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Progesterone-Induced Inhibition of Chemokine Receptor Expression on Peripheral Blood Mononuclear Cells Correlates with Reduced HIV-1 Infectability In Vitro

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Recent studies have shown that progesterone, a sex steroid hormone, enhances the sexual transmission of various pathogens, including SIV. The goal of this study was to determine whether progesterone affects mechanisms underlying the sexual transmission of HIV-1. We first studied the effects of various physiologic concentrations of progesterone on the expression of chemokines and chemokine receptors by T cells and macrophages. Chemokines are involved in leukocyte recruitment to peripheral sites; in addition, the chemokine receptors CCR5 and CXCR4 are HIV-1 coreceptors, and their ligands can block HIV-1 infection. Progesterone treatment had no effect on constitutive expression of CCR5 and CXCR4 by nonactivated T cells and macrophages, but significantly inhibited IL-2-induced up-regulation of CCR5 and CXCR4 on activated T cells (p < 0.05). Progesterone also inhibited both mitogen-induced proliferation and chemokine secretion (macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, RANTES) by CD8⁺ T lymphocytes. Control and progesterone-treated PBMC cultures were also tested for susceptibility to infection by T cell-tropic (HIV-1MN) and macrophage-tropic (HIV-1JR-CSF) viral strains in vitro. Infection with low titers of HIV-1MN, was consistently inhibited in progesterone-treated cultures; progesterone effects on infection with the HIV-1JR-CSF strain were more variable, but correlated with progesterone-induced reductions in CCR5 levels. These results indicate that progesterone treatment can inhibit mechanisms underlying HIV-1 transmission, including infection of CD4⁺ target cells via CXCR4/CCR5 coreceptors and effects on chemokine-mediated recruitment of lymphocytes and monocytes to mucosal epithelia. The Journal of Immunology, 1999, 162: 7510–7518.

Research over the past few years has linked HIV-1 infection with the chemokine-chemokine receptor system. Chemokines, a family of low m.w. proteins with a major role in leukocyte chemotaxis, emigration, and activation (1, 2), were shown to suppress HIV-1 infection (3–6). It is now established that chemokine receptors are used by HIV-1 for entry into CD4-expressing cells.

Chemokines have been classified into four groups according to the pattern of cysteine residues near the N-terminus; the CXC chemokine family has an amino acid between these two cysteine residues, whereas the CC family has none. CXCR4 was the first of the HIV-1 coreceptors discovered (7). CXCR4 is expressed on neutrophils, monocytes, and T and B lymphocytes, and its primary ligand is the CXC chemokine stromal cell-derived factor-1. T cell-tropic (T-tropic), syncytium-inducing HIV-1 isolates preferentially use CXCR4 for cell entry (7–9). CCR5, another receptor for the CC chemokines RANTES, macrophage inflammatory protein-1α (MIP-1α), and MIP-1β, mediates entry of macrophage-tropic (M-tropic) HIV-1 strains (5, 6, 8–10). The importance of CCR5 has been underscored by reports of resistance to HIV-1 infection of individuals who are homozygous for a nonfunctional CCR5 allele that contains a 32-bp coding region deletion (Δ32) (11–14). CCR5 is expressed on cells in mucosal epithelia and is thought to be the most important chemokine receptor mediating HIV-1 transmission across mucosal surfaces (15–17).

Progesterone is a sex steroid hormone, naturally produced in the ovary and placenta. High levels are detected in serum of women during the secretory phase of the menstrual cycle and pregnancy. A bidirectional interrelationship between sex steroids and the immune system has been established over the years (18–21). Progesterone affects lymphocyte migration and proliferation in the female genital tract (22, 23) and inhibits a number of immunologic responses, including macrophage phagocytosis, NK cell activity, CTL activity, T cell proliferation, and the secretion of Th1-type cytokines (24–27). On the other hand, cytokines produced by lymphocytes and macrophages can regulate progesterone secretion by placental trophoblast and ovarian granulosa cells (28–30). The cellular and molecular mechanisms by which sex steroids manipulate immune responses are incompletely understood. Recent evidence indicates that human CD8⁺ T cells, but not CD4⁺ cells, express estrogen receptors (31). Human lymphocytes and monocytes apparently do not express the classical progesterone receptor (32, 33); they do, however, express the glucocorticoid receptor, which has a distinct progesterone binding domain (34).

