Secretion of cytokines and chemokines by polarized human epithelial cells from the female reproductive tract

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BACKGROUND: Pro-inflammatory chemokines that attract and cytokines that activate immune cells contribute to normal physiological homeostasis in the female reproductive tract, and are needed to deal effectively with potential pathogenic microbes. Mucosal epithelial cells are capable of producing these factors that communicate with cells of the innate and adaptive immune systems. METHODS: Epithelial cells from Fallopian tube, endometrium and endocervix were isolated and grown to high transepithelial resistance in cell inserts from seven patients who had hysterectomies. Interleukin (IL)-8, IL-6, granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1), granulocyte–macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor-α (TNF-α) and macrophage inflammatory peptide-1β (MIP-1β) were assessed by Luminex bead analysis or enzyme-linked immunosorbent assay (ELISA) in epithelial cell conditioned media from the apical and basolateral compartments. RESULTS: With the exception of MCP-1, the seven chemokines/cytokines constitutively produced by the polarized epithelial cells were preferentially secreted apically. A concentration pattern was found in all cases, with IL-8 and IL-6 produced in the greatest quantity. CONCLUSIONS: The concentrations of IL-8, IL-6, G-CSF and MCP-1 are similar to the levels found in reproductive tract fluids of patients with infection. The constitutive secretion and compartmentalization of large quantities of bioactive chemokines and cytokines provide additional evidence for the role of epithelial cells as gatekeepers of innate immune protection in the female reproductive tract.

Key words: chemokines/cytokines/female reproductive tract/innate immunity/polarized epithelial cells

Introduction

Female reproductive tract (FRT) epithelial cells, in addition to forming an uninterrupted mucosal barrier between the lumen and underlying cells, produce a variety of substances that promote health and reproduction. For example, epithelial cells secrete natural microbicides and mucus, as well as a variety of peptide mediators that regulate the traffic (chemokines) and activity (cytokines) of immune cells (for a review see Quayle, 2002). In addition, uterine epithelial cells can differentiate to promote attachment of the conceptus. Thus, as integral participants in innate and acquired immunity as well as the physiology of reproduction, FRT epithelial cells facilitate the survival of the mother, fetus and species (Wira et al., 2002).

Tight junctions between columnar epithelial cells maintain the integrity of the mucosal monolayer in the Fallopian tube, endometrium and endocervix. With an apical surface to the lumen and a basolateral surface to the basement membrane and underlying cells, epithelial cells have a structural, and often functional, polarized orientation. This permits epithelial cells to respond to different stimuli and serve as a directional conduit for different factors. For example, the epithelial cell pIgR transverses the epithelial cell from the basolateral side to the apical side to release IgA immunoglobulins into the uterine lumen (Wira and Stern, 1992). Also, in intestinal epithelial cells, Toll-like receptor 5 (TLR5), the innate immune receptor for bacterial flagellin, is preferentially expressed on the basolateral side (Hershberg, 2002). In other studies, uterine epithelial cells reportedly secrete cytokines such as transforming growth factor-β (TGF-β) preferentially at the basolateral surface and tumour necrosis factor-α (TNF-α) at the apical surface (Grant and Wira, 2003).

Uterine epithelial cells in culture express TLRs that are capable of recognizing specific structural components of bacterial, fungal and viral pathogens (Schaefer et al., 2004; Young et al., 2004). Addition of TLR agonists to epithelial cells often, but not always, elicits the induction and secretion of chemokines and cytokines, usually through intracellular signalling of NF-κB. The secreted pro-inflammatory mediators attract and stimulate the immune cells, often provoking further inflammation. Some epithelial secreted factors have multiple effects in innate and adaptive immunity. For
example, the microbicide human β defensin-2, which we have shown is produced by primary polarized uterine epithelial cells in culture (Schaefer et al., 2005), is also a chemokine for memory T cells and immature dendritic cells (Yang et al., 1999) and neutrophils (Niyonsaba et al., 2004). Similarly, the chemokine macrophage inflammatory peptide-3α (MIP-3α), which is secreted by uterine epithelial cells in response to lipopolysaccharide (LPS) (Crane-Godreau and Wira, 2004) and attracts immature dendritic cells, has also been shown to be microbicidal for bacteria (Hoover et al., 2002).

