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Keratinocyte Growth Factor Stimulates Macrophage Inflammatory Protein 3 α and Keratinocyte-derived Chemokine Secretion by Mouse Uterine Epithelial Cells

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Abstract

Problem—Communication between uterine epithelial cells and the underlying stromal fibroblasts is critical for proper endometrial function. Stromal fibroblast-derived growth factors have been shown to regulate epithelial immune functions. The purpose of this study was to determine whether keratinocyte growth factor (KGF) regulates uterine epithelial cell chemokine and antimicrobial secretion.

Method of study—Uterine epithelial cells were isolated from Balb/c mice and cultured in either 96-well plates or transwell inserts. Epithelial cells were treated with KGF, epidermal growth factor (EGF), or hepatocyte growth factor (HGF). Macrophage inflammatory protein 3 (MIP3) and keratinocyte-derived chemokine (KC) levels were measured by ELISA.

Results—Keratinocyte growth factor stimulated the secretion of MIP3 and KC. The effects on MIP3 by KGF were specific because EGF and HGF had no effect. In contrast, KGF, EGF, and HGF had similar effects on KC. Furthermore, KGF administered to the apical side of epithelial cells had no effect on MIP3 or KC secretion, indicating that the KGF receptor is located on the basolateral surface of uterine epithelial cells.

Conclusion—We demonstrate that KGF plays a role in uterine epithelial cell secretion of MIP3 and KC, key immune mediators involved in the protection of mucosal surfaces in the female reproductive tract.

Keywords

Epithelial; immunology; KC; KGF; MIP3 ; uterus

Introduction

The innate immune system is the first line of protection against pathogens that encounter and breach the surface of mucosal sites.^{1,2} In the female reproductive tract, epithelial cells act as sentinels by protecting women from invading pathogens that may initiate a reproductive infection, pelvic inflammatory disease, and in some cases lead to infertility and/or death.³ Furthermore, in the upper portion of the tract, uterine epithelial cells maintain a delicate balance between sensing pathogens present in the lumen while still remaining tolerant of sperm, blastocyst implantation, and an immunologically distinct fetal-placental unit.⁴ Uterine epithelial cells are connected by tight junctions; thus forming a physical

barrier that prevents pathogens from accessing the underlying mucosa.^{3,5} In addition to providing a physical barrier, uterine epithelial cells actively participate in a number of other mucosal immune responses.^{6–14} Epithelial cells constitutively secrete cytokines, chemokines, and antimicrobials that are important for normal physiological processes such as tissue regeneration and remodeling of the endometrium during each reproductive cycle.^{15–17} Some of the cytokines, chemokines, and antimicrobials produced by epithelial cells include tumor necrosis factor (TNF), interleukin-8 (IL-8), macrophage inflammatory protein 3 (MIP3), transforming growth factor (TGF), beta-defensins, and secretory leukocyte protease inhibitor (SLPI).^{11,13,18–21} In addition to constitutive secretion, uterine epithelial cell secretion of cytokines, chemokines, and antimicrobials can be induced upon pathogen exposure.^{11–14} Epithelial cells express Toll-like receptors (TLRs) that recognize highly conserved molecular patterns shared by many microorganisms. Engagement of a TLR by its cognate ligand can lead to the induction of cytokines, chemokines, and antimicrobials.^{11–14,19,22,23}

In the upper portion of the tract, the lining of the uterus is composed of a simple columnar epithelium that sits atop a stromal layer.⁵ Normal endometrial development and function are dependent on precise bidirectional communication between uterine epithelial cells and underlying stromal fibroblasts.²⁴ This intricate communication begins during embryonic development and continues through adulthood.^{24,25} Importantly, uterine stromal fibroblasts influence the differentiation of epithelial cells in processes specific to each area of the tract.^{25–29} Regulation of epithelial cell proliferation and secretory activity by uterine stromal fibroblasts is accomplished through the release of soluble factors (i.e., growth factors) turnover of extracellular matrix and direct cell–cell contact.^{24,30,31}