It has been known for some time that animal models of sexually transmitted diseases require priming with progesterone (35–37). It was recently shown that rhesus macaques with s.c. progesterone implants were more susceptible to SIV vaginal transmission (38). It has been postulated that enhanced infection of the female genital tract after progesterone treatment is due to thinning of the vaginal
barrier, but other factors, such as progesterone-induced immunosuppression or enhanced availability of HIV-1 target cells, due to recruitment and/or effects on HIV-1 receptor expression, may also be important. The goal of our study was to investigate the effects of various physiologic concentrations of progesterone on mechanisms of HIV-1 transmission. As a first step, we studied the effects of progesterone on CCR5 and CXCR4 chemokine receptor expression and on chemokine secretion. Subsequently, we challenged progesterone-treated PBMC cultures with HIV-1 to determine the overall effects of progesterone treatment on HIV-1 infection.

Materials and Methods

Study design

Healthy women (n = 8) with normal menstrual cycles, between 22–36 yr of age, and not taking hormonal contraceptives were recruited for the study. All the protocols involving use of human material were approved by the Brigham and Women’s Hospital institutional review board. To standardize progesterone effects in our experiments, peripheral blood was obtained during the early proliferative phase of the menstrual cycle (days 6–8), when circulating progesterone levels are lowest. Plasma was separated from heparinized blood and stored at –70°C until tested to determine estrogen and progesterone levels. The hormone assays were performed at the Department of Reproductive Endocrinology, Massachusetts General Hospital (Charlestown, MA), using the IMx estradiol assay and the AxxSYM progesterone assay (Abbott Laboratories, North Chicago, IL). PBMCs were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation (Amersham, Piscataway, NJ). Serum from each individual was also prepared from nonheparinized whole blood using standard techniques and was stored at –20°C until use.

All study participants were genotyped by PCR (see below) to determine whether they contained the Δ32 mutant CCR5 allele. This allele has a profound role in resistance to HIV-1 acquisition by PBMCs, and recent studies have also indicated that Δ32/Δ32 and Δ32/Δ32 individuals express markedly reduced levels of CCR5 that cannot be up-regulated even after IL-2 treatment (39); only individuals homozygous for the wild-type CCR5 allele were included in the study.

Progesterone (Sigma, St. Louis, MO) was used at 10⁻³, 10⁻⁶, and 10⁻⁹ M final concentrations. These concentrations were chosen because they can be found in different physiologic states; 10⁻⁶ M corresponds to levels present in the circulation during the secretory phase of the cycle (40), 10⁻⁶ M corresponds to peripheral blood levels during pregnancy (41), and concentrations of progesterone as high as 10⁻³ M have been detected at the fetal-maternal interface (42).

RNA isolation and RT-PCR analysis

Total RNA was extracted from PBMCs using Trizol reagent (Life Technologies, Grand Island, NY), according to the manufacturer’s instructions. Total RNA was extracted from PBMCs using Trizol reagent (Life Technologies), according to the manufacturer’s instructions. The DNA was treated with 1 U/mg of RNA (Bacterial DNAse I; Promega, Madison, WI). DNase digestion was followed by PCR negative control, test cDNA was replaced with deionized water. To control for the presence of genomic DNA, PCR reactions were also performed using DNAse-treated RNA instead of cDNA for each case. PCR products were subjected to electrophoresis on a 1% agarose gel in the presence of 0.25 μg/ml ethidium bromide. PCR reactions were performed using a 1°C increase in primer extension.

The method of Wu et al. (39) was followed to up-regulate CCR5 protein expression in PBMCs. Films were purchased from Life Technologies, using the IMx estradiol assay and the AxxSYM progesterone assay (Abbott Laboratories, North Chicago, IL). PBMCs were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation (Amersham, Piscataway, NJ). Serum from each individual was also prepared from nonheparinized whole blood using standard techniques and was stored at –20°C until use.

