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Pregnancy hormones, estrogen and progesterone, prevent HIV-1 synthesis in monocytes but not in lymphocytes

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Increase in levels of estrogen and progesterone during pregnancy may affect intra-uterine HIV-1 infection through their effect on maternal immunocompetent cells. These hormones were examined for containing HIV-1 production from ACH-2 lymphocytes and U1 monocytes. Neither of the hormones has an effect on ACH-2, but with U1, the physiological concentrations (0.1 μg–0.1 ng) of progesterone and estrogen demonstrate significant inhibition of HIV-1 release. Except for the highest dose of 1 μg/ml, the dose–response to progesterone and estrogen was not correlated with the negative influence on proliferation of both types of cells. The results suggest that in vivo low doses of female steroids may display specific antiviral activity in monocytes but not in lymphocytes.

HIV; Estrogen, Progesterone; Monocyte; Lymphocyte; Pregnancy

1. INTRODUCTION

The alterations that occur in female hormone production during pregnancy may have an important impact on transmission of HIV-1 from mother to child. Human trophoblasts composing the outer surface of the placenta produce a wide range of placental proteins and hormones, including estrogenic and progestational steroids. Although it is not clear how the increase in levels of estrogen and progesterone may affect HIV status, it undoubtedly affects maternal virus-carrying immune cells. There is ample evidence of interaction of these hormones with lymphocytes and monocytic cells [1,2], the main circulating vectors of HIV-1. In this study two pregnancy hormones, progesterone and 17β-estradiol, were tested for their effect on HIV-1 synthesis in virus-carrying monocytes and lymphocytes.

2. MATERIALS AND METHODS

The effect of pregnancy steroid hormones on HIV-1 synthesis in lymphocytic ACH-2 and monocytic U1 cells (courtesy of AIDS Research and Reference Reagent Program, NIH Rockville, MD; contributor: Thomas Folks) was measured by evaluating the levels of p24 *gag* gene product of HIV-1 in the culture fluid of cells (initial concentration 5×10^5 cells/ml) exposed for 3 days to serial ten-fold dilutions (from 1 μg/ml to 0.1 ng/ml) of 17β-estradiol and progesterone (Sigma, St. Louis, MO).

Viral production from infected cells resulting in release of HIV-1 gag p24 protein was tested by ELISA. Viral core p24 antigen ELISA kit was purchased from Cellular Products (Buffalo, NY) and the assay was performed according to manufacturer's instructions. The values below 50 O.D. (cut off value = mean O.D. of wells containing culture medium only) were considered as negative values.

The effect of pregnancy hormones on proliferation of ACH-2 and U1 cells (5×10^5 cells/ml) following 3 days of continuous exposure was measured simultaneously by [^{3}H]thymidine uptake. Cells were grown in RPMI 1640 medium with 10% fetal calf serum in the presence or absence of $\log_{10}$ concentrations of steroids for three days. Four h before assay, cells were pulsed with 1 μCi of [^{3}H]thymidine (Amersham, Arlington Heights, IL), and incorporation of the label was determined by scintillation spectroscopy.

Student's *t*-test was used to compare control values to obtained data from described experiments.

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3. RESULTS

HIV-1-producing ACH-2 lymphocytes and U1 monocytes were incubated for three days in the presence of serial ten-fold dilutions of estrogen and progesterone. Fig. 1 shows, that except for 1 μg dose, the pregnancy hormones do not modify viral production from lymphocytes. The dose–effect as tested by p24 ELISA on U1 cells is shown in Fig. 2. The suppression of viral release was observed for all tested doses starting from the lowest concentration of 0.1 ng/ml. The action of both progesterone and estrogen was characterized by a slope indicating a direct dose–response.

To determine whether the observed inhibition of virus production was not related to the growth suppression of cells, we evaluated the effect of steroids on [^{3}H]-labeled thymidine incorporation (Fig. 2). There was no significant dose-dependence for progesterone and estrogen except at the maximal dose of 1 μg/ml. It is, therefore, likely that lower doses of estrogen and progesterone have a specific antiviral effect that is unrelated to the suppression of cell proliferation.

