Polarized Uterine Epithelial Cells Preferentially Present Antigen at the Basolateral Surface: Role of Stromal Cells in Regulating Class II-Mediated Epithelial Cell Antigen Presentation

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Polarized Uterine Epithelial Cells Preferentially Present Antigen at the Basolateral Surface: Role of Stromal Cells in Regulating Class II-Mediated Epithelial Cell Antigen Presentation

Charles R. Wira,2* Richard M. Rossoll,* and Roger C. Young†

To study Ag presentation in the female reproductive tract, DO11.10 TCR transgenic mice specific for the class II MHC-restricted OVA1323–339 peptide and nontransgenic BALB/c mice were used. We report here that freshly isolated uterine epithelial cells, uterine stromal, and vaginal APCs present OVA and OVA1323–339 peptide to naive- and memory T cells, which is reduced when cells are incubated with Abs to CD80 and 86. To determine whether polarized primary epithelial cells present Ags, uterine epithelial cells were cultured on cell inserts in either the upright or inverted position. After reaching confluence, as indicated by high transepithelial resistance (>2000 ohms/well), Ag presentation by epithelial cells incubated with memory T cells and OVA1323–339 peptide placed on the basolateral surface (inverted) was 2- to 3-fold greater than that seen with epithelial cells in contact with T cells and peptide on the apical surface (upright). In contrast, whereas freshly isolated epithelial cells process OVA, polarized epithelial cells did not. When epithelial cells grown upright on inserts were incubated with T cells and OVA1323–339 peptide, coculture with either hepatocyte growth factor or conditioned stromal medium increased epithelial cell Ag presentation (~90% higher than controls). These studies indicate that uterine stromal cells produce a soluble factor(s) in addition to a hepatocyte growth factor, which regulates epithelial cell Ag presentation. Overall, these results demonstrate that polarized epithelial cells are able to present Ags and suggest that uterine stromal cells communicate with epithelial cells via a soluble factor(s) to regulate Ag presentation in the uterus. The Journal of Immunology, 2005, 175: 1795–1804.

Epithelial cells at mucosal surfaces throughout the body are the first line of protection against potential bacterial and viral pathogens (1, 2). Acting as sentinels of immune protection, epithelial cells have evolved to provide a physical barrier (3), recognize and respond as a part of the innate immune system by secreting chemokines and cytokines that recruit and activate immune cells (4, 5), secrete microbicidal and virucidal agents that inactivate and/or kill pathogens (6–8), as well as process and present Ags as part of the adaptive immune system (9, 10). Unique to epithelial cells in the female reproductive tract is the additional demand of responding to potential pathogens, while at the same time, allowing sperm and a fetal placental unit, both of which are allogeneic, to survive (11–13).

Ag presentation by epithelial cells has been demonstrated at several mucosal surfaces. For example, numerous reports have described the expression of HLA class II Ags on epithelial cells of the intestine and reproductive tract (9, 14–17). Using intestinal epithelial cell (IEC)3 lines to measure Ag processing and presentation, IECs were shown to internalize Ags and present processed peptides in the context of class II to CD4+ T cells (9). Kaiserlian et al. (18) demonstrated in a murine model that IECs could present Ags to a CD4+ T cell hybridoma and that an anti-class II mAb blocked IL-2 production by T cells. Subsequent studies with other Ags confirmed these results, although most Ags were inefficiently presented (19). Hershberg et al. (20, 21) used a polarized IEC line transfected to express HLA-DR to demonstrate class II-tetanus toxoid Ag presentation to restricted CD4+ T cells. These studies demonstrated that epithelial cells preferentially presented Ag at the basolateral surface (21). We more recently demonstrated that uterine cell suspensions consisting of human epithelium and stromal cells were capable of presenting Ags to autologous T cells (17). These studies further demonstrated that isolated, purified human uterine epithelial cells can process and present Ags to autologous T cells (17).