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Progesterone effects on constitutive CCR5 and CXCR4 expression in nonactivated T cells

PBMCs were cultured at a cell density of 10⁶/ml in AIM V phenol red-free and serum-free medium (Life Technologies) or phenol red-free RPMI supplemented with 1000 U/ml penicillin, 1 mg/ml streptomycin, 2 mM l-glutamine, 0.1 mM MEM nonessential amino acids (all purchased from Fisher Scientific, Springfield, NJ), and 10% autologous serum. Progesterone (10⁻⁵ M) was added at the initiation of the cultures; nonadherent cells were harvested from progesterone and control medium (alone) wells at 24 and 72 h for CCR5 and CXCR4 quantification.

Progesterone effects on IL-2-induced up-regulation of CCR5 in activated T cells

PBMCs were cultured at a cell density of 10⁶/ml in AIM V medium. Cells were maintained in culture for up to 4 wk. Parallel cultures without progesterone (10⁻⁵, 10⁻⁶, and 10⁻⁷ M) from day 1 of culture, whereas in group B cells were cultured with progesterone at various time points after CCR5 up-regulation had started and also when maximum up-regulation had been achieved for varying periods of time (24, 48, and 72 h). Cells were maintained in culture for up to 4 wk. Parallel cultures without progesterone served as negative controls for each sample.

PEG progestrone effects on IL-2-induced up-regulation of CXCR4 in activated T cells

Cells (10⁶/ml in AIM V medium) were cultured in the presence of PHA (Boehringer Mannheim; final concentration, 1.2 μg/ml) and progesterone at three different final concentrations (10⁻³, 10⁻⁴, and 10⁻⁵ M). After 3 days cells were fed with fresh RPMI supplemented with antibiotics, 10% autologous serum, and IL-2 (2 U/ml) and cultured for an additional 4 days. Cells from each individual were split into two groups. In group A cells were cultured in the presence of progesterone (10⁻³, 10⁻⁴, and 10⁻⁵ M) from day 1 of culture, whereas in group B cells were cultured with progesterone at various time points after CCR5 up-regulation had started and also when maximum up-regulation had been achieved for varying periods of time (24, 48, and 72 h). Cells were maintained in culture for up to 4 wk. Parallel cultures without progesterone served as negative controls for each sample.

Progesterone effects on constitutive CCR5 and CXCR4 expression in monocytes/macrophages

PBMCs were cultured for 3–4 days in AIM V medium or RPMI supplemented with antibiotics and 10% autologous serum in the presence of or absence of progesterone (10⁻³ and 10⁻⁴ M). Following culture, nonadherent cells were removed, and culture wells were treated with 0.5 mM EDTA (two times, 10 min each time) to detach the adherent macrophage-enriched cell population.

mAbs and FACS analysis

mAbs specific for CCR5 (5C7, LeukoSite, Cambridge, MA) (39) and CXCR4 (12G5, J. Hoxie) (43) were obtained through the AIDS Research
and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health (Bethesda, MD), CD3-PE (Coulter, Hialeah, FL), CD4-PE, CD8-PE, and CD14-PE (Becton Dickinson) mAbs were also used.

Cells (0.4 × 10⁶) were resuspended in PBS, pH 7.4, containing 2% heat-inactivated human serum (Scantibodies Laboratory, Santee, CA), 0.1% sodium azide, and 10 μg/ml CR5R or CXCR4 mAbs. After 25 min at 4°C, cells were washed twice in wash buffer (PBS, pH 7.4, containing 0.2% BSA and 0.1% sodium azide) and incubated for 20 min in the dark with goat anti-mouse Ig conjugated with FITC (Becton Dickinson). Following a brief wash in buffer, cells were analyzed on the FACSkan (single labeling) or were incubated with a second mAb directly conjugated with PE for 20 min at 4°C washed, and then analyzed on the FACScan (double labeling) using CellQuest software (Becton Dickinson). Propidium iodide was used to exclude dead cells.