Several growth factor and their receptors, including epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), hepatocyte growth factor (HGF), and keratinocyte growth factor (KGF), have been identified within the uterus and shown to be involved in stromal fibroblast–epithelial cell interactions.^{32–34} KGF, also known as fibroblast growth factor (FGF)-7, has been found in many mucosal sites including the female reproductive tract and shown to induce proliferation of uterine and vaginal epithelial cells.^{35–39} More recently, KGF was found to play a key role in wound healing via re-epithelialization as well as protecting lung epithelium from oxidant damage.^{40–44} The KGF receptor (KGFR) is a transmembrane tyrosine kinase receptor expressed by epithelial cells.^{45–47} Owing to the specific ligand and receptor expression patterns, KGF is thought to function as a paracrine mediator of stromal fibroblast–epithelial cell interactions.^{36,48–50}

Previous studies in our laboratory have demonstrated that HGF increases uterine epithelial cell transepithelial resistance (TER) while decreasing TNF secretion.⁵¹ HGF neutralization of conditioned stromal fibroblast media (CSM) abolished the increase in TER but unexpectedly failed to reverse CSM effects on TNF secretion. These findings led to the conclusion that HGF has a role in regulating the integrity of the uterine epithelial cell barrier but other soluble factors play a role in TNF secretion.⁵¹ These results also prompted further examination of the effects of other growth factors, such as KGF, on epithelial cell immune function. Two molecules that are important in innate immunity of the female reproductive tract are MIP3 and IL-8.^{17,52–55} Because neutrophils and dendritic cells (DCs) play an important role in host protection through phagocytosis (neutrophils) and pathogen sensing (DCs),^{56–58} identifying the role and regulation of MIP3 and IL-8 becomes important. MIP3, also known as CCL20, recruits immature DCs, memory T cells, and B cells by binding to CCR6.^{59–65} MIP3 functions not only as a chemokine, but also as an antimicrobial.^{55,66} Previously, our laboratory examined MIP3 secretion and found that polarized rat, mouse, and human uterine epithelial cells constitutively secrete MIP3.^{19,52,55,67} Furthermore, mouse uterine epithelial cell secretion of MIP3 was

increased following treatment with TLRs 1, 2, 6, 4, 7, and 9 agonists, while treatment with a TLR3 agonist had no effect.⁶⁷ IL-8 primarily functions to recruit neutrophils, and to a lesser extent T cells.^{54,68–70} IL-8 is present in the human endometrium and has been shown to be involved in many aspects of normal human endometrial physiology including proliferation, angiogenesis, menstruation, implantation, cervical ripening, and parturition.^{54,71–73} IL-8 has not been identified in the rodent, but chemokines similar to IL-8 have been detected.^{74–76} KC (keratinocyte-derived chemokine) is a potent neutrophil chemoattractant that has come to be known as the functional mouse homolog of human IL-8^{74,77–79} and is expressed in the rodent female reproductive tract.^{58,77,80–83} Recently, studies in our laboratory have demonstrated that mouse uterine epithelial cells constitutively secrete KC. In addition, treatment with TLR1/6, 2, and 4 agonists significantly increased KC secretion.¹¹

Knowing that the secretion of chemokines and antimicrobials is essential in endometrial protection, the goal of this study was to determine whether KGF regulates mouse uterine epithelial cell chemokine and antimicrobial secretion. Specifically, our objectives were to determine whether KGF affects uterine epithelial cell secretion of MIP3 and KC and to compare the effects of KGF on uterine epithelial cells to those of EGF and HGF. We show that KGF stimulates uterine epithelial cell secretion of MIP3 and KC, suggesting that KGF plays a previously unrecognized role in female reproductive tract immune responses.

Material and methods

Animals

Female, sexually mature, 8-week-old Balb/c mice from the National Cancer Institute colony at Charles River Laboratories (Kingston, NY, USA) were housed at the Dartmouth Animal Resources Center and kept in a constant-temperature environment with controlled light–dark intervals of 12 hr each. Animals were allowed food and water *ad libitum*. At 10–12 weeks of age, animals were killed by CO₂, and uteri were removed. Uteri were pooled from 15–30 animals at various stages of the estrous cycle. All procedures involving animals were conducted after approval from the Dartmouth College Institutional Animal Care and Use Committee.

