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Inhibitory effect of human chorionic gonadotropin (hCG) on HIV-1 transmission from lymphocytes to trophoblasts

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It has been demonstrated that human chorionic gonadotropin (hCG) inhibits HIV production in vitro, suggesting that this soluble placental glycoprotein can control viral replication and spread in vivo. hCG – the major product of fetal trophoblasts – was tested on an in vitro model consisting of choriocarcinoma-derived ENAMI trophoblasts exposed to HIV-infected MOLT-4 lymphocytes. The results show a U-shaped antiviral dose–effect and suggest that hCG may contribute to protection against intrauterine transmission of HIV-1.

hCG; HIV; Lymphocyte; Trophoblast; Placenta

1. INTRODUCTION

Recent observations seem to indicate that CD4-negative placental trophoblasts – the only fetal cells in direct contact with maternal blood – can be susceptible to HIV infection depending on the mode of virus delivery [1–3]. Trophoblast choriocarcinomic lines were resistant to infection by cell-free virus [3]. Furthermore, there were no signs of infection when trophoblasts were exposed to HIV-carrying cells with impaired adhesion capacity [2]. However, the exposure to lymphocytes that adhere to substrate cells invariably resulted in infection of placental cells [1,2]. Nevertheless, the low transmission rate of HIV across the placenta cannot be accounted for solely by such an explanation [4]. The possibility that pregnancy proteins and hormones may regulate retroviral infection has been recently explored [2] and it was found that human chorionic gonadotropin (hCG) can inhibit viral production in HIV-infected cells [5]. hCG is the most abundant glycoprotein hormone produced by fetal trophoblasts and is known as a potent suppressor of cell-mediated allogeneic reactions involving the interaction of maternal lymphocytes with fetal implant [6,7]. In this study we evaluated the effect of hCG on viral transmission using an in vitro model of placental infection occurring upon lymphocyte-to-trophoblast contact.

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2. MATERIALS AND METHODS

2.1. *Antiviral assay*

The seeding concentration of choriocarcinoma-derived ENAMI trophoblasts (kindly from Dr. N. Matsuzaki, Osaka University Medical School, Japan) was 1×10^5 cells per well (1 cm^2) and plates were cultured in a humidified incubator with 5% CO_2 at 37°C for 24 h prior to co-incubation with lymphocytes. Serial ten-fold dilutions of purified preparation of hCG (specific activity 4,000 IU/mg; Sigma, St. Louis, MO) were added to washed target ENAMI trophoblast cells for 0.5 h at 4°C, followed by the addition of 10^5/ml of MOLT-4/YH5 cells (HIV-1 strain YH5 infected MOLT-4 T cell line was provided by Dr. Jun Minowada, Fujisuki Cell Center, Okayama, Japan); the co-culture was continued in the presence of hCG for 1 h at 37°C. By the end of incubation suspension-grown donor lymphocytic cells were thoroughly washed from the plastic-adherent trophoblasts by repeated rinsing with ice-cold Ca^{2+}, Mg^{2+} free PBS. Following the removal of non-absorbed HIV and adherent HIV-infected lymphocytes, the virus-exposed trophoblasts were trypsinized and replated into new culture dishes. Residual lymphocytes which could persist after washing were eliminated by further culture in a medium (Serumless Medium, Neuman & Tytell, Gibco) that has no effect on trophoblast viability. One month post-infection mock-infected and HIV infected trophoblasts remained morphologically identical and no cytopathic effect could be observed in trophoblast cells exposed to HIV-1. The infection of trophoblasts was persistent, with very low spontaneous viral production, as there was no detectable p24 release or RT activity.

2.2. *Recovery of virus from latently infected trophoblasts by coculture with cord-blood-derived MT-4 lymphocytes*

The coincubation of low-producing trophoblasts with non-infected indicator MT-4 lymphocytes (from Dr. J. Minowada) always resulted in a heavy viral production accompanied by syncytium formation and lymphocyte death by day 7. Therefore, the recovery and amplification of the virus from low-producing placental cells was achieved by coincubation with HIV-susceptible MT-4 lymphocytes carried out two weeks after the initial inoculation. MT-4 cells (10^6 cells/ml) were washed twice and added to virus-carrying ENAMI cells for 1 h. Infected-by-contact MT-4 cells were gently removed, washed and cultured at 37°C in 5% CO_2 for 7 days. Samples of the culture medium were tested on day 4 with p24 Ag ELISA (Cellular Products, Buffalo, NY).