Whereas the induction and secretion of chemokines and cytokines from immune cells and FRT epithelial cells by TLR agonists have been demonstrated, the production, significance and interaction of these factors without microbial challenge has received less attention. Kaysili and associates reported on the importance of interleukin-8 (IL-8) and monocytic chemoattractant protein-1 (MCP-1) in normal uterine physiology, particularly in proliferation, angiogenesis, menstruation, implantation, cervical ripening and parturition (Kayish et al., 2002). The chemokines and cytokines regulate their own production as well as other chemokines/cytokines by autocrine and paracrine mechanisms (Haddad, 2002). Also, sex hormones exert control over many chemokines/cytokines in the FRT (Wira et al., 2002). For example, progesterone withdrawal results in upregulation of MCP-1 and IL-8, leading to chemotaxis and activation of monocytes and neutrophils, which results in release and activation of matrix metalloproteinases that contribute to initiation of menstruation (Crichtley et al., 2001). Thus, concentrations of chemokines and cytokines will vary in FRT tissues and fluids during normal physiological processes, as well as pathological conditions, such as infection and endometriosis.

Our objective in this study was to ascertain the constitutive production of several chemokines and cytokines by pure populations of FRT (Fallopian tube, endometrium and endocervix) epithelial cells grown in culture. By culturing the epithelial cells to high transepithelial resistance on cell inserts, epithelial factors may contribute to the quantity and type of immune cells trafficking in FRT tissues (Givan et al., 1997), the formation of uterine lymphoid aggregates (Yeaman et al., 1997) and the sampling of luminal fluids for pathogenic microbes by leukocytes (Rescigno et al., 2001). Finally, by assessing a number of chemokines and cytokines in each sample through Luminex analysis, we may find interesting patterns or networks of their production and regulation.

### Materials and 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 by disease upon inspection by a trained pathologist. Pathologists also determined the menstrual status, as well as the stage in the cycle of pre-menopausal patients. Tissues were transported from Pathology and procedures to prepare purified epithelial sheets began within 2 h of surgery. Approval to use tissues was obtained previously from the IRB. The tissues from seven patients, menstrual status, reason for surgery and age are listed in Table I.

#### Isolation of uterine epithelial cells

Epithelial cells were isolated as previously described (Fahey et al., 1998, 1999). Briefly, tissues were minced under sterile conditions into 1–2 mm fragments and subjected to enzymatic digestion using a ‘PHC’ enzyme mixture that contained final concentrations of 3.4 mg/ml pancreatin (Invitrogen, Grand Island, NY), 0.1 mg/ml hyaluronidase (Workhington Biochemical Corporation, Freehold, NJ), 1.6 mg/ml collagenase (Worthington) and 2 mg/ml D-glucose; in 1× Hank’s balanced salt solution (HBSS, Invitrogen). 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’s medium (DMEM)/F12 complete medium, and analysed for cell number and viability. Complete medium was supplemented with 20 μM/l HEPES, 50 U/ml penicillin, 50 μg/ml streptomycin, 1 μg/ml fungizone, 2 μM/l 1-glutamine (all from Invitrogen) with 10% heat-inactivated defined fetal bovine serum (HyClone, Logan, UT); this medium did not contain phenol red.

Epithelial cell sheets were separated from stromal cells by filtration through a 20 μm nylon mesh filter (Small Parts Inc., Miami Lakes, FL). Epithelial sheets were retained on the filters, while stromal cells passed through the filters. Washing and back-washing the filters with complete medium recovered the epithelial sheets. Epithelial sheets were collected, centrifuged at 500 g for 5 min and resuspended in a small volume of complete medium. Using this procedure, we have isolated epithelial cells, which stain positive for the epithelial antigens Ber-EP4 and cytokeratin and negative for CD4,