Using the rat as a model system, we demonstrated that uterine epithelial and stromal cells process and present OVA Ags and that the Ag presentation to CD4+ T cells is mediated through class II (22, 23). In other studies, we found that Ag presentation in the uterus and vagina is under the control of sex hormones and varies with the stage of the reproductive cycle. When given to ovariec-tomized rats, estradiol enhanced Ag presentation in the uterus (23) and inhibited Ag presentation in the vagina (24, 25). In addition to sex hormones, Ag presentation in the uterus increases when ovariec-tomized rats are treated with IFN-γ or IL-6 (22). More recently we found that, in response to estradiol, uterine and vaginal epithelial cells inhibit Ag presentation in the uterine stroma by secreting TGF-β (26, 27). These findings indicated that epithelial cells affect stromal cell function through the release of soluble factors such as TGF-β.
Less recognized is the important role of soluble factors, which are produced by underlying stromal cells that regulate uterine epithelial cell function in the female reproductive tract. In the uterus, for example, stroma and epithelium function as a unit with each cell type producing factors that regulate the growth, differentiation, and function of the other (28, 29). The stroma, in particular, heavily influences the epithelium during organ development and in the maintenance of adult homeostasis (30–32). Previously, we found that underlying stromal cells from the uterine endometrium decreased the barrier function of human polarized epithelial cells (33). More recently, we focused on the role of uterine stromal cells in regulating mouse epithelial cell monolayer electrical integrity and cytokine release (34). These studies indicated that uterine stromal cells produce a soluble factor(s) that increases epithelial cell TER and decreases TNF-α release (34). More recently, we found that hepatocyte growth factor (HGF), a known mesenchymal growth factor that mediates mammary gland development (34), more recently we found that hepatocyte growth factor (HGF), a known mesenchymal growth factor that mediates mammary gland development (34), increases epithelial cell TER and decreases TNF-α release (34). More recently, we found that hepatocyte growth factor (HGF), a known mesenchymal growth factor that mediates mammary gland development (34), increases epithelial cell TER and decreases TNF-α release (34).

Preparation of CD4+ naïve T cells

The naïve population of T cells was further isolated after the CD4 column by triple-stain sorting with a FACStar-plus Flow Cytometer (BD Biosciences). The cells (30–35 × 10^6) in 1 ml of IMDM with 10% FBS (heat inactivated, defined) were incubated withAbs to CD4 (final concentration at 5 μg/ml, FITC-labeled), CD26 (2 μg/ml, PE), and CD44 (2 μg/ml, CY5; all directly labeled monoclonals from BD Biosciences/Pharminigen) in IMDM for 30 min on ice in the dark, washed, then resuspended in fresh medium for flow cytometric analysis. Naïve T cells were characterized as CD4+,CD62Lhigh,CD44low as described previously (39).

Preparation of CD44+ memory T cells

To prepare isolated epithelial cells, uteri were removed, cut open lengthwise, and incubated with 46,500 U/ml of trypsin (Sigma-Aldrich) in 2.5% pancreatin (Invitrogen Life Technologies), at 20°C/g tissue, for 60 min at 4°C and 60 min at 22°C as described previously (34). Following transfer to ice-cold (3°C) HBSS, uteri were vortexed to release sheets of epithelial cells. Uterine tissues were rinsed and vortexed an additional two times, and resulting cell suspensions were combined. Pooled epithelial sheets were recovered by passing the cell suspension through a 20-μm mesh nylon screen (Small Parts), collected, and centrifuged (500 × g) for 5 min. Epithelial sheets were resuspended in culture medium consisting of DMEM/Ham’s F-12 nutrient mixed 1:1 (without phenol red; Invitrogen Life Technologies) containing 10% defined FBS and supplemented with 20 mM HEPES (Invitrogen Life Technologies), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Sheets were separated through 20-g needles to prepare isolated cells for experimentation.

To culture epithelial sheets, ~2 × 10^6 cells (without needle disruption) were seeded in the inside compartment of 6.4 mm in diameter Falcon cell culture inserts with 0.4-μm pore membranes (Fisher Scientific) coated with diluted Matrigel (growth factor reduced, without phenol red; Collaborative Biomedical Products). Cells were seeded in a 300-μl volume of DMEM: Ham’s F-12 with 10% charcoal-dextran-stripped FBS (HyClone) at a ratio of three to four culture inserts per uterus and placed in 24-well tissue culture companion plates (Fisher Scientific) containing 850 μl of the appropriate culture medium in the outside compartment and incubated at 37°C with 5% CO2. Alternatively, Nunc inserts (10 mm, 0.4-μm pore; Nalge Nunc International) were coated and seeded at the same cell ratio with 500 μl of medium (either DMEM/Ham’s F-12 with 10% stripped FBS or Cellgro complete medium (Mediatech) containing l-glutamine, penicillin, and streptomycin) inside and 500 μl outside in 24-well Nunclon plates (Nalge Nunc International) to adjust for the different volume capacities of the inserts. The purity of epithelial cell cultures prepared as described above was evaluated by three criteria: 1) epithelial cells used in these experiments were examined immunohistochemically and found to consist of 99.5% epithelial cells; 2) mouse uterine epithelial cells achieve high TER (>2000 ohms/well; background resistance 150 ohms/well), which would not be possible if there was stromal cell contamination; and 3) staining of cell inserts with anti-CD45 Ab indicated that epithelial cells account for >99.5% cells present on each insert.

