Medroxyprogesterone Acetate Regulates HIV-1 Uptake and Transcytosis but Not Replication in Primary Genital Epithelial Cells, Resulting in Enhanced T-Cell Infection

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Although clinical and experimental evidence indicates that female sex hormones and hormonal contraceptives regulate susceptibility to human immunodeficiency virus type 1 (HIV-1) infection, the underlying mechanism remains unknown. Genital epithelial cells (GECs) are the first cells to encounter HIV during sexual transmission and their interaction with HIV may determine the outcome of exposure. This is the first report that HIV uptake by GECs increased significantly in the presence of the hormonal contraceptive medroxyprogesterone acetate (MPA) and progesterone and that uptake occurred primarily via endocytosis. No productive infection was detected, but endocytosed virus was released into apical and basolateral compartments. Significantly higher viral transcytosis was observed in the presence of MPA. In GEC and T-cell cocultures, maximum viral replication in T cells was observed in the presence of MPA, which also broadly upregulated chemokine production by GECs. These results suggest that MPA may play a significant role in regulating susceptibility to HIV.

**Keywords.** HIV-1; genital epithelial cells; female sex hormones; hormonal contraceptives; HIV transmission; HIV replication; transcytosis; inflammation; medroxyprogesterone acetate.

Approximately 40% of all new human immunodeficiency virus type 1 (HIV-1) infections originate in the female genital tract [1], which is lined by genital epithelial cells (GECs), the first cells to encounter HIV during sexual transmission. Significant controversy exists whether HIV can replicate in GECs. In vitro studies demonstrate that primary and immortalized GECs can support HIV replication [2–5], while others have been unable to show evidence of productive infection [6–8]. HIV virions have been proposed to traverse the epithelium via transcytosis [9–14], a vesicular transcellular pathway whereby cargo is transported from the apical side to the basolateral side of a polarized epithelium.

The primary site of HIV transmission in the female genital tract remains unknown. Postcoital microabrasions and specialized cells such as dendritic cells have been implicated in HIV acquisition in the lower genital tract [1, 15]. However, studies on simian immunodeficiency virus (SIV) infection in nonhuman primates suggest that HIV invades through the upper genital tract [16–18], consisting of the endocervix, endometrium, and Fallopian tubes, which is lined by a columnar epithelium. In humans, a large number of activated CD4+ T cells populate the cervical transformation zone, making it a prime target site for HIV [19]. Recent
studies have also demonstrated that HIV penetrates the cervical columnar epithelium and the vaginal squamous epithelium of humans to depths where target cells reside [20] and that simian immunodeficiency virus can target multiple tissues within the genital tract of female nonhuman primates [18].

Regardless of the site of infection, fluctuating levels of 17β-estradiol (E2) and progesterone (P4) regulate the morphology and function of the lower genital tract and upper genital tract throughout the menstrual cycle. Medroxyprogesterone acetate (MPA; Pfizer, New York, New York) and its injectable homologue, depot medroxyprogesterone acetate (DMPA; Depo-Provera), is a popular first generation synthetic progestin used by >100 million women [21]. A number of epidemiological studies have noted a significant increase in HIV susceptibility and transmission with hormonal contraceptive use, particularly DMPA [22, 23]. Furthermore, studies in nonhuman primates and mice have found that DMPA significantly enhances the risk of acquiring herpes simplex virus type 2 and SIV [24, 25].

Given the lack of understanding of the early events in the female genital tract following sexual transmission, we investigated the role of female sex hormones and MPA on regulating HIV infection in GECs. Our results show that MPA increased uptake of HIV and enhanced transcytosis, but not replication, HIV infection in GECs. Our results show that MPA increased uptake of HIV and enhanced transcytosis, but not replication, in GECs, resulting in increased infection of T cells. These results suggest that MPA may regulate GEC susceptibility to HIV, which may have significant implications for HIV acquisition in the female genital tract.

METHODS

Source of Tissues and Epithelial Cell Preparation
Female genital tract tissues were obtained from women undergoing hysterectomies for nonmalignant gynecological purposes at McMaster University Medical Centre in Hamilton, Canada. Written informed consent was received with the approval of the McMaster University Research Ethics Board. The detailed protocol for isolation and culture is described elsewhere [28]. Briefly, epithelial sheets were isolated from the endometrium and endocervix after enzymatic digestion and filtration steps. Approximately 10^5 GECs were seeded onto Matrigel (BD, Mississauga, Canada) coated transwell inserts until they formed confluent monolayers, as measured by a transepithelial resistance >1 kΩ/cm. The purity of GEC monolayers was between 95% and 98%, with no trace of hematopoietic cells, including leukocytes, as seen by lack of CD45 staining [26]. E2, P4, or MPA (Sigma-Aldrich, Oakville, Canada) were prepared in phenol-red-free Dulbecco’s modified Eagle’s medium/F12 (Life Technologies, Burlington, Canada) primary cell media [27].