Analysis of CXCR4 expression was also performed in whole blood samples. Briefly, 100 μl of blood was incubated with CXCR4 mAb (20 min, room temperature). RBC were lysed using Pharm Lyse (Pharmingen, San Diego, CA). Following RBC lysis, samples were washed twice, incubated with FITC-labeled goat anti-mouse Ig, and analyzed in the FACSkan as detailed above.

For each sample, 10,000 cells were analyzed. Isotype-matched mAb controls (Sigma), used at the same concentration as the mAbs, served as negative controls to determine the amount of fluorescence due to nonspecific binding.

**Immunofluorescence staining for confocal microscopy**

Fluorescence intensity of CXCR4 cell surface expression in the progestosterone-treated PBMC cultures was also assessed using confocal microscopy. Cells were maintained for 4–5 days in the presence of PHA with and without progesterone (10⁻⁷ M). On day 3 cells were fed with RPMI supplemented with antibiotics, 10% autologous serum, and IL-2 as detailed above. Following incubation, cells were prepared for confocal imaging according to the method of Amara et al. (44). Briefly, cells were fixed in 3.7% paraformaldehyde in PBS for 20 min, washed in PBS, incubated with 0.1 M glycine in PBS to quench free aldehydes, permeabilized with 0.05% saponin in PBS supplemented with 0.2% BSA for 15 min, and sequentially stained with CXCR4 mAb and FITC-conjugated goat anti-mouse Ig as described. Following staining, cells were mounted in Vectashield mounting medium (Vector, Burlingame, CA) and viewed in a Leica TCSNT confocal laser scanning microscope (Leica, Exton, PA). Fluorescence confocal micrographs were recorded by exposing selected fields of view to 488 nm light. A 510-nm long-pass filter was used to select light emitted from the fluorochrome. ImageSpace software (Molecular Dynamics, Sunnyvale, CA) was used to measure the average pixel intensity of individual cells; an average of three fields were analyzed per sample.

**Progesterone effects on chemokine secretion by activated T cells**

CD4⁺ and CD8⁺ T cells were purified using the MidiMACS magnetic separation system (Miltenyi Biotec, Auburn, CA). Briefly, PBMCs were incubated with CD4 or CD8 MACS microbeads for 15 min at 4°C, and the magnetically labeled cell suspension was passed through a MidiMACS separation column. Positive cells remained attached to the column and were recovered using a plunger supplied with the column. Purified cells (10⁵/ml) were cultured in AIM V medium in the presence of PHA with and without progesterone (10⁻⁷ M). Irradiated (5000 rad) allogeneic macrophages (adherent cells remaining after adding 10⁵ irradiated PBMCs) were also present in the CD8⁺ T cell cultures. Cell-free supernatants were collected after 24 h and at −70°C until use.

RANTES, MIP-1α, and MIP-1β secretion was assessed using commercially available immunoassay kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s protocols. Results were calculated employing DeltaSoft 3 software (Bio-Tek Instruments, Winooski, VT), using a four-parameter logistic curve fit.

**Progesterone effects on CD4⁺ and CD8⁺ T lymphocyte proliferation**

CD4⁺ and CD8⁺ purified T cells (0.2 × 10⁶) were dispensed into U-bottom 96-well plates in duplicate and cultured in AIM V medium in the presence of PHA with and without progesterone (10⁻⁷ M) for 72 h. Feeder cells (as described above) were also present in the CD8⁺ pure T cell cultures. Cells cultured in AIM V medium alone served as controls for background proliferation. Cultures were pulsed with ³⁵S]thymidine (1 μCi/well; Amersham, Arlington Heights, IL) 5 h before the termination of the culture period. After 72 h, cell cultures were harvested into glass-fiber filters (Wallac, Turku, Finland) and counted in a beta counter. The results were expressed as mean counts per minute.

