



Estradiol and progesterone regulate HIV-1 replication

in peripheral blood cells

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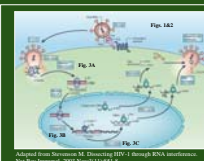
Abstract

Background: Endogenous levels of estradiol and progesterone fluctuate in the peripheral blood of pre-menopausal women during the reproductive cycle. We studied the effects of these sex hormones on HIV-1 replication in peripheral blood mononuclear cells (PBMC). **Methods:** We compared HIV-1 replication in PBMC infected in the presence of mid-secretory (high concentrations of estradiol plus progesterone), mid-proliferative (low concentrations of estradiol plus progesterone), or in the absence of sex hormones. **Results:** Our findings demonstrate that HIV-1 replication is decreased under mid-secretory phase conditions and enhanced under mid-proliferative phase conditions. To determine if sex hormones affect specific stages of the viral life cycle, we performed real-time PCR and found decreased levels of viral integration in PBMC exposed to mid-secretory phase conditions and enhanced levels of HIV-1 transcription in PBMC exposed to mid-proliferative phase conditions. No significant effects on CCR5 expression or on HIV-1 reverse transcription were found. Moreover, we assessed hormonal regulation of the HIV-1 LTR in the absence of the viral regulatory protein Tat. We observed that mid-secretory phase conditions reduced activity of the LTR and mid-proliferative phase conditions enhanced activity of the LTR. **Conclusions:** These findings demonstrate that in HIV-1 infected cells, estradiol and progesterone regulate HIV-1 replication most likely by directly altering HIV-1 transcriptional activation or indirectly by regulation of cytokine and chemokine expression.

Introduction

Estradiol and progesterone, two sex steroid hormones produced by the ovary and the placenta, impact the functional properties of circulating immune cells, regulate the expression of cellular receptors, the secretion of chemokines and cytokines, as well as the production of anti-microbial peptides and protective antibodies. Because of the profound effects of estradiol and progesterone on immune cells, fluctuations in hormone levels are thought to play an important role in the susceptibility and immune responses to HIV-1 infection in women. Despite these observations, there is contradictory evidence regarding the impact of fluctuations in estradiol and progesterone on levels of plasma viral RNA, vaginal shedding, as well as HIV-1 pathogenesis. Overall, HIV-1 infected women have a lower plasma viral load and higher CD4 cell count compared to HIV-1 infected men, and women have a greater risk of developing AIDS with the same viral load and CD4 count as men.

To address the potential impact of estradiol and progesterone on HIV-1 replication in women, we studied HIV-1 infection in peripheral blood mononuclear cells (PBMC) under conditions that mimicked those found during a woman's menstrual cycle. The menstrual cycle is divided into the proliferative phase (days 1-14, low hormone levels) and the secretory phase (days 15-28, high hormone levels). PBMC obtained from female donors were cultured under mid-proliferative, mid-secretory, or in the absence of exogenous hormones prior to and throughout infection with an R5-tropic isolate of HIV-1 (HIV-1_{BR}). We assessed sex hormone effects on cellular expression of the HIV-1 receptor CD4 and co-receptor CCR5, as well as on reverse transcription (DNA copy number), integration (proviral DNA), HIV-1 DNA transcription (HIV-1 transcripts), and release of virus (HIV-1 p24 levels). These analyses permitted us to assess effects of different levels of estradiol and progesterone on early and transcriptional events of the virus life cycle.



HIV-1 replication cycle in susceptible target cells. Following viral interaction with the cellular receptor CD4 and the chemokine receptor CXCR4 or CCR5, fusion is initiated and results in uncoating and entry of virus into the target cell. After internalization, viral RNA is reverse transcribed to cDNA. Following reverse transcription, the viral cDNA is transported to the nucleus to form the integrated provirus. The virus encoded protein Tat promotes the expression of genomic and subgenomic viral transcripts, which are exported from the nucleus by the protein Rev. Subgenomic RNAs are translated into structural and regulatory proteins. Genomic viral RNAs are transported to sites of virus assembly for incorporation into progeny virions. New virions are assembled and released from the infected cell.