HIV Infection of Primary GECs
HIV-ADA (an R5 strain) and HIV-IIIB (an X4 strain) were prepared by infection of adherent monocytes from human peripheral blood mononuclear cells and from the chronically infected H9 cell line, respectively, as described previously [28]. Stocks were purified and concentrated by the Amicon Ultra-15 ultracentrifugation filtration system (Millipore, Billerica, Massachusetts). Viral stocks were checked for cellular factors by a multiplex bead-based sandwich immunoassay (Luminex, Austin, Texas). Tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), interleukin 8 (IL-8), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), macrophage inflammatory protein 1β (MIP-1β), regulated on activation normal T-cell expressed and secreted (RANTES), interleukin 1α (IL-1α), and interleukin 1β (IL-1β) were not detected in any viral stock (lower limit of detection, 0.1–4.5 pg/mL). Confluent monolayers of GECs were exposed apically to HIV-ADA or HIV-IIIB (2 ng/mL p24) at 37°C for 2 or 4 hours; the inoculum was removed, and the cells were treated with cold 1% acetic acid in 0.5M NaCl for 1 minute to remove virus attached to the cell surface [29]. The cells were washed, and fresh hormone medium was added to the cells. At designated time points, GECs were disrupted, and protein and nucleic acids were collected. To inhibit endocytosis, GECs were pretreated with 80 µM Dynasore (Sigma-Aldrich) for 30 minutes at 37°C [30]. To ensure that the virus crossed GECs via transcytosis and not paracellular leakage due to TNF-α, GECs were treated with anti–human TNF-α–neutralizing antibody for the duration of the experiment (25 µg/mL; Rockland Immunochemicals, Limerick, Pennsylvania) [28] or with 10 µM colchicine (Sigma-Aldrich) [31] for 1 hour before virus exposure. Cell viability was assessed by a trypan blue exclusion assay. To measure HIV-1 within disrupted cells or in cell culture supernatants, a commercial HIV-1 p24 antigen enzyme-linked immunosorbent assay (ELISA) kit was used (ZetaToxMetrix, Buffalo, New York). The sensitivity of the p24 antigen ELISA was 3 pg/mL, and the linearity ranged from 7.8 to 125 pg/mL. Infectious virus was titrated using the TZM-bl cell (ATCC, Manassas, Virginia) infection assay, as previously described [32].

Confocal Microscopy
GECs were fixed in 4% paraformaldehyde (PFA; Electron Microscopy Sciences, Hatfield, Pennsylvania), permeabilized, and stained for ZO-1, and immunofluorescence microscopy was performed, as previously described [28]. As a positive control for mucosal epithelial barrier disruption, GECs were exposed to 10 or 20 ng/mL recombinant TNF-α (R&D Systems, Minneapolis, Minnesota) for 24 hours.

Real-Time Reverse-Transcription Polymerase Chain Reaction (RT-PCR) for HIV RNA Splicing
HIV-exposed GECs were disrupted, and RNA was extracted and purified using an RNeasy Mini Kit (Qiagen, Toronto, Canada). Complementary DNA was synthesized using the High Capacity Reverse Transcription Kit (Applied Biosystems, Carlsbad, California). Real-time RT-PCR was performed using primers
(Supplementary Table 1) for unspliced, single-spliced, and multispliced RNA according to a previously established protocol [33], and findings were compared to data for the housekeeping gene RPL13a. Chronically infected Jurkat cells (ATCC) served as a positive control for all PCR assays.

**TaqMan Real-Time PCR for HIV DNA Integration or HIV Reverse Transcription**

A previously described real-time nested PCR approach was used to measure integrated proviral HIV DNA [34], using extracted DNA and Alu and Gag M661 primers. PCR products were subsequently diluted and subjected to real-time PCR targeting the HIV R/U5 promoter region, using the M667 and AA55 primer pair. To measure HIV reverse-transcription products, a TaqMan real-time RT-PCR was performed using extracted GEC DNA and specific primers targeting strong-stop (R/U5), Env, Gag, and RPL13a DNA [35]. Fluorogenic probes (Biosearch Technologies, Novato, California) were used in all real-time PCR reactions, to increase the specificity of the reactions (Supplementary Table 1).