**Progesterone effects on HIV infection of activated T cells**

PBMCs were incubated with PHA or anti-CD3, as detailed above, in the presence of the absence of progesterone (10⁻⁷ M). IL-2 was added on day 3 of culture. Cell-free isolates of the T-tropic HIV-1Lu, the M-tropic HIV-1Lai, and the M-tropic HIV-1Lai-CRF were obtained through the AIDS Research and Reference Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, from Dr. Robert Gallo (45, 46) and Dr. Irvin Chen (47, 48), respectively. Nonadherent mononuclear cells (10⁶) were infected with the viral strains at inoculation titers ranging from 2 to 1000 TCID₅₀ (titers determined in preliminary experiments by end-point dilution on the H9 cell line and activated PBMCs). Infected cultures were carried for approximately 15 days. Cell viability was routinely checked in both control and progesterone-treated cultures following infection and was always >98%. HIV p24 levels in supernatants of both control and progesterone-treated cultures were measured by commercial ELISA (DuPont-New England Nuclear, Boston, MA).

**Data analysis**

All data were tested and determined to be normally distributed by the Kolmogorov-Smirnov normality test (p > 0.05). As a result, analysis of datasets containing more than two groups was achieved by repeated measures ANOVA followed by Scheffe’s tests for post-hoc analysis. Similarly, analysis of datasets containing two groups was performed by two-tailed paired t test. All tests were considered statistically significant when p < 0.05.

**Results**

Two of the women enrolled were found to be heterozygous for the mutant CCR5 allele and were not used. Plasma estrogen and progesterone levels were obtained for the remaining six women during the early proliferative stage of the menstrual cycle. Concentrations ranged from 0.031 ± 0.001 (± SEM) to 0.174 ± 0.032 ng/ml for estrogens and from 0.203 ± 0.022 to 0.500 ± 0.104 ng/ml for progesterone (values represent the mean of three independent experiments) and were consistent with previous reports of estrogen and progesterone levels during the early proliferative phase (40).

**Progesterone effects on constitutive chemokine receptor expression in nonactivated T cells**

Investigation of chemokine receptor expression in nonactivated T cells was performed at the protein level by double labeling, using CXCR4 or CCR5 mAbs and PE-conjugated anti-CD3, anti-CD4, and anti-CD8, and at the RNA level by RT-PCR. Cells were analyzed just after PBMC isolation and after 24 and 72 h in culture. Approximately 5% of CD4⁺ and CD8⁺ T cells were CCR5⁺ in both control and progesterone-treated (10⁻⁵ M) wells at all three time points.

There was a distinct pattern of CXCR4 protein expression in PBMCs. In freshly isolated PBMCs, CCRX4 protein expression ranged from 3 to 15%; only 2% of peripheral blood CD3⁺ T cells expressed CXCR4 immediately after PBMC isolation (Fig. 1b). Analysis of CXCR4 expression in whole blood confirmed the low expression of this Ag on circulating PBMCs. CXCR4 expression was rapidly up-regulated in T cells following in vitro incubation, reaching a peak at 20–24 h (Fig. 1, a and d). After 72 h CXCR4 was still expressed in 50% of T cells. Progesterone treatment from the initiation of the cultures did not affect CXCR4 mRNA or protein expression in these cells (Fig. 1, d and f).

**Progesterone effects on IL-2 induced up-regulation of chemokine receptor expression in activated T cells**

Studies of CCR5 and CXCR4 expression in activated T cells were performed in the nonadherent PBMC fraction by single labeling for CCR5 or CXCR4 and gating on lymphocytes during FACS...
analysis or by double labeling using CXCR4 or CCR5 mAbs and PE-conjugated anti-CD4 or anti-CD8.