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Disease</th>
<th>Menstrual status</th>
<th>Age</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>2464</td>
<td>Ovarian mass</td>
<td>Proliferative</td>
<td>53</td>
<td>Endometrium</td>
</tr>
<tr>
<td>2488</td>
<td>Endometriosis</td>
<td>Secretory</td>
<td>46</td>
<td>Endometrium</td>
</tr>
<tr>
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<td>Fibroid</td>
<td>Proliferative</td>
<td>51</td>
<td>Endometrium</td>
</tr>
<tr>
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<td>Pelvic pain</td>
<td>Secretory</td>
<td>22</td>
<td>Endometrium</td>
</tr>
<tr>
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<td>Tubal metaplasia</td>
<td>Proliferative</td>
<td>39</td>
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</tr>
<tr>
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</tr>
<tr>
<td>2552</td>
<td>Menorrhagia</td>
<td>Inactive</td>
<td>53</td>
<td>Fallopian tube</td>
</tr>
</tbody>
</table>
CD45 and vimentin. Purity of epithelial cells was determined by flow cytometry. When stained with the pan-leukocyte marker CD45, non-epithelial cells were <0.1% of the total cells added to cell inserts (P. Wallace and C. Wira, unpublished data).

**Cell culture**

To establish a cell culture system of polarized human uterine epithelial cells with both apical and basolateral compartments, the human uterine epithelial cells were seeded into the apical compartment of human extracellular matrix- (BD Biosciences, Bedford, MA) coated Falcon cell culture inserts in 24-well culture dishes designed for these cell inserts (Fisher Scientific, Pittsburgh, PA). Typically, six, nine or 12–24 cell inserts could be seeded with Fallopian tube, cervix or uterine tissue epithelial cells, respectively. For these experiments, apical and basolateral compartments had 300 and 600 µl of complete medium, respectively. The medium was changed every 2 days, and fungizone was removed from the complete medium 4 days after seeding. Maximum transepithelial resistance (TER) was achieved within 8 days for all tissues, and conditioned medium for assay was obtained after maximal TER was attained. Media from cell inserts with no cells were used as control conditioned medium for assay was obtained after maximal TER was achieved within 8 days for all tissues, and conditioned medium for assay was obtained after maximal TER was attained. Media from cell inserts with no cells were used as control blanks for the assays. Following each 48 h culture period, the apical and basolateral conditioned medium was removed, centrifuged at 10 000 g for 5 min in a microfuge (Eppendorf/Brinkman) to remove any cell debris and the supernatants were stored at −80°C until assayed.

**Measurement of transepithelial resistance**

As an indicator of tight junction formation in epithelial cell monolayers, TER was assessed periodically using an EVOM electrode and Voltohmimeter (World Precision Instruments, Inc., Sarasota, FL), as described previously (Richardson et al., 1995). TER values were measured from a minimum of six inserts per patient and the means were calculated. These values were used to determine the mean TER for each tissue type as shown in Figure 1.

**Cytokine/chemokine analysis By Bio-Plex assay**

Cytokines and chemokines measured in this study were IL-8, IL-6, IL-4, IL-17, MCP-1, granulocyte–macrophage colony-stimulating factor, granulocyte colony-stimulating factor, tumour necrosis factor-α (TNF-α) and MIP-1β. At least four cell inserts per patient were assayed. The Bio-plex suspension array system using fluorescently dyed Luminex microspheres (beads) was used (Bio-Rad, Hercules, CA) as it is ideally suited to measure multiple cytokines from one sample. Cytokine/chemokine standards were prepared in the same fresh medium that was used for culturing the cells and were assayed in triplicate. Spiked controls accurately portrayed the added cytokine or chemokine concentration. Assays were performed in a 96-well filtration plate (Millipore, Billerica, MA). To each well, 5000 beads coated with antibody to each chemokine or cytokine were mixed with either a standard, sample, spiked control or blank in a final volume of 100 µl and incubated for 30 min with continuous shaking at room temperature. After washing the beads three times, biotinylated antibodies were added for 30 min with shaking. Beads were again washed three times and streptavidin–phycoerythrin (PE) was added for 10 min. After further washes, the fluorescence intensity of the beads was measured using the Bio-Plex array reader, and Bio-Plex Manager software with five-parametric-curve fitting was used for data analysis. The level of detection for each chemokine or cytokine was 7.8 pg/ml. Chemokine and cytokine values were measured from a minimum of four inserts per patient tissue and the means were calculated. These values were used to determine the mean chemokine and cytokine concentrations for each tissue type.