**Electron Microscopy**

GECs were exposed to $10^5$ infectious viral units of HIV for 4 hours, fixed in 4% PFA in PBS, and postfixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 hour at room temperature. Next, GECs were dehydrated in ascending concentrations of ethanol, infiltrated, and embedded in Spurr resin (Sigma-Aldrich). Sections with a thickness of 100 nm were mounted on 200-mesh copper palladium grids and counterstained with uranyl acetate and lead citrate. Samples were examined with a TEMSCAN 1200 (JEOL, Peabody, Maryland) at 80 kV.

**GEC and T-Cell Coculture Assay**

Primary GECs were grown to confluence with or without hormones and exposed to HIV-IIIB for 4 hours, at which point the inoculum and basolateral supernatants were removed and cells washed. The transwell was transferred to a new well containing $5 \times 10^4$ uninfected Jurkat T cells and incubated for 7 days. Supernatants from the basolateral chamber were collected, and the p24 antigen level was measured by ELISA.

**Multiplex Cytokine Assay**

GEC supernatants were analyzed for cytokines and chemokines, using the 64-Plex Discovery Luminex Assay from Eve Technologies (Calgary, Canada). Analytes included in this panel are accessible online (available at: http://www.evetechinologies.com/discoveryAssayListHuman.php).

**Statistical Analysis**

GraphPad Prism, version 5, was used to compare $\geq 3$ mean values, using the Kruskal–Wallis nonparametric analysis of variance test. When an overall statistically significant difference was detected (defined on the basis of a $P$ value of $<.05$), the Dunn test was used to correct for multiple comparisons.

**RESULTS**

**MPA and P4 Increase HIV Uptake Into Primary GECs**

To determine whether female sex hormones regulate HIV uptake, primary endometrial GEC cultures, free of contaminating leukocytes [26], were grown in the presence or absence of E2 ($10^{-9}$ M), P4 ($10^{-7}$ M), or MPA ($10^{-9}$ M) and exposed to HIV-ADA or HIV-IIIB. Sex hormone and MPA levels correspond to peak serum levels during the menstrual cycle and following administration of contraceptive, respectively [36, 37]. Cells were disrupted, and HIV uptake was measured by an HIV p24 antigen ELISA. Two hours after exposure, uptake of HIV-1 was significantly increased in cells grown in the presence of MPA for HIV-ADA (mean p24 concentration, 108.6 pg/mL; Figure 1A), and in the presence of MPA (mean p24 concentration, 404.4 pg/mL) or P4 (mean p24 concentration 306.40 pg/mL) for HIV-IIIB (Figure 1B). Twenty-four hours after exposure, HIV uptake in endometrial GECs was higher under all conditions, but a significant increase was detected only in cells grown in MPA (Figure 1C and 1D).

Studies implicate the endocervix as a preferential site for HIV transmission [16, 19, 20]. Similar to endometrial epithelial cells, HIV uptake was significantly increased 2 hours after exposure in endocervical epithelial cells grown in the presence of P4 (for HIV-ADA) or MPA (for HIV-ADA and HIV-IIIB; Figure 1E and 1F). Mean p24 antigen concentrations for HIV-exposed hormone naive cells were 50 pg/mL for HIV-ADA and 108.8 pg/mL for HIV-IIIB, relative to mean p24 antigen concentrations for cells grown in P4 (170.3 pg/mL for HIV-ADA and 284.4 pg/mL for HIV-IIIB) or MPA (181.8 pg/mL for HIV-ADA and 466.4 pg/mL for HIV-IIIB). As with endometrial GECs, HIV uptake 24 hours after exposure was significantly increased in endocervical GECs grown in P4 (for HIV-ADA) or MPA (for HIV-ADA and HIV-IIIB; Figure 1G and 1H).

No evidence of epithelial cell disruption was observed when GECs were exposed to HIV for 4 hours, as evidenced by the presence of intact organelles in cytoplasm, intact cilia on the apical side of cells, intact tight junctions, and round nuclei with no indication of blebbing, located toward the basolateral side of the cell (Supplementary Figure 1A).

