Effect of Menstrual Status on Antibacterial Activity and Secretory Leukocyte Protease Inhibitor Production by Human Uterine Epithelial Cells in Culture

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The objective of this study was to examine the production of antibacterial factor(s) by uterine epithelial cells from pre- and postmenopausal women. Apical rinses from polarized epithelial cells recovered from women at the proliferative and secretory stages of the menstrual cycle were equally effective in killing Staphylococcus aureus and Escherichia coli, but those from postmenopausal women were not. Secretory leukocyte protease inhibitor (SLPI) concentrations of apical washes from premenopausal women were significantly higher than those obtained from postmenopausal women. SLPI production correlated with bactericidal activity with respect to menstrual status and time in culture. Anti-SLPI antibody significantly decreased bactericidal activity of premenopausal epithelial cell rinses. The endometrial epithelial cell line HEC-1A did not have a bactericidal effect, nor did it produce SLPI. In contrast, HEC-1B cells produced SLPI and a factor that inhibited bacterial growth. These results indicate that menstrual status (pre- vs. postmenopausal) influences the production of SLPI and bactericidal activity by uterine epithelial cells.

The uterine epithelium provides an effective physical barrier to infection in both pre- and postmenopausal women. In addition, studies have demonstrated that epithelial cells in the female reproductive tract have an active role in initiating and regulating local immune responses to potential microbial pathogens. For example, uterine epithelial cells produce a secretory component, the polymeric immunoglobulin receptor, for transporting of mucosal IgA antibodies from tissues into secretions [1]. Mucosal epithelial cells produce cytokines that initiate and regulate immune responses [2–6]. We recently demonstrated that rat [7] and human [8] uterine epithelial cells present antigen to T cells.

The growth, differentiation, and function of the luminal and glandular epithelial cells in the endometrium are regulated by endocrine changes [9–11]. We have demonstrated that menstrual status and/or sex steroid hormones affect the participation of human endometrial epithelial cells in a variety of immune functions. For example, sex steroid hormones regulate the movement of mucosal IgA antibody and its transporter, polymeric immunoglobulin receptor, from uterine tissues into secretions [12–14]. Estradiol administered to ovariectomized rats increased uterine epithelial cell antigen presentation [5, 7] and IgA and IgG antibody levels in uterine secretions [15]. Uterine epithelial cell inhibition of the peripheral blood mononuclear cell responses to mitogens and antigens is influenced by the phase of the menstrual cycle [16].

In addition to these direct effects on epithelial cells, we have also reported menstrual cycle–dependent changes relating to immune cell architecture and function in the female reproductive tract. Lymphoid aggregates consisting of a central core of B cells surrounded by numerous CD8+ T cells, which in turn are circumscribed by monocytes or macrophages, are small or absent at the early proliferative stage, large at mid-cycle and during secretory stage, and absent in postmenopausal women [17]. In the uterine endometrium, CD3+ T cell cytolytic activity is present during the proliferative phase and absent during the secretory phase; postmenopausal women retain cytolytic capability [18]. These findings contribute to our understanding of the mechanism by which the uterus accepts a semiallogeneic fetus while providing resistance to infectious organisms.

Since the inductive arm of the immune response may take hours or days to be activated by and to be effective against pathogens, initial innate protective mechanisms are available and are essential for health. One such mechanism is the production of microorganism growth–inhibiting soluble factors by female reproductive tract epithelial cells. Among the epithelial cell secretions with known bactericidal effects are defensins, secretory leukocyte protease inhibitor (SLPI), the enzymes lysozyme and lactoferrin, tracheal antimicrobial peptide, and numerous other small peptides (for review, see [19]). SLPI is produced by macrophages and epithelial cells, including epithelial cells of the intestinal tract and female reproductive tract [6, 20], and has broad-spectrum activity against a variety of potential pathogens [21], including human immunodeficiency virus type 1 (HIV-1) [22–26]. Of interest, levels and/or expression of SLPI varies in cervical mucus (but not in serum) during the menstrual cycle [27] and increases in amniotic fluid during gestation and labor [28], and, in response
to progesterone, SLPI is released by cervical tissue [29]. King et al. [30] showed that the primary site of SLPI synthesis in the endometrium and decidua was the glandular epithelium, and tissues derived from women in the late secretory phase produced higher SLPI levels than tissues obtained from women in the proliferative phase.