For some experiments, epithelial cultures were grown on the bottom of coated Nunc inserts. Cell sheets were added in 200 μl of medium to chambers on inverted inserts made by attaching sterile Tygon tubing “collars” around the bottom of the insert. The inside compartments of the inserts had been filled with medium and immersed upside down to just below the collar lip in medium in 6-well Costar culture plates (Corning Glass). Cultures were grown for 2–3 days before collagen was removed, and inserts were washed enough in medium in 24-well plates. In all cases, epithelial cell monolayers were monitored daily by TER as an indication of tight junction formation using an Evom epithelial voltohmmeter and electrode (World Precision Instruments) and fed fresh culture medium in both compartments every other day.

Uterine stromal cells

To isolate stromal cells, pooled uteri, following the removal of epithelial cells, were incubated for 30 min at 37°C in 0.05% trypsin and 530 μM EDTA in HBSS (Mediatech) with 400 U/ml deoxyribonuclease I (Worthington Biochemical), using 2 ml/tissue as described previously. (34). Tissues were digested by gentle rubbing in culture medium on a 40-μm mesh nylon screen (Small Parts), and the resulting cell suspension was centrifuged (500 × g) for 10 min. Stromal cells were resuspended in DMEM/Ham’s F-12 with 10% defined FBS and plated at 5 × 10^6 cells in 850 μl/well of 24-well plates (Fisher Scientific). To prepare conditioned stromal medium (CSM), culture medium from stromal cells was collected and replaced at 48-h intervals, centrifuged (10,000 × g) for 5 min, stored at −80°C, and diluted 1:1 with fresh DMEM/Ham’s F-12 with 10% stripped FBS when used.

Materials and Methods

General procedures

Sexually mature, BALB/cAnNcr, female mice weighing between 15 and 20 g were obtained from the National Cancer Institute colony at Charles River Laboratories. Transgenic BALB/c-Tg(DO11.10)10Loh mice at 20–25 g were purchased from The Jackson Laboratory. Transgenic mice express the mouse α- and β-chain TCR that pairs with the CD4 coreceptor and is specific for chicken OVA232–339 (OVA232–339 peptide) in the context of I-A^d (class II restricted). Animals were maintained in a constant-temperature room with fixed light/dark intervals (12 h) and allowed food and water ad libitum. Animals were sacrificed by CO2, and uteri and/or vaginae were pooled from 8 to 16 animals at all stages of the estrous cycle. All procedures involving animals were conducted after approval of the Dartmouth College Institutional Animal Care and Use Committee.

Preparation of CD44+ memory T cells

CD4+ T cells from TCR transgenes homozygous mice were prepared from spleens and peripheral lymph nodes of 6- to 10-wk-old mice as described previously (37, 38). Splenic cells were dispersed by gentle grinding between two sterilized glass slides in HBSS (Invitrogen Life Technologies). Following centrifugation for 8 min at 500 × g, erythrocytes were lysed by NH4Cl treatment. The CD4+ subpopulation of T cells was purified by negative selection using a Mouse T Cell CD4 Subset Column kit (R&D Systems). To prepare memory cells, T cells were stimulated with 0.3 μM OVA peptide and incubated with irradiated BALB/c splenocytes added at 20:1 as APCs in IMDM (Sigma-Aldrich) containing 10% heat-inactivated (56°C for 30 min) FBS (HyClone) supplemented with 100 μM nonessential amino acids, 1 mM sodium pyruvate (both from Invitrogen Life Technologies), 50 μM 2-ME (Sigma-Aldrich), 2 mM L-glutamine, 100 μM penicillin, and 100 μg/ml streptomycin (all from Mediatech). Two-milliliter cultures (5 × 10^6 cells/well) were expanded at 72 h by 3-fold dilution into fresh medium. Between days 7 and 10, when T cells were considered to be resting as determined by minimal [3H]thymidine uptake, they were harvested, washed, and frozen at −80°C in IMDM containing 50% FBS and 10% DMSO (Sigma-Aldrich) until used.
**Vaginal cells.** Vaginal cells from BALB/c mice were prepared by incubation of tissues, which were hemi-sectioned lengthwise, with 46,500 U of trypsin/ml, 2.5% pancreatin, using 2 ml/vagina, for 60 min at 4°C and 60 min at 22°C with constant rotation (100 rpm) before separation by gentle rubbing in DMEM:Ham’s F-12 culture medium on a 40-μm mesh nylon screen. Cells were aspirated through 18- and 20-gauge needles to prepare isolated cells for experimentation.