Uterine Epithelial Cell Preparation

For isolation of epithelial cells, uteri were removed, cut open lengthwise, pooled, and incubated in a solution of 46,500 units trypsin (Sigma-Aldrich, St Louis, MO, USA) per 1 mL of 2.5% pancreatin (Invitrogen, Carlsbad, CA, USA) at 20 mL/g of tissue for 1 hr at 4°C followed by 1 hr at 22°C. Uteri were then transferred to ice-cold Hanks balanced salt solution (HBSS; Invitrogen) and vortexed with the Vortex-Genie on setting #6 (Scientific Industries Inc., Springfield, MA, USA) to release sheets of epithelial cells. Uterine tissues were rinsed, vortexed two more times, and the resulting cell suspensions combined. Epithelial cells were passed through a 20-μm-mesh nylon screen (Small Parts, Inc., Miami Lakes, FL, USA) to recover epithelial cell sheets. Epithelial cell sheets were removed from the 20-μm screen, centrifuged at 500 × *g* for 5 min, and 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, USA) supplemented with 20 mM Hepes (Invitrogen), 2 mM L-glutamine (Mediatech, Herndon, VA, USA), and 100 μg/mL Primocin (Invivo-Gen, San Diego, CA, USA). This complete medium will be referred to as DMEM/F-12 + 10% stripped FBS. In some experiments, freshly isolated uterine epithelial cells were incubated in Cellgro Complete Medium (Mediatech) supplemented with 15 mM Hepes (Invitrogen) and 100 μg/mL Primocin (Invivo-Gen). This complete medium will be referred to as Cellgro. The purity of cell cultures was more than 99% epithelial cells as previously reported.⁸⁴

Epithelial Cell Transwell Culture

For studies with polarized cells, epithelial cell sheets were seeded in the upper (apical) compartment of 10-mm-diameter Nunc tissue culture inserts with 0.4- μ m pore membranes (Nalge Nunc, Rochester, NY, USA) coated with diluted Matrigel (1:4 dilution; growth factor reduced, without phenol red; BD Bio-sciences, Bedford, MA, USA). Uterine epithelial cells were seeded in a volume of 300 μ L per insert at a ratio of 3–4 culture inserts per uterus. Inserts containing epithelial cells were placed in 24-well Nuclon plates (Nalge Nunc) containing 500 μ L of DMEM/F-12 + 10% stripped FBS. Plates and inserts containing epithelial cells were incubated at 37°C with 5% CO₂ for 5–7 days to allow cells to grow to confluence and form tight junctions (TER = 2000 ohms/well). Medium was collected from the apical and basolateral compartments and replaced at 48-hr intervals.

Transepithelial Resistance Measurement

Transepithelial resistance of epithelial cells grown on inserts was monitored daily with an EVOM™ epithelial voltohmmeter and electrode (World Precision Instruments Inc., New Haven, CT, USA). Background TER of Matrigel-coated cell culture inserts was approximately 250 ohms/well. Epithelial cells were considered confluent and polarized when high TER (> 2000 ohms/well) was reached.

Epithelial Cell Fresh Preparation

For studies with freshly isolated epithelial cells, epithelial cell sheets were resuspended in Cellgro, and sheets were then sheared by passage through a 20-gauge needle resulting in the formation of a single cell suspension. Epithelial cells were counted using 0.4% trypan blue (Gibco/Invitrogen, Carlsbad, CA, USA), centrifuged at 400 $\times g$ for 8 min, resuspended in Cellgro at a final density of 2×10^5 cells/100 μ L, and plated into 96-well tissue culture plates (Nalge Nunc). Epithelial cells were incubated overnight at 37°C with 5% CO₂ prior to treatment.

Growth Factor Treatment

Recombinant human KGF (R&D Systems, Minneapolis, MN; PeproTech Inc., Rocky Hill, NJ, USA), HGF (R&D Systems; PeproTech, Inc.), and EGF (R&D Systems) were added to the basolateral compartment for 48 hr (unless otherwise noted) of polarized, uterine epithelial cells that had reached high TER (> 2000 ohms/well). For experiments with freshly isolated uterine epithelial cells, growth factors were added directly to the cells in 96-well plates.