We next performed a dose-response analysis to measure uptake of HIV by GECs over a range of physiological and supra-physiological concentrations of E2 ($10^{-7}$–$10^{-11}$ M), P4 ($10^{-6}$–$10^{-10}$ M), and MPA ($10^{-7}$–$10^{-11}$ M). Uptake of HIV was consistently lower in GECs grown in E2, compared with those grown in P4 and MPA (Figure 2). A dose-dependent decrease in HIV uptake was observed as E2 concentrations increased. HIV uptake into GECs did not vary much with escalating concentrations of P4, but a significant increase in uptake was detected for several MPA concentrations, including known circulating serum levels in women receiving Depo-Provera ($10^{-9}$ M) [37].
Figure 1. Effect of female sex hormones on uptake of human immunodeficiency virus type 1 (HIV) in columnar genital epithelial cells (GECs). Primary endometrial (A–D) and endocervical (E–H) GECs were grown to confluence on transwell inserts in the presence or absence of estradiol (E2; 10^{-9} M), progesterone (P4; 10^{-7} M), or medroxyprogesterone acetate (MPA; 10^{-9} M) and exposed to HIV-ADA (A and E) or HIV-IIB (B and F) at 2 ng/mL of p24 antigen. Two hours later, GECs were disrupted, and HIV p24 antigen was measured by enzyme-linked immunosorbent assay. Alternatively, primary GECs were exposed to HIV-ADA (C and G) or HIV-IIB (D and H), and 24 hours after exposure, cells were disrupted and p24 antigen was measured. Data shown represent the mean ± standard error of the mean of 3 experiments. A minimum of 2 replicates per experimental condition were included in every experiment performed. All groups were compared to GECs infected in the presence of no hormone (NH + HIV). *P < .05, **P < .01.
HIV Entry Into Primary GECs Takes Place Primarily via Endocytosis

Evidence indicates that canonical HIV receptors that lead to HIV fusion with the host cell membrane (CD4, CXCR4, and CCR5) are poorly or not expressed on human GECs [6, 10, 38]. Others have shown that HIV enters/crosses epithelial cells through endocytosis and transcytosis [9–14]. Thus, we decided to examine the mechanism of HIV uptake in our study. When GECs were treated with Dynasore, an inhibitor of dynamin and Drp1-mediated endocytosis [30], uptake of HIV was significantly abrogated, with the largest decrease (approximately 80%) detected in cells exposed to MPA (Figure 3A); cell viability was not affected by Dynasore (Supplementary Figure 1B). Transmission electron micrographs showed that internalized HIV localized to secretory/endocytic vesicles within GECs (Figure 3B). Off-target effects of Dynasore, such as disruption of the epithelial barrier, were ruled out by an absence of changes in ZO-1 expression relative to medium controls (Figure 3C). These results indicate that endocytosis plays a significant role in HIV entry into primary human GECs and that MPA may regulate endocytic uptake of HIV into GECs.

HIV-1 Exposure to Primary GECs Results in a Nonproductive Infection

Since we observed that approximately 80% of HIV entry into primary GECs takes place via endocytosis, we next decided to measure whether this uptake resulted in a productive infection and whether sex hormones influenced HIV replication in GECs. Twenty-four hours after exposure, integrated HIV proviral DNA was not detected in primary GECs, regardless of hormone exposure or viral strain, but was detected within infected T cells (Figure 4A). HIV integration in GECs was also not detected 48 or 120 hours after exposure (data not shown). Although genomic/unspliced HIV RNA was detected under all conditions in GECs, we were unable to detect single-spliced or multispliced RNA transcripts (Figure 4B), which are produced during productive infection. Last, 24 hours (Figure 4C) or 48 hours (data not shown) after exposure, we were unable to detect early (strong stop DNA), intermediate (Env), or late (Gag) reverse-transcription products in GECs exposed to HIV. Together, these results indicate that, despite successful entry, HIV does not replicate in primary GECs and that female sex hormones/MPA do not influence GEC permissivity to HIV.