**Ag presentation assays**

To measure Ag presentation by freshly isolated cells, OVA peptide-specific T cells (1 × 105 cells/100 μl) in DMEM:Ham’s F-12 culture medium were seeded in triplicate wells in 96-well flat-bottom microtiter plates (Nunc) with irradiated uterine epithelial or stromal cells as well as vaginal APCs (1 × 105 cells/100 μl) in the presence of OVA (APC+T+OVA) or OVA323–339 peptide (APC+T+peptide). APCs were irradiated before the start of Ag presentation with 5000 rad to prevent their proliferation. Controls included in all experiments were APCs incubated with T cells in the absence of Ag (APC+T), APCs incubated with Ag (APC+OVA/peptide), and T cells incubated with Ag (T+OVA/peptide). Following 48 h of incubation at 37°C, T lymphocyte proliferation was measured by [3H]thymidine uptake as described previously (27). Each well received 1 μCi (50 μl of medium) 20–24 h before the termination of each experiment. Cells in wells were transferred individually onto glass fiber filtermats with a cell harvester (Skatron). Radioactivity incorporated into cells was measured in a liquid scintillation counter (Packard Instrument).

**Ag presentation assays in culture inserts**

Once epithelial cells achieved high TER (>2000 ohms/well) after ~7 days in culture, inserts of polarized upright and inverted epithelial monolayers were used in Ag presentation assays. T cells were added to the inside chamber (3 × 105 cells/300 μl of final volume of appropriate medium) in the presence of 1200 μg/ml OVA or 5 μg/ml peptide in the inside and/or outside chamber as indicated in the figure legends and incubated as above. Incorporation of [3H]thymidine was measured by pipette resuspension of T cells in individual inserts without disrupting epithelial monolayers, removing the cell suspension to centrifuge away bubbles, then transferring it to 96-well plates to be harvested as above.

**Abs and cytokines**

Abs and isotype control Abs to CD4, CD62L, and CD44, all directly labeled monoclonals, were purchased from BD Biosciences/Pharmingen. Recombinant human HGF and goat anti-human HGF Ab and isotype control were purchased from R&D Systems. Purified rat anti-mouse B7-1 (CD80) and B7-2 (CD86) mAbs (BD Biosciences/Pharmingen) were added alone or in combination, each at a final concentration of 2.5 μg/ml, to inhibit Ag presentation/proliferation. Rat IgG2a,K isotype was used at the same concentration as a control.

**Statistics**

Data were compared by one-way ANOVA, followed by a Tukey multiple comparison post test. Differences of p ≤ 0.05 were considered significant. All values are expressed as mean ± SEM.

**Results**

**Presentation of OVA323–339 peptide by uterine epithelial cells to memory T cells**

Previous studies from our laboratory have shown that rat uterine epithelial cells, as well as APCs in the uterine stroma and in the vagina, are able to present Ags to T cells (23, 27). To more fully characterize Ag presentation in the female reproductive tract, studies were undertaken to extend our Ag presentation system to the mouse. To study Ag presentation in the uterus and vagina, DO11.10 TCR transgenic mice were used as a source of T cells that are specific for the class II MHC–restricted OVA323–339 peptide (323–339), as reported previously (38). Uterine epithelial cells were prepared from nontransgenic BALB/c mice as described previously (34, 40). As shown in Fig. 1, when memory T cells, prepared by culture in vitro with OVA peptide (see Materials and Methods for details), are incubated with uterine epithelial cells, increasing concentrations of OVA323–339 peptide resulted in increased Ag presentation, measured as the proliferation of T cells by [3H]thymidine incorporation into OVA-specific memory T cells incubated with irradiated uterine epithelial cells. In the absence of OVA323–339 peptide, very little [3H]thymidine was incorporated when epithelial cells were incubated with T cells or with OVA323–339 peptide or when T cells were incubated with OVA323–339 peptide. Ag presentation by uterine cells was significantly greater with 2.5 and 5 μg/ml than with 0.5 and 1.0 μg/ml Ag. These results indicate that this model system can be used to measure Ag presentation in the female reproductive tract.

**Ag presentation by uterine and vaginal cells to memory T cells**

To establish that epithelial cells as well as APCs in the uterine stroma and vagina are able to process as well as present Ags, isolated cells from intact animals were prepared and incubated with DO11.10 memory T cells in the presence of OVA or OVA323–339 peptide. As shown in Fig. 2, when OVA323–339 peptide (upper panel) or OVA (lower panel) was added to the incubation medium, Ag presentation by uterine epithelial and stromal cells as well as vaginal cells was observed. The incorporation of [3H]thymidine seen with OVA and OVA323–339 peptide was significantly greater than that seen in the absence of OVA (APC+T). These studies indicate that epithelial cells as well as APCs in the uterus and vagina are able to both process and present Ags to memory T cells.
presentation is inhibited relative to that seen with isotype controls. Ag presentation of OVA to memory T cells was reduced by 30–40% when anti-B7-1 and B7-2 Abs were added to the assay wells.