Supernatant Collection & Chemokine Analysis

Following treatment, supernatants were collected and centrifuged at 10,000 $\times g$ for 5 min at 4°C, transferred, and stored at –80°C until assayed. Supernatants from either polarized or freshly prepared uterine epithelial cells were diluted prior to analysis for MIP3 and KC secretion by commercially available ELISA kits (R&D Systems). Supernatants collected from apical and basolateral compartments of polarized epithelial cells were diluted in media at 1:20 for MIP3 and 1:10 for KC detection. Supernatants from freshly isolated epithelial cells were diluted in Cellgro at 1:10 for both MIP3 and KC analysis. Standards were diluted into the appropriate media, and ELISAs were carried out according to manufacturer's protocol.

Statistics

Data were calculated as the mean $\pm$ standard error of the mean. Prism 4 for Macintosh (GraphPad Software, Inc. La Jolla, CA, USA) was used to calculate *t*-tests and one-way

repeated measures analysis of variance (ANOVA). When the statistical test indicated that there was a significant difference between means, Bonferroni post-tests were used to adjust *P*-values. A *P*-value of <0.05 was used as an indication of statistical significance.

Results

KGF Stimulated Freshly Isolated Uterine Epithelial Cell MIP3 α and KC Secretion

Growth factors have emerged as possible stromal-derived mediators based on their ability to diffuse across the basement membrane and elicit rapid changes in cell morphology and function.³² One such growth factor, KGF, is a stromal-derived paracrine mediator known to increase epithelial cell proliferation in many tissues including mammary gland, uterus, and vagina.^{36,39,49,50} To test the hypothesis that KGF regulates uterine epithelial cell MIP3 and KC secretion, freshly isolated uterine epithelial cells were plated onto 96-well plates and treated with KGF (50 ng/mL) for 48 hr. As seen in Fig. 1, KGF treatment significantly increased both MIP3 (Fig. 1a) and KC (Fig. 1b) secretion by uterine epithelial cells. To further define the effects of KGF on MIP3 and KC release, dose-response and time course studies were carried out. Fig. 2 demonstrates that KGF treatment increased secretion of MIP3 (Fig. 2a) and KC (Fig. 2b) in a dose-dependent manner. KGF doses of 0.00005–5 ng/mL had no significant effect on MIP3 secretion; although there was a trend toward increased secretion as KGF dose increased. When epithelial cells were treated with 50 ng/mL of KGF, MIP3 secretion was significantly greater than controls. This same dose-dependent pattern was observed with KC secretion. As seen in Fig. 2b, increasing doses of KGF increased KC secretion, with 50 ng/mL, resulting in a statistically significant increase in KC levels. To investigate the time course of responsiveness of epithelial cells, epithelial cells were incubated with KGF (50 ng/mL), and secretions were collected at various time points. Fig. 3a demonstrates that MIP3 was undetectable prior to 24 hr but then increased significantly above controls at 48 and 72 hr. In contrast, when secretions were analyzed for KC (Fig. 3b), levels were detectable at 12 hr, and the effect of KGF was first measurable at 24 hr. This increase in KC, relative to controls, persisted at 48 and 72 hr. Overall, these findings indicate that freshly isolated epithelial cells secrete MIP3 and KC in a dose- and time-dependent manner in response to KGF treatment.

Polarized Uterine Epithelial Cell Secretion of MIP3 α and KC Increased in Response to KGF

