrogen Life technologies, Grand Island, New York) for *in vitro* studies or in sterile saline for *in vivo* experiments.

In vivo estradiol and SERM treatment

To control for normal circulation of reproductive hormones, female BALB/c 6 week old mice were ovariectomized 7 days prior to three daily subcutaneous injections (50µl) of sterile saline (vehicle control), 17-β estradiol (0.5µg/mouse), ICI 182,780 (10µg/mouse), Y134 (10µg/mouse) or PHTPP (10µg/mouse). A total of eight mice were used for each treatment group. Vaginal and uterine lavage fluids were collected as above 24 hrs after third injection.

In vitro stimulation of freshly isolated FRT cells

Uterine epithelial, uterine stromal and vaginal cells were isolated upon sacrifice from normal cycling animals. Briefly, vaginal and uterine tissue were cut lengthwise, and incubated separately with 46,500 U/ml of trypsin (Sigma-Aldrich) in 2.5% pancreatin (Invitrogen Life Technologies), at 20 ml/g tissue, for 60 min at 4°C and 60 min at 22°C as described previously ²².

Isolation of vaginal cells—Following digestion the mixture was passed through 40µm mesh nylon screen using mechanical disruption (Small Parts, Miami Lakes, FL). Flow through vaginal cells were collected, centrifuged and resuspended in complete Cellgro® low serum culture medium without phenol red (Mediatech, Manassas, Virginia) supplemented

with 20mM HEPES (Invitrogen Life Technologies) and primocin (InvivoGen, San Diego, California).

Isolation of uterine epithelial cells—Epithelial sheets were released from underlying stroma by vortexing in cold HBSS. Epithelial cells were captured from the suspension on a 20µm mesh nylon screen (Small Parts) and collected, centrifuged and resuspended in complete Cellgro®. Sheets were aspirated ~5 times through 20-g needle resulting in single cell suspension.

Isolation of uterine stromal cells—Following removal of epithelial sheets (see above), stromal tissues were digested in an enzyme mixture containing 0.5 g pancreatin (Sigma-Aldrich), 0.2 g trypsin (Sigma-Aldrich) and 400 U of Dnase I/mL (Roche, Indianapolis, Indiana) in 20 mL of HBBS per 1 g tissue for 30min at 37°C before being passed through 40µm mesh nylon screen (Small Parts) using mechanical disruption and flow through cells were collected. Cells were centrifuged and resuspended in complete Cellgro®. Single cell suspensions of primary uterine epithelial, uterine stromal and vaginal cells at 1×10^5 cells in 100µl complete Cellgro® were plated into NUNC 96 well culture plates (Thermo Fisher Scientific, Rochester, New York). Following plating, cells were incubated at 37°C for 24hrs with medium alone (control), 17-β estradiol or SERMS (10^{-9} to 10^{-7} M). Supernatants from each treatment group were prepared by centrifugation at 500g and stored at -80°C. For time-course experiments, treated uterine epithelial cell supernatants were collected at 6, 12, 18 and 24 hr.

CCL20 and CXCL1 ELISA

Concentration (pg/ml) of CCL20 and CXCL1 in *in vitro* cultures and *in vivo* secretions (vaginal, uterine and serum) were determined using Quantitative Duo Set ELISA (R&D Systems, Minneapolis, Missouri) as per manufacturer instructions.

ERα immunohistochemistry

Whole uterine horn and vaginal tissues were fixed in buffered formalin prior to processing and embedding in paraffin with 4µm thick longitudinal sections cut. Tissues sections were stained for estrogen receptor α expression using estrogen receptor AbII (clone 1D5) antibody as per manufacturers instructions (Thermo Fisher Scientific). Tissues sections were counterstained with Hematoxylin and eosin (H&E) and ERα staining was visualized under light microscopy.

Statistical analysis

Data are presented as the mean ± standard error of the mean (SEM) for mice in each *in vivo* group (n=8). *In vitro* results show combined data from triplicate wells from two separate experiments. Unless specified, One-Way analysis of variance (ANOVA) followed by Bonferroni's post-test was used to examine the differences between each stage of the estrous cycle and also for *in vitro* and *in vivo* treatments, and Two-Way analysis of variance (ANOVA) followed by Bonferroni's post-test used for time-course. The significance level was set at $P < 0.05$ for all tests. Statistical analysis was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California, www.graphpad.com).

