Modulation of HIV replication by steroid hormones in monocyte derived macrophages

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Introduction

- Early studies on HIV transmission were focused mainly on males, typically transmission between males who have sex with males (MSM) and majority of the outcomes and conclusions about pathogenesis, disease progression and therapeutic options were derived from studies conducted on male populations.

- At present, there is very little information on HIV pathogenesis, transmission and disease progression that impacts the female population.
Introduction

- Population based studies have shown that there may be gender differences in the pathogenesis of HIV-1 infection.
- Women appear to be more susceptible to HIV-1 infection and have a more diverse population of HIV-1 variants.
- HIV viral load in blood is generally lower in women than in men at similar stages of HIV infection.
Introduction

- Currently, viral load is used in conjunction with other parameters (e.g., CD4 counts, drug resistance genotyping, therapy history, appearance of side effects) to decide whether to initiate or modify anti-viral therapy.

- Therefore, lower HIV-1 viral load seen in HIV-1 positive women prompts the concern that it may not be accurately quantitated or detected by current assays.

- Could lead to inappropriate delays in their admission to anti-retroviral therapy under standard protocols, resulting in suboptimal efficacy in female patients.
Several studies have implicated female sex hormones in influencing HIV transmission, modulating HIV replication, influencing disease progression and interfering with antiretroviral therapy.


Progesterone and Zidovudine (AZT) synergistically inhibited HIV-1 replication in primary placental macrophages (Lee et al 1997).

Beta-estradiol attenuates the anti-HIV-1 efficacy of Stavudine (D4T) and inhibits HIV-1 replication in primary human PBL (Zhang M et al 2008).

Therefore, it becomes important to determine the role of sex hormones and emerging genetic diversity in the increased spread of HIV-1 infection, in female vs. male populations.
AIM: To study the effects of HIV subtypes, gender and steroid hormones on the kinetics and level of HIV replication in macrophages isolated from male and female donors.
Monocytes

- Monocytes play a key role in the pathogenesis of HIV-1 disease
- Monocytes serve as a major viral reservoir in infected tissues
- Vectors for virus transmission to target cells
- Source of potent cytokines that can affect cell function and virus replication
Background

- Preliminary experiments were carried out with MDMs isolated from 5 females and 5 males that were treated with physiologically relevant concentrations of steroid hormones for females and males and infected with HIV-1 Ba-L and primary isolates representing different HIV-1 subtypes.

- Results indicated that high concentrations of estrogen and progesterone downregulated HIV-1 replication and low concentrations of estrogen and progesterone upregulated HIV-1 replication.

- This apparent modulation of HIV-1 replication was not gender specific.

- To further investigate the effect of steroid hormones and gender on modulation of HIV-1 replication we decided to use a wider range of hormone concentration, reflective of physiological concentrations of both males and females.
Methods

- Monocytes were isolated from 3 female and 3 male donors under 50 years old.

- The cells were cultured for 5-7 days and infected with HIV-1 (HIV-1 primary isolates subtype B-NSI (B9697) and subtype C-NSI (SE/364/90) equivalent to 5 ng/ml p24 units).

- After a two-hour exposure, virus particles were removed and the cells were washed 3 times in 1x PBS. Fresh culture medium was added with appropriate additions of the steroid hormones and the cells cultured at 37° C until further use. The cells were treated with physiologically relevant concentrations of steroid hormones for females and males.

- The following concentrations of steroid hormones were used:
  Estrogen: 1.75 µM, 110 nM, 140 pM, 40 pM;
  Progesterone: 64 nM, 32 nM, 2.5 nM, and 1 pM;
  Testosterone: 10 ng/mL, 5 ng/mL, 2 ng/mL, and 1 ng/mL.

- Culture supernatants were harvested 3 days, 6 days, and 9 days post infection and HIV-1 replication quantitated by p24 ELISA. Assays were performed in triplicate.
Effect of Estrogen on HIV-1 Clade B Replication in Females

![Graph showing the effect of estrogen on HIV-1 replication](image)

- **Day 3:**
  - Estrogen 175 mM
  - Estrogen 110 nM
  - Estrogen 140 pM
  - Estrogen 40 pM
  - Control

- **Day 6:**
  - Estrogen 175 mM
  - Estrogen 110 nM
  - Estrogen 140 pM
  - Estrogen 40 pM
  - Control

- **Day 9:**
  - Estrogen 175 mM
  - Estrogen 110 nM
  - Estrogen 140 pM
  - Estrogen 40 pM
  - Control
Effect of Progesterone on HIV-1 Clade B Replication in Females