Having observed that KGF increased MIP3 and KC secretion by freshly isolated epithelial cells, we wanted to test the hypothesis that KGF would increase apical and basolateral secretion of both MIP3 and KC by polarized uterine epithelial cells. Polarized uterine epithelial cells (TER = 2000 ohms/well) were treated with KGF (50 ng/mL) in the basolateral compartment for 48 hr. As seen in Figs 4 and 5 and consistent with previous data,^{11,85,86} polarized mouse uterine epithelial cells constitutively secreted MIP3 and KC into both apical and basolateral compartments (see control bars in Figs 4 and 5). Fig. 4 demonstrates that KGF significantly stimulated both the apical and the basolateral secretion of MIP3. Moreover, these data indicate that MIP3 is released equally into apical and basolateral compartments. In contrast, KGF treatment induced only basolateral KC but had no effect on apical KC secretion relative to controls (Fig. 5). Whereas MIP3 was secreted bidirectionally, these findings indicate that KC is preferentially secreted into the basolateral compartment. Consistent with our findings with freshly prepared epithelial cells (Fig. 1), KGF stimulates MIP3 and KC secretion by polarized uterine epithelial cells.

Specificity of KGF Effects on MIP3 α and KC Secretion by Polarized Mouse Uterine Epithelial Cells

Numerous studies have shown that a number of growth factors are produced in the endometrium and contribute to homeostasis and growth through-out the reproductive

cycle.^{32,49,87–93} To test the hypothesis that the effects of KGF on MIP3 and KC secretion were specific, polarized uterine epithelial cells were treated either with KGF (50 ng/mL), EGF (4 ng/mL), or HGF (50 ng/mL) in the basolateral compartment for 48 hr. Fig. 6 shows that KGF significantly increased MIP3 secretion both apically and basolaterally. In contrast, EGF had no effect on either apical and basolateral secretion of MIP3. Interestingly, HGF had no effect on apical MIP3 secretion but significantly inhibited basolateral MIP3 secretion. When the same secretions were analyzed for KC levels, we unexpectedly found that all three growth factors examined had similar effects on KC secretion. As seen in Fig. 7, of those growth factors tested, only EGF significantly increased apical KC secretion. In contrast, basolateral KC secretion was significantly increased not only by KGF, but also in response to EGF and HGF. These findings indicate that the effects on MIP3 are specific to KGF, while effects on KC may be similar in response to other growth factors.

KGF Receptor is Located on the Basolateral Surface of Polarized Mouse Uterine Epithelial Cells

The receptor for KGF has been located on the basolateral surface of Caco-2 cells, an intestinal epithelial cell line.⁹⁴ To determine whether mouse uterine epithelial cells express KGFR on the basolateral surface, polarized uterine epithelial cells were incubated with KGF placed either in the apical compartment or basolateral compartment for 48 hr prior to media collection and analysis. As seen in Fig. 8, when added to the basolateral surface, KGF significantly increased both apical and basolateral MIP3 secretion relative to control values. In contrast, when KGF was placed in the apical compartment at a concentration identical to that used in the basolateral compartment, no effect on either apical or basolateral MIP3 secretion was observed. As a part of these studies, we analyzed KC and found that basolateral addition of KGF significantly increased basolateral KC secretion (Fig. 9). Similar to that seen with MIP3, apical KGF administration had no effect on either apical or basolateral KC secretion. Together, these data suggest that KGFR is located on the basolateral surface of polarized uterine epithelial cells.

Discussion

The data presented in this study demonstrate that KGF, a stromal fibroblast-derived factor, affects mouse uterine epithelial cell secretion of both MIP3 and KC, key immune mediators involved in the protection of mucosal surfaces in the female reproductive tract. Our studies indicate that when added to the basolateral surface of polarized uterine epithelial cells, KGF stimulated apical and basolateral secretion of MIP3 as well as the basolateral secretion of KC. When added to the apical surface, KGF had no effect on either MIP3 or KC secretion, suggesting that the receptor for KGF is located on the basolateral surface of uterine epithelial cells. The effects on MIP3 by KGF are specific in that EGF and HGF had no effect on MIP3 secretion. In contrast, KGF, EGF, and HGF had similar effects on KC release by uterine epithelial cells. Overall, our findings suggest that female reproductive tract immune protection against bacterial, fungal, and viral pathogens may be indirectly mediated via secretions from underlying stromal fibroblasts.