Resistance or susceptibility to infection varies with the stage of the reproductive cycle and the administration of exogenous sex steroid hormones [31]. Uteri from rats at different stages of the reproductive cycle could inhibit bacterial growth, whereas uteri from castrated and pseudopregnant rats exhibited no bactericidal activity; uterine flushings from rats in proestrus demonstrated antibacterial activity [32]. Weischer et al. [33] showed that uterine fluids from premenopausal women at different stages of the menstrual cycle inhibit bacterial growth. The purpose of this study was to determine whether normal human uterine epithelial cells derived from premenopausal women at various stages of the menstrual cycle and from postmenopausal women produce an antibacterial factor(s) in culture, and, if a factor(s) was produced, we intended to begin studies to identify the factor(s).

**Methods**

**Source of uterine tissue.** Uterine mucosal tissue was obtained immediately following surgery from women who had undergone hysterectomies at Dartmouth-Hitchcock Medical Center. Tissues used in this study were distal to the sites of pathology and were determined to be unaffected with disease after inspection by a trained pathologist. Pathologists also determined the menstrual status and the stage in the cycle of premenopausal patients. Tissues were transported on ice, and procedures to prepare purified epithelial sheets began within 2 h of surgery.

**Isolation of uterine epithelial cells.** Epithelial cells were isolated as described elsewhere [1, 34]. In brief, tissues were minced under sterile conditions into 1–2-mm fragments and were subjected to enzymatic digestion, using a pancreatin-hyaluronidase-collagenase (PHC) enzyme mixture that contained final concentrations of 3.4 mg/mL pancreatin (Life Technologies), 0.1 mg/mL hyaluronidase (Worthington Biochemical), 1.6 mg/mL collagenase (Worthington), and 2 mg/mL d-glucose in 1× Hanks’ balanced salt solution (HBSS; Life Technologies) containing 50 U/mL penicillin and 50 µg/mL streptomycin. Enzymes were chosen to maximize digestion of the extracellular matrix while minimizing digestion of cell-surface antigens. After incubating in PHC-HBSS for 1 h at 37°C, cells were dispersed through a 250-µm mesh screen, washed, resuspended in Dulbecco’s modified Eagle medium (DMEM/F12 complete medium), and analyzed for cell numbers and viability. Complete medium was supplemented with 20 mM HEPES, 50 U/mL penicillin, 50 µg/mL streptomycin, 1 µg/mL Fungizone, 2 mM L-glutamine (all from Life Technologies), and 10% heat-inactivated defined fetal bovine serum (Hyclone), and it did not contain phenol red.

Epithelial cell sheets were separated from stromal cells by serial filtration through 40- and 20-µm nylon mesh filters (Small Parts). Epithelial sheets were retained on the filters, whereas stromal cells passed through the filters. Epithelial sheets were recovered by washing and back-washing the filters with complete medium. Epithelial sheets were collected, centrifuged at 500 g for 10 min, and resuspended in a small volume of complete medium. Using this procedure, we isolated epithelial cells that stained positive in response to antibodies (Calbiochem) for the epithelial antigens Ber-EP4 and cytokeratin and negative in response to CD4, CD45, and vimentin. Although it is not possible to state categorically that there were no cells other than epithelial cells present in our transwell chambers, the antibody studies and the attainment of high trans-epithelial resistance (TER) indicate that the cultures contained purified epithelial cells.