![Graph showing the effect of different concentrations of progesterone on HIV-1 p24 levels over days 3, 6, and 9.](image-url)
Effect of Testosterone on HIV-1 Clade B Replication in Females

![Graph showing the effect of testosterone on HIV-1 Clade B replication.](image-url)
Effect of Estrogen on HIV-1 Clade B Replication in Males

Graph showing the effect of estrogen on p24 pg/ml over different days (Day 3, Day 6, Day 9) with different concentrations of estrogen (1.75 mM, 110 nM, 140 pM, 40 pM) compared to control.
Effect of Progesterone on HIV-1 Calde B Replication in Males

![Graph showing the effect of progesterone on HIV-1 replication in males. The x-axis represents days (Day 3, Day 6, Day 9), and the y-axis represents p24 pg/ml. The graph compares different progesterone concentrations: 64 nM, 32 nM, 2.5 nM, and 1 pM, along with a control.](image-url)
Effect of Testosterone on HIV-1 Clade B Replication in Males

![Chart showing the effect of testosterone on HIV-1 Clade B replication in males.](chart.png)
Effect of Estrogen on HIV-1 Clade C Replication in Females

![Graph showing the effect of estrogen on HIV-1 replication over days 3, 6, and 9.](Image)

- Day 3: Estrogen 175 mM, Estrogen 110 nM, Estrogen 140 pM, Estrogen 40 pM, Control
- Day 6: Estrogen 175 mM, Estrogen 110 nM, Estrogen 140 pM, Estrogen 40 pM, Control
- Day 9: Estrogen 175 mM, Estrogen 110 nM, Estrogen 140 pM, Estrogen 40 pM, Control

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Effect of Progesterone on HIV-1 Clade C Replication in Females

![Graph showing the effect of progesterone on HIV-1 Clade C replication in females. The x-axis represents days (Day 3, Day 6, Day 9), and the y-axis represents HIV-1 p24 levels (pg/ml). The graph compares different concentrations of progesterone: 64 nM, 32 nM, 2.5 nM, 2 nM, and 1 pM, with a control group. The graph indicates a decrease in HIV-1 p24 levels with increasing progesterone concentration.]
Effect of Testosterone on HIV-1 Clade C Replication in Females

![Graph showing the effect of testosterone on HIV-1 p24 levels over 9 days.](image-url)
Effect of Estrogen on HIV-1 Clade C Replication in Males

[Graph showing the effect of estrogen on HIV-1 p24 levels over days 3, 6, and 9. The x-axis represents days (3, 6, 9), and the y-axis represents HIV-1 p24 levels (pg/ml) from 0 to 3500. The graph compares different estrogen concentrations: 175 mM, 110 nM, 140 pM, 40 pM, and control.}
Effect of Progesterone on HIV-1 Clade C Replication in Males

![Graph showing the effect of progesterone on HIV-1 Clade C replication in males.](image-url)
Effect of Testosterone on HIV-1 Clade C Replication in Males

![Graph showing the effect of testosterone on HIV-1 p24 levels over days 3, 6, and 9.](image)
Summary

- Low concentrations of estrogen (140 pM and 40 pM) upregulated HIV-1 replication by 28-33% in MDMs isolated from females and by 20-48% in MDMs isolated from males compared to untreated control.

- Low concentrations of progesterone (2.5 nM and 1 pM) upregulated HIV-1 replication by 23-30% in MDMs isolated from females and by 32-46% in MDMs isolated from males compared to untreated control.

- High concentrations of estrogen and progesterone had no effect or marginally downregulated HIV-1 replication.

- The effect of estrogen was more pronounced in MDM isolated from females, while the effect of progesterone was more pronounced in MDMs isolated from males.

- No significant effect was observed with testosterone treatment of MDMs isolated from males or females.
Conclusion

Our results show that the levels of steroid hormones, estrogen and progesterone modulate HIV-1 replication in MDMs suggesting that these hormones may exert a significant biological effect on HIV transmission and replication.
Future Studies

- Further investigations will be necessary to determine the exact molecular mechanisms that contribute to the observed hormonal and gender effects.

- Determine the synergistic effect of estrogen and progesterone in modulating HIV-1 replication in vitro.

- To systematically determine the effects of female steroid hormones on HIV-1 replication, anti-retroviral drugs and combinations of hormones and anti-retroviral drugs.
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