Results

Estrous cycle regulation of CCL20 and CXCL1 in FRT secretions *in vivo*

To determine changes in CCL20 and CXCL1 levels during the reproductive cycle, we measured their concentrations in uterine and vaginal secretions at proestrus, estrus and

diestrus stages of the estrous cycle. We further compared the secretion of FRT CCL20 and CXCL1 with concentrations found in serum at each stage to determine if chemokines are regulated locally in the FRT or are derived directly from serum. As seen in Figure 1A, CCL20 in the vaginal secretions was higher at diestrus when compared to both estrus ($p<0.001$) and proestrus ($p<0.001$). Similarly, uterine CCL20 was elevated at diestrus relative to estrus ($p<0.05$). In contrast, no detectable CCL20 was observed in serum at any stage of the mouse estrous cycle (Figure 1B). These findings indicate that CCL20 production occurs locally in FRT and is suppressed during the estrogen dominant phases of the estrous cycle.

CXCL1 levels in uterine and vaginal secretions differ from that of CCL20. Vaginal CXCL1 production was lowest at diestrus, increased at proestrus and peaked at estrus ($p<0.05$) (Figure 1C). Uterine production, however, was highest at proestrus relative to diestrus ($p<0.001$) and estrus ($p<0.01$). In contrast to the FRT (Figure 1D), serum CXCL1 was significantly higher at diestrus compared to proestrus ($p<0.05$) and estrus ($p<0.01$). Variations of CXCL1 production in serum, vaginal and uterine secretions indicate that CXCL1 is differentially regulated by sex hormones at each site. Overall, FRT CXCL1 is consistently enhanced in the estrogen dominant phases of the estrous cycle.

Estradiol regulates the secretion of CCL20 and CXCL1

Estrous cycle changes in chemokine production suggested that hormones regulate the secretion of CCL20 and CXCL1. Using a hormonally unopposed mouse model, we examined the effect of estradiol given *in vivo* on luminal chemokine secretion. Ovariectomized mice were treated subcutaneously with 17β -estradiol daily for 3 days. Figure 2 indicates the changes in vaginal and uterine levels of secreted CCL20 and CXCL1. When compared to saline controls, estradiol inhibited the concentration of CCL20 in vaginal washes ($p<0.05$) but had no effect on CCL20 in uterine washes (Figure 2A). In contrast, CXCL1 levels in both vaginal and uterine secretions increased in response to estradiol relative to saline control ($p<0.05$) (Figure 2B). Overall, these results show that estradiol regulates the secretion of CCL20 and CXCL1 *in vivo* in the FRT.

To extend our *in vivo* results, we sought to determine the specific cellular target of estradiol mediated chemokine production. Vaginal, uterine stromal and uterine epithelial cells isolated from non-ovariectomized mice were treated with 10^{-7} M 17β -estradiol for 24hrs. In vaginal cultures, control levels of CCL20 or CXCL1 were not detected at 24hrs (Figure 2C and D) or following 3 days in culture (data not shown) in the presence or absence of estradiol. Therefore the source of estradiol mediated CCL20 and CXCL1 secretion in the vaginal tract is not derived from cells lining the vaginal tract. In contrast, isolated uterine epithelial cells constitutively secrete both CCL20 and CXCL1. Furthermore, treating uterine epithelial cells with estradiol significantly decreased CCL20 ($p<0.001$) (Figure 2C) but enhanced CXCL1 secretion ($p<0.05$) (Figure 2D) relative to control cultures. This was consistent with results observed during the estrous cycle and in other *in vitro* studies⁸, but in contrast to our *in vivo* ovariectomy results. Similar to vaginal cells, uterine stromal cell secretion of CCL20 was undetectable, however CXCL1 was secreted, albeit at lower levels than uterine epithelium. Treating stromal cells with estradiol significantly increased CXCL1 production ($p<0.05$) (Figure 2D). These results indicate that CCL20 and CXCL1 secretion by FRT cells is site specific and regulated by estradiol.

ER α but not ER β antagonists mediate CCL20 and CXCL1 secretion by uterine epithelial cells *in vitro*

To more fully define the mechanism of estrogen action in the FRT, we investigated the specific role of ER α and ER β in mediating chemokine secretion by uterine epithelial cells *in*

vitro. Freshly isolated uterine epithelial cells were treated with SERMs with specific antagonistic affinity to estrogen receptors. Using increasing doses of SERMs (Figure 3), we found that at 10^{-7} M the ER α and β antagonist ICI 182,780 increased CCL20 and decreased CXCL1 secretion relative to control cells. Unexpectedly, we found that these effects were opposite to that seen with estradiol, which decreased CCL20 and increased CXCL1 production compared to controls ($p < 0.05$) (Figure 3 A and B). When ER α and ER β specific SERMs (Y134 and PHTPP respectively) were analyzed, we found that ER α but not ER β receptors mediated the cytokine responses seen with ICI 182,780. Figure 3 indicates that 10^{-7} M Y134 enhanced CCL20 ($p < 0.05$) and inhibited CXCL1 ($p < 0.05$) production, whereas PHTPP had no effect at any doses examined. Interestingly, SERM treatment did not have any effect on CCL20 or CXCL1 secretion by vaginal epithelial or uterine stromal fibroblast cells *in vitro* (data not shown).

