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Estradiol Regulation of Constitutive and Keratinocyte Growth Factor-induced CCL20 and CXCL1 Secretion by Mouse Uterine Epithelial Cells

Severina N. Haddad, Ph.D.[*] and **Charles R. Wira, Ph.D.**[†]

[†]Department of Physiology and Neurobiology, Geisel School of Medicine at Dartmouth, One Medical Center Drive, Lebanon, NH 03756

[*]Department of Biology and Environmental Science, University of New Haven, 300 Boston Post Road, West Haven, CT 06516

Abstract

Problem—Estradiol can directly affect epithelial cells or indirectly affect epithelial cells via stromal fibroblast secretion of growth factors, such as keratinocyte growth factor (KGF). The purpose of the present study was to determine if estradiol regulates constitutive as well as KGF-induced uterine epithelial cell secretion of CCL20 and CXCL1.

Method of Study—Freshly isolated and polarized uterine epithelial cells from Balb/c mice were cultured with estradiol in the presence or absence of KGF. CCL20 and CXCL1 were measured by ELISA.

Results—Estradiol inhibited CCL20 secretion by freshly isolated and polarized uterine epithelial cells in the presence or absence of KGF. Unexpectedly, it enhanced KGF-induced CXCL1 secretion beyond that seen with KGF alone. Estradiol increased CXCL1 secretion at 24 hours and inhibited CCL20 at 48 hours. The effects of estradiol are specific in that progesterone, cortisol, dihydrotestosterone, and aldosterone had no effect on either CCL20 or CXCL1 secretion. The inhibitory effect of estradiol on CCL20 secretion was reversed with ICI 182,780, an estrogen-receptor antagonist, indicating that this effect is estrogen receptor-mediated.

Conclusions—Our data indicate that estradiol is important in regulating the effects of KGF on mouse uterine epithelial cell secretion of CCL20 and CXCL1.

Keywords

CCL20; CXCL1; epithelial; estradiol; KGF; uterus

INTRODUCTION

The mucosal immune system of the female reproductive tract (FRT) protects against invading pathogens that threaten reproductive health and often cause harmful pathological

[†]Correspondence: Charles R. Wira, Department of Physiology and Neurobiology, Geisel School of Medicine at Dartmouth, One Medical Center Drive, Lebanon, NH 03756, Telephone: +1 603 650 7733, Fax: +1 603 650 6130, charles.r.wira@dartmouth.edu.

conditions.[1, 2] Among the challenges faced by the immune system of the FRT are sexually transmitted diseases (STDs), reproductive cancers, endometriosis and pelvic inflammatory disease, many of which lead to infertility or death.[3–7] The mucosal lining of the uterus is composed of simple columnar epithelial cells joined together by tight junctions, which form a physical barrier preventing pathogens from accessing the underlying mucosa.[1, 8] Uterine epithelial cells not only provide a physical barrier, but also have other mucosal functions, such as secretion of cytokines, chemokines and antimicrobials.[9–18] Underneath the epithelial lining is a layer of connective tissue (stroma) composed of an extracellular matrix and a heterogeneous population of cells, including fibroblasts and various immune cells (macrophages, dendritic cells, neutrophils, NK cells, and T and B lymphocytes).[8, 19, 20] Epithelial cells have an intricate bidirectional communication with these various stromal cells in order to orchestrate an immune response.[20]

Within the FRT, estradiol is a crucial regulator of uterine growth, differentiation, and secretory function.[21–24] Furthermore, estradiol has been shown to regulate many aspects of FRT mucosal immunity, including epithelial cell monolayer permeability, recruitment of immune cells into the tract, epithelial cell and stromal fibroblast antigen presentation in the uterus and vagina, as well as epithelial cell production of the IgA receptor, known as the polymeric immunoglobulin receptor (pIgR).[10, 25–36] For example, ovariectomized rats treated with estradiol have increased IgA and IgG levels in uterine secretions, while immunoglobulin concentrations are decreased in vaginal secretions.[26, 37–39] In addition, estradiol can alter uterine epithelial cell production of cytokines, chemokines, and antimicrobials.[11, 18, 40–45] The effects of estradiol are mediated through the estrogen receptor (ER).[46–48] Grant-Tschudy & Wira[31] demonstrated that estradiol decreases transepithelial resistance of uterine epithelial cells by binding to the ER expressed by epithelial cells. Furthermore, estradiol has also been shown to directly affect uterine epithelial cell secretion of secretory leukocyte protease inhibitor (SLPI) and CCL20.[44, 49, 50]

Estradiol can also regulate epithelial cell function indirectly via the underlying ER-positive stromal fibroblasts.[21, 51, 52] Indirect effects of estradiol include uterine epithelial cell proliferation and tumor necrosis factor α (TNFα) secretion.[51–53] These studies suggest that estradiol regulates uterine stromal fibroblast secretion of soluble mediators, which in turn regulate epithelial cell proliferation and cytokine secretion. Growth factors have emerged as possible mediators of estradiol effects, since they can diffuse across the basement membrane and elicit changes in cell morphology and function.[54] For example, in the mammary gland, epithelial cell proliferation in response to estradiol is mediated by stromal fibroblast secretion of hepatocyte growth factor (HGF).[55]

Previously, we demonstrated that keratinocyte growth factor (KGF), a stromal fibroblast-derived factor, regulates uterine epithelial cell innate immune function by increasing secretion of CCL20 (also known as macrophage inflammatory protein 3α (MIP3α)) and CXCL1 (also known as keratinocyte-derived chemokine (KC)).[56] Others have demonstrated that KGF expression in the FRT fluctuates with stage of the menstrual cycle.[57–59] In the uterus, KGF expression is lowest during the proliferative phase (predominantly estradiol) and highest during the secretory phase (predominately progesterone).[58, 59] Previous work from our laboratory has shown that, *in vitro*, estradiol inhibits mouse uterine epithelial cell

constitutive, as well as LPS-induced, CCL20 secretion.[44] More recently, our laboratory has demonstrated that CCL20 and CXCL1 fluctuate during the estrous cycle of the mouse. Uterine lavages from mice in diestrous (low levels of estradiol) have high levels of CCL20 relative to estrus and proestrus. In contrast, CXCL1 is highest at proestrus (high levels of estradiol) relative to other stages of the cycle.[60, 61]

The following studies were conducted to test the hypothesis that estradiol inhibits constitutive and KGF-induced CCL20 and CXCL1 secretion by mouse uterine epithelial cells. In addition, studies were undertaken to determine if the effects of estradiol on CCL20 and CXCL1 secretion are specific for estradiol and mediated via the epithelial cell ER. As a part of these studies we compared the effects of estradiol on freshly prepared uterine epithelial cells to its effects on polarized uterine epithelial cells.

