

Progesterone Inhibits HIV-1 Replication in Human Trophoblast Cells through Inhibition of Autocrine Tumor Necrosis Factor Secretion

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Background. Progesterone levels are higher in placental barriers during pregnancy, but the effect of progesterone on human immunodeficiency virus type 1 (HIV-1) infection in placental cells has not been addressed. We hypothesize that progesterone may affect HIV infection.

Methods. Purified trophoblastic cells and trophoblastic cell lines were infected or transfected with HIV-1, and the effect of progesterone was analyzed. Viral replication was measured by viral p24 or viral load quantification. Nuclear factor κ -B (NF- κ B) or long terminal repeat (LTR)–dependent transcription was measured by luciferase assays. Expression of chemokine receptors was analyzed by flow cytometry. Tumor necrosis factor (TNF) messenger RNA was assessed by reverse-transcription polymerase chain reaction (RT-PCR) and quantitative RT-PCR.

Results. Progesterone inhibits HIV-1 replication in placental cells at the concentration found in the placental interface, at a postentry step, and does not affect cell surface expression of chemokine receptors. Progesterone did not inhibit basal or induced LTR transcription or NF- κ B activation. TNF synthesis in placental cells is induced by HIV-1 infection that, in an autocrine manner, activates viral replication, because neutralizing anti-TNF antibodies block it. Progesterone inhibits the induction of TNF synthesis by viral infection and virus or gp-120–induced TNF transcription.

Conclusion. Our results demonstrate that progesterone inhibits HIV-1 replication in placental cells by reducing TNF levels, which are required for optimal viral replication.

Vertical transmission of HIV-1 is a serious public health issue. It is estimated that 1600 infants acquire HIV-1 infection every day worldwide. The HIV-1 transmission rate is increasing in developing countries, ranging from 10% to 40% in the absence of antiretroviral treatment,

but it can be strongly decreased if antiretroviral therapy is supplied to mothers [1].

HIV-1 vertical transmission to the infant in utero is thought to occur mostly through the placenta. Viral antigens and nucleic acids have been identified in placental tissue samples obtained from HIV-1–infected women, by means of immunocytochemical analysis and in situ hybridization [2, 3]. There are several possible (although not mutually exclusive) mechanisms involved. First, transmission could involve a direct passage of infected maternal cells into the fetus through holes in a damaged placental barrier [4]. Second, the placenta may allow transcytosis of the virus from the maternal to the fetal circulation either directly or complexed with maternal antibodies through placental Fc receptors [5, 6]. Finally, direct infection of placental cells with HIV-1, either by endocytosis [7] or by lymphocyte-trophoblast contact [8, 9], could be implicated. The presence of the virus has been detected in vivo in trophoblasts, Hofbauer cells, and villous endothelial cells [2, 9, 10].

Received 9 February 2006; accepted 4 November 2006; electronically published 23 March 2007.

Potential conflicts of interest: none reported.

Financial support: M.A.M.-F. received support from Fundación para la Investigación y Prevención del SIDA en España (FIPSE) (grant 36536-05), Fondos para la Investigación Sanitaria (grant 040883), and Salud y Farmacia (grant 06778); M.F. received support from SAF (grants 0920-03 and 05109-04) and Comunidad Autónoma de Madrid (grant 08.3/0023.1/2001); M.A.M.-F. and M.F. received support from FIPSE (grant 36514-05) and Red Temática Cooperativa de Investigación en SIDA (grant RIS G03/173); Asociación Española de Pediatría 2005-54 Congreso de la Asociación Española de Pediatría, Murcia (poster no. P322).

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The Journal of Infectious Diseases 2007;195:1294–302

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0022-1899/2007/19509-0010\$15.00

DOI: 10.1086/513434

Human choriocarcinoma cell lines (i.e., BeWo, JAR, and JEG-3), as well as isolated primary trophoblast and Hofbauer cells, were shown to be permissive to HIV-1 infection *in vitro* and to sustain a low level of virus replication [5, 7, 9, 11]. The mechanism of entry is controversial. HIV-1 may enter via a CD4-dependent mechanism, a coreceptor, or a DC-specific intracellular adhesion molecule–grabbing nonintegrin (DC-SIGN)–dependent but CD4-independent mechanism, by endocytosis; through contact between peripheral blood mononuclear cells and placental cells; or by a combination of several of these mechanism [7, 8, 11, 12].