MPA and P4 Increase Transcytosis of HIV Across GECs

We next determined whether female sex hormones/MPA influenced HIV transport through GECs. Apical and basolateral supernatants were collected from HIV-exposed GECs grown in female sex hormones or MPA. Apically recycled HIV-ADA was significantly increased in MPA-exposed cells relative to hormone naive controls (Figure 5A). Furthermore, the amount of HIV-ADA p24 antigen in basolateral supernatants of MPA-exposed GECs (mean level, 57.8 pg/mL) was significantly greater than that measured in cells grown in hormone-naive conditions (mean level, 5.34 pg/mL; Figure 5C). HIV-IIIB p24 antigen was also significantly higher in basolateral supernatants of GECs grown in P4 (mean level, 136.92 pg/mL) or MPA (mean level, 202.80 pg/mL), relative to infected cells grown in hormone-naive conditions (mean level, 74.38 pg/mL; Figure 5D). Together, these results indicate that HIV taken up via endocytosis is recycled apically, as well as transcytosed to
the basolateral compartment, and that P4 and MPA enhance HIV transcytosis across the epithelium.

Transcytosed HIV Is Infectious, Amplified in T Cells, and Enhanced in the Presence of MPA

To confirm that the virus detected in apical and basolateral compartments was released via endocytic machinery, GECs were pretreated with Dynasore, which inhibited >99% of p24 released into both the apical (Figure 6A) and basolateral (Figure 6B) compartments. Next, we decided to confirm HIV transcytosis through primary GECs. Previous studies have shown that, following longer exposures to HIV, TNF-α produced by GECs mediates epithelial barrier disruption and paracellular leakage of HIV; neutralization of TNF-α prevents this paracellular leakage [28, 39]. Despite TNF-α neutralization, we detected HIV in the basolateral supernatant (Figure 6C). In contrast, no HIV was detected in basolateral supernatants of GECs treated with colchicine, a previously validated inhibitor of HIV transcytosis in GECs [9, 13, 31]; cell viability was not affected by colchicine or TNF-α (Supplementary Figure 1B and 1C).

Infectious virus was found in basolateral supernatants under all conditions, using the TZM-b1 indicator cell assay, and an increase in the amount of infectious virus transcytosed was noted in the presence of MPA (Figure 6D). Further confirmation that transcytosed virus was infectious was obtained by coculturing
GECs with uninfected T cells. After 7 days of coculture, a >5-fold increase in HIV replication was detected in T cells cocultured with GECs and grown in MPA, relative to hormone-naive controls (Figure 6E). Together, these results indicate that HIV that is transcytosed across GECs is infectious and capable of replicating in target cells and that the level of virus transcytosis is increased by MPA.

**Figure 4.** Effect of female sex hormones and medroxyprogesterone acetate (MPA) on human immunodeficiency virus type I (HIV) replication in genital epithelial cells (GECs). Primary human GECs grown in the presence or absence of sex hormones were exposed apically to HIV-ADA or HIV-IIIB at 10^5 infectious viral units. Twenty-four or 48 hours after exposure, the cells were disrupted, and RNA and DNA were collected. HIV DNA integration (A) was assessed using a 2-step, Alu-Gag TaqMan real-time polymerase chain reaction (PCR) assay. Extracted RNA from primary GECs was used to measure RNA splicing, using real-time reverse-transcription PCR (B). Last, DNA was collected to measure HIV reverse transcription (C). In all experiments, chronically infected H9 T cells were used as a positive control, and uninfected GECs were used as negative controls. Relative differences in product expression between samples were visualized using gel electrophoresis. For each assay, 4 separate cultures were used, with a minimum of 2 replicates used per experimental condition. Abbreviations: E2, estradiol; NH, no hormone; P4, progesterone.

**DISCUSSION**

Our results support epidemiological and experimental studies showing an increased risk of acquiring HIV due to use of hormonal contraceptives such as MPA [22, 24] and provide insight into the biological mechanism by which this may occur. MPA increased the uptake and transcytosis of HIV by primary GECs, resulting in increased infection of and replication in T cells.
Furthermore, GECs increased production of chemokines that could recruit HIV target cells, such as macrophages, dendritic cells, and CD4+ T cells, in the presence of MPA [40]. Thus, in addition to increasing the amount of virus that traverses the epithelium, MPA may also induce the recruitment of target cells to the genital mucosa, potentially facilitating acquisition.

Several recent studies provide compelling clinical evidence that MPA may induce significant immunomodulatory effects that may lead to increased susceptibility to HIV in women. A recent study showed that DMPA use was associated with increased RANTES levels in African women, and this was associated with HIV seroconversion [41]. In a different study, MPA suppressed levels of inflammatory cytokines and interferon α in mononuclear cells and plasmacytoid dendritic cells [42]. Our studies support these observations, since we also observed MPA-mediated suppression of apical proinflammatory cytokines, as well as a general increase in levels of apical and basolateral chemokines, including RANTES. Although RANTES can inhibit HIV infection by competing with HIV gp120 for the CCR5 chemokine coreceptor [43], RANTES has also been shown to be a potent chemotactic factor for HIV target cells [44], which could potentially undermine its HIV-inhibiting capacity. Decreased CD3+ T cell counts and vaginal H2O2-producing lactobacilli have also been documented among DMPA users [45], suggesting that MPA may play various roles in regulating the female genital tract immune system.