**Cell culture.** To establish a cell culture system of polarized human uterine epithelial cells with both apical and basolateral compartments, the cells were cultured in Matrigel (Collaborative Biomedical Products)—coated Falcon cell culture inserts in 24-well culture dishes designed for these cell inserts (Fisher Scientific). Two human uterine epithelial cell lines, HEC-1A and HEC-1B (American Type Culture Collection), were also cultured under these conditions. For these experiments, apical and basolateral compartments had 300 and 600 µL of complete medium, respectively. The medium was changed every 2 days.

**Measurement of TER.** As an indicator of tight junction formation in epithelial cell monolayers, TER was periodically assessed by use of an EVOM electrode and Voltohmeter (World Precision Instruments), as described elsewhere [35].

**Experimental incubations.** After the cells were determined to have reached a maximal TER, apical and basolateral compartments were washed 4 times with serum- and antibiotic-free incomplete medium (DMEM/F12 supplemented with HEPES and 1-glutamine). Cells were cultured in incomplete medium for 24 h and retained maximal TER. After an additional 4 washings with incomplete medium, only the basolateral compartment received incomplete medium; the apical compartment was left at surface air conditions, as described by Smith et al. [36]. At indicated times, the surface of the apical chamber was gently covered with 100 µL of sterile water (Sterile Water for Irrigation; Abbott Laboratories) and was immediately removed. Recovered apical rinses were frozen at −80°C until they were assayed for antibacterial activity. In many cases, the water rinse was centrifuged at 500 g for 5 min to remove cell debris before the supernatant was frozen. Centrifugation had no effect on the presence of antibacterial activity.

**Antibacterial assay.** A 20-µL suspension of Staphylococcus aureus 502A (gift from Michael Selsted, College of Medicine, University of California at Irvine) was mixed with an equal volume of uterine epithelial cell rinse for 75–90 min at 37°C in microfuge tubes. Viable bacteria were counted by standard procedures, as described elsewhere [32]. In brief, the contents of each tube was spread on a Luria broth agar plate by a bacteria spreader. After overnight incubation, colony-forming units were counted. There was no difference between control counts, which included water alone, and rinses recovered from cell inserts with no cells. Unless otherwise indicated, the results reported here were done with S. aureus. Escherichia coli (obtained from Jack Bodwell, Dartmouth Medical School) was also used in some experiments.

**Measurement of SLPI.** Concentrations of SLPI in the apical rinses from polarized epithelial cells were determined with an
ELISA test kit (Hycult Biotechnology SLPI; Caltag Laboratories) according to the manufacturer’s protocol. The SLPI ELISA has a minimum detection level of 20 pg/mL, and calculations of SLPI were determined from a standard curve after optical density measurements at 450 nm on an ELISA reader (Dynex).

Neutralization of antibacterial activity with antibody to SLPI. Apical washes of epithelial cells derived from 3 premenopausal patients (patients 1365, 1407, and 1453) and washes from cell inserts coated with Matrigel in the absence of cells were pooled, divided into 15-μL aliquots, and incubated with 5 μL of water (positive control), 5 μL of normal goat antibody (1 mg/mL; isotype control), or 5 μL of goat anti–human SLPI antibody (1 mg/mL; anti–human SLPI antibody) for 1 h at 37°C. After incubation, S. aureus was mixed with an equal volume of treated uterine epithelial cell rinse for 75–90 min at 37°C. Viable bacteria were counted by standard procedures, as described above. Antibody and isotype control were purchased from R&D Systems.

Statistical analysis. The significance of the differences between experimental and control groups was calculated by Student’s t test. P < .05 was considered to be significant.

Results

Inhibition of S. aureus growth by human uterine epithelial cells in culture. We have found that human uterine epithelial cells from patients at different phases of the menstrual cycle, when recovered as cell sheets and cultured on cell inserts, as described elsewhere [1, 34], attain high values of TER 2–7 days after primary culture [34]. To develop a procedure for determining whether epithelial cells produce bactericidal factors, epithelial cells on cell inserts were washed, before exposure to surface air conditions, in the apical compartment for different time periods before a single wash with sterile water. As seen in figure 1, when apical rinses were recovered at 1, 4, and 24 h from epithelial cells derived from a patient in the proliferative phase and a patient in the late secretory phase of the menstrual cycle and were cultured with S. aureus, significant inhibition of bacterial growth was seen at 4 h. Analysis of apical washes at 24 h indicated a >90% reduction in colony-forming units at 24 h from both patients.