MATERIAL AND METHODS

Animals

Sexually mature, female Balb/c mice from the National Cancer Institute colony at Charles River Laboratories (Kingston, NY) were used. Animals were housed at the Dartmouth Animal Resources Center and allowed food and water *ad libitum*. Animals were kept in a constant-temperature environment with controlled light-dark intervals of 12 hr each. When animals reached 10–12 weeks of age, they were sacrificed by CO_2, and uteri were removed and pooled from 15–30 animals at various stages of the estrous cycle. Approval from the Dartmouth College Institutional Animal Care and Use Committee was established prior to conducting any procedures involving animals.

Uterine Epithelial Cell Preparation

Mouse uterine horns were removed, slit lengthwise, pooled and incubated in a solution of 46,500 units trypsin (Sigma-Aldrich, St Louis, MO) per 1 ml of 2.5% pancreatin (Invitrogen, Carlsbad, CA) at 20 ml/g of tissue for 1 hr at 4°C followed by 1 hr at 22°C. Uterine tissues were then transferred to ice-cold Hanks Balanced Salt Solution (HBSS; Invitrogen), vortexed three times to release sheets of epithelial cells, after which the resulting cell suspensions were combined. The cell suspension was then filtered through a 20 μm mesh nylon screen (Small Parts, Inc., Miami Lakes, FL) to recover epithelial cell sheets. Epithelial sheets were washed off of the screen and centrifuged at 500×*g* for 5 min. Epithelial sheets were resuspended in complete medium consisting of Dulbecco's Modified Eagle Medium (DMEM)/Ham F-12 nutrient mixed 1:1 (without phenol red; Invitrogen) containing 10% stripped fetal bovine serum (FBS; Hyclone, Logan, UT) and supplemented with 20 mM Hepes (Invitrogen), 2 mM L-glutamine (Mediatech, Herndon, VA), and 100 μg/ml Primocin (InvivoGen, San Diego, CA). Complete medium will be referred to as DMEM/F-12 + 10% stripped FBS in Results. As indicated below, for experiments with freshly isolated uterine epithelial cells, Cellgro Complete Medium (Mediatech) supplemented with 15 mM Hepes (Invitrogen) and 100 μg/ml Primocin (InvivoGen) was used (referred to as Cellgro). The purity of cell cultures was more than 99% epithelial cells as previously described in Grant-Tschudy and Wira (2005).[62]

Epithelial Cell Transwell Culture

For experiments conducted with polarized cells, epithelial cell sheets were seeded onto 0.4 μm pore membrane/10 mm diameter Nunc tissue culture inserts (Nalge Nunc, Rochester, NY) that had been coated with diluted Matrigel (1:4 dilution; growth factor reduced, without phenol red; BD Biosciences, Bedford, MA). Uterine epithelial cells (approximately 1×10^5 cells/insert) in 300 μl DMEM/F-12 + 10% stripped FBS were added to the top of each insert at a ratio of 3–4 culture inserts per uterine horn. Inserts were placed in 24-well Nuclon plates (Nalge Nunc) containing 500 μl of DMEM/F-12 + 10% stripped FBS and incubated at 37°C with 5% CO_2 for 5–7 days to allow cells to grow to confluence and form tight junctions (TER ≥ 2000 ohms/well). For all polarized epithelial cell experiments, medium was collected from the apical and basolateral compartments and replaced at 48-hr intervals.

Transepithelial Resistance Measurement

Transepithelial resistance (TER) of polarized epithelial cells on transwell inserts was monitored daily using an EVOM™ epithelial voltohmmeter and electrode (World Precision Instruments Inc., New Haven, CT). Once epithelial cells had reached high TER (≥ 2000 ohms/well), they were considered to be a polarized, confluent monolayer.

Epithelial Cell Fresh Preparation

For experiments using freshly isolated epithelial cells, epithelial cell sheets were re-suspended in Cellgro prior to passage through a 20-gauge needle resulting in a preparation of a single cell suspension. The epithelial cell suspension was centrifuged at 400×*g* for 8 min, resuspended in Cellgro at a density of 2×10^5 cells/100 μl Cellgro per well of 96-well tissue culture plates (Nalge Nunc) and incubated overnight at 37°C with 5% CO_2 prior to treatment.

Hormone and Antagonist Preparation and Treatment

Estradiol-17β (E_2; Calbiochem, La Jolla, CA), progesterone (P_4; Calbiochem), dihydrotestosterone (DHT; Steraloids, Inc., Wilton, NH), cortisol (Steraloids, Inc.), aldosterone (Sigma-Aldrich), ICI 182,780 (Tocris Bioscience, Ellisville, MO) were each dissolved in 100% ethanol (Sigma-Aldrich), evaporated to dryness, and resuspended in either DMEM/F-12 + 10% stripped FBS or Cellgro. An equivalent amount of 100% ethanol (Sigma-Aldrich) was evaporated in vials prior to the addition of media to control for residues present in the ethanol. When polarized uterine epithelial cells reached high TER, media was removed and replaced with fresh media either alone or containing E_2 or P_4. In experiments with freshly isolated uterine epithelial cells, E_2, P_4, DHT, cortisol, or aldosterone was added directly to the epithelial cells in the 96-well plates. In some studies, hormones were added concurrently with KGF to determine their effect on KGF-mediated effects on uterine epithelial cell CCL20 and CXCL1 secretion. In experiments blocking the estrogen receptor, ICI 182,780 was added prior to the addition of estradiol. ICI 182,780 was used at a concentration 100-fold greater than the concentration of estradiol.