Results

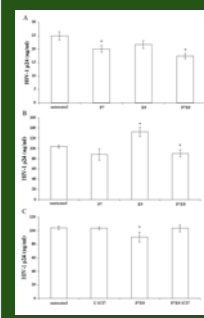


Figure 1. Mid-secretory phase concentrations of estradiol plus progesterone reduce HIV-1 replication in PBMC from women. PBMC were isolated from HIV-1-seronegative, pre-menopausal women and were maintained with or without sex hormones for 48 hrs, followed by the addition of PHA for 48 hrs, and infection with 50 TCID₅₀/ml of HIV-1_{BR} for 2 hours. HIV-1 replication was monitored by p24 ELISA of the culture supernatants collected on days 6 and 10 post-infection. Results shown are the mean \pm SEM from three replicates from a single representative donor. * Indicates statistically significant differences from the untreated cultures.
(A) HIV-1 replication is reduced by mid-secretory phase concentrations of sex hormones by day 6. HIV-1 p24 levels were measured in PBMC without sex hormones (untreated), or in PBMC treated with progesterone at 10⁻⁹ M (P7), estradiol at 10⁻⁹ M (E9), or the combination of estradiol plus progesterone (P7E9) on day 6 post-infection.
(B) The reduction of HIV-1 replication is retained through day 10 post-infection.
(C) An estrogen receptor antagonist reverses the inhibitory effect of mid-secretory phase conditions. HIV-1 p24 levels from PBMC treated as in (A) with the addition of ICI 182,780 (10⁻⁹ M), an estrogen receptor antagonist, to untreated cells (U ICI7), or to cells treated with the combination of estradiol plus progesterone (P7E9 ICI7) on day 10 post-infection.

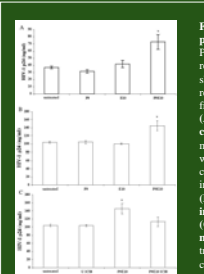


Figure 2. Mid-proliferative phase concentrations of estradiol plus progesterone enhance HIV-1 replication in PBMC from women. PBMC were isolated and handled as in Figure 1, then assayed for HIV-1 replication with p24 ELISA on days 6 and 10 post-infection. Results shown are the mean \pm SEM from three different replicates from a single representative donor. * Represents statistically significant differences from the untreated cultures.
(A) HIV-1 replication is enhanced by mid-proliferative phase concentrations of sex hormones by day 6. HIV-1 p24 levels were measured in PBMC without sex hormones (untreated), in PBMC treated with progesterone at 10⁻⁹ M (P9), estradiol at 10⁻¹⁰ M (E10), or the combination of estradiol plus progesterone (P9E10) on day 6 post-infection.
(B) The increase in HIV-1 replication is retained through day 10 post-infection.
(C) An estrogen receptor antagonist reverses the stimulatory effect of mid-proliferative phase conditions. HIV-1 p24 levels from PBMC treated as in (A) with the addition of ICI 182,780 (10⁻⁹ M) to untreated cells (U ICI8), or to cells treated with the combination of estradiol plus progesterone (P9E10 ICI8).