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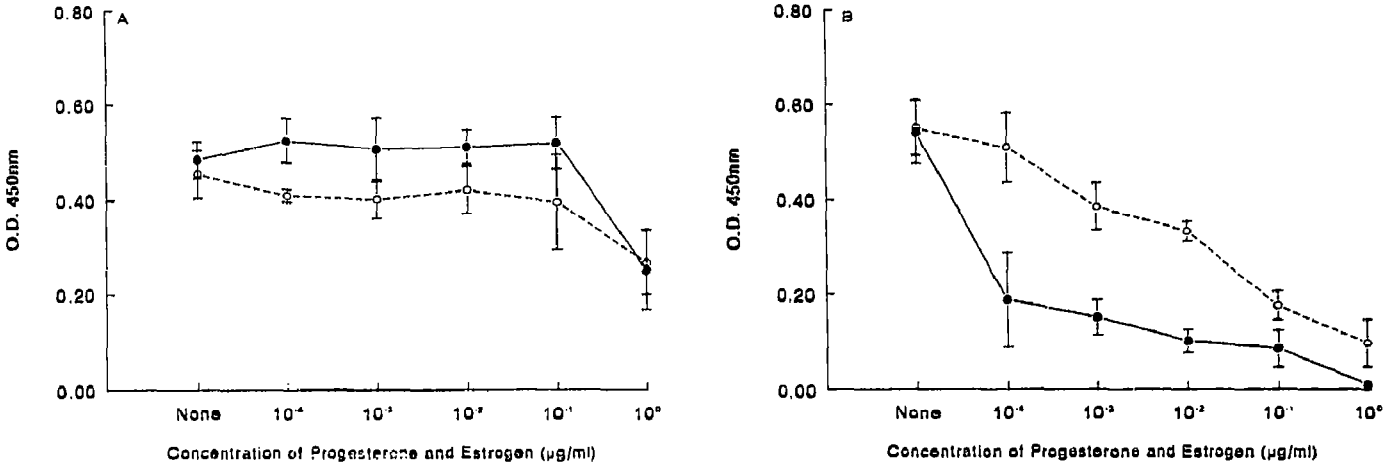

Fig. 1. Effect of progesterone (●) and estrogen (○) on HIV-1 production from lymphocytic ACH-2 (A) and monocytic U1 cells (B) following 3 days of continuous exposure as measured by p24 ELISA. Typical results from triplicate wells are shown. Neither steroid hormone has any effect on HIV-1 replication in ACH-2 lymphocytes except for the cytostatic dose of 1 μg/ml. Virus production from U1 monocytes in response to estrogen exposure exhibits a dose-dependent mode. There is 90%–8% inhibition across the tested range of estrogen from 1 μg to 0.1 ng per ml. Progesterone appears to display a more potent antiviral effect. According to the O.D. values there is 100%–73% reduction in p24 levels resulting from exposure to 1 μg–0.1 ng of progesterone per ml of monocyte culture.

4. DISCUSSION

Placental cells appear to resist HIV-1 infection, but the mechanism of such impregnability is not known. Our investigations [3,4] and studies by others [5] demonstrated that the infection of CD4-negative epithelial cells and placental trophoblasts results predominantly from physical contact with HIV-1 infected lymphocytes or monocytes but not from exposure to free virus. Therefore, the possible mechanism of placental resistance could be attributed, at least in part, to the prevention of viral production from infected blood cells by secreted placental constituents like pregnancy hormones.