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3. RESULTS

The antiviral effect of hCG against cell-contact-mediated transmission was evaluated by measuring the levels of p24 from MT-4 lymphocytes after coincubation with ENAMI trophoblasts exposed beforehand to MOLT-4/YH5 in the presence of the hCG. This indirect procedure is time consuming. However, considering that HIV-1 replication in trophoblasts results in very low spontaneous viral production that is at the sensitivity limit of ELISA, the simplest mean of characterizing the degree of infection is the amplification of the virus by co-culture with MT-4. Productive HIV infection in MT-4 lymphocytes is characterized by a formation of syncytial cells and extensive cell death. Instead of relying on visual evaluation based on the manual counting of multinucleated cells, we quantitated the dose–response by standardized ELISA for p24 viral antigen. The results shown in Fig. 1 seem to indicate that hCG can prevent cell-mediated HIV-1 infection in a dose-dependent fashion. A U-shaped dose–response curve was observed, implying that the intermediate doses of hCG appear to prevent cell-mediated HIV-1 infection more efficiently than the lower or higher doses at the edges of the curve. Cell-to-cell HIV-1 transmission was blocked most efficiently within a dose range of 0.1–100 IU/ml. Lower and higher concentrations of hCG, to 0.01 and 1,000 IU/ml, resulted in approximately 10% and 50% inhibition, respectively. Despite such a peculiar dose–response all tested concentrations of hCG were at least partially protective against HIV infectivity.

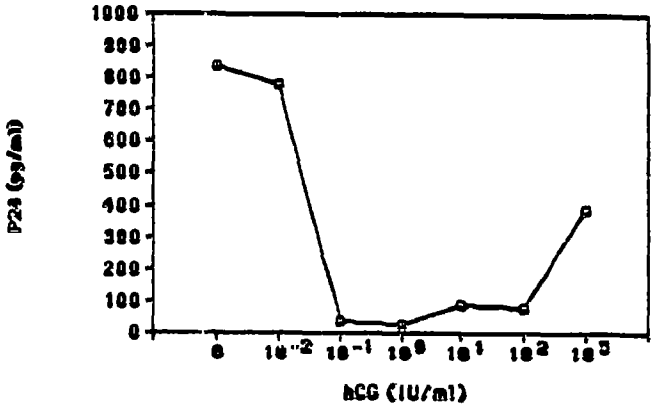

Fig. 1. Effect of hCG (Sigma) on cell-mediated HIV transmission occurring as a result of lymphocyte-to-trophoblast contact in the presence or absence of concentrations of hCG ranging from 10^{-2} IU/ml to 10^{3} IU/ml. The experiments were repeated three times and means from duplicate wells of a representative experiment are shown. Note that levels of p24 below 10 pg/ml are beyond the reliable sensitivity limit of the ELISA kit.

4. DISCUSSION

In previous studies we have obtained two sets of data. First, trophoblasts were permissive to HIV delivered by physical contact with infected lymphocytes but not when exposed to cell-free HIV or infected cells with impaired adhesion capacity [2]. Second, low doses of hCG can inhibit reverse transcriptase activity and release of p24 HIV gag antigen from infected lymphocytes; the dose–response was U-shaped. hCG seemed to have a specific antiviral effect since there was no discernible cytotoxic effect [5]. Based on these observations we decided to evaluate the effect of hCG in cell–cell infection using an in vitro system representing the model of placental infection. The results presented here indicate that hCG inhibits the onset of viral spread via cell–cell contact and the dose–response curve appeared to be of the same U-shape observed previously [5].

hCG is the earliest and most abundant polypeptide hormone produced during gestation and is mostly known for its involvement in hormonal interplay at the establishment and maintenance of pregnancy. The function of hCG as an immunoregulator is not well understood – it is possible that locally secreted hCG interferes with the cell–cell interaction required for lymphocyte-mediated rejection of the semi-allogeneic fetal implant [6,7]. Although there is still a considerable controversy regarding this issue [8,9], purified hCG preparations have been demonstrated to inhibit the reactivity of lymphocytes [11–14] in an alloreactive environment. We have suggested earlier that hCG may have an inhibitory effect on release of cytoplasmic content, i.e. viral particles from lymphocytes triggered by contact with the substrate cell [5].

The normal levels of hCG in plasma fluctuate from 160 IU/ml in the first trimester to under 80 IU/ml during the rest of the pregnancy [6]. It was recently reported that HIV-1 infection of placental explants results in a ten-fold decline of hCG production and it has been proposed that this may decimate normal fetal development [15]. Effective antiviral concentrations of hCG in our study were within the range that may correspond to the decreased levels of hCG in plasma of HIV-1-positive pregnant women. However, there are no clinical data that correlate hCG concentrations in virus-carrying pregnant women and the incidence of fetal infection. It is difficult to predict whether clinical applications of this glycoprotein hormone can be projected. The possibility that exogenous pregnancy products may prevent fetal infection and/or control viral infection in vivo cannot be excluded.

Except for interferon alpha [16], hCG is one of the few proteins secreted by the human body that has been claimed to have antiviral activity [5]. Although hCG was shown to inhibit reverse transcriptase activity and p24 production in infected cells, the mechanism of its action is not yet known. Further studies are needed to establish the mechanism of hCG and other pregnancy factors such as steroid hormones [2] and placental interferon against HIV replication and spread in vivo.

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