Our results suggest that HIV entry into primary GECs of the upper genital tract occurs in a noncanonical fashion primarily via endocytosis and that, despite uptake, HIV does not productively infect GECs. Whether HIV productively infects epithelial cells remains a highly contentious issue, with many past studies showing conflicting results, likely due to differences in models, viruses, infectious doses, and other components that vary from study to study. A previous study by Asin et al found that primary uterine GECs grown in transwells were productively infected by HIV-IIIB [2], but they were unable to detect productive infection with an R5 strain (HIV-BaL) and showed that uterine GECs transcytosed HIV-BaL, which went on to successfully infect T cells. While our results are in agreement with their R5-tropic virus observations, we did not find productive infection with X4-tropic virus. A possible explanation may be that Asin et al exposed their cells to HIV for a greater duration (overnight).

Figure 5. Effect of female sex hormones and medroxyprogesterone acetate (MPA) on apical recycling and transcytosis in primary genital epithelial cells (GECs). Primary GECs were grown to confluence on transwell inserts in the presence or absence of estradiol (E2; 10^{-9} M), progesterone (P4; 10^{-7} M), or MPA (10^{-9} M) and exposed to human immunodeficiency virus (HIV)–ADA (A and C) or HIV-IIIB (B and D) at 2 ng/mL of p24 antigen. Twenty-four hours after exposure, apical (A and B) and basolateral (C and D) supernatants were collected and HIV p24 antigen was measured. Data shown represent the mean ± standard error of the mean of 3 experiments. A minimum of 2 replicates per experimental condition were included in every experiment performed.

*P < .05, **P < .01. Abbreviation: NH, no hormone.
and at a higher infectious dose (100 ng/mL) than used in our study (2–4 hours and 2 ng/mL, respectively). We designed our experiments to recapitulate the exposure of upper genital tract epithelium to a low dose of viral inoculum for a short duration. Other studies that have shown productive infection of vaginal, cervical, or uterine GECs used transformed or immortalized cell lines [3–5, 38], which likely have altered characteristics, compared with primary cultures. In concordance with our results, many other studies have failed to detect productive HIV infection in GECs and have reported HIV transcytosis across the epithelium [6–11]. A limitation of our study was that we only used 2 laboratory-adapted strains of HIV-1 to

Figure 6. Primary human genital epithelial cells (GECs) transcytose infectious virus, which results in enhanced infection and replication in T cells. To measure the effect of Dynasore on apical recycling or transcytosis, cells were or were not pretreated with Dynasore. Twenty-four hours after exposure, apical (A) and basolateral (B) supernatants were collected, and human immunodeficiency virus type I (HIV) p24 antigen was measured via an enzyme-linked immunosorbent assay. To determine whether basolateral virus was a result of transcytosis and/or paracellular leakage (C), primary GECs were treated with colchicine or incubated with a neutralizing tumor necrosis factor α (TNF-α) antibody and exposed to HIV-ADA at 2 ng/mL of p24 antigen. Twenty-four hours after exposure, primary GECs were disrupted, and HIV p24 antigen was measured. Furthermore, 24 hours after exposure, basolateral supernatants were collected and placed onto a TZM-b1 cell indicator system to measure infectious HIV (D). Lastly, primary GECs were grown to confluence in the presence of absence of hormones and exposed to HIV-IIIB for 4 hours. Next, transwells were transferred to new wells containing 5 × 10⁵ uninfected Jurkat T cells and incubated for 7 days. Supernatants from the basolateral chamber (containing the T cells) were collected, and p24 antigen was measured. Controls included uninfected cocultures and a culture containing only epithelial cells and no T cells (E). Data shown represent the mean ± standard error of the mean of 3 experiments. A minimum of 2 replicates per experimental condition were included in every experiment performed. *P < .05, ***P < .001. Abbreviations: E2, estradiol; MPA, medroxyprogesterone acetate; NH, no hormone; P4, progesterone.
Table 1. Proinflammatory Cytokine and Chemokine Profiles for Primary Genital Epithelial Cells (GECs) Exposed to Female Sex Hormones and HIV