After establishment of the pregnancy, the levels of female steroids rise considerably, reaching a maximum of 0.5 μg and 1 μg per ml of plasma for estrogen and progesterone respectively. A number of observations support the concept that female steroids influence immunity [1,2,6,7]. Estrogen receptors were identified on T-cytotoxic/suppressor but not on T-helper/inducer cells. Steroid hormones at pharmacological concentrations display a potent immunosuppressive effect on lymphocytes in autologous immune reactions [8–11]. Since most of the studies involving female steroids were conducted on T lymphocytes, little is known on the interaction of these hormones with monocytes. However, it is known that decidual maternal cells at the materno-fetal

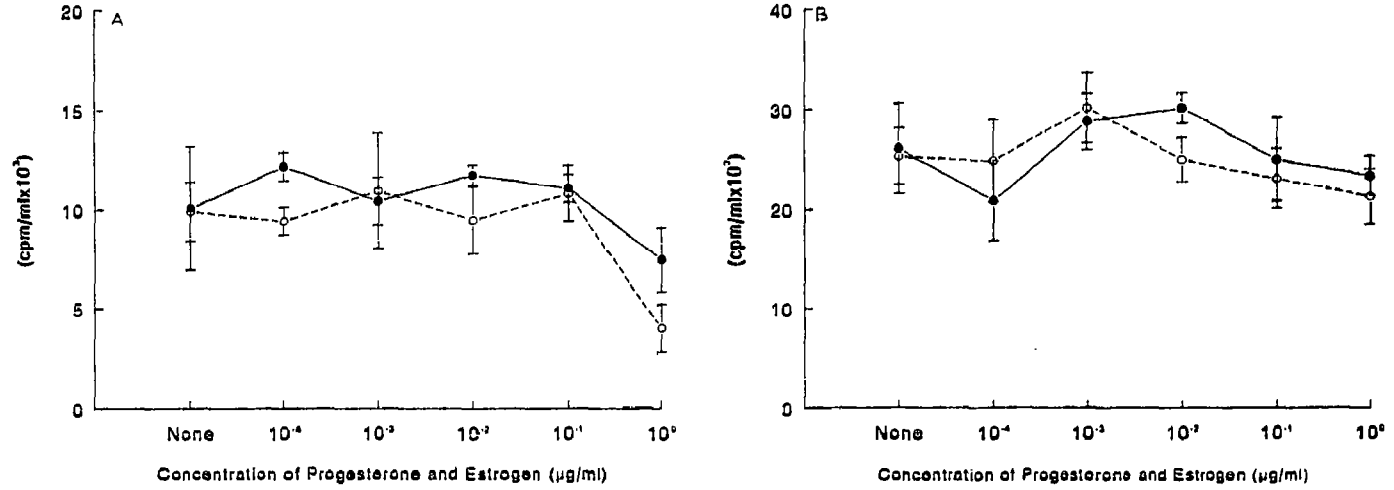

Fig. 2. Effect of 3 days exposure to progesterone (●) and estrogen (○) on [³H]thymidine uptake by ACH-2 (A) and U1 cells (B) as determined in two separate experiments. Inhibition of cellular growth is observable only at 1 μg concentration. Statistical analysis of combined results from the proliferation assays indicates that observed suppression of p24 release from HIV-infected monocytes and lymphocytes at concentrations of steroid hormones below 1 μg/ml is not correlated to the cell growth pattern as there is no discernible dose effect.

interface are mainly composed of macrophages and not of T lymphocytes like ACH-2 [12,13]. The differential effect of steroids on the immune cells of monocyte/ macrophage lineage may be relevant to the fact that the antiviral action of progesterone and estrogen is demonstrated in monocytic cells and not on lymphocytes. Contrary to the often ambiguous effect of estrogen on immunity, progesterone has been consistently reported as a potent immunosuppressor [14]. Incidentally, in our study estrogen appears to inhibit the production of infectious virions to a lesser degree than progesterone. Complete inhibition of p24 release by progesterone was observed at the noncytotoxic concentration of 0.1 μg/ml, whereas a similar dose of estrogen contributed to 75% inhibition. Considering the paucity of available anti-HIV drugs, these two hormones with established safety records of clinical use represent remarkably good candidates for clinical trials.

Evidence of endocrine involvement in the course of HIV-1 infection had been documented [15–17]. However, at the present time the role of female hormones on viral status remains largely obscure [18]. Our study in vitro supports the notion that female steroid hormones control the replication of HIV-1 in monocytes and therefore, may contribute to the prevention of HIV-1 transmission from mother to child. The observations described here urge for examining the antiviral role of pregnancy hormones in vivo.

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