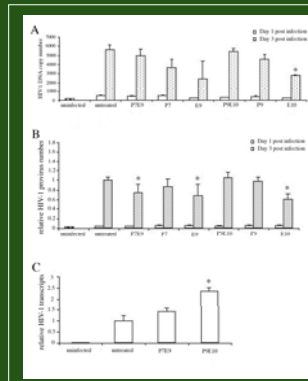


Figure 3. Effects of mid-secretory and mid-proliferative phase concentrations of sex hormones on the intracellular life cycle of HIV-1 in PBMC. PBMC were infected with HIV-1 in the absence of sex hormones (untreated), or in the presence of the combinations of progesterone plus estradiol at mid-secretory phase concentrations (P7E9), or at mid-proliferative phase concentrations (P9E10) and the individual hormones as in Figures 1 and 2. DNA was extracted from uninfected and HIV-infected PBMC on day 1 (clear bars) and day 3 (dotted bars) post-infection. HIV-1 DNA was quantified by real-time PCR using SYBR Green and normalized to the human β -actin gene. DNA copy number was estimated by comparison with a standard curve generated by serial dilutions of genomic DNA extracted from ACH2 cells and uninfected cells. All results shown are the mean \pm SD from nine replicates from a single representative donor. * Indicates statistically significant differences from the untreated cultures.
(A) HIV-1 reverse transcription was unaffected by either combination of sex hormones. Full length viral DNA was detected by primers annealing to the HIV-1 LTR and to the gag sequences. The results are presented as HIV-1 DNA copy number per 40,000 cells.
(B) HIV-1 integration was suppressed by mid-secretory concentrations of sex hormones. Integrated virus was detected by a two-step PCR in which the first round PCR amplifies the DNA sequence between the HIV-1 proviral sequence and the nearest chromosomal ALU elements, and the second, nested real-time PCR specifically amplifies HIV-1 PCR products pre-amplified in the first round. The values are expressed relative to the quantity of provirus detected in untreated PBMC on day 3 post infection.
(C) HIV-1 transcription is stimulated by mid-proliferative concentrations of sex hormones. HIV-1 transcription was detected by quantitative real-time PCR with primers complementary to the flanking sequence of the common splice donor and acceptor sites of the Tat and Rev genes. Total cellular RNA was extracted on day 3 post-infection from all PBMC, and values of HIV-1 RNA were normalized to expression of the human GAPDH gene, and shown relative to levels of HIV-1 RNA of untreated PBMC.

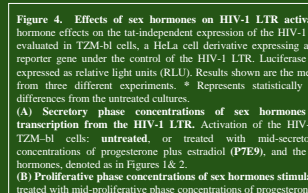


Figure 4. Effects of sex hormones on HIV-1 LTR activation. Sex hormone effects on the tat-independent expression of the HIV-1 LTR were evaluated in TZM-bl cells, a HeLa cell derivative expressing a luciferase reporter gene under the control of the HIV-1 LTR. Luciferase activity is expressed as relative light units (RLU). Results shown are the mean \pm SEM from three different experiments. * Represents statistically significant differences from the untreated cultures.
(A) Secretory phase concentrations of sex hormones decrease transcription from the HIV-1 LTR. Activation of the HIV-1 LTR in TZM-bl cells: untreated, or treated with mid-secretory phase concentrations of progesterone plus estradiol (P7E9), and the individual hormones, denoted as in Figures 1 & 2.
(B) Proliferative phase concentrations of sex hormones stimulate transcription from the HIV-1 LTR. Activation of the HIV-1 LTR in TZM-bl cells: untreated, or treated with mid-proliferative phase concentrations of progesterone plus estradiol (P9E10), or the individual hormone concentrations.

Conclusion

1. Our results demonstrate that estradiol and progesterone regulate HIV-1 replication in the peripheral blood, with mid-secretory phase conditions being inhibitory and mid-proliferative phase conditions being stimulatory.
2. Although an indirect mechanism of sex hormone regulation of cytokine and chemokine secretion can not be excluded, our results suggest that the regulatory effect is most likely direct, due to the regulation of HIV-1 transcriptional activation.
3. As enhanced detection of HIV-1 in cervico-vaginal secretions is often correlated with increased plasma viral load, further characterization of the molecular mechanisms of estradiol and progesterone regulation of HIV-1 replication will broaden our understanding of the kinetics of viral replication in the peripheral blood, which may in turn, impact HIV-1 genital shedding and female-to-male transmission of HIV-1.