<table>
<thead>
<tr>
<th>Variable</th>
<th>No Hormone + HIV</th>
<th>Estradiol + HIV</th>
<th>Progesterone + HIV</th>
<th>MPA + HIV</th>
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<tbody>
<tr>
<td></td>
<td>Apical Basolateral</td>
<td>Apical Basolateral</td>
<td>Apical Basolateral</td>
<td>Apical Basolateral</td>
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<td>Proinflammatory cytokines</td>
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<tr>
<td>TNF-α</td>
<td>305.4 ± 73.5 9.925 ± 0.025</td>
<td>760.6 ± 196.3 23.49 ± 0.24</td>
<td>556.1 ± 228.5 9.310 ± 0.040</td>
<td>14.22 ± 2.316a 45.58 ± 1.86</td>
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<td>IL-15</td>
<td>16.78 ± 0.881 1.655 ± 0.215</td>
<td>19.57 ± 1.004 0.705 ± 0.185</td>
<td>20.55 ± 2.064 2.260 ± 1.00</td>
<td>13.44 ± 0.405 1.805 ± 0.975</td>
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<td>IL-1α</td>
<td>142.8 ± 30.0 0.325 ± 0.325</td>
<td>149.9 ± 17.52 1.370 ± 0.110</td>
<td>90.71 ± 29.66 0.475 ± 0.015</td>
<td>1968 ± 274.3b 5.285 ± 0.185b</td>
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<td>IL-1β</td>
<td>2.65 ± 0.272 0.910 ± 0.120</td>
<td>3.045 ± 0.200 0.715 ± 0.135</td>
<td>2.390 ± 0.268 0.975 ± 0.255</td>
<td>2.328 ± 0.028 0.9250 ± 0.275</td>
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<td>IL-6</td>
<td>6940 ± 1912 413.0 ± 7.17</td>
<td>10 000 ± 0 423.0 ± 2.445</td>
<td>2319 ± 112.6 420.4 ± 9.315</td>
<td>88.89 ± 0.4939a 350.5 ± 8.66a</td>
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<td>G-CSF</td>
<td>2950 ± 79.52 4.075 ± 2.125</td>
<td>7369 ± 1596 5.670 ± 0.130</td>
<td>6708 ± 2493 5.080 ± 0.405</td>
<td>511.9 ± 41.97a 5.405 ± 0.265</td>
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<td>GM-CSF</td>
<td>770.7 ± 132.7 12.79 ± 1.445</td>
<td>1431 ± 143.4 25.16 ± 0.240b</td>
<td>988.7 ± 313.8 15.03 ± 1.290</td>
<td>68.10 ± 2.37a 42.95 ± 1.125b</td>
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<td>IL-10</td>
<td>0.643 ± 0.042 1.110 ± 0.290</td>
<td>1.99 ± 0.154b 1.30 ± 0.0</td>
<td>2.02 ± 0.253b 1.270 ± 0.03</td>
<td>2.198 ± 0.092b 1.205 ± 0.275</td>
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<td>IL-1RA</td>
<td>94.09 ± 5.73 43.75 ± 0.285</td>
<td>163.0 ± 3.592 35.00 ± 1.480</td>
<td>79.78 ± 4.734 28.72 ± 3.020</td>
<td>90.88 ± 0.820 33.45 ± 6.530</td>
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<td>IL-8</td>
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<td>11 406 ± 1371 3010 ± 23.78</td>
<td>13 552 ± 443.4 5129 ± 141.3b</td>
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<td>IP-10</td>
<td>258.1 ± 82.93 10.59 ± 3.260</td>
<td>213.2 ± 33.37 31.30 ± 0.610b</td>
<td>358.3 ± 102.1 30.80 ± 0.350b</td>
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<td>MCP-1</td>
<td>1908 ± 126.1 1073 ± 68.26</td>
<td>4414 ± 1333 1019 ± 27.61</td>
<td>9321 ± 687.6b 943.0 ± 14.31</td>
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<tr>
<td>MIP-1α</td>
<td>42.90 ± 6.525 0.695 ± 0.695</td>
<td>47.68 ± 1.412 4.220 ± 0.360</td>
<td>33.46 ± 4.334 1.870 ± 1.30</td>
<td>68.08 ± 7.516 2.740 ± 1.050</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>15.18 ± 1.39 1.115 ± 0.715</td>
<td>15.00 ± 0.277 1.120 ± 0.0</td>
<td>15.03 ± 0.4618 3.40 ± 0.760</td>
<td>37.33 ± 4.345 4.855 ± 0.395b</td>
</tr>
<tr>
<td>MIP-2β</td>
<td>18 024 ± 2792 3207 ± 153.9</td>
<td>27 288 ± 4585 3232 ± 253.4</td>
<td>18 451 ± 446.7 3515 ± 99.34</td>
<td>75.9 ± 91.37a 3562 ± 1.240</td>
</tr>
<tr>
<td>RANTES</td>
<td>36.30 ± 7.41 2.090 ± 2.090</td>
<td>103.2 ± 13.30b 2.990 ± 0.520</td>
<td>73.72 ± 26.18 2.090 ± 2.09</td>
<td>208.4 ± 13.64b 4.220 ± 0.250b</td>
</tr>
<tr>
<td>Eotaxin-1</td>
<td>24.90 ± 0.870 Not detected</td>
<td>27.59 ± 1.875 Not detected</td>
<td>20.48 (1.200 Not detected</td>
<td>21.88 ± 0.295 Not detected</td>
</tr>
<tr>
<td>Eotaxin-2</td>
<td>51.91 ± 1.08 2.775 ± 0.465</td>
<td>53.39 ± 1.757 1.640 ± 0.40</td>
<td>55.56 ± 5.610 1.155 ± 1.155</td>
<td>109.7 ± 6.64b 1.775 ± 0.535</td>
</tr>
<tr>
<td>Fractalkine</td>
<td>849.1 ± 152.3 49.13 ± 2.435</td>
<td>73.10 ± 10.18a 32.93 ± 0.305</td>
<td>1072 ± 179.6 33.53 ± 7.675</td>
<td>1638 ± 584.6 39.37 ± 1.225</td>
</tr>
</tbody>
</table>