**CCR5.** Addition of IL-2 led to marked CCR5 protein up-regulation in control cultures. Investigation of the effect of progesterone started as soon as up-regulation was detected. Progesterone, added on day 1 of culture and remaining for the entire culture period (group A), significantly suppressed CCR5 protein expression at both $10^{-5}$ M ($p < 0.001$) and $10^{-6}$ M ($p < 0.05$; Fig. 2, a and b). Although the time point at which peak CCR5 up-regulation occurred and the proportion of CCR5$^+$ cells detected varied among individuals, progesterone treatment ($10^{-5}$ M) resulted in at least a 35% reduction in the proportion of CCR5-expressing T cells (range, 35–87%; Fig. 2a). This progesterone-mediated effect could be seen only after IL-2-induced CCR5 up-regulation; when progesterone-treated and control cultures from each individual were tested for CCR5 expression before CCR5 up-regulation had started (days 7–10; Fig. 2a), they both demonstrated a similar percentage of CCR5$^+$ cells, in agreement with the results obtained regarding the effect of progesterone on the constitutive CCR5 protein expression. There was no significant suppression of CCR5 expression when progesterone was added to the cultures at $10^{-7}$ M (Fig. 2b), except for one individual who showed consistent suppression even with the $10^{-7}$ M concentration. Interestingly, this individual had the highest percentage of CCR5$^+$ cells in control cultures after IL-2-induced up-regulation (Fig. 2b), except for one individual who showed consistent suppression even with the $10^{-7}$ M concentration. Interestingly, this individual had the highest percentage of CCR5$^+$ cells in control cultures after IL-2-induced up-regulation. Double labeling studies demonstrated that both CD4$^+$ and CD8$^+$ subpopulations expressed CCR5, and that progesterone inhibited CCR5 expression to the same extent in both subpopulations. Progesterone treatment did not affect CD4 or CD8 Ag expression (data not shown). The mean fluorescence intensity (MFI) of the CCR5 signal in positive cells was also significantly decreased in progesterone-treated ($10^{-5}$ M) cultures vs
control cell cultures (mean MFI ± SEM: control, 13.22 ± 1.33; progesterone-treated, 5.94 ± 1.41; p < 0.01; Fig. 3).

Semiquantitative analysis of mRNA expression at the same time points used for protein studies revealed a significant up-regulation in CCR5 mRNA levels in anti-CD3/IL-2-activated PBMCs compared with nonactivated cells (p < 0.01). However, progesterone had no effect on CCR5 mRNA expression levels in activated T cells (data not shown).

Incubation of PBMCs with progesterone (10⁻⁵ and 10⁻⁷ M) for varying periods of time (24, 48, and 72 h) during CCR5 up-regulation and when maximum CCR5 expression had been achieved (group B) had no effect on CCR5 mRNA expression levels in activated T cells (data not shown).

CXCR4. PHA activation and subsequent IL-2 addition resulted in CXCR4 up-regulation. CXCR4 protein expression was studied at a minimum of three time points for each individual, starting from days 3 to 4 of culture. Peak CXCR4 protein expression in PHA/IL-2-activated T lymphocytes was detected between days 5–8. All three concentrations of progesterone caused a significant reduction in the number of PBMCs expressing CXCR4 (p < 0.001; Fig. 3a).

The MFI of CXCR4⁺ cells was also significantly reduced in the progesterone-treated cultures at both 10⁻⁵ and 10⁻⁶ M compared with that in the control cell cultures (mean ± SEM: control cultures, 17.94 ± 2.20; 10⁻⁵ M progesterone, 6.69 ± 1.74; 10⁻⁶ M progesterone, 11.37 ± 1.68; p < 0.01). Confocal microscopy also indicated that CXCR4 expression in progesterone-treated PBMCs was significantly lower than that in the control samples (average pixel intensity ± SEM: control, 51.89 ± 8.34; 10⁻⁵ M progesterone-treated samples, 23.69 ± 6.09; p = 0.04; mean of three independent experiments; Fig. 4b). Double labeling of control and progesterone-treated (10⁻⁵ M) PBMC cultures indicated that the effect of progesterone on CXCR4 protein expression could be seen in both CD4⁺ and CD8⁺ T cell subsets.

Progesterone did not have a detectable effect on CXCR4 mRNA levels in activated PBMCs. In addition, no significant differences were found in mRNA expression between nonactivated and PHA/IL-2-activated PBMCs (data not shown).

Progesterone effects on constitutive chemokine receptor expression in monocytes/macrophages

Expression of CCR5 and CXCR4 by the adherent monocyte/macrophage population after 3–4 days in culture was investigated by double labeling cells with CXCR4 or CCR5 mAbs and PE-conjugated CD14 and setting the gates on monocytes/macrophages.

