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Effect of sex steroid hormones on replication and transmission of major HIV subtypes

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ABSTRACT

Background: The HIV epidemic is expanding worldwide with an increasing number of distinct viral subtypes and circulating recombinant forms (CRFs). Out of 34 million adults living with HIV and AIDS, women account for one half of all HIV-1 infections worldwide. These gender differences in HIV pathogenesis may be attributed to sex hormones. Little is known about the role of sex hormone effects on HIV Subtypes pathogenesis. The aim of our study was to determine sex hormone effects on replication and transmissibility of HIV subtypes.

Methods: Peripheral blood mononuclear cells (PBMC) and monocyte derived dendritic cells (MDDC) from male and female donors were infected with HIV subtypes A–D and CRF02_AG, CRF01_AE, MN (lab adapted), Group-O, Group-N and HIV-2 at a concentration of 5 ng/ml of p24 or p27. Virus production was evaluated by measuring p24 and p27 levels in culture supernatants. Similar experiments were carried out in the presence of physiological concentrations of sex steroid hormones. R5/X4 expressions measured by flow cytometry and transmissibility was evaluated by transfer of HIV from primary dendritic cells (DC) to autologous donor PBMC.

Results: Our results from primary PBMC and MDDC from male and female donors indicate in the absence of physiological concentrations of hormone treatment virus production was observed in three clusters; high replicating virus (subtype B and C), moderate replicative virus (subtype A, D, CRF01_AE, Group_N) and least replicative virus (strain MN). However, dose of sex steroid hormone treatment influenced HIV replication and transmission kinetics in PBMC, DCs and cell lines. Such effects were inconsistent between donors and HIV subtypes. Sex hormone effects on HIV entry receptors (CCR5/CXCR4) did not correlate with virus production.

Conclusions: Subtypes B and C showed higher replication in PBMC from males and females and were transmitted more efficiently through DC to male and female PBMC compared with other HIV-1 subtypes, HIV-1 Group O and HIV-2. These findings are consistent with increased worldwide prevalence of subtype B and C compared to other subtypes. Sex steroid hormones had variable effect on replication or transmission of different subtypes. These findings suggest that subtype, gender and sex hormones may play a crucial role in the replication and transmission of HIV.

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1. Introduction

The HIV epidemic is expanding worldwide with an increasing number of distinct viral subtypes and circulating recombinant forms (CRFs) [1]. Until now, research has been focused primarily on subtype B, the most prevalent subtype in North America and Europe. However, the global distribution of subtypes and CRFs is varied and dynamic in nature and could contribute to the emerging diversity of HIV variants. These variants potentially affect rates of disease progression [2]. According to UNAIDS [3] almost half of all HIV-1 infected individuals worldwide are women. Studies comparing the course of HIV-1 infection between women and men have demonstrated considerable sex differences in the manifestations of HIV-1 disease [4]. Although the rate of progression to AIDS is similar among men and women, plasma HIV-1 RNA levels in women are significantly lower than in men [5,6]. These

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gender based disparities were confirmed in a long-term follow up study [7] from seroconversion to clinical outcomes through subsequent follow up. This study revealed significant statistical racial differences present in the earliest stages of infection and disappeared at 6 months of follow up for patients on HAART. In this same study untreated women had lower viral loads compared with men after 6 months and reported fewer symptoms associated with the acute retroviral syndrome, which may delay or complicate early diagnosis in this population.

Physiological hormonal levels fluctuate in the peripheral blood and reproductive organs of women during the menstrual cycle [8]. It has been hypothesized that during the follicular phase high estrogen levels and higher immunity may have protective effects against invading pathogens, while during the luteal phase, high progesterone levels and reduced immunity may favor microbial invasion [9]. These fluctuations in hormone levels are thought to play an important role in susceptibility and immune responses to HIV-1 infection in women. In vitro studies demonstrate sex hormones, estrogen and progesterone, prevent HIV-1 infection in monocytes but not in lymphocytes suggesting cell specific effects of female sex hormones [10]. In another study estradiol and progesterone were shown to regulate HIV-1 replication in PBMC during the mid-proliferative phase by increasing replication, while decreased HIV-1 replication was observed during the mid-secretory phase [11]. Another in vitro study demonstrated that beta-estradiol inhibited both HIV-1 replication in primary human peripheral blood lymphocytes (PBL) and the antiretroviral efficacy of stavudine (D4T) in PBL cultures [12]. These studies suggested that HIV-1 infection could be modulated either by hormones or host genetic factors.

It is apparent that women are more susceptible to HIV-1 infection, and prognosis rates vary with diverse population of HIV-1 variants and that these gender differences may likely be a selective factor for the evolution of HIV-1 variants. Hence a better understanding of the biologic factors leading to these differences may give insight into the potential impact of sex-specific differences including hormonal effects on the replication and transmission of HIV. In this study, we attempted to determine whether the replication of specific major HIV subtypes would differ in pathogenesis and modulated by sex hormone levels using susceptible in vitro cell culture systems including PBMC and MDDC (monocyte derived dendritic cell).