KGF is a stromal fibroblast-secreted peptide that is a member of the FGF family.^{35,36} The production of KGF by stromal fibroblasts as well as the mitogenic, morphogenic, and motogenic effects of KGF on epithelial cells suggests that KGF functions as a paracrine mediator of stromal fibroblast–epithelial cell interactions.^{39,48–50,95} Others have shown that uterine and vaginal epithelial cell proliferation increases approximately 5-fold in neonatal mice following treatment with KGF.³⁸ In the lung, KGF has been shown to influence epithelial cell secretory function.^{43,83,96} When alveolar type II cells were treated with KGF, surfactant protein (SP)-A and SP-D expression and secretion increased.⁹⁶ Other studies have

investigated the role of KGF in neutrophil recruitment in the lung and found that KGF administration prior to acid aspiration injury decreases the secretion of KC into bronchoalveolar lavage fluid.⁸³ Our studies extend these findings by demonstrating that KGF regulates the secretion of MIP3 and KC by primary mouse uterine epithelial cells. To the best of our knowledge, this is the first report demonstrating that *in vitro* treatment of uterine epithelial cells with KGF results in increased MIP3 and KC secretion.

This study compared KGF-induced MIP3 and KC secretion by non-polarized and polarized uterine epithelial cells to determine whether time in culture affects KGF responsiveness. Several studies have compared polarized epithelial cells with non-polarized cells under a variety of conditions and found functional differences. For example, in a study comparing polarized and non-polarized human endometrial monolayer culture systems on murine embryo development, two-cell embryos cultured on a polarized monolayer had a significantly higher developmental rate than those from non-polarized cells.⁹⁷ When the KGFR, a transmembrane tyrosine kinase receptor expressed on epithelial cells,^{45–47} was measured in the mammary gland, epithelial cell expression of KGFR was greatest in freshly isolated cells with levels decreasing as cells were kept in culture.⁹⁸ Our findings show no difference in KGF-induced chemokine secretion, suggesting that polarized uterine epithelial cells in culture for 5–7 days are as responsive to KGF as freshly isolated cells. Moreover, when receptor localization was determined by addition of KGF to apical versus basolateral surface of polarized cells, we found that basolateral but not apical administration altered MIP3 and KC secretion. These findings extend the immunohistochemical observations of Visco et al. who reported that the KGFR is located on the basolateral surface of intestinal epithelial cells.⁹⁴

KGF treatment of freshly isolated epithelial cells for various lengths of time indicated that MIP3 and KC have different secretory profiles. Constitutive secretion of KC was detected sooner than MIP3 and the length of time for the effects of KGF to be observed was also different between MIP3 and KC. From a mechanistic standpoint, these data suggest that KC and MIP3 are regulated and/or processed differently in response to KGF. Previously, we found that cytokine secretion varies with the cytokine measured and the TLR agonist used.¹⁹ Time course studies of MIP3 and TNF secretion in response to Pam₃Cys and LPS (lipopolysaccharide; a component of the outer membrane of Gram-negative bacteria) indicated that MIP3 release peaked between 4 and 6 hr after treatment, whereas TNF release was gradual over the length of the 12-hr incubation.¹⁹ Our findings that KGF increases KC and MIP3 at 24 and 48 hr, respectively, indicate that KGF regulates cytokine secretion by uterine epithelial cells via different mechanisms. Further studies are needed to determine the regulation (i.e., transcriptional, translational, and exocytic) of MIP3 and KC production and secretion by uterine epithelial cells.

Our results indicate that KGF regulates the apical and basolateral MIP3 secretion by uterine epithelial cells. Considering the dual functions of MIP3 as a pro-inflammatory chemokine and an antimicrobial,^{55,62–66} MIP3 is an important molecule in both the innate and adaptive immune systems.⁹⁹ MIP3 has antimicrobial effects against *Escherichia coli*, *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, *Streptococcus pyogenes*, *Enterococcus faecium*, *Staphylococcus aureus*, *Candida albicans*, and HIV-1.^{55,66} The observed increase in apical secretion of MIP3 in response to KGF most likely relates to its function as an antimicrobial.^{66,100} Having MIP3 available in the uterine lumen would allow for the direct killing of pathogens that are introduced into the tract at the time of mating, when pathogens are most likely to be present. KGF also stimulated the basolateral secretion of MIP3, suggesting that MIP3 may have a role in the recruitment of immature DCs into the endometrium. DCs are present throughout the estrous cycle, with highest numbers present at estrus relative to other stages of the cycle.⁵⁷ These findings suggest that MIP3 may play an