To determine whether menopausal status influences antibacterial activity, uterine epithelial cells derived from 12 pre- and 4 postmenopausal (inactive) patients were cultured to maximal TER in cell inserts. The age and menstrual status of the patients in this study are shown in table 1. As reported elsewhere, no significant differences in the maximal TER or the time to attain maximal TER were observed for epithelial cells obtained from premenopausal (proliferative and secretory) and postmenopausal (inactive) patients [34]. Maximal TER values varied from patient to patient and ranged from 800 to 1300 ohms/well. Membrane perturbation with washes or rinses temporarily reduced TER. Since epithelial cells reattained maximal TER within hours of the addition of medium after a 24-h incubation at surface air conditions (not shown), we concluded that viability was not adversely affected and that the temporary reduction in TER was probably due to the addition of medium. As shown in figure 2, a 24-h wash of uterine epithelial cells derived from patients in the proliferative and secretory phases of the menstrual cycle inhibited S. aureus viability by >90%. In contrast, washes from epithelial cells derived from patients with inactive endometrial status (postmenopausal women) had virtually no effect on bacterial growth. Titration of 48- and 72-h recovered washes from premenopausal patients showed either no difference or a small increase in antibacterial activity, compared with matched titrations of washes recovered after 24 h (data not shown).

To determine whether the numerous washes with incomplete medium, surface air incubation, and final water rinse affected cell viability, complete medium was added to the epithelial cells in the apical chamber after the water rinse and after TER was measured. In all experiments, TER values measured within 30 min of the addition of medium were significantly higher than values for controls, indicating that the epithelial cells remained viable and

Figure 1. Time course of antibacterial activity of human uterine epithelial cells obtained from a patient in the proliferative phase (A) and a patient in the late secretory phase (B) of the menstrual cycle. Epithelial cells were grown to confluence on cell inserts to transepithelial resistance > 800 mohms/well, as described in Methods. Before collection of apical secretions, cells were incubated in antibiotic- and serum-free medium. To collect apical secretions, cells were incubated for the times indicated, under surface air conditions, before a single rinse with 100 μL of sterile water, as indicated in Methods. Water rinses from ≥4 cell inserts per time point per patient were each incubated with Staphylococcus aureus and then were plated to count bacterial colonies. The effect of apical secretions on mean colony-forming units ± SD is shown. Control represents 24-h incubation in cell inserts without cells. *P < .001, compared with control.
maintained their tight junctions over the course of the collection period (data not shown).

**Inhibition of E. coli growth by human uterine epithelial cells in culture.** To define more fully the antibacterial activity of uterine epithelial cells, uterine washes were also incubated with *E. coli*. Antibacterial activity against *E. coli* and *S. aureus* from epithelial cells derived from patients with proliferative, late secretory, and inactive menstrual status is shown in figure 3. Recovered rinses from the premenopausal patients were effective against both gram-positive and gram-negative bacteria. In contrast, washes from the epithelial cells of a postmenopausal patient did not significantly inhibit the growth of either bacterium.

**Production of SLPI by human uterine epithelial cells.** SLPI has broad-spectrum bactericidal activity [21], is produced by female reproductive tract epithelial cells [6], and has been shown to vary in concentration in human cervical mucus during the menstrual cycle [27]. To explore the possibility that uterine epithelial cells in culture produce SLPI, concentrations were measured in 24-h apical washes of polarized uterine epithelial cells derived from 3 pre- and 3 postmenopausal (inactive) patients. As shown in figure 4, SLPI levels were measurable and significantly higher in the washes of cells from premenopausal patients, compared with those from patients with inactive menstrual status.