Ag presentation to naive T cells

To determine whether uterine and vaginal cells are able to present Ags to naive T cells, epithelial cells as well as uterine stromal and vaginal APCs were incubated with naive T cells recovered from DO11.10 mice. As seen in Fig. 3, reproductive tract APCs were able to process and present Ags to naive T lymphocytes. To confirm these findings, our second approach was to prepare naive T cells by flow cytometry because naive DO11.10 mice can have low numbers of memory OVA-reactive T cells (J. Gorham, unpublished observation). To eliminate the possibility that Ags were being presented to these cells, flow cytometry purification and isolation of naive T cells was undertaken. In the current study, naive, Ag-specific CD62L/highCD4+/H11001CD44/lowDO11.10 T cells were isolated from the spleens of transgenic mice. These cells were used to study Ag processing and presentation by uterine epithelial and stromal cells using our T cell proliferation assay. As shown in Fig. 4, when epithelial cells and stromal cells containing APCs were incubated with OVA, both processed and presented OVA as well as the OVA323–339 peptide to naive T cells.

Table I. Influence of accessory molecules on Ag presentation by uterine (UT) epithelial cells and APCs in the uterine stroma and vagina

<table>
<thead>
<tr>
<th>Ab</th>
<th>Isotype Control (%)</th>
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<tbody>
<tr>
<td></td>
<td>UT epithelial</td>
</tr>
<tr>
<td>Isotype control (IgG2a,κ)</td>
<td>100 ± 3.1</td>
</tr>
<tr>
<td>Anti-B7-1 Ab</td>
<td>97.4 ± 5.0</td>
</tr>
<tr>
<td>Anti-B7-2 Ab</td>
<td>74.0 ± 8.1*</td>
</tr>
<tr>
<td>Anti-B7-1/B7-2 Abs</td>
<td>59.4 ± 2.9**</td>
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</tbody>
</table>

* p < 0.05.
** p < 0.01.
Ag presentation by polarized uterine epithelial cells grown on culture inserts

To determine whether polarized primary epithelial cells present Ags at the apical and basolateral surfaces, uterine epithelial cells were isolated and grown to confluence on cell inserts in either the upright or inverted position. We found that under the culture conditions used in these studies, TER (mean ± SE) values of both insert sets were the same irrespective of whether epithelial cells were grown in the upright or inverted position (2690 ± 581 vs 2227 ± 701 ohms/well). After forming tight junctions, epithelial cells were incubated with DO11.10 TCR memory T cells and OVA323–339 peptide placed either on the apical surface (upright) or basolateral surface (inverted) for 48 h before the addition of [3H]thymidine to the upper chamber for an additional 24 h. As seen in Fig. 5, Ag presentation by epithelial cells in contact with T cells at the basolateral surface (inverted) was 2- to 3-fold greater with OVA323–339 peptide than that seen with epithelial cells in contact with T cells and OVA323–339 peptide on the apical surface (upright). The differences seen between apical and basolateral Ag presentation, as shown in Fig. 5, are representative of our findings in three of three experiments. In contrast, as seen in Fig. 6, whereas freshly isolated epithelial cells presented intact OVA to T cells (Fig. 2), polarized epithelial cells, irrespective of whether T cells were in contact with basolateral or apical (data not shown) surfaces, failed to process and present OVA.

In a series of experiments (data not shown), we found that variations in the pore size of inserts (0.4–3.0 μm), coating inserts with different dilutions of Matrigel (1:1–1:4) as well as varying the concentration of OVA used (300–1200 μg/ml), had no effect on the ability of polarized cells to present Ags at either surface. In other studies (data not shown), we found by flow cytometry that whereas fresh epithelial cells and splenocytes are positive for B7.1 and 4–13 and 4–9%, respectively, we were unable to detect

FIGURE 4. Presentation of Ags by uterine epithelial and stromal cells to purified naive DO11.10 T cells. Naive T cells (CD4+/CD62Lhigh, CD44high) from spleen cells (two to four animals) of adult DO11.10 mice were recovered by flow cytometer-positive selection as described in Materials and Methods. Naive T cells were incubated (1 × 10^5 cells/well) with epithelial (upper graph) or stromal (lower graph) cells (1 × 10^5 cells/well) prepared from a pool of 12 BALB/c mice along with OVA323–339 peptide (5 μg/ml) or OVA (1200 μg/ml). Following 2 days of incubation, [3H]thymidine was added for an additional 24 h (three wells/group). **, Significantly (p < 0.01) greater than controls.