**Measurement of IL-8**

Since concentrations of IL-8 in some samples exceeded the maximum on the IL-8 standard curve from the Luminex analysis, IL-8 in the apical and basolateral conditioned media from polarized epithelial cells was determined with an enzyme-linked immunosorbent assay (ELISA) Duoset test kit (R&D Systems). IL-8 standards were prepared in culture media. Calculations of IL-8 were determined from a standard curve after optical density measurements at 450 nm on an ELISA reader (Dynex). The level of detection for IL-8 was 15.6 pg/ml.

**Statistics**

The data for the uterine and Fallopian tube cells are presented as the mean ± SEM; cervical cell data are shown as the range. InSTAT Software was used to perform a one-way repeated-measures analysis of variance (ANOVA). When an ANOVA indicated that significant differences existed among means, pre-planned paired comparisons were made using the Dunnet method to adjust P-values. A P-value of <0.05 was considered statistically significant.

**Results**

**TER of epithelial cells derived from FRT tissues**

Eleven FRT tissues were obtained from seven patients (see Table I). Epithelial cells from all tissues achieved a maximum TER within 8 days and maintained this TER for as long as 2 months. Figure 1 shows the mean maximal TER for uterine (six patients), cervical (two patients) and Fallopian tube (three patients) epithelial cells from all patients.
Fallopian tube TER was consistently lower than TER from the uterus and cervix, even when isolated from the same patient. For example, the mean maximal TER values for patient 2488 for Fallopian tube, uterine and cervical epithelial cells were 1325, 3563 and 3020 Ω/insert, respectively. The TER of inserts without cells or of monolayers of ectocervical cells that do not form tight junctions was < 150 Ω/insert.

**Cytokine and chemokine constitutive production by uterine epithelial cells**

Figure 2 shows the apical and basolateral concentrations of IL-8, IL-6, G-CSF, MCP-1, GM-CSF, TNFα and MIP-1β in 48 h conditioned media of polarized human uterine epithelial cells derived from six patients. IL-4 and IL-17 were below the level of detection in these analyses (not shown). For comparison with other studies that assessed FRT lavage fluids or cell culture media, data are shown as pg/ml; however, the basolateral medium in this investigation contains twice the volume of the apical medium (300–600 μl). Therefore, this difference in volume must be considered when determining whether or not there is a preferential secretion of a particular cytokine/chemokine. The relatively small number of sample tissues does not permit a correlation between responses and menstrual status, patient age or reason for surgery.

IL-8, IL-6, G-CSF and MCP-1 were produced in relatively copious quantities by the uterine epithelial cells (Figure 2A). Even accounting for the 2-fold greater volume in the basolateral compartment, there is a preferential release of IL-8, IL-6 and G-CSF to the apical compartment. In contrast, although the apical and basolateral concentrations (pg/ml) were significantly different, the total quantities of MCP-1 in each compartment were not dissimilar. Therefore, MCP-1 is not preferentially secreted. Relative to the aforementioned cytokines/chemokines, GM-CSF, TNF-α and MIP-1β were produced in low concentrations (Figure 2B); these cytokines/chemokines are primarily secreted apically.

All cytokines/chemokines tested were consistently produced during weeks of culture, indicating that secretion is a constant aspect of the uterine epithelial cells. As shown in Figure 3, four successive 48 h conditioned apical media collections from uterine epithelial cells from one patient (2464) had comparable values of IL-6. Similar results were obtained with the other chemokines and cytokines, as well as from FRT epithelial cells derived from other patients (not shown).

**Constitutive cytokine and chemokine production by cervical and Fallopian tube epithelial cells**

Figure 4A and B shows the chemokine and cytokine production of human primary epithelial cells derived from cervical tissue of two patients. Cervical epithelial cells produced chemokine and cytokine concentrations that were similar to those of the uterine epithelial cells and appeared to be preferentially secreted to the apical compartment. Fallopian tube

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**Figure 2.** The 48 h accumulation of chemokines and cytokines secreted by polarized uterine epithelial cells derived from six patients. The apical (hatched bar) and basolateral (clear bar) media from a minimum of four inserts of uterine epithelial cells derived from each patient were collected separately and analysed according to procedures outlined in Materials and methods. The mean values for (A) IL-8, IL-6, G-CSF and MCP-1 and (B) GM-CSF, TNF-α and MIP-1β from each patient’s cells were then used to determine the mean concentrations ± SEM from uterine epithelial cells from six patient tissues. *Significantly different from apical, P < 0.05.