Apical washes from patient 1407 (proliferative) were assessed over time for SLPI levels and antibacterial activity. As shown in figure 5, there was a direct correlation between antibacterial activity (reduction in colony-forming units) and SLPI concentrations in uterine epithelial cells derived from this patient. Specifically, we found that, as SLPI concentrations increased, antibacterial activity, measured as the inhibition of *S. aureus* colony-forming units, also increased. SLPI was not detected (<20 pg/mL) in water rinses of cell inserts without cells or in cell inserts with epithelial cells at time zero.

**Neutralization of antibacterial activity with antibody to SLPI.** Because these experiments suggested that bactericidal activity might be due to epithelial cell production of SLPI, apical washes were incubated with goat anti–human SLPI antibody before incubation with bacteria, to determine whether this would reduce bactericidal activity. As shown in figure 6, when apical washes of epithelial cells from 3 premenopausal patients (patients 1365, 1407, and 1453) were pooled and preincubated with goat anti–human SLPI antibody, bactericidal activity was significantly inhibited. Preincubation with isotype control antibody or no antibody had no effect on the antibacterial activity. Similar results were obtained in 2 other experiments with individual and/or pooled rinses. These results indicate that SLPI produced by human uterine epithelial cells in culture is responsible for the bactericidal activity measured in apical secretions.

**Production of an antibacterial factor(s) by the human uterine epithelial cell line HEC-1B.** To explore the possibility that uterine epithelial cell lines also have antibacterial activity and/or produce SLPI, HEC-1A and HEC-1B cells were analyzed as described above. The cells were grown to confluence in cell inserts and were assessed for their ability to release a substance(s) in the apical compartment that would inhibit bacterial growth. Similar to the normal uterine epithelial cells, the HEC-1B cells produced

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**Table 1.** Age and menstrual status of 16 subjects in a study to determine the effect of menstrual status on antibacterial activity and secretory leukocyte protease inhibitor production by uterine epithelial cells.

<table>
<thead>
<tr>
<th>Menstrual status, patient no., phase (if known)</th>
<th>Age, years</th>
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<tbody>
<tr>
<td>Proliferative 1226</td>
<td>46</td>
</tr>
<tr>
<td>1280</td>
<td>35</td>
</tr>
<tr>
<td>1369</td>
<td>44</td>
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<tr>
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</tr>
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<td>1407</td>
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<tr>
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<td>45</td>
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<tr>
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<td>1293, early</td>
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<td>1397</td>
<td>48</td>
</tr>
<tr>
<td>1341</td>
<td>72</td>
</tr>
</tbody>
</table>

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**Figure 2.** Effect of menstrual status on antibacterial activity by human uterine epithelial cells derived from 16 women. Epithelial cells were grown to confluence on cell inserts before incubation for 24 h without medium in the apical chamber. After rinsing with 100 µL of sterile water, rinses were incubated with *Staphylococcus aureus* and then were plated to count bacterial colonies. The mean of colony-forming units from >4 cell inserts from each patient was compared with that for controls to determine percentage of inhibition for that patient. The mean inhibition ± SD of each menstrual status group is shown. *Significantly lower (P < .001) than antibacterial activity of proliferative and secretory groups.
an antibacterial factor(s) in a time-dependent manner, with maximum effect at 24 h (data not shown). As shown in table 2, HEC-1A cells, which share a common origin with HEC-1B cells, not only did not produce an inhibitor of bacterial growth at 24 h but also failed to secrete SLPI. The presence of antibacterial activity was accompanied by an increase in SLPI in water rinses from HEC-1B cells (table 2).