Growth Factor Treatment

Recombinant human KGF (R&D Systems, Minneapolis, MN; PeproTech Inc., Rocky Hill, NJ), epidermal growth factor (EGF) (R&D Systems) and hepatocyte growth factor (HGF) (R&D Systems; PeproTech, Inc.) were placed in the basolateral compartment for 48 hr (unless otherwise noted) of confluent, polarized, mouse uterine epithelial cells. For experiments using freshly isolated uterine epithelial cells, KGF was added directly to the cells in 96-well plates (Nalge Nunc).

CCL20 and CXCL1 Analysis

The concentrations of CCL20 and CXCL1 in conditioned media (CM) from cell cultures were determined using commercially available ELISA kits (R&D Systems) according to manufacturer's protocol. CM from polarized and freshly prepared uterine epithelial cells was diluted prior to being analyzed for CCL20 and CXCL1 concentrations. Apical and basolateral CM from polarized epithelial cells were diluted in DMEM/F-12 + 10% stripped FBS at 1:20 for CCL20 ELISA and 1:10 for CXCL1 ELISA. CM collected from freshly isolated epithelial cells was diluted in Cellgro at 1:10 for both CCL20 and CXCL1 analysis. ELISA standards were diluted in the appropriate media rather than reagent diluent as the commercial protocol suggests.

Statistics

The mean and standard error of the mean were determined using Prism 4 for Macintosh (GraphPad Software, Inc. La Jolla, CA) to calculate t-tests and one-way repeated measures analysis of variance (ANOVA). Bonferroni post-tests were used when the statistical test indicated that there was a significant difference between means. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Estradiol Regulation of CCL20 and CXCL1 Secretion by Freshly Isolated Uterine Epithelial Cells

Since estradiol is an essential regulator of endometrial function, the goal of these studies was to determine if estradiol regulates uterine epithelial cell innate immune function, measured as CCL20 and CXCL1 secretion. Freshly isolated mouse uterine epithelial cells were plated onto 96 well plates and treated with KGF (50 ng/ml) in the presence or absence of estradiol (1×10^{-7}M) for 48 hr. Figure 1 demonstrates that KGF alone significantly increased uterine epithelial cell secretion of both CCL20 and CXCL1. Similarly, we found that estradiol alone inhibited ($p<0.001$) constitutive CCL20 secretion (Figure 1A). Interestingly, when incubated in combination with KGF, estradiol inhibited ($p<0.001$) KGF-induced secretion of CCL20. Estradiol inhibited CCL20 secretion by approximately the same percentage in the presence or absence of KGF. In contrast to CCL20, estradiol had no effect on constitutive CXCL1 secretion (Figure 1B). However, when added along with KGF, estradiol increased ($p<0.05$) CXCL1 secretion beyond that seen with KGF alone (Figure 1B).

Time course studies were conducted to determine the onset of estradiol effects on CCL20 and CXCL1 secretion. As seen in Table I, constitutive as well as KGF-induced CCL20 and

CXCL1 secretion increased over time regardless of treatment group. Table I also demonstrates that estradiol significantly ($p<0.001$) inhibited constitutive CCL20 as well as KGF-induced CCL20 secretion at 48 and 72 hr. In contrast to CCL20, constitutive CXCL1 secretion increased slightly at 48 hr of hormone treatment ($p<0.05$); but was absent at 72 hr. When added along with KGF, estradiol significantly ($p<0.001$) enhanced KGF-induced CXCL1 secretion at 12, 24, 48, and 72 hr compared to KGF treatment alone.

Specificity of Estradiol Effects on CCL20 and CXCL1 Secretion by Freshly Isolated Uterine Epithelial Cells

To test the hypothesis that the effects on CCL20 and CXCL1 were specific to estradiol, freshly isolated uterine epithelial cells were treated with estradiol, progesterone, cortisol, dihydrotestosterone (DHT), or aldosterone alone or in combination with KGF for 48 hr. Consistent with data shown in Figure 1A and Table I, estradiol significantly inhibited constitutive and KGF-induced secretion of CCL20 (Figure 2A). Figure 2A also demonstrates that progesterone, cortisol, DHT, and aldosterone had no effect on either constitutive or KGF-induced CCL20 secretion by uterine epithelial cells. As shown in Figure 2B, when CXCL1 was measured, progesterone, cortisol, DHT, and aldosterone also had no effect on either constitutive or KGF-induced CXCL1, while estradiol in combination with KGF significantly increased KGF-induced CXCL1 secretion similar to results shown in Figure 1B and Table I.

Estradiol Regulation of CCL20 and CXCL1 Secretion by Polarized Uterine Epithelial Cells

Thus far, the studies presented were conducted using freshly isolated mouse uterine epithelial cells. To better mimic *in vivo* conditions, mouse uterine epithelial cells were grown to confluence on transwell cell culture inserts. Following polarization and formation of tight junctions with high TER, uterine epithelial cells were treated with KGF and/or estradiol (1×10^{-7} M) for 48 hr. Consistent with previously reported results,[56] when added to polarized cells KGF significantly ($p<0.001$) stimulated apical and basolateral CCL20 secretion (Figure 3A and 3B). Figure 3 also demonstrates that estradiol inhibited ($p<0.001$) both apical and basolateral constitutive CCL20 secretion, similar to the findings by Soboll *et al.*[44] In addition, we found that estradiol significantly inhibited KGF-induced apical and basolateral secretion of CCL20 (Figure 3A and 3B). In contrast to CCL20, estradiol had no effect on either constitutive or KGF-induced apical CXCL1 secretion by polarized uterine epithelial cells (Figure 4A). Furthermore, as seen in Figure 4B shows that constitutive basolateral secretion of CXCL1 was not affected by estradiol. Unexpectedly, we found that estradiol in combination with KGF enhanced KGF-induced basolateral CXCL1 release beyond that seen with KGF alone.