FIGURE 5. Apical and basolateral Ag presentation by polarized uterine epithelial cells to CD4+/ DO11.10 memory T cells. Isolated uterine epithelial cells from 12 intact animals were cultured for 8 days on cell inserts (three to four inserts/uterus). Epithelial cells were plated on inserts either above or below the insert membrane as described in Materials and Methods. Following the attainment of high TER values, memory T cells (3.5 × 10^5 cells/well) along with OVA323–339 peptide (5 μg/ml) were placed inside each cell insert as indicated in the diagrams of cell orientation. [3H]Thymidine was added for the last 24 h of a 3-day incubation. At the end of each experiment, T cells were gently aspirated from inserts for measurement of [3H]thymidine incorporation. Values shown are [3H]thymidine incorporation for APC+T+Ag323–339 peptide as well as control groups APC+T and T+Ag323–339 peptide incubations as mean ± SE (three wells/group). **, Ag presentation at the basolateral surface was significantly (p < 0.01) greater than that seen at the apical surface. Representative of three experiments.
any evidence of staining by confocal microscopy of polarized epithelial cells grown to confluence on cell inserts. Cells were B7.1 and B7.2 negative irrespective of whether they were incubated with Abs at the apical or basolateral surface as either live or fixed cells. These findings suggest that in the absence of underlying stromal cells and/or estradiol and progesterone, epithelial cell Ag processing is compromised, possibly due to deficiencies in co-stimulatory molecule expression.

**Influence of CSM on Ag presentation by polarized uterine epithelial cells**

In previous studies with polarized uterine epithelial cells, we found that the presence of mouse uterine stromal cells or CSM had pronounced effects on epithelial cell TER and TNF-α secretion in culture (34). When stromal cells and/or CSM were placed in the basolateral chamber below epithelial cells for 24 – 48 h, epithelial cell TER increased (70% higher than control) and TNF-α release decreased into both the apical and basolateral chambers (30 – 50%). These studies indicated that uterine stromal cells produce a soluble factor(s) that regulates epithelial cell TER and release of TNF-α without effecting TGF-β release. To determine whether uterine stromal cells communicate with epithelial cells via a soluble factor(s) to affect epithelial Ag presentation, epithelial cells were grown upright on cell culture inserts before being transferred to plates containing CSM in the basolateral compartment. As seen in Fig. 7, culture for 24 h before addition of memory T cells and OVA323–339 peptide increased epithelial cell Ag presentation (90% higher than control). These studies indicate that uterine stromal cells produce a soluble factor(s), which enhances epithelial cell Ag presentation. In other experiments (data not shown), CSM, when added to epithelial cells along with OVA, failed to reverse the inability of polarized cells to present Ags. Overall, these results suggest that uterine stromal cells communicate with epithelial cells via a soluble factor(s) to regulate Ag presentation at apical surfaces in a way similar to that seen with its effect on TER (34, 36).

![FIGURE 6.](http://www.jimmunol.org/)

**FIGURE 6.** Presentation of OVA323–339 peptide and OVA Ags to memory T cells by inverted polarized uterine epithelial cells. Isolated uterine epithelial cells from 12 intact animals were cultured for 8 days on cell inserts (three to four inserts/group). Following the attainment of high TER values (2020 ± 248 ohms/well), epithelial cells were incubated with memory T cells (3.5 × 10⁵ cells/well) along with OVA323–339 peptide (5 μg/ml) or OVA (1200 μg/ml) placed inside each cell insert. [3H]Thymidine was added for the last 24 h of a 3-day incubation. Values shown are [3H]thymidine incorporation for APC+T+Ag as well as control groups, including APC+T and T+Ag incubations as mean ± SE. ***, Ag presentation at the basolateral surface was significantly (p < 0.01) greater than that seen in controls. Representative of five experiments.

![FIGURE 7.](http://www.jimmunol.org/)

**FIGURE 7.** Effect of epithelial cell culture with CSM on Ag presentation at the apical surface. Epithelial cells from 12 intact animals were grown on cell inserts (three to four inserts/treatment group) to confluence and transferred to wells containing CSM (500 μl; diluted 1:1 with fresh medium) in the basolateral compartment, as described in Materials and Methods. Coincident with insert transfer, memory T cells (3.5 × 10⁵ cells/well) along with OVA323–339 peptide (5 μg/ml) were placed inside each cell insert (300 μl). Following 2 days of incubation, [3H]thymidine was added, and cells were incubated for an additional 24 h. At the end of each experiment, T cells were gently aspirated from inserts for measurement of [3H]thymidine incorporation. Results are shown as mean ± SE. ***, Ag presentation by epithelial cells incubated with CSM in the basolateral compartment was significantly (p < 0.01) greater than that seen with control groups. Representative of six experiments.
Effect of HGF on uterine epithelial cell Ag presentation in culture