important role in the implantation of a blastocyst.^{101,102} Synthesis of MIP3 can be induced in response to inflammatory mediators such as IL-1 and TNF as well as LPS.^{19,67,103–105} In the female reproductive tract, MIP3 secretion by epithelial cells can be induced following treatment with pathogen-associated molecular patterns (PAMPs) or *Escherichia coli*.^{19,52,67} Our studies extend these findings by demonstrating that, in addition to PAMPs and bacteria, growth factors produced by underlying stromal fibroblasts regulate epithelial cell immune protection.

The data presented here demonstrate that KGF increases uterine epithelial cell basolateral KC secretion. Thus far, KC appears to function exclusively as a chemokine for neutrophils; therefore basolateral secretion of KC is most likely responsible for the infiltration of neutrophils into the endometrium.^{56,58,77,82} Others have shown that neutrophils are essential for remodeling and regeneration of the endometrium during each reproductive cycle in the human and rodent.^{16,56,58,106–109} Our data showing preferential secretion of KC into the basolateral compartment in response to KGF demonstrate the complexity of the immune system in the female reproductive tract and suggest a level of cell–cell communication that has not previously been appreciated.

Our studies indicate that the KGF-induced secretion of MIP3 is specific in that EGF had no effect on MIP3 secretion while HGF inhibited basolateral MIP3. In contrast, EGF stimulated both apical and basolateral KC secretion, while HGF stimulated only basolateral KC secretion. Others have shown that KGF, EGF, and HGF have potent mitogenic effects on epithelial cells,^{87,110,111} but their actions on cytokine/chemokine secretion by uterine epithelial cells, to the best of our knowledge, have not been examined. Our study indicates that each growth factor has its own pattern of action on uterine epithelial cell secretion of innate immune molecules. Previously, our laboratory found that HGF placed in the basolateral compartment of polarized uterine epithelial cells increased TER and decreased TNF secretion.⁵¹ This study expands these findings by demonstrating that KGF and EGF also affect epithelial cell secretion of MIP3 and KC, both of which are critical to innate immune protection.

In summary, the findings presented from this study indicate that KGF is an important regulator of mouse uterine epithelial cell immune function. To the best of our knowledge, this is the first demonstration that KGF stimulates both apical and basolateral secretion of MIP3 and basolateral secretion of KC by polarized mouse uterine epithelial cells *in vitro*. It remains to be determined whether estradiol and/or progesterone regulates KGF and the extent to which KGF effects are critical to immune protection, successful fertilization, and reproduction.

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Fig. 1. Keratinocyte growth factor (KGF) increases MIP3 and keratinocyte-derived chemokine (KC) secretion by freshly prepared mouse uterine epithelial cells. Freshly isolated mouse uterine epithelial cells were incubated overnight prior to treatment with control medium or KGF (50 ng/mL) for 48 hr. Supernatants were collected and analyzed for MIP3 (a) and KC (b). The results are shown as the mean + S.E.M. of eight separate experiments. ***MIP3 or KC significantly ($P < 0.001$) greater with KGF treatment than controls.

Fig. 2.

Keratinocyte growth factor (KGF) dose-dependently stimulates freshly prepared uterine epithelial cell release of MIP3 and keratinocyte-derived chemokine (KC). Freshly isolated mouse uterine epithelial cells were incubated overnight prior to treatment with KGF (0.00005–50 ng/mL) for 48 hr. Supernatants were collected and analyzed for MIP3 (a) and KC (b). The results are shown as the mean + S.E.M. ***MIP3 significantly ($P < 0.001$) or *KC significantly ($P < 0.05$) greater with KGF treatment than controls.