To more fully determine the effects of estradiol on the secretion of CCL20 and CXCL1, a dose-response experiment was carried out. Figure 5 demonstrates that as the dose of estradiol increased, apical (Figure 5A) and basolateral (Figure 5B), constitutive as well as KGF-induced apical CCL20 secretion decreased. Also shown (solid vs. hatched lines) is that uterine epithelial cells treated with KGF, regardless of the presence or absence of estradiol, epithelial cells secreted significantly more CCL20 than control cells. When CXCL1 levels

were measured, estradiol alone or in combination with KGF had no effect on apical or basolateral CXCL1 secretion (data not shown).

Estradiol Inhibition of CCL20 is Estrogen Receptor Mediated

To determine whether the effect of estradiol on uterine epithelial cell CCL20 secretion was estrogen receptor mediated, polarized epithelial cells were incubated with estradiol and/or ICI 182,780, an estradiol receptor antagonist [63, 64]. Polarized uterine epithelial cells were treated with or without estradiol (1×10^{-8} M), ICI 182,780 (1×10^{-6} M), or estradiol in combination with ICI 182,780 for 48 hr. As shown in Figure 6A estradiol inhibition of apical CCL20 production was reversed in the presence of ICI 182, 780. Additionally, Figure 6B indicates that estradiol-mediated inhibition of basolateral CCL20 secretion was also reversed in the presence of ICI 182,780, indicating that the effect of estradiol on CCL20 is mediated through the estrogen receptor. Unexpectedly, we found that epithelial cells, when incubated with ICI 182,780 in the absence of estradiol, tended to increase basolateral CCL20 secretion beyond that seen with medium alone.

DISCUSSION

The studies presented here demonstrate that uterine epithelial cells are responsive to estradiol treatment *in vitro*. Specifically, estradiol inhibited both constitutive as well as the KGF-induced secretion of CCL20 in a dose-dependent manner. Moreover, we show that polarized uterine epithelial cell inhibition of CCL20 secretion by estradiol is estrogen receptor mediated. Furthermore, when CXCL1 secretion was measured, estradiol had no effect on polarized uterine epithelial cell constitutive apical or basolateral CXCL1 secretion; though, unexpectedly, we found that estradiol did increase KGF-induced basolateral CXCL1 secretion. Finally, these studies show that the effects of estradiol on CCL20 and CXCL1 are specific, since treatment with progesterone, cortisol, DHT, and aldosterone had no effect on either CCL20 or CXCL1 secretion. Overall, these studies indicate that estradiol and KGF act directly on epithelial cells to regulate their innate immune function, measured as CCL20 and CXCL1 secretion.

Estradiol is known to regulate uterine epithelial secretion of cytokines, chemokines and antimicrobials.[44, 49, 50, 53] Previously, Soboll *et al.*[44] demonstrated that estradiol inhibited the constitutive as well as pathogen associated molecular pattern (PAMP)-induced CCL20 secretion by polarized mouse uterine epithelial cells. Our findings extend the work of Soboll *et al.*[44], first by demonstrating that estradiol inhibits constitutive apical and basolateral CCL20 secretion in a dose-dependent manner and that inhibition by estradiol is ER-mediated. Second, the studies of Soboll *et al.*[44] demonstrated that, when added to the culture medium along with LPS, estradiol inhibited LPS-induced CCL20 secretion; similarly, our studies show that estradiol inhibits KGF-induced CCL20 secretion by polarized uterine epithelial cells. Third, when freshly isolated uterine epithelial cells were examined for hormone responsiveness, we found that estradiol inhibited constitutive and KGF-induced CCL20 secretion similar to that seen with polarized epithelial cells. Taken together, these studies suggest that estradiol plays a central role in regulating uterine epithelial cell responsiveness to Toll-like receptor (TLR) agonists (pathogens) as well as growth factors

produced by the underlying stromal fibroblasts. Moreover, since CCL20 is chemotactic for immature dendritic cells, B cells and memory T cells whereas CXCL1 is chemotactic for neutrophils, these studies suggest that epithelial cell chemokine production, an important part of innate immune defense in the FRT, is under hormonal control and is responsive to underlying stromal fibroblasts.

In addition to its chemotactic activity, CCL20 is known to have antimicrobial properties. Others have demonstrated that CCL20 has pronounced inhibitory effects on a number of potential pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*.[65] In other studies, our laboratory found that CCL20 has anti-HIV activity.[66] When incubated with HIV-1, we found that recombinant CCL20 inhibits both X4/IIIB and R5/Bal HIV-1 in a dose-dependent manner.[66] The inhibitory activity was observed only when the virus was incubated with CCL20 suggesting that the mechanism of inhibition is likely to be a direct interaction between HIV-1 and CCL20. Our findings in the present study indicate that CCL20 is precisely regulated by the interactions of stromal fibroblast secretion of KGF and estradiol. What remains to be elucidated is the net effect of KGF and estradiol on CCL20 and CXCL1 secretion in the uterus during the reproductive cycle. As discussed by Wira & Fahey,[67] antimicrobials, such as SLPI and defensins, found in human cervical-vaginal secretions are suppressed at midcycle when ovulation occurs. The extent to which CCL20 and CXCL1 fluctuate within the upper reproductive tract (uterus and Fallopian tubes) during the menstrual cycle remains to be determined. However, it is clear that regulation, based on the present findings, is more complicated than previously thought and most probably involves estradiol and progesterone acting in concert at several levels to elicit a net overall effect.