HGF, a mesenchymal growth factor made by stromal fibroblasts (44), is known to stimulate mammary epithelial cell proliferation (35). More recently, we found that HGF, when added to the basolateral chamber of polarized uterine epithelial cells, increased TER, while at the same time, decreased apical TNF-α release (36). When epithelial cells were incubated with anti-HGF or anti-HGFR Ab before HGF or CSM, HGF effects on TER, but not TNF-α release, were blocked. These findings prompted us to ask whether HGF might be the factor in CSM that affects the ability of epithelial cells to present OVA323–339 peptide to OVA-specific memory T cells. As seen in Fig. 8, when HGF was added to the basolateral surface of epithelial cells grown to high TER on inserts, we found that Ag presentation of OVA323–339 peptide to memory T cells increased significantly beyond that seen with control cells (no HGF). The stimulatory effect of HGF was observed when T cells were placed on the apical as well as the basolateral surfaces of epithelial cells.

To determine whether HGF has a stromal cell-like effect on epithelial cell Ag presentation, epithelial cells were grown to confluence before being incubated in the presence of HGF or CSM. Fig. 9 demonstrates that basolateral addition of HGF (100 ng/ml) for 48 h significantly increases Ag presentation by epithelial cells at the apical surface, as does CSM. As seen in Fig. 9, preincubation of CSM with anti-HGF Ab (5 μg/ml) for 30 min before placement in the basolateral compartment of polarized epithelial cells failed to block the stimulatory effect of CSM on Ag presentation. Addition of anti-HGF Ab to HGF (50 ng/ml) and CSM completely blocked the stimulatory effect of each on TER (data not shown). Overall, these studies indicate that, whereas HGF is the growth factor important for regulating epithelial cell integrity as measured as TER, stimulation of Ag presentation by polarized epithelial cells appears to involve a stromal cell signal(s) other than HGF.

Discussion

The research presented demonstrates that uterine epithelial cells, as well as APCs in the uterine stroma and vagina, process and present recall Ags to memory T cells. Furthermore, using naive T cells from DO11.10 mice, these studies indicate that epithelial cells and APCs in the uterine stroma are able to process and present Ags to naive T cells.
naive CD4+ T cells. These findings also indicate that polarized epithelial cells present Ags and that Ags are preferentially presented at the basolateral surface relative to that seen when memory T cells are in contact with the apical surface. Lastly, we found that CSM and HGF stimulate epithelial cell Ag presentation but that factor(s) in CSM, in addition to HGF, play a regulatory role in epithelial cell Ag presentation. These findings suggest a key role for stromal cells in regulating Ag presentation by uterine epithelial cells.

In the current study, we asked whether epithelial cells in the uterus are able to present Ags to naive T cells. We found that freshly isolated epithelial cells, as well as APCs in the uterine stroma, are able to process and present Ags as determined by co-incubation of APCs with naive T cells from non-OVA-primed mice as well as T cells selected by flow cytometry. Others have shown that lung dendritic cells stimulate naive Ag-specific T cells (39). What was unexpected was their finding that lung B cells were unable to present Ags. These findings indicate that APCs at mucosal surfaces must be analyzed on a case-by-case basis to evaluate Ag presentation function.

Our findings that uterine epithelial cells process and present Ags to naive and memory T cells take on a new importance with the recognition that the uterine lumen is not a sterile environment but rather a site at which bacteria and viruses move continuously from vagina to uterus to the Fallopian tubes (45). This conclusion is based in part on migration studies showing that sperm deposited in the vagina reach the Fallopian tubes within minutes (46). More recently, Parsons et al. (45) demonstrated that radio-opaque dye is seen in the uterine lumen within 2 h of being placed in the vagina. In this study, dye traveled from vagina to uterus irrespective of stage of the menstrual cycle, the use of oral contraceptives, or whether women were pre- or postmenopausal (45). Our studies suggest that in addition to functioning as a part of the innate immune system, epithelial cells have the capacity to initiate an adaptive immune response under conditions in which pathogenic challenge exceeds the innate immune protection afforded by epithelial cells. For review, see Ref. 47.

One of the principal aims of this study was to examine the ability of polarized uterine epithelial cells to present Ag to CD4+ memory cells. To test the capacity of epithelial cells to present Ags, epithelial cells were grown to confluence on either the upper or lower surface of cell inserts. Following formation of tight junctions, T cells and OVA323–339 peptide were added to one surface or the other. We initially used OVA323–339 peptide to bypass processing to directly measure Ag presentation to memory T cells. We found that presentation of Ags was significantly greater at the basolateral surface than at the apical surface. These findings extend the important observations of Hershberg et al. (21) who demonstrated preferential basolateral presentation with a human IEC line that expressed HLA-DR. To the best of our knowledge, our findings represent the first demonstration of Ag processing by primary epithelial cells.