The pronounced effect of ER α antagonists on uterine CCL20 and CXCL1 prompted us to examine the onset of this response. As seen in Figure 3 (C and D), Y134 treatment of uterine epithelial cells rapidly increased CCL20 ($p < 0.05$) and inhibited CXCL1 production of both chemokines compared to all other treatment. Significant changes in CCL20 and CXCL1 secretion were seen initially at 12–18 hr ($p < 0.05$) compared to all other treatment (estradiol and ICI 182,780) responses that were measurable initially at 24 hr post treatment.

ER α antagonists mediate vaginal but not uterine CCL20 secretion *in vivo*

Using ovariectomized animals, we examined if SERMs with ER subtype specific antagonistic properties also regulate chemokine secretion *in vivo*. In vaginal secretions, CCL20 was increased with ICI 182,780 and Y134 treatment compared to saline controls ($p < 0.05$) (Figure 4A) whereas PHTPP had little effect. As observed earlier with estradiol, SERM treatment did not significantly alter the concentration of CCL20 in uterine secretions compared to saline controls (Figure 4A). Furthermore, SERMs did not alter CXCL1 in both vaginal and uterine secretions compared to saline controls (Figure 4B).

Ovariectomy lowers the expression of ER α in epithelial cells lining the FRT

To understand the discrepancies between our uterine CCL20 *in vitro* and *in vivo* results, we compared the expression of ER α in FRT tissue isolated from normal cycling and ovariectomized animals using immunohistochemistry (Figure 5). As previously reported²³ we observed diminished ER α staining in uterine epithelial cells following ovariectomy (Figure 5B) compared to intact animals (Figure 5A). In contrast, ER α expression in cells lining the vaginal lumen was greater following ovariectomy (Figure 5D) compared to intact animals (Figure 5C).

Discussion

There is compelling evidence that estradiol regulates the secretion of antimicrobial chemokines by uterine epithelial cells *in vitro* and that under *in vivo* conditions, the innate immune system in the FRT is hormonally controlled. Our studies demonstrate that the secretion of CCL20 and CXCL1 are regulated during the estrous cycle in both the upper and lower FRT of mice. We show that CCL20 levels are lowest in the FRT at proestrus and estrus, when blood estradiol levels are known to be elevated, relative to that seen at diestrus. In contrast, CXCL1 levels are highest in secretions at proestrus and estrus. We show that in the upper FRT, estradiol is a key regulator of CCL20 and CXCL1 as it decreases CCL20 and increases CXCL1 secretion directly by uterine epithelial cells. We further demonstrate that estradiol-mediated chemokine secretion is altered specifically by ER α ligands, such as ICI 182,780 and Y134, but not the ER β antagonist PHTPP. Overall, these studies show that

changes to CCL20 and CXCL1 levels seen during the estrous cycle are due in part to cyclic changes in the concentration of estradiol mediated directly by ER α .

Our findings show that uterine epithelial cells are a major source of chemokines in the upper FRT and that changes in the secretion of CCL20 and CXCL1 are regulated through ER α . The diminished expression of ER α by uterine epithelial cells following ovariectomy provides an explanation as to why uterine tissue is less responsive to estradiol and SERMs given *in vivo* in these animals. In contrast to uterine secretion, following ovariectomy the concentration of vaginal CCL20 following ER α stimulation was 4-fold greater than peak expression during the estrous cycle. Moreover, increased staining of ER α in cells lining the vaginal lumen in ovariectomized mice was observed. These findings suggest that uterine and vaginal epithelial cell ER α staining correlates with SERM mediated CCL20 secretion *in vivo*. Furthermore, it suggests that changes in ER α expression following ovariectomy may alter the chemokine environment of the upper and lower FRT in a site-specific manner.

An interesting finding in our study was that although uterine stromal cells responded to estradiol with enhanced CXCL1 production, SERM treatment with both ER α and β antagonists had no effect. In contrast, isolated uterine epithelial cells were responsive to both estradiol and SERMs. This highlights differences in SERM-mediated responses at a cellular level within the upper FRT. Cell specific transcriptional activity to SERMs is dependent on multiple factors. For example, SERM-ER binding initiates receptor conformational changes that combine with cell specific co-activators and/or co-repressors to form a unique receptor-transcription complex. In combination with specific promoter characteristics of estrogen responsive genes, a number of cell specific responses may result^{24, 25}. Our findings indicate that responsiveness to estradiol and SERMs, while mediated through ER α , leads to unique immune cell responses that vary with the cell type analyzed. Identifying these differences in FRT tissues will be essential for elucidating the potential use of SERMs as mediators for antimicrobial molecules in the FRT.