Our finding that estradiol and KGF regulate the secretion of CCL20 has important implications when considered in the context of reproductive success. In the FRT, there exists a delicate balance between immunoprotection and immunosuppression. On the one hand, the immune system must be dampened to allow survival of an allogeneic conceptus, while on the other hand, some level of antimicrobial activity in secretions is essential to protect against microorganisms associated with semen as well as vaginal commensal bacteria.[68] Our discovery that secretion of CCL20 is dampened, but not totally suppressed by estradiol suggests that the innate immune system is finely tuned to protect the fetus and mother at a time when adaptive immunity is suppressed.[69] Alternatively, whereas our findings in the rodent uterine epithelial cells indicate that estradiol inhibits CCL20, other studies by us using human uterine epithelial cells have shown that estradiol upregulates other antimicrobials, including SLPI, human beta defensin 2 (HBD2), and elafin.[11, 49, 70, 71] This suggests that multiple antimicrobials in the reproductive tract have evolved to inhibit pathogens and that each is under hormonal control.

Glucocorticoids have been shown to inhibit CCL20 expression by human lung epithelial cells.[72] Similarly, CXCL1 gene expression was down-regulated by glucocorticoid treatment of mouse embryonic fibroblasts and rat mesangial cells.[73–75] In contrast, dexamethasone had no effect on mouse lung epithelial cell CXCL1 expression.[76] Our studies demonstrate that, unlike epithelial cells at other mucosal surfaces,[72–75, 77] cortisol had no effect on either CCL20 or CXCL1 secretion regardless of the presence or absence of KGF. Having shown

that cortisol had no effect on CCL20 and CXCL1 secretion, we asked if other hormones had an effect and found that progesterone, DHT and aldosterone had no effect on either CCL20 or CXCL1 secretion by uterine epithelial cells. Overall, these studies demonstrate the specificity of estradiol regulation of CCL20 and CXCL1 secretion.

In conclusion, our studies suggest a mechanism through which estradiol, by modulating the actions of KGF on epithelial cells, regulates the presence of uterine neutrophils and dendritic cells, both of which are essential for the repair, remodeling and regeneration of the endometrium during each reproductive cycle in the human and rodent.[18, 54, 78–83] Neutrophils and dendritic cells also play an important role in host protection during mating and the latter part of the reproductive cycle, when endometrial sloughing occurs in primates.[84–86] These studies indicate that estradiol regulation of CCL20 and CXCL1 secretion by epithelial cells in the uterus is most likely mediated through the complex interactions of stromal fibroblasts and epithelial cells that utilize growth factors rather than exclusively through the direct action of estradiol on its target cell. These findings suggest that epithelial-stromal cell communication along with endocrine balance are important determinants in the response of the mucosal immune system to the challenges of sexually transmitted infections, gynecological cancers, endometriosis, and infertility.

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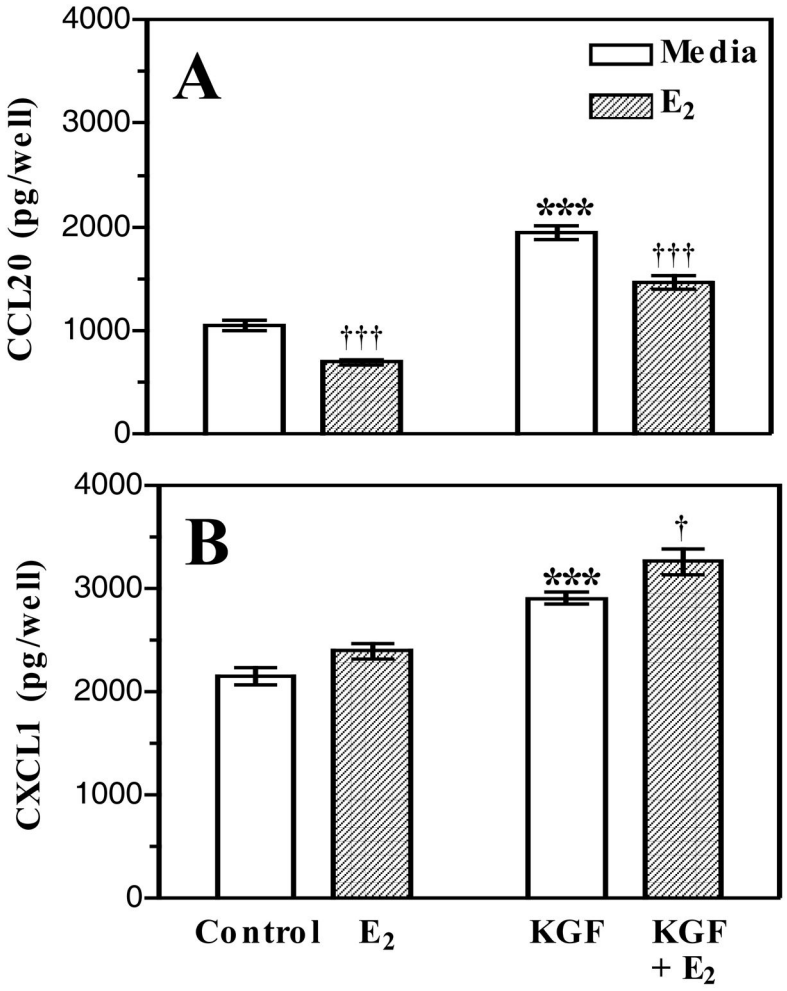

Figure 1.

Estradiol regulates constitutive and KGF-induced CCL20 and CXCL1 secretion by uterine epithelial cells. Freshly isolated mouse uterine epithelial cells were incubated overnight prior to treatment with control medium or estradiol (E_2; 1×10^{-7}M) with or without KGF (50 ng/ml). Supernatants were collected at 48 hr and analyzed for CCL20 (A) and CXCL1 (B). The results are shown as the mean ± SEM of 4 pooled experiments. ***, CCL20 or CXCL1 significantly ($p<0.001$) greater with KGF treatment than controls. †, CXCL1 significantly ($p<0.05$) greater with estradiol and KGF treatment together than KGF alone. †††, CCL20 significantly ($p<0.001$) less with estradiol than controls.