**Figure 3.** Four consecutive 2 day apical accumulations of IL-8 from the polarized epithelial cells isolated from one patient (2464). There was no significant difference in the mean values of IL-8 obtained from four inserts of uterine epithelial cells on each day over the time period. Brackets represent the SEM.
epithelial cell secretion of chemokines and cytokines derived from three patients is shown in Figure 5A and B. A similar quantitative hierarchy of chemokines and cytokines from IL-8/IL-6 to MIP-1β was observed with the Fallopian tube cells as with the uterine and cervical epithelial cells. In contrast to the uterine and cervical cells, Fallopian tube cells did not routinely demonstrate a preferential apical secretion; indeed, in one case, when expressed as pg/well, MCP-1 secretion was primarily to the basolateral chamber.

**Cumulative chemokine and cytokine secretion by FRT epithelial cells**

Figure 6 shows the total (apical + basolateral) accumulation in pg/ml of the seven chemokines and cytokines produced by epithelial cells derived from the cervix, uterus and Fallopian tube. IL-8 and IL-6 were the predominant chemokine and cytokine produced by cells from all three tissues, respectively. G-CSF secretion by epithelial cells from the uterus

![Figure 4](image1.png)

**Figure 4.** The 48 h accumulation of chemokines and cytokines from polarized endocervical epithelial cells derived from two patients. The apical (hatched bar) and basolateral (clear bar) media from a minimum of four inserts of endocervical epithelial cells derived from each patient were collected separately and analysed according to procedures outlined in Materials and methods. The mean values for (A) IL-8, IL-6, G-CSF and MCP-1 and (B) GM-CSF, TNF-α and MIP-1β from each patient’s cells were used to determine the mean concentrations ± range from endocervical epithelial cells isolated from two patient tissues. *Significantly different from apical, P < 0.05.

![Figure 5](image2.png)

**Figure 5.** The 48 h accumulation of chemokines and cytokines from polarized Fallopian tube epithelial cells derived from three patients. The apical (hatched bar) and basolateral (clear bar) media from a minimum of four inserts of Fallopian tube epithelial cells derived from each patient were collected separately and analysed according to procedures outlined in Materials and methods. The mean values for (A) IL-8, IL-6, G-CSF and MCP-1 and (B) GM-CSF, TNF-α and MIP-1β from each patient’s cells were used to determine the mean concentrations ± SEM from Fallopian tube epithelial cells from three patient tissues. *Significantly different from apical, P < 0.05.

![Figure 6](image3.png)

**Figure 6.** Total secretion (apical + basolateral) of IL-8, IL-6, G-CSF, MCP-1, GM-CSF, TNF-α and MIP-1β over 48 h from polarized epithelial cells isolated from the cervix, uterus and Fallopian tube. Media from a minimum of four inserts of epithelial cells were collected separately and analysed according to procedures outlined in Materials and methods. The average of mean values for each set of epithelial cells from each patient’s tissues is shown ± SEM for uterus and Fallopian tube or the range for cervix.
was 6-fold greater than that secreted from the other two tissues. MCP-1 was produced by cells from all three tissues in equal amounts.

Discussion
These results demonstrate that polarized human epithelial cells in culture derived from the cervix, endometrium and Fallopian tube constitutively produce seven chemokines/cytokines; some mediators were found at concentrations that could modulate innate immune effector cell function. All seven mediators are generally considered proinflammatory due to their presence at inflammatory sites, their induction by proinflammatory stimuli such as LPS and their association with acute and chronic inflammatory disease. All the peptide factors produced by the FRT epithelial cells have multiple effects on immune cells, yet they are often classified as either chemokines or cytokines based on their purported primary function.