Cytokines involved in placental and fetal growth could also be important. Enhanced production of inflammatory cytokines by trophoblast cells has been reported in HIV-1–infected pregnant women [13, 14], as has placental inflammation [15]. Inflammatory cytokines may cause damage to the placental barrier, allowing transfer of the virus [16], and could stimulate viral replication [17] (like tumor necrosis factor [TNF], which increases HIV-1 replication [18, 19]), because they are potent inducers of nuclear factor- κ B (NF- κ B), which is required for HIV-1 long terminal repeat (LTR) transcription [20].

Progesterone, a sex steroid hormone naturally produced in the ovary and placenta [21], is essential for pregnancy to reach full term. Its concentration (normally in the range of 1–10 nmol/L) increases during pregnancy (up to 0.3 μ mol/L) in humans and mice [22], and it appears to have anti-inflammatory properties. It inhibits T lymphocyte proliferation and function, as well as synthesis of Th1-type cytokines [22, 23], and favors the development of human Th2-type cells [24]. Progesterone also inhibits the expression of inducible nitric oxide synthase in activated macrophages [25] and natural killer cells [26] and inhibits cytotoxic T lymphocyte function [27] and TNF synthesis [28, 29].

Despite the influence of progesterone on immune function and pregnancy, its influence on HIV-1 replication has been scarcely studied. We studied the effect of progesterone on HIV-1 replication in trophoblast cells and placental cell lines, as well as the cellular and molecular mechanisms involved. We found that progesterone inhibits HIV-1 replication in infected or transfected placental cells. Progesterone affects neither the expression of chemokine receptors nor LTR transcription; rather, it inhibits HIV-1–induced TNF production, which is required, in an autocrine fashion, for HIV-1 replication in those cells.

MATERIALS AND METHODS

Isolation and culture of primary and trophoblastic cell lines. Cytotrophoblasts were purified from normal full-term human placentas, after vaginal delivery. Several cotyledons were minced and digested in a calcium- and magnesium-free Hanks' (CMF Hanks') solution layered over fetal calf serum (FCS) centrifuged at 800g for 10 min and were resuspended in Dulbecco's MEM

(DMEM) containing 25 mmol/L *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid and 25 mmol/L glucose. This suspension was layered over a preformed discontinuous Percoll gradient (Pharmacia) made up in CMF Hanks' solution (from 70% to 5% Percoll in 5% steps) centrifuged at 1200g for 20 min. Cells were collected at densities between 1.051 and 1.063, as determined by running parallel gradients (Pharmacia). Cytotrophoblasts were purified by immunomagnetic separation, by use of monoclonal antibodies against human HLA-ABC (class I) and HLA-DP, DQ, DR (class II). The purity was >97%, as confirmed by flow cytometric analysis with anticytokeratin antibodies (Dako AIS). The cells were cultured in DMEM-HG 20% FCS at a concentration of 10^6 cells/ml.

To create polarized cells, we used Millicell filter inserts (Millipore) that were coated with 100 μ g/mL of a basement membrane matrix (Matrigel; BD Biosciences) and placed within a well of a 24-well plate, as described elsewhere [30]. This system allows confluent cells to develop a polarized structure in which apical and basal domains are segregated and tight junctional complexes are formed.

Transformed trophoblastic cell lines JAR (ATCC HTB 144) [31] and JEG-3 (ATCC HTB 36) [32] were routinely grown in 10% FCS RPMI 1640 at 37°C in a humidified atmosphere of 5% CO₂. Every 3 days, the cells were trypsinized and plated according to the experiment being performed.