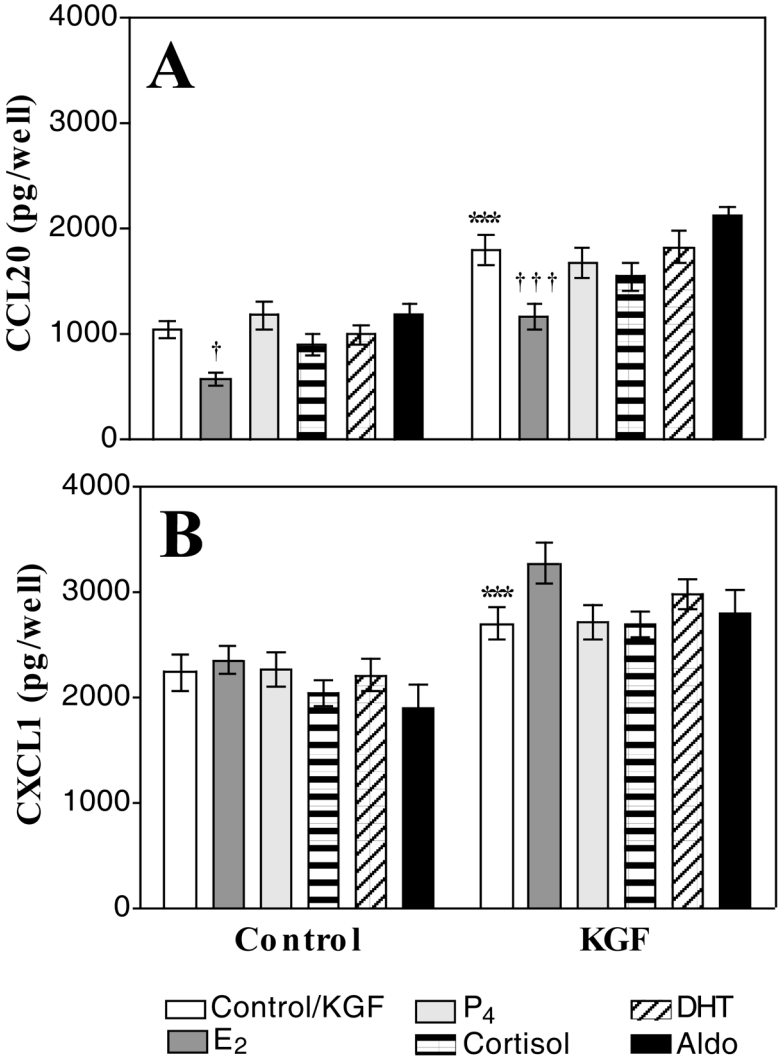

Figure 2.

The effect of estradiol is specific for CCL20 and CXCL1 secretion. Freshly isolated mouse uterine epithelial cells were incubated overnight prior to treatment with control medium or KGF (50 ng/ml) with or without estradiol (E_2), progesterone (P_4), cortisol, dihydrotestosterone (DHT), or aldosterone (1×10^{-8} M) for 48 hr. Supernatants were collected and analyzed for CCL20 (A) and CXCL1 (B). The results are shown as the mean ± SEM of 3 pooled experiments. ***, CCL20 or CXCL1 significantly ($p<0.001$) greater with KGF treatment than controls. †, CCL20 significantly ($p<0.05$) less with estradiol treatment than controls. Δ, CXCL1 significantly ($p<0.05$) greater with estradiol in combination with KGF than KGF alone.

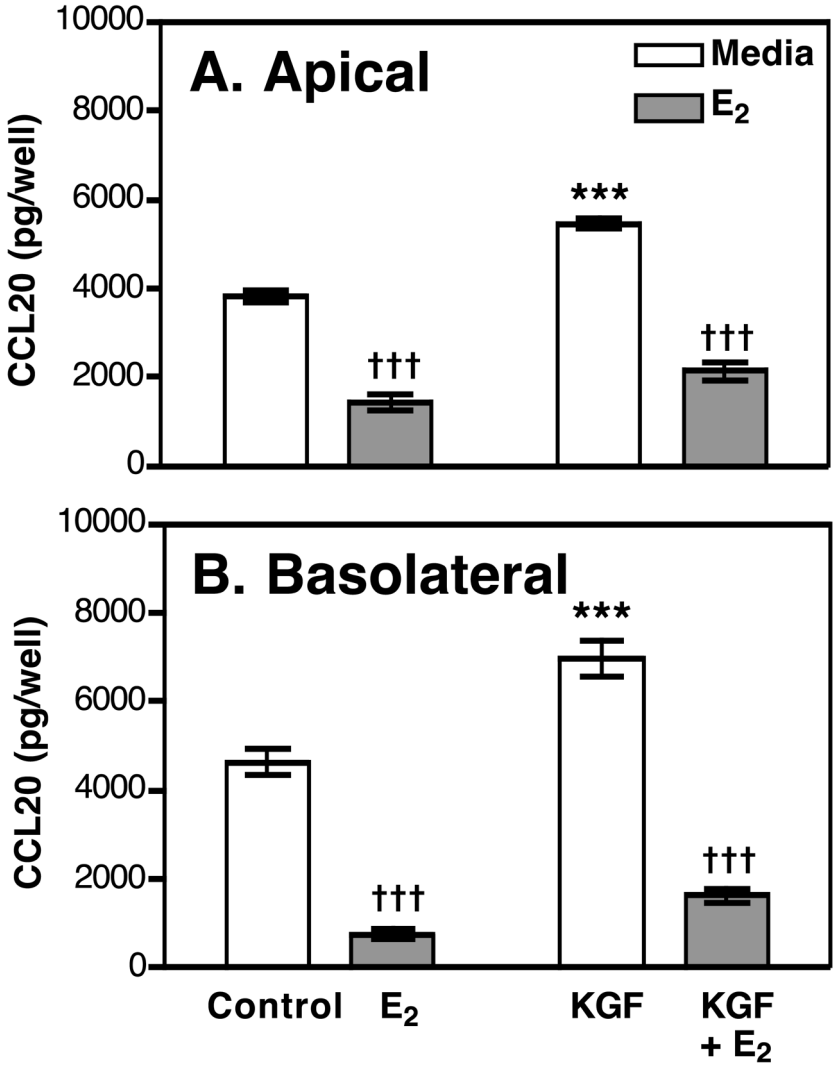

Figure 3.