Of the seven mediators secreted by the FRT epithelial cells, several are predominantly known for their chemotactic effect on leukocytes. For example, IL-8 and MCP-1 are potent chemokines for neutrophils (Baggiolini, 1995) and monocytes, (Yoshimura and Leonard, 1992), respectively. Most pertinent is that we have demonstrated that the secretions from uterine epithelial cells in culture measured in this study have been shown to attract neutrophils (Shen et al., 2004) and monocytes (R.Meter et al., unpublished data). The concentrations of IL-8 and MCP-1 in the FRT epithelial cell secretions were sufficient to achieve maximum effect in standard chemotaxis assays compared with recombinant chemokine. In addition, the chemotactic activity for neutrophils or monocytes was effectively removed by pre-incubation of the epithelial secretions with specific neutralizing antibodies to IL-8 or MCP-1, respectively. GM-CSF, which is chemotactic for neutrophils (Gomez-Cambronero et al., 2003), can greatly synergize the IL-8 effect; potentiation is best shown when GM-CSF is added at the same time as IL-8 (Shen et al., 2004). MIP-1β is chemotactic for T cells, monocytes, neutrophils and natural killer (NK) cells (Menten et al., 2002). Unlike IL-8 and MCP-1, the MIP-1β concentrations produced by the FRT epithelial cells in these studies are unlikely to have a strong direct chemotactic effect. The data presented in these studies underscore the critical role that epithelial cells have in controlling migration of leukocytes and lymphocytes in the FRT tissues.

In addition to the chemokines, the pro-inflammatory cytokines IL-6, G-CSF, GM-CSF and TNF-α were constitutively produced by FRT epithelial cells. These mediators have significant effects on proliferation and differentiation of haematopoietic cells, as well as activation once the cells mature. IL-6 and TNF-α have multiple additional effects on immune cells, and these pleiotropic factors may even be considered as regulating inflammation when other pro-inflammatory factors are increasing without restraint.

In general, all FRT tissues tested produced a similar pattern of constitutive expression, with regard to both the type and relative quantity of chemokine and cytokine. The neutrophil chemokine IL-8 was secreted in the highest quantity by the epithelial cells of the three tissues. The values of ~50 ng/ml for the FRT cells over 48 h are in the range of those found in normal and pathological cervical fluids (Simhan et al., 2003). In addition to chemotaxis, high concentrations of IL-8 have been associated with proliferation and angiogenesis during early to mid-secretory phase, as well as with apoptosis during menstruation (Kayisli et al., 2002), a process in which neutrophils contribute prominently. IL-6 was also produced in copious quantities by epithelial cells from all three tissues, with Fallopian tube cells producing approximately half the levels of the cervix and endometrium. Interestingly, G-CSF production was >6-fold higher from the uterine epithelial cells compared with the others. In addition, uterine GM-CSF and TNF-α were also produced in greater quantities relative to Fallopian tube and cervix. Since GM-CSF and TNF-α stimulate the production of G-CSF (Sieff et al., 1988; Cavaillon, 1994), it is possible that the former factors contribute to the latter’s abundance by a positive feedback loop. Indeed, since production of cytokines and chemokines is often upregulated in an autocrine and/or paracrine manner, a simultaneous and/or sequential pattern of pro-inflammatory peptides may be produced by the FRT epithelial cells in order to marshal neutrophil, monocyte/macrophage, dendritic cell, T-cell and B-cell forces against a potential infective microbe. Chemokines are known to attract immune cells to tissues where cytokines affect differentiation and activation of immune cells. Vaday and colleagues have reported on the combination of inflammatory signals that can influence leukocyte actions, providing a variety of interactions (Vaday et al., 2001). In addition to direct upregulation of their production, chemokines and cytokines can also modulate pro-inflammatory responses by increasing the number of chemokine and cytokine receptors on leukocytes. Finally, since there were no other cell types to respond to the plethora of chemokines and cytokines produced by the epithelial cells, feedback mechanisms and receptors on other cell types that might exist in vivo are not available in our culture system to attenuate (or even enhance) production.