CCR5. There was heterogeneity among individuals in the proportions that CCR5⁺ CD14⁺ cells formed in relation to either the gated cell population or the CD14⁺ cell population (mean percentage of CCR5⁺ CD14⁺ cells: range, 5.05–34.14%; n = 3). Nevertheless, within each sample, treatment with progesterone, even at the maximum 10⁻⁵ M concentration, did not alter the proportion of CCR5⁺ CD14⁺ cells.

CXCR4. Progesterone did not affect expression of CXCR4 in monocytes/macrophages at any of the concentrations tested (mean...
Progesterone effects on chemokine secretion by activated T lymphocytes

CD4⁺ cells secreted significantly more MIP-1α than MIP-1β and RANTES (p < 0.04), whereas there was no significant difference among the chemokine secretion levels in CD8⁺ cells (Fig. 5, a and b). CD4⁺ T cells secreted significantly more MIP-1α than did CD8⁺ T cells (p = 0.028).

Progesterone at the maximum concentration significantly inhibited the secretion of all three chemokines by CD8⁺ cells (p < 0.04), whereas it had no effect on chemokine secretion by CD4⁺ cells (Fig. 5, a and b). To determine whether the reduced chemokine secretion by progesterone-treated CD8⁺ cells was due to a negative effect of progesterone on cell proliferation, we tested the proliferative responses of both CD4⁺ and CD8⁺ T cells to PHA in the presence and the absence of progesterone. Progesterone significantly suppressed the proliferation of CD8⁺ cells (mean ± SEM: control, 123,311 ± 30,227 cpm; progesterone-treated, 72,149 ± 19,061 cpm; p = 0.013), whereas it did not significantly affect the proliferation of CD4⁺ T cells (control, 91,104 ± 17,849; progesterone-treated, 77,615 ± 13,574; p = 0.16). Since chemokine levels were reduced by 50–70% in progesterone-treated CD8⁺ cell cultures (Fig. 5b), and the mean counts per minute value of CD8⁺ progesterone-treated cells was 58% that of untreated cells, it is possible that the suppressive effect of progesterone on chemokine secretion by CD8⁺ cells was due to the inhibition of cell proliferation and the lower cell numbers in these cultures.

Progesterone effects on HIV infection of activated T cells

PHA-activated PBMCs were cultured from day 1 with or without progesterone (10⁻⁸ M); nonadherent cells were infected on day 3 of culture with the T-tropic HIV-1MN viral strain. At low viral dose (2 TCID₅₀) there was profound resistance to infection in the progesterone-treated cultures, whereas the control cultures were easily infected with HIV-1 (Fig. 6). This effect was highly reproducible; p24 inhibition was consistently observed in progesterone-treated cultures from four individuals (representative experiments shown in Fig. 6). Anti-CD3/IL-2-activated nonadherent PBMCs were infected with the M-tropic HIV-1JR-CSF strain as soon as CCR5 up-regulation was detected in control cultures by flow cytometry, approximately on days 10–12 of culture. Progesterone effects on infection of these cultures by HIV-1JR-CSF were variable; in experiments performed with low viral inoculations (1–25 TCID₅₀), progesterone did not affect the infection of T cell cultures from two women, but significantly and consistently suppressed infection in cultures from a third woman (Fig. 6). When these data were compared with CCR5 expression levels, it was found that the individual that was protected from HIV-1JR-CSF infection by progesterone was also the one that showed a dramatic reduction of CCR5 expression by progesterone (Fig. 3; individual 3; 74% decrease). The two individuals that were not protected from HIV-1JR-CSF infection by progesterone was also the one that showed a dramatic reduction of CCR5 expression by progesterone (Fig. 3; individuals 5 and 6; 35% and 50% reduction, respectively).

Discussion

Considerable research effort has been directed toward the identification and development of molecules that can prevent or regulate HIV-1 infection. It is well established that adrenal steroid hormones (glucocorticosteroids) promote HIV-1 infection via activation of a steroid response element in the HIV long terminal repeat region (49, 50). Preliminary studies provide evidence that other steroid hormones, such as estrogens and progesterone, also modulate HIV infection in vitro (51, 52). However, given the complexity of the interactions between the immune and endocrine systems and limited understanding at the molecular level of sex hormone-lymphocyte interactions, the roles of these hormones in HIV-1 infection are unclear. The present study, using an in vitro system, addressed whether progesterone influences HIV-1 infection mechanisms via effects on the chemokine-chemokine receptor system.