Discussion

Mucosal surfaces are the first line of defense against toxic particles and potentially pathogenic microorganisms. In response to infection, mucosal uterine epithelial cells can initiate local immune responses by stimulating, either directly or indirectly, through cytokines, the function of immune cells. However, the result, such as antibody production, of inducing an immune response by epithelial cells or professional antigen-presenting cells may take days; this is a critical time in which an infecting microorganism may grow and cause significant morbidity. Epithelial cells have evolved innate immune mechanisms that can immediately inhibit microorganism growth. In this study, we demonstrated that human uterine epithelial cells in culture produce an antibacterial factor(s) that is equally effective in inhibiting the growth of 2 types of gram-positive and gram-negative bacteria. In addition, we showed that the ability of epithelial cells to produce the antibacterial activity is related to menstrual status, since cells from premenopausal women inhibit bacterial growth, whereas cells derived from postmenopausal women do not. Last, we demonstrated that, in culture, human uterine epithelial cells derived from premenopausal women produce SLPI, the production of which appears to be responsible for the antibacterial activity seen in uterine epithelial cell secretions.

HEC-1A and HEC-1B cells are human endometrial epithelial cell lines that have been used as models for human uterine epithelial cell function [37]. HEC-1B cells produce SLPI and demonstrate bactericidal activity. In contrast, HEC-1A cells grown under identical conditions produced neither SLPI nor bactericidal activity. These results support our findings of SLPI production and bactericidal activity by normal human epithelial cells, in that HEC-1B cells act similarly to premenopausal cells, and HEC-1A cells are similar to postmenopausal cells. Furthermore, our studies with HEC cells indicate that secretion of SLPI and a bactericidal factor(s) is not dependent on tight junction formation, since HEC-1B cells, which fail to form tight junction, nevertheless produce SLPI and bactericidal factor(s).

Endocrine changes during the menstrual cycle control uterine cell growth and differentiation as well as the composition and function of immune cells, to ensure effective immune protection against pathogens and protection of the fetus [38, 39]. We have demonstrated that a variety of afferent and efferent immune responses in the female reproductive tract are under endocrine control, including antigen presentation [5, 7, 40], lymphocyte proliferation [16], IgA transport, and the presence of IgA and IgG in uterine secretions [11, 14, 15, 35, 41–44]. In humans, lymphoid aggregate formation [17] and cytotoxicity due to CD8+ cells [18] are influenced by the stage of the menstrual cycle. Our findings extend these studies by demonstrating that an important arm of the innate immune system varies with menstrual status, in that bactericidal activity was measurable in cells derived from premenopausal but not postmenopausal women.

The reason why uterine epithelial cells from postmenopausal women cannot inhibit bacterial growth is unknown but may be related to loss of endocrine responsiveness. Loss of receptors...
in the absence of sex steroid hormones is one possibility. Alternatively, whereas epithelial cells from premenopausal women comprise both glandular and luminal epithelial cells, uteri from postmenopausal women contain only luminal epithelial cells. Our findings suggest that glandular but not luminal epithelial cells may be the source of the bactericidal activity observed in this study. Consistent with this hypothesis is the finding of King et al. [30], who demonstrated in premenopausal women that SLPI is preferentially associated with the glandular epithelium in the endometrium. These investigators also reported that SLPI secretion by endometrial explants from women in the secretory phase was greater than that by explants from women in the proliferative phase of the menstrual cycle. Our findings of no differences in SLPI production by epithelial cells from proliferative and secretory phase tissues differ from those of King et al. [30]. Whether epithelial cells grown on cell inserts for 7–10 days lose the influence of endogenous hormones remains to be determined. Alternatively, since King et al. [30] observed increased SLPI secretion with tissues from late secretory phase tissues, it may be that their measurements reflect the combined production of SLPI by glandular epithelial cells and by macrophages and neutrophils, which are known to infiltrate the uterus during the last 4–5 days of the menstrual cycle [45–48].

Since women with inactive uteri are generally older than premenopausal women, age could be a factor in the secretion of an antibacterial factor(s). In other studies, immune function both systemically and at mucosal surfaces has been reported to decline with age [49, 50]. Our finding of a lack of bactericidal activity in postmenopausal women is consistent with these results and suggests that, under certain conditions, an antibacterial response in postmenopausal women may be impaired to a greater extent than in premenopausal women. White et al. [18] have shown that cytotoxic T lymphocyte (CTL) activity is significantly higher in postmenopausal women than in premenopausal women. Increased CTL activity could serve as an effective counterbalance to the reduction in antibacterial activity and other immune responses in postmenopausal women.