HIV-1 infection of placental cells. The established viral strain HIV-1_{NL4.3} and primary isolates from HIV-1–infected pregnant women (i.e., HIV-1₂₀₆₉₁ [macrophage-tropic, R5] and HIV-1₁₄₉₈₁ [lymotropic, X4]) were used. The virus was added to individual cultures (MOI, 0.1 or 1) and was maintained at 37°C for 4 h. Cultures were extensively washed (between 10 and 20 times) with warm RPMI 1640, to remove all free virus. In some experiments, single cells were transfected with plasmid pNL4.3 encoding for the entire HIV-1_{NL4.3} sequence and were stimulated with the indicated stimuli. At various times after infection, virus titer was quantified in the supernatant by p24 antigen production (Innotest HIV Antigen mAb; Innogenetics) or by the quantification of HIV RNA (i.e., the viral load) (Amplicor-HIV Monitor test; Roche). Peripheral blood mononuclear cells (PBMCs) were isolated from blood by Ficoll-Paque (Pharmacia) density gradient centrifugation and were infected by HIV-1 as described elsewhere [18].

Flow cytometry. Cell surface expression of CCR5 and CXCR4 was evaluated by flow cytometry. Placental cells were recovered by treatment of the monolayers with 0.02% EDTA-containing RPMI 1640 medium and were incubated with either fluorescein isothiocyanate anti-CXCR-4 or phycoerythrin anti-CCR5 (clone 12G5 and CTC5, respectively; R&D Systems) monoclonal antibodies or with irrelevant fluorescein isothiocyanate or phycoerythrin labeled as negative controls, at 4°C for 30 min. Surface fluorescence was determined using a FAC-