2. Results

2.1. Dynamics of HIV-1 subtypes in peripheral blood mononuclear cells (PBMCs)

It is known that subtypes could differ in infectivity and may account for geographic variations in distribution of HIV subtypes. In our experiments cells were obtained from normal blood donors of Caucasian origin. To evaluate the replication of different HIV subtypes, cells were infected with similar concentrations of virus based on p24 antigen or p27 antigen levels. Least square measure (Fig. 1) revealed that subtype B replicated well in cells from both males and females followed by subtype C and other subtypes. We identified 3 clusters based on statistically significant differences in replication capacity. Subtype B and C yielded high p24 values and were classified as a high replicating cluster 1, subtype A, D, CRF01_AE, Group-N had moderate p24 values compared to cluster 1 ($p < 0.05$) and were classified as cluster 2 and MN (strain), a lab adapted strain was cluster 3 and showed the least p24 production compared to cluster 1 ($p < 0.001$) in PBMC. Other subtypes such as F2, CRF02_AG, Group-O and HIV-2 did not replicate efficiently (data not shown) suggesting that either higher input of virus or longer duration of infection was required.

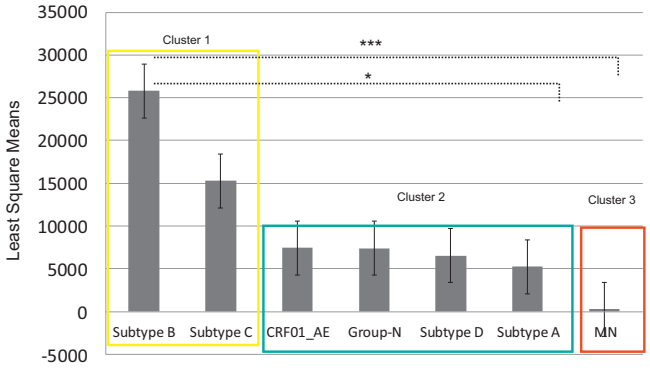

Fig. 1. Dynamics of HIV-1 subtypes in peripheral blood mononuclear cells (PBMCs). HIV subtypes replication in four male and two female donor PBMCs forms three clusters. Replication of HIV subtypes expressed as least square means varies significantly. $^{*}p < 0.05$ cluster 1 vs 2; $^{***}p < 0.001$ cluster 1 vs 3.

2.2. Estrogen and progesterone effect are consistent in female donor cells with laboratory adapted strain MN

Even though the lab adapted MN strain replicated less efficiently in primary cells than T cell lines, this strain has been extensively used for studies of HIV pathogenesis. We therefore chose to further study hormonal effects using this strain because of measurable p24 levels at different time points. Infection of cells with similar levels of virus resulted in a significant 1 log reduction in replication in female cells treated with progesterone ($p = 0.0021$, Fig. 2A), while estrogen treatment had no effect. On the contrary, estrogen, progesterone and testosterone treated male cells showed 1–2 logs increase in HIV replication ($p < 0.05$, Fig. 2A).

2.3. Hormone effect showed inconsistency between donors and subtypes

The three primary strains subtype A, B, and C replicated efficiently with 1–4 logs of virus production in all donors as indicated, however they were differentially regulated by female and male hormones. Hormone effect varied between donors for subtype A where low concentrations of progesterone suppressed subtype A replication by 2 logs in one donor and favored replication for donor W1 ($p < 0.001$, Fig. 2B). This may be due to differences in host factors. In one male and female donor, subtypes B and C had no effect with hormone treatment except for subtype C where virus production was reduced by one log in cells treated with high estrogen ($p = 0.041$, Fig. 2B). CRF01_AE, Group-O, Group-N also showed inconsistent variation in replication kinetics with or without progesterone and estrogen hormone treatment (data found in the Additional file 1).

2.4. Significant donor to donor variation in HIV subtypes transmission from dendritic cells (DCs) to autologous peripheral blood mononuclear cells

Because varied responses were observed in PBMC with or without hormone treatment we wanted to study whether hormones had any effect on dendritic cells which are located in the mucosae (including the oral and vaginal mucosal surfaces) and the lymphoid tissues as they have been proposed to be first target cells for sexual transmission of HIV or SIV [13]. Dendritic cells were first infected with virus concentrations similar to that used for PBMC and the monolayer was washed and co-cultured with autologus PBMC (uninfected) cells. Day 4 and 7 culture sups were analyzed for virus production by measuring HIV-1 p24 and HIV-2 p27 levels. We observed statistically significant ($p < 0.001$) donor to donor