Polarized monolayers of uterine epithelial cells from premenopausal women had significantly higher SLPI production than cells derived from women with an inactive uterus. In addition to its well-known action as an inhibitor of neutrophil elastase activity [51], SLPI has been shown to be a potent and potentially significant broad-spectrum inhibitor of bacterial growth [21]. Our finding of a correlation of SLPI levels with antibacterial activity suggested that bacterial growth inhibition might be due to SLPI production by epithelial cells from the uterus of premenopausal women. Since the antibacterial activity was neutralized by specific antibody to SLPI, these findings further suggest that the antibacterial activity is probably due to SLPI. Our finding that premenopausal epithelial cell bactericidal activity was equally effective against *S. aureus* and *E. coli* indicates potential effectiveness against 2 microorganisms that might be associated with ascending genitourinary infections.
Table 2. Antibacterial activity and secretory leukocyte protease inhibitor (SLPI) concentrations in water rinses from 2 uterine epithelial cell lines, HEC-1A and HEC-1B, which were grown to confluence in inhibitor (SLPI) concentrations in water rinses from 2 uterine epithelial cell lines, HEC-1A and HEC-1B, which were grown to confluence in

<table>
<thead>
<tr>
<th>Cells</th>
<th>Staphylococcus aureus, cfu ± SD</th>
<th>SLPI concentration ± SD, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>562.7 ± 1.1</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>HEC-1A</td>
<td>533.3 ± 54.3</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>HEC-1B</td>
<td>12.7 ± 6.7</td>
<td>11.8 ± 0.9</td>
</tr>
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*Antibacterial activity (i.e., the presence of an antibacterial factor) was determined by inhibition of *S. aureus*, as measured by a reduction in colony-forming units.

*Significantly different (P < .001) from control and HEC-1A cells.

In addition to being a potent antibacterial agent, SLPI has been shown to be a potent virucidal agent that interferes with HIV-1 infection. Several studies have demonstrated that SLPI inhibits HIV infection by blocking HIV-1 internalization rather than by binding to CD4+-expressing cells in vitro [22–26]. In the reproductive tract, an association between higher SLPI concentrations in vaginal fluid samples and a reduced rate of perinatal HIV-1 transmission has been reported [52]. Although many factors, such as virus load and frequency of exposure, all contribute to the likelihood of perinatal and heterosexual transmission of HIV-1, our findings in the present study indicate that SLPI, which is produced by uterine epithelial cells throughout the menstrual cycle, may play a central role in diminishing the risk of infection as a consequence of sexual intercourse. Previous studies from our laboratory have shown that human uterine epithelial cells express HIV-specific chemokine receptors in the greatest numbers during the secretory phase of the menstrual cycle (authors’ unpublished data). Since CTL activity is absent at this time [18] and HIV can infect uterine epithelial cells directly [53], SLPI might play a central role in diminishing the risk of infection at this mucosal surface. If so, this may partly explain why the risk of infection (1:1000–1:10,000 per act of sexual intercourse) remains low under conditions of repeated viral exposure. Studies are needed to define the role of SLPI and other innate virucidal agents in limiting and possibly preventing the spread of HIV-1.

In conclusion, these studies demonstrate that normal uterine epithelial cells derived from premenopausal women secrete an antibacterial factor(s) and SLPI in culture. In contrast, uterine epithelial cells derived from postmenopausal women, when cultured under identical conditions, have little or no bactericidal activity or SLPI. These results emphasize the central role of uterine epithelial cells in protecting the female reproductive tract from bacterial sexually transmitted diseases. In addition, this study suggests that endocrine balance may influence uterine epithelial cell function in innate immune responses.

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