All seven inflammatory mediators secreted by uterine epithelial cells showed a preferential secretion to the apical compartment of inserts, even after accounting for volume differences. Some factors, particularly IL-8 and IL-6, were also preferentially secreted apically by Fallopian tube and cervical epithelial cells; an apical tendency was observed with several others. Whether or not the relative lack of apical secretion by Fallopian tube epithelial cells is due to the relatively lower TER (see Figure 1) remains to be determined. Regardless, it is evident that epithelial cells from all three FRT sites produce chemokines and cytokines, and some of these secretions are preferentially released to the lumen. There are several potential benefits of this. These epithelial factors, which are modulated by sex hormones, undoubtedly contribute to the resident and temporary populations of immune cells in the subepithelial layers of the FRT tissues. For example, the chemokines produced by the epithelial cells could account for the influx of leukocytes and lymphocytes that form lymphoid aggregates observed during the secretory
phase of normal endometrium (Yeaman et al., 1997). The immune cells, also under hormonal control, participate in architectural changes that occur during the menstrual cycle, as well as formation of the trophoblast in the presence of a conceptus. Should a breach in the epithelial lining occur, pre-formed chemokines and cytokines would fill the injured area and have immediate effects on immune cell trafficking and activation. Since some of the factors produced by epithelial cells [β-defensins, MIP, secretary leukocyte protease inhibitor (SLPI), etc.] are microbicidal, their luminal presence resists the development of microbial infection. In addition, luminal secretions of natural microbicides in the upper FRT tissues can most probably wash down to bathe the surface of the ectocervix and vagina to protect against potential pathogens. Finally, the relatively higher levels of chemokines and cytokines would attract macrophages and dendritic cells to the epithelial lining to sample the lumen for microbes. Thus, the preferential secretions contribute to innate immune surveillance.

The fact that the isolation of epithelial cell sheets results in epithelial cells that achieve high transepithelial resistance indicates that the cell population is essentially devoid of non-epithelial cells. In prior studies (Prabhala et al., 1998), we have shown by immunohistochemistry that the cells recovered are purified epithelial cells. This does not completely eliminate the possibility that a few inter-epithelial lymphocytes and/or leukocytes are present in our cultures. However, it is unlikely that a few non-epithelial cells could produce the concentration levels of cytokines and chemokines that we have found. In addition, we have shown that our epithelial cells do not secrete these chemokines and cytokines in response to LPS (T.M.Schaeffer et al., unpublished data). Since LPS routinely stimulates leukocytes to produce these pro-inflammatory factors, this is further evidence for the epithelial purity of our cultures.

The recruitment of specific populations of cells is essential for embryo implantation. For example, uterine NK cells increase in number rapidly during the secretory phase of the menstrual cycle and early pregnancy, and it is possible that the chemokines are responsible for these changes (Kitaya et al., 2003). The expression of chemokines that attract leukocytes is hormonally controlled, upregulated during the endometrial receptive period and associated with endometrial epithelia (Garcia-Velasco and Arici, 1999; Caballero-Campo et al., 2002; Dominguez et al., 2003; Jones et al., 2004). Our results showing constitutive production of several chemokines also suggest that epithelial cell production of chemokines could contribute to infiltration of NK cells and other leukocytes involved in altering endometrial architecture for embryo implantation.

Chemokines, cytokines, microbicides and other agents can act as autocrine and paracrine regulators in modulating human immunodeficiency virus (HIV). For example, chemokines, such as RANTES, MIP-1β and MCP that are produced by FRT epithelial cells, can act as CCR-5 antagonists to interfere with HIV uptake (Blanpain et al., 1999). In a recent paper, we demonstrated increases in expression of the intracellular antiviral compounds interferon (IFN)-β, and the IFN-β-stimulated genes myxovirus resistance gene 1 and 2′,5′ oligoadenylate synthetase induced by poly(I:C) in pure populations of uterine epithelial cells (Schaefer et al., 2005). In addition, we have shown that FRT epithelial cells constitutively produce SLPI (Fahey and Wira, 2002), a known inhibitor of HIV infection (Pillay et al., 2001). Studies are underway in our laboratory to elucidate the intracellular and secreted compounds from FRT epithelial cells with anti-HIV activity.

In conclusion, these studies suggest that FRT epithelial cells, through their secretions, contribute to maintaining physiological homeostasis, as well as preparedness in case of infectious insult. The constitutive secretion and compartmentalization of large quantities of bioactive chemokines and cytokines provide additional evidence for the role of epithelial cells as gatekeepers of innate immune protection in the female reproductive tract (Wira and Fahey, 2004). Further studies will explore the ways that sex hormones modulate both the constitutive and TLR-induced production and secretion of the epithelial chemokines and cytokines.

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