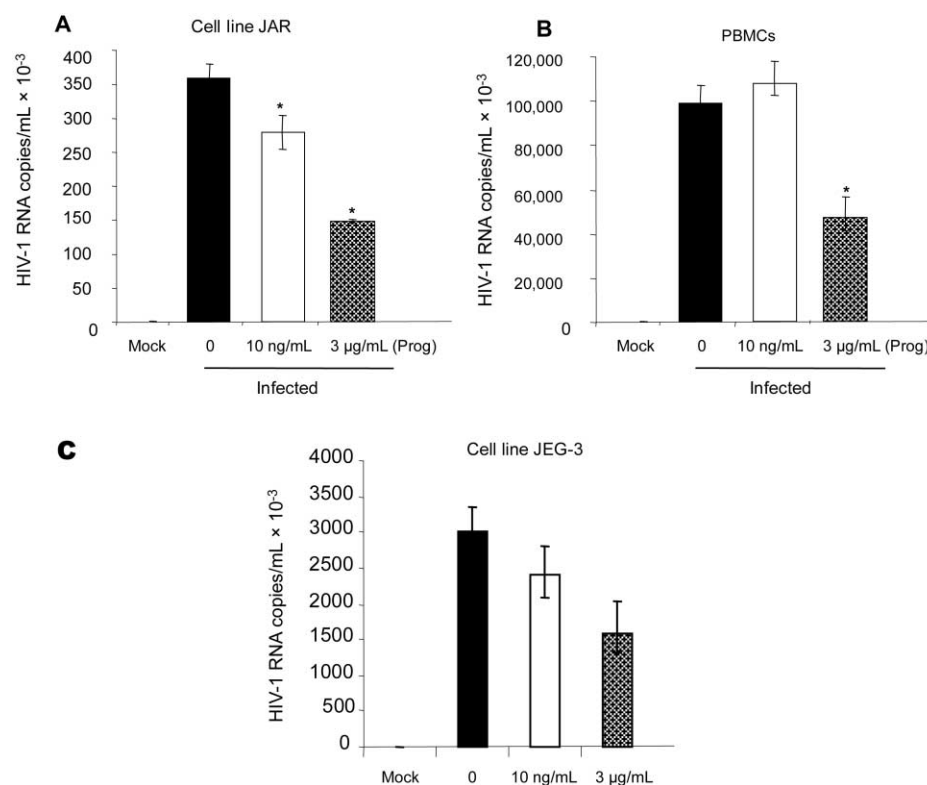


Figure 1. Inhibition of HIV-1 replication in human placental trophoblast and peripheral blood mononuclear cells (PBMCs) by progesterone (Prog). *A*, Infection of cell line JAR with HIV-1 (MOI, 1) in the presence or absence of Prog, 10 ng/mL or 3 µg/mL, as indicated. HIV-1 replication was measured as the no. of HIV-1 RNA copies noted 72 h later. *B*, HIV-1 infection of PBMCs (0.5 ng/10⁶ cells) obtained from healthy women. **P* < .001. *C*, Infection of polarized JEG.3 cells, as noted in panel *A*. Results are the mean ± SD from replicate assays of the 3 independent experiments performed.

Scan cytometer (Becton-Dickinson). A minimum of 10,000 cells/point was analyzed.

TNF mRNA detection. mRNA from 10⁵ placental cells was isolated with oligo (dT)-coated magnetic beads. DNA amplification was performed using 5 µL of cDNA. Primers used for TNF amplification were as follows: sense 5'-GAGTGACAAGC-CTGTAGCCCATGTTGTAGCA-3' and antisense 5'-GCAAT-GATCCCAAAGTAGACCTGCCAGACT-3' (obtained from Clontech Laboratories).

Real-time polymerase chain reaction (PCR) using 200 ng of total RNA was performed using a Roche Light Cycler PCR instrument and a Light-Cycler reverse-transcription PCR (RT-PCR) kit (Roche Perkin-Elmer). The standard used to make the control curve was the commercial plasmid pCRTM II (Invitrogen) into which a fragment of the TNF gene was inserted. The number of molecules of the plasmid was quantified by measuring the quantity of DNA in a spectrum photometer. Because the molecular weight of the standard (plasmid plus sequences of TNF) was known, the exact number of molecules could be extrapolated.

Transcription assays. The reporter pLTRWT-luc expression plasmid was a gift from J. L. Virelizier [33]. It carries the

U3⁺R of the LTR of the HIV-1_{LAI} strain of HIV-1 from nt -644 to 78+. The reporter pNF-κB-luc expression vector contains 3 tandem copies of the NF-κB site of the conalbumin promoter driving the luciferase reporter gene [34]. Human TNF promoter construct from nt -1311 (pTNFluc) coupling to luciferase has been described elsewhere [35] and was a gift from Dr. K. Rhoades.

For transfection assays, uninfected single trophoblast cells and JAR and JEG-3 cell lines were cultured in 12-well plates at 2 × 10⁵ cells/well, in a free FCS medium (Opti-Men; Life-Technologies), and were transfected with 0.5 µg of plasmid DNA by use of 1 µg of lipofectin (Invitrogen)/well for 12 h. After 12 h, 10% FCS culture medium was added, and, after another 12 h, the medium was replaced by 2% FCS medium. The cells were stimulated for 4 h, trypsinized, washed with PBS, and resuspended in lysis buffer (Promega). Luciferase activity was measured in a luminometer and was expressed as relative luciferase units (RLU), which were calculated by use of the following equation: [light emission from the experimental sample minus light emission from untransfected cells]/10⁶ cells. Data are also represented as fold induction, expressed in the following equation: [observed experimental RLU/basal RLU in the absence of any stimulus].

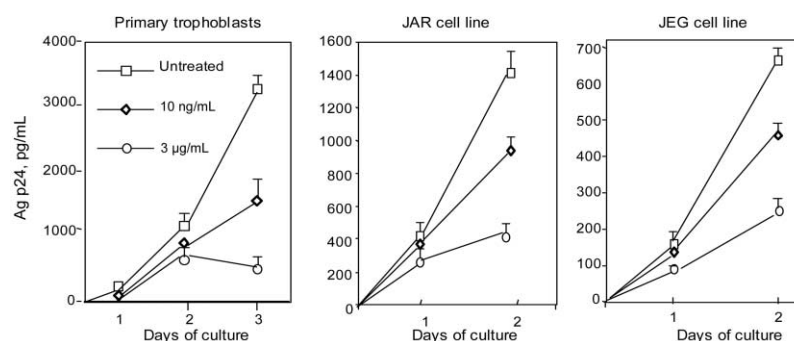


Figure 2. Inhibition of HIV-1 replication in HIV-1-transfected trophoblast cells by progesterone. Human trophoblast JAR or JEG-3 cell lines were transfected with pNL4.3, and Ag p24 was released to the supernatants monitored at the indicated times. Results are the mean \pm SD from replicate assays of the 4 independent experiments performed.