In nonactivated PBMCs, CCR5 expression remained low and was unaffected by progesterone treatment. Addition of IL-2 following anti-CD3 activation of T cells resulted in marked up-regulation of CCR5 mRNA and protein expression, consistent with previous reports (39, 53). Progesterone exerted a dose-dependent
inhibitory effect on CCR5 protein expression in these activated cultures. This inhibitory effect of the hormone could be seen only when progesterone was added before cell activation and was present for the entire culture period; CCR5 expression was unaltered when progesterone was added after activation of the cells, including the time of peak CCR5 expression.

In contrast to CCR5, CXCR4 protein expression was rapidly and markedly up-regulated in nonactivated PBMCs. This noninduced up-regulation of CXCR4 in freshly isolated peripheral blood T cells suggests that expression of this receptor may be negatively regulated in situ. Treatment with progesterone had no effect on CXCR4 mRNA or protein expression in these cells. On the other hand, progesterone suppressed IL-2-induced CXCR4 protein up-regulation in PHA-activated PBMCs even at the lowest (10^{-7} M) concentration; both the percentage of CXCR4-positive cells and the level of CXCR4 per positive cell were significantly reduced in progesterone-treated cultures.

No significant differences were detected between control and progesterone-treated cultures when mRNA levels for CXCR4 and CCR5 were measured by semiquantitative PCR. It is possible that the method employed was not sensitive enough to detect differences between control and test samples. Alternatively, it is possible that progesterone effects occur at the post-transcriptional level.

Progesterone suppressed IL-2-induced CCR5 and CXCR4 protein expression in PHA-activated PBMCs even at the lowest (10^{-7} M) concentration, both the percentage of CXCR4-positive cells and the level of CXCR4 per positive cell were significantly reduced in progesterone-treated cultures.

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reduction in the number of CD4+ cells recruited by chemokines to the mucosal epithelium, thereby decreasing the number of HIV-1 host cells at sites of transmission.

Considering the effects of progesterone in the chemokine-chemokine receptor system, it was of interest to directly assess the effects of progesterone on HIV-1 infection in vitro. Treatment with progesterone dramatically and consistently inhibited infection of activated PBMCs with low titers of the HIV-1MN T-tropic strain, indicating that the effect of the hormone in this model system was protective against HIV-1 infection. Since T cell-tropic viruses primarily use the CXCR4 coreceptor for entry, and CXCR4 expression (but not CD4 expression) was reduced in progesterone-treated cultures at the time of HIV-1 inoculation, our study suggests that progesterone may inhibit transmission of T-tropic strains of virus by decreasing CXCR4 expression on CD4+ host T cells. Our preliminary experiments using an M-tropic HIV strain also suggested that progesterone may inhibit infection of activated T cells with CCR5-tropic strains of virus by decreasing CCR5 expression.

Enhanced transmission of SIV and other sexually transmitted diseases in progesterone-treated experimental animals may be due to significant thinning of the vaginal epithelium (38, 57) and/or suppression of cytotoxic T cell and NK cell antiviral functions. Recent studies in women indicate that progesterone does not affect vaginal epithelial thickness to the same extent (D. J. Anderson et al., unpublished observations); furthermore, our data suggest that progesterone suppresses the expression of chemokine receptors in activated lymphocytes and inhibits HIV-1 infection by T-tropic and M-tropic viral strains in vitro. This information and meta-analyses of recent epidemiologic studies of HIV-1 acquisition in women on progestin-based contraceptives (58) suggest that progesterone does not enhance HIV-1 infection in women. More research is needed at the molecular level to further elucidate the effects of progesterone on mechanisms of viral entry and intracellular events leading to down-regulation of chemokine receptors. Furthermore, it will be important to determine whether progesterone-associated changes in chemokine-chemokine receptor profiles observed in PBMCs correlate with altered expression of these molecules at mucosal sites and affect patterns of T cell homing and T cell/monocyte recruitment to sites of infection.

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