Data are mean apical and basolateral cytokine and chemokine levels in pg/mL (±standard error of the mean) from 3 pooled cultures. Apical and basolateral supernatants were collected from cultures of primary GECs grown in estradiol, progesterone, MPA, or hormone-naive conditions and exposed to HIV. Supernatants and were then analyzed for multiple cytokines and chemokines, using a 64-Plex assay.

Abbreviations: G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; HIV, human immunodeficiency virus; IL, interleukin; IP-10, inflammatory protein 10; MPA, medroxyprogesterone acetate; MCP-1, monocyte chemotactic protein-1; MIP, macrophage inflammatory protein; RANTES, regulated on activation normal T-cell expressed and secreted; TNF-α, tumor necrosis factor α.

a Statistically significantly (P < .05) decreased production, relative to No hormone + HIV.

b Statistically significantly (P < .05) increased production, relative to No hormone + HIV.
perform our studies. A recent study detected productive infection in Ect-1 and End-1 epithelial cells exposed to HIV carrying primary transmitted/founder envelope genes [5]. Since founder viruses are widely believed to play an essential role in the early events of HIV infection in the female genital tract, future studies examining primary epithelial cell infection by transmitted/founder viruses need to be done.

Our previous and current results imply that interactions between HIV and GECs may vary under inflammatory and hormone-enriched conditions. Previously, we found that primary human columnar epithelial cells directly interacted with gp120, leading to the production of TNF-α, which disrupted the epithelial barrier, causing bacterial and viral translocation across the epithelium [28, 39]. In these studies, HIV was continuously exposed to GECs and TNF-α levels of >1 ng/mL [28]. Thus, in the presence of a high viral load and inflammation, paracellular leakage may be the preferred mode of crossing the barrier. In contrast, our current results suggest that, in the presence of MPA, small amounts of virus can still cross the barrier by transcytosis, despite a lack of inflammation. The specific mechanism by which these processes are regulated needs further examination.

In conclusion, our results provide a cellular mechanism by which MPA may enhance susceptibility to HIV. These results will provide valuable knowledge to HIV researchers regarding the early events of HIV transmission in the female genital tract and will have important implications regarding the safety of progestin-based hormonal contraceptive formulations.

Supplementary Data
Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


42. Huijbregts RP, Michel KG, Hdl Z. Effect of progestins on immunity: medroxyprogesterone but not norethisterone or levonorgestrel suppresses the function of T cells and pDCs. Contraception 2014; 90:123–9.