Understanding the mechanisms by which estrogen receptors alter the production of chemokines with broad-spectrum antimicrobial activity is important for developing potential therapies to protect the FRT against sexually transmitted pathogens. Ghosh *et al* showed that recombinant CCL20 directly inhibits HIV infection of target cells *in vitro*⁵. Further, because of structural similarities with β -defensins²⁶, it has been hypothesized that CCL20 may also be effective against other STI's including *Chlamydia trachomatis*, *Neisseriae gonorrhoea* and Herpes simplex virus^{27, 28}. Our finding that ER α antagonists enhance the local production of CCL20 in the vaginal tract *in vivo* highlights their potential use as local stimulators of protective innate immunity. Further studies are required to determine if the up-regulation of CCL20 by ER α antagonists can protect the reproductive tract against STI's.

This work provides a foundation for understanding the role for ER in the induction of innate immunity in the upper and lower FRT. Our findings suggest that CCL20 and CXCL1 production in the FRT is primarily mediated through ER α ; therefore SERMs with a high affinity to ER α subtype should be further explored as potential mediators of local innate immune protection in the FRT. Furthermore, this work highlights a caveat in studying the direct effects of hormones *in vivo*. As ovariectomized animals are widely used to study the effects of compounds in a hormonally unopposed manner, we are reminded that FRT functions throughout estrous cycle are controlled by multiple hormones, growth factors and cytokines. Changing the normal highly coordinated presence of hormones by ovariectomy alters these functions and may contribute to selective unresponsiveness of cells throughout the FRT. This observation is of clinical importance to post-menopausal women in which the absence of normal cycling hormones may result in alterations in innate immunity that lead to susceptibility to infection.

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Figure 1. Estrous cycle regulation of CCL20 and CXCL1 secretion. The concentration of CCL20 (A) and CXCL1 (B) present in vagina, uterus and serum. Graph shows combined data from individual mice collected at diestrus (n=11), proestrus (n=8) and estrus (n=12). *p<0.05, **p<0.01, ***p<0.001. Data represent mean $\pm$ standard error.

Figure 2.

Estradiol alters the secretion of CCL20 and CXCL1 in FRT lavages in vivo and by FRT epithelial and stromal cells in vitro. In vivo concentration (pg/ml) of CCL20 (A) and CXCL1 (B) in vaginal and uterine luminal secretions collected from ovariectomized mice (n=8) following 17 β -estradiol treatment (grey bars) compared to saline control (black bars). In vitro secretion of CCL20 (C) and CXCL1 (D) from isolated vaginal epithelial (Vag Ep), uterine epithelial (Ut Ep) and uterine stromal fibroblast (Ut Str) cells 24hrs following treatment with 10 -7 M 17 β -estradiol (grey bars) compared to medium control (black bars). *p<0.05, *** p<0.001 significance compared to saline control. Data represent mean $\pm$ standard error.

Figure 3.

The effect of estrogen receptor antagonists in mediating the secretion of chemokines from uterine epithelium in vitro. Freshly isolated primary uterine epithelial cells were incubated for 24 hr with medium, 10⁻⁹M, 10⁻⁸M and 10⁻⁷M of ICI 182,780 (ER α/β antagonist), Y134 (ER α antagonist) or PHTPP (ER β antagonist). Secretion of CCL20 (A) and CXCL1 (B) directly compared to saline and 10⁻⁷M 17 β -estradiol controls. Time course of CCL20 (C) and CXCL1 (D) secretion from uterine epithelium in vitro following treatment with 10⁻⁷M 17 β -estradiol (filled triangle), 10⁻⁷M ICI 182,780 (open triangle), 10⁻⁷M Y134 (open diamond) and 10⁻⁷M PHTPP (closed diamond) compared to medium control (filled square). Media was collected and replaced at 6 hr intervals for 24 hr. Data represent two

separate experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significance compared to medium control. Data represent mean $\pm$ standard error.

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Figure 4.

The effect of estrogen receptor antagonists on the luminal secretion of CCL20 and CXCL1 in vivo. The concentration (pg/ml) of CCL20 (A) and CXCL1 (B) in vaginal and uterine luminal secretions collected from ovariectomized mice (n=8) following ICI 182,780 (ER α / β antagonist), Y134 (ER α antagonist) or PHTPP (ER β antagonist) treatment compared to saline control. * p<0.05 significance compared to medium control. Data represent mean $\pm$ standard error.

Figure 5.

ERα staining in mouse reproductive tissue counterstained with Hematoxylin and eosin (H&E) staining visualized under light microscopy (20×). Upper panels show ERα staining in uterine tissue from normal cycling (A) compared to ovariectomized animals (B). Note the loss of ERα staining localized to uterine columnar epithelial cells in ovariectomized animals (B). Lower panels show ERα staining in vaginal tissue from normal cycling (C) compared to ovariectomized animals (D). Note the greater degree of ERα staining and a reduced width of the epithelium lining the vaginal tract in ovariectomized animals (D). Scale bars, 100μm.