What was unexpected in our study was the failure of polarized epithelial cells to process Ags. In contrast, Hershberg et al. (21) reported that polarized IEC process as well as present Ags to HLA-DR-restricted T cells. One explanation for this difference may be that because epithelial cells from the reproductive tract are under hormonal control, the presence of estradiol and/or progesterone may be essential for processing to occur. Previously, we reported that Ag presentation by epithelial cells from estradiol-treated rats is 2- to 3-fold greater than that seen with epithelial cells from ovariectomized (hormone depleted) controls (26, 27). Because polarized cells growth to confluence takes 7–10 days and occurred in our studies in the absence of CSM or physiological concentrations of hormone, the presence of these hormones or CSM factors may be critical for processing to occur.

Our findings in the present study that Ag presentation by polarized epithelial cells increases when CSM is added to the basolateral chamber, to the best of our knowledge, is the first demonstration that stromal cells play an important role in regulating Ag presentation by epithelial cells in the uterine lumen. Recognition of epithelial-stromal communication grew out of the pioneering studies of Cunha and Korach (30, 48). Using estradiol receptor knockout mice in uterine cell reconstitution experiments, they demonstrated that estradiol-induced uterine epithelial cell proliferation is mediated through estradiol receptors in stromal fibroblasts rather than through receptors in epithelial cells (48). More recently, we found that epithelial cell integrity, measured as high TER, and cytokine secretion by epithelial cells are influenced by underlying stromal cells and by CSM (34, 49, 50). Just how Ag presentation is affected remains to be determined and is under investigation in our laboratory.

In addition to CSM stimulating epithelial cell Ag presentation, we found that HGF added to the basolateral chamber had a pronounced stimulatory effect on Ag presentation. Molecules such as HGF are produced by the uterine stroma and act via epithelial receptors to affect changes in epithelial cells (48, 51, 52). Studies by Zhang et al. (35) identified HGF as the stromal mediator of estrogen-induced epithelial proliferation in the mouse mammary gland. More recently, we found that HGF increases TER, while at the same time, decreases apical TNF-α release (36). When epithelial cells and/or stromal cells were incubated with anti-HGF or anti-HGF Ab before HGF, HGF effects were blocked, indicating that epithelial cells express the receptor for HGF and that HGF mediates the effects of HGF on TER and TNF-α. Based on the neutralization of CSM with HGF- and HGF-R Abs, we concluded that stromal cell regulation of epithelial cell TER, but not TNF-α secretion, is mediated through the secretion of stromal HGF. In the present study, we tested HGF in our polarized Ag presentation assay and found that it stimulates Ag presentation. Because anti-HGF Ab added to CSM had no effect, these findings indicate that factor(s), in addition to HGF, affect epithelial cell Ag presentation and as well as cytokine secretion by epithelial cells. Others have shown that stromal cells produce a number of growth factors, including keratinocyte growth factor, epidermal growth factor, and insulin-like growth factor 1, which regulate epithelial cell proliferation (53–56). These studies, as well as our own findings, suggest that epithelial cells are responsive to multiple stromal signals that act either alone or in combination to affect cell proliferation, barrier function, cell secretion, and Ag presentation.

The present studies demonstrate that uterine epithelial cells present Ag. What remains to be determined is to whom Ag is presented in situ. That epithelial cells make contact with T cells as well as other immune cells in the stroma was demonstrated by Komuro (57, 58) and Hashimoto (58), who identified fenestrations (pores) in the basement membrane immediately below the epithelia in the intestine. Scanning electron microscopy indicated that these fenestrations, which are circular to oval in shape and are 0.5–5 μm in diameter, were passages for migrating cells of the immune system such as lymphocytes, eosinophils, and macrophages. Of equal importance was the identification of protrusions from the basal parts of epithelial cells that passed through these fenestrations into the lamina propria to make physical contact with underlying immune cells. In other studies, they demonstrated the penetration of the epithelial processes and close contact between lymphocytes and so forth and the epithelial cells (59). Our finding
that polarized epithelial cells present Ags to T cells placed in contact with the basolateral surface suggest that the anatomical associations seen by Komuro represent a intercellular communication between these different cell types.

In conclusion, our studies indicate that underlying stromal cells markedly influence Ag presentation by epithelial cells in the uterus. These findings further demonstrate that epithelial cells are able to present Ags to naive as well as memory CD4+ T cells. Given the ability of uterine epithelial cells to recruit and activate immune cells through the secretion of cytokines and chemokines in response to potential pathogens, our findings suggest that epithelial cells are in a unique position to recruit cells as part of the innate immune system as well as to present Ags as part of the adaptive immune system.

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Disclosures

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