Fig. 3. Time course of the effect of keratinocyte growth factor (KGF) on MIP3 and keratinocyte-derived chemokine (KC) secretion by uterine epithelial cells. Freshly isolated mouse uterine epithelial cells were incubated overnight prior to treatment with control medium or KGF (50 ng/mL) for 6, 12, 24, 48, or 72 hr. Supernatants were collected and analyzed for MIP3 (a) or KC (b). The results are shown as the mean + S.E.M. ***MIP3 or KC significantly ($P < 0.001$) greater with KGF treatment than controls.

Fig. 4.

Keratinocyte growth factor (KGF) increases apical and basolateral MIP3 secretion by polarized uterine epithelial cells. Mouse uterine epithelial cells were isolated and polarized on transwell cell culture inserts. Polarized epithelial cells were treated with control medium or KGF (50 ng/mL) in the basolateral compartment for 48 hr. Apical (a) and basolateral (b) supernatants were collected and analyzed for MIP3. The results are shown as the mean + S.E.M. of 10 separate experiments. ***MIP3 significantly ($P < 0.001$) greater with KGF treatment than controls.

Fig. 5. Keratinocyte growth factor (KGF) stimulates basolateral keratinocyte-derived chemokine (KC) secretion by polarized uterine epithelial cells. Mouse uterine epithelial cells were isolated and polarized on transwell cell culture inserts. Polarized epithelial cells were treated with control medium or KGF (50 ng/mL) in the basolateral compartment for 48 hr. Apical (a) and basolateral (b) supernatants were collected and analyzed for KC. The results are shown as the mean + S.E.M. of 10 separate experiments. ***KC significantly ($P < 0.001$) greater with KGF treatment than controls.

Fig. 6.

Specificity of keratinocyte growth factor (KGF) effect on MIP3 secretion. Mouse uterine epithelial cells were isolated and polarized on transwell cell culture inserts. Polarized epithelial cells were treated with control medium, KGF (50 ng/mL), epidermal growth factor (4 ng/mL), or HGF (50 ng/mL) in the basolateral compartment for 48 hr. Apical (a) and basolateral (b) supernatants were collected and analyzed for MIP3. The results are shown as the mean + S.E.M. of four separate experiments. *MIP3 significantly ($P < 0.05$) greater with KGF treatment than with control medium. **MIP3 significantly ($P < 0.01$) less with HGF treatment than with control medium.

Fig. 7. Specificity of keratinocyte growth factor (KGF) effect on keratinocyte-derived chemokine (KC) release. Mouse uterine epithelial cells were isolated and polarized on transwell cell culture inserts. Polarized epithelial cells were treated with control medium, KGF (50 ng/mL), epidermal growth factor (4 ng/mL), or HGF (50 ng/mL) in the basolateral compartment for 48 hr. Apical (a) and basolateral (b) supernatants were collected and analyzed for KC. The results are shown as the mean + S.E.M. of four separate experiments. *KC significantly ($P < 0.05$) greater with growth factor treatment than with control medium. ***KC significantly ($P < 0.001$) greater with growth factor treatment than with control medium.

Fig. 8.

Effect of keratinocyte growth factor (KGF) apical treatment on polarized uterine epithelial cell MIP3 secretion. Mouse uterine epithelial cells were isolated and polarized on transwell cell culture inserts. Polarized epithelial cells were treated with control medium, KGF (50 ng/mL) in the apical or basolateral compartment for 48 hr. Apical (a) and basolateral (b) supernatants were collected and analyzed for MIP3. The results are shown as the mean + S.E.M. of two separate experiments. **MIP3 significantly ($P < 0.01$) greater with KGF treatment than with control medium. ***MIP3 significantly ($P < 0.001$) greater with KGF treatment than with control medium.

Fig. 9.

Effect of keratinocyte growth factor (KGF) apical treatment on keratinocyte-derived chemokine (KC) release by polarized uterine epithelial cells. Mouse uterine epithelial cells were isolated and polarized on transwell cell culture inserts. Polarized epithelial cells were treated with control medium, KGF (50 ng/mL) in the apical or basolateral compartment for 48 hr. Apical (a) and basolateral (b) supernatants were collected and analyzed for KC. The results are shown as the mean + S.E.M. of two separate experiments. ***KC significantly ($P < 0.001$) greater with KGF treatment than with control medium.