Estradiol inhibits constitutive and KGF-induced CCL20 secretion by polarized uterine epithelial cells. Polarized uterine epithelial cells were cultured with or without KGF (50 ng/ml) and/or with or without estradiol (E_2; 1×10^{-7} M) for 48 hr. Results are shown as the mean ± SEM of 4 pooled experiments. ***, CCL20 significantly ($p<0.001$, respectively) greater with KGF treatment than epithelial cells cultured without KGF. †††, CCL20 significantly ($p<0.001$) lower with estradiol treatment than epithelial cells cultured without estradiol.

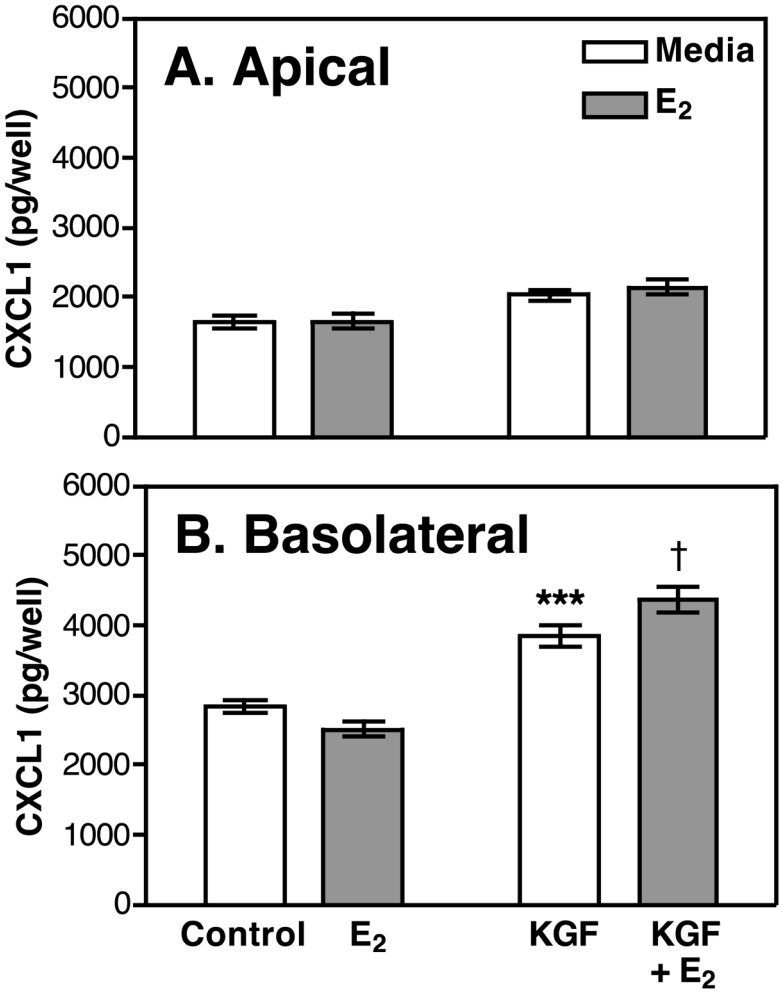

Figure 4.

Estradiol increases KGF-induced CXCL1 basolateral secretion by polarized uterine epithelial cells. Polarized mouse uterine epithelial cells were cultured with or without KGF (50 ng/ml) and/or with or without estradiol (E_2; 1×10^{-7} M) for 48 hr. Results are shown as the mean ± SEM of 4 pooled experiments. ***, CXCL1 significantly ($p<0.001$) greater with KGF treatment than epithelial cells cultured without KGF. †, KGF significantly ($p<0.01$) greater with KGF in combination with estradiol treatment than epithelial cells cultured with KGF alone.

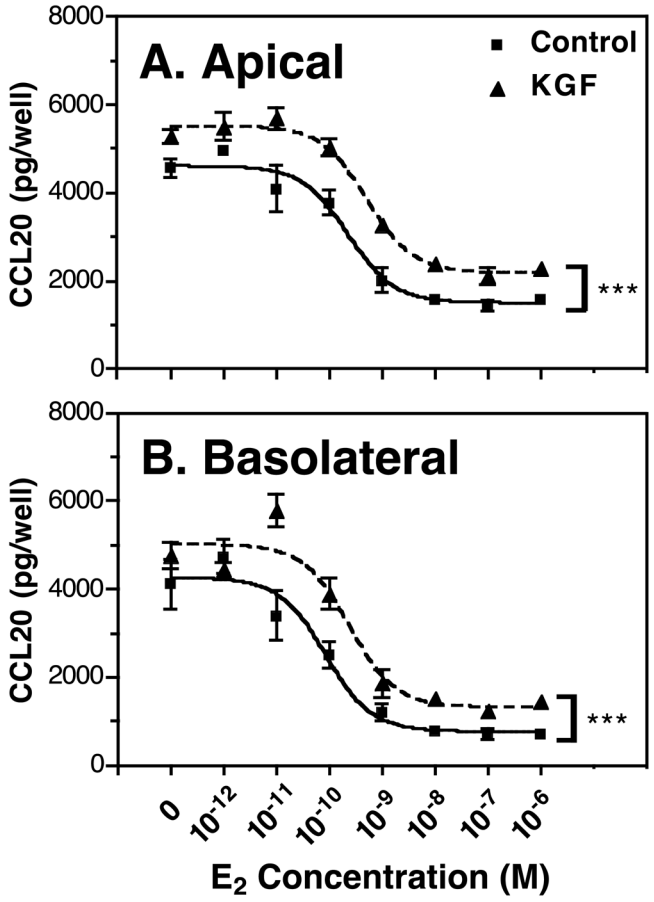

Figure 5.

Estradiol dose-dependently inhibits constitutive and KGF-induced CCL20 secretion by polarized uterine epithelial cells. Polarized mouse uterine epithelial cells were cultured with or without KGF (50 ng/ml) and/or with or without estradiol (E_2; 1×10^{-12}, 1×10^{-11}, 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, or 1×10^{-6} M) for 48 hr. Results are shown as the mean ± SEM. ***, CCL20 significantly ($p<0.001$) greater with KGF treatment than controls.

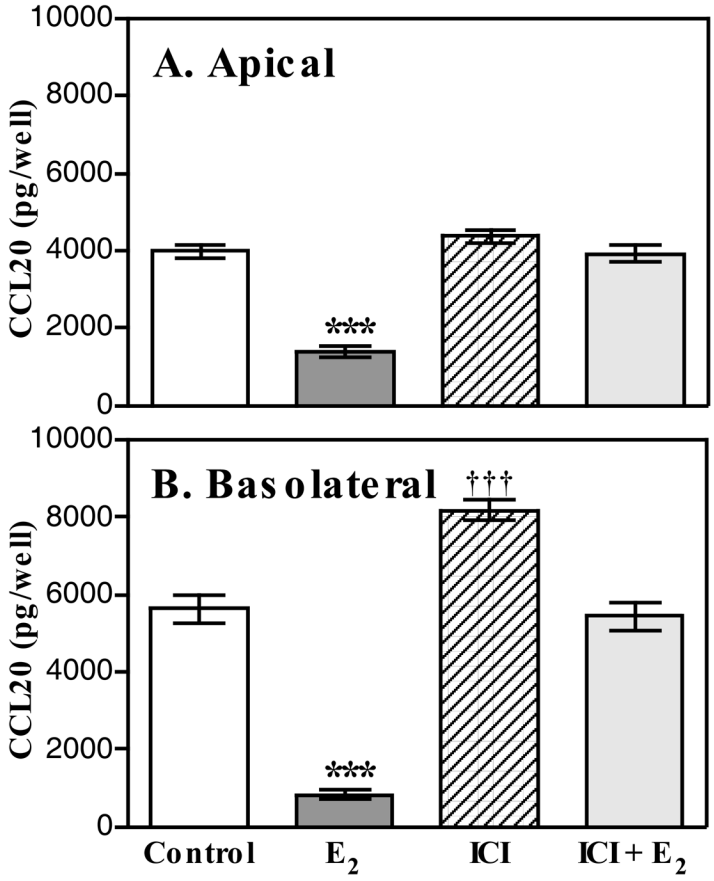

Figure 6.

Estradiol inhibition of CCL20 secretion is mediated via the ER. Polarized mouse uterine epithelial cells were cultured with or without estradiol (E_2; 1×10^{-8} M) and/or with or without ICI 182, 780 (1×10^{-6} M) for 48 hr. Results are shown as the mean ± SEM of 3 pooled experiments. **, ***, CCL20 significantly ($p<0.01$, $p<0.001$, respectively) less with estradiol treatment than epithelial cells cultured without estradiol. ††, CCL20 significantly ($p<0.01$) greater with ICI treatment than epithelial cells cultured without ICI.

Table I

Effect of Incubation Time on Uterine Epithelial Cell Secretion of CCL20 and CXCL1.

	CCL20 (pg/well)					CXCL1 (pg/well)				
	6h	**12h**	**24h**	**48h**	**72h**	**6h**	**12h**	**24h**	**48h**	**72h**
Control	6.3 ± 2.1	39.4 ± 0.59	199.29 ± 8.8	994.695 ± 18.9	2018.52 ± 87.5	165.98 ± 5.6	433.47 ± 7.9	1081.16 ± 57.3	2014.06 ± 38.4	2978.8 ± 58.8
E_2	7.99 ± 0.83	22.370 ± 1.6	131.23 ± 2.16	611.41 ± 5.17†††	1285.9 ± 47.07†††	144.38 ± 4.22	407.76 ± 19.99	1198.66 ± 16.7	2237.8 ± 45.02†	2993.34 ± 79.62
KGF	10.6 ± 0.9	38.79 ± 2.7	286.28 ± 10.2	1788.263 ± 72.8***	2935.0 ± 80.2***	251.65 ± 46.7	494.21 ± 32.4	1508.4 ± 38.5***	2722.5 ± 83.9***	3540.1 ± 45.4***
KGF + E_2	7.21 ± 2.6	34.77 ± 4.73	227.08 ± 7.4	1241.2 ± 49.8†††	2213.7 ± 84.9†††	218.952 ± 8.53	759.7 ± 51.67$^{\Delta\Delta\Delta}$	1862.57 ± 53.5$^{\Delta\Delta\Delta}$	3030.9 ± 132.67$^{\Delta\Delta\Delta}$	4108.15 ± 105.87$^{\Delta\Delta\Delta}$

***CCL20 or CXCL1 significantly ($p<0.001$) greater with KGF treatment than controls.

†††CCL20 significantly ($p<0.001$) less with E_2 treatment than media alone or KGF alone.

†CXCL1 significantly ($p<0.05$) greater with E_2 treatment than media alone.

$^{\Delta\Delta\Delta}$CXCL1 significantly ($p<0.001$) greater with E_2 and KGF treatment than KGF alone.