RESULTS

Inhibition of HIV-1 replication in placental trophoblasts by progesterone.

We and other investigators have previously reported that placental cells can be infected by HIV-1 [5, 7, 9, 11]. However, the role of progesterone in HIV-1 infectivity has not been addressed. To address this, we treated placental cells with progesterone, with the lowest concentration used (10 ng/mL) similar to levels found in the serum of nonpregnant women, and with the highest concentration used (3 μ g/mL) the same as that noted at the placental interface [22]. Trophoblasts were infected with HIV-1_{NL4.3} and were cultured over 3 days in the absence or presence of progesterone, and production of RNA-HIV-1 was quantified in the culture supernatants. We detected proviral DNA by use of nested PCR (not shown) [8]. HIV-1 replication was detected by quantification of the HIV-1 RNA load (figure 1A). The released viral particles were infectious. Progesterone significantly reduced replication in a dose-dependent manner, with the greatest effect occurring with the use of a concentration of 3 μ g/mL.

As a control, we tested progesterone on T cell blasts that were infected with HIV-1_{NL4.3} and were cultured over 3 days in the absence and presence of progesterone, and we then quantified the viral load. As expected, T cell blasts show much higher viral replication than do placental cells. Only the high dose of progesterone produced a statistically significant reduction (figure 1B). Similar results were obtained with primary viral isolates HIV-1₂₀₆₉₁ (macrophage-tropic, R5) and HIV-1₁₄₉₈₁ (lymphotropic, X4) (not shown). This effect of progesterone was not the result of a toxic effect in placental cells, because it does not affect cell viability at that concentration. Moreover, progesterone does not affect cell proliferation or alter the cell cycle (data not shown).

Next, we tested whether the effect of progesterone takes place with polarized trophoblast to more closely parallel in vivo conditions. For this, we used polarized cells in Millicell filter inserts

coated with Matrigel and added to the apical surface of the cells. As shown in figure 1C, HIV somewhat more efficiently inhibited polarized JEG-3 cells, but progesterone also inhibited HIV infection.

Although direct infection with HIV-1 has been reported, the virus may enter trophoblasts in a receptor-independent mechanism via cell-to-cell contact or through endocytosis [6–8]. To mimic cells infected in this way, we transfected single primary trophoblast cultures and single JAR and JEG-3 cell lines with the pNL4.3 provirus [36]. We found a time- and dose-dependent inhibition of the viral replication in all transfected cells (figure 2), indicating that progesterone could affect viral replication regardless of the mechanism of viral entry. Moreover, the inhibitory effect of progesterone was more pronounced in primary trophoblasts than in cell lines.

Chemokine receptor expression in placental trophoblasts not inhibited by progesterone.

Some reports have suggested that HIV-1 infects placental cells via a coreceptor-dependent and CD4-independent mechanism [37], and up-regulation of CCR5 in the placenta has been associated with vertical transmission [38]. Moreover, progesterone inhibits CXCR4 and CCR5 expression in PBMCs, and this correlates with reduced susceptibility to HIV-1 infection [39]. As previously shown elsewhere [8], the trophoblastic JAR cell line expresses low levels of CXCR4 and somewhat higher levels of CCR5, but progesterone did not significantly inhibit their expression (figure 3). Similar results were found in primary trophoblast cells (not shown). In contrast, progesterone decreased CXCR4 expression in T lymphocytes (data not shown), in agreement with the findings of previous reports [39].

No effect of progesterone on LTR transcription. The aforementioned results suggested that progesterone affects postentry steps. To test the effect of progesterone on viral transcription, we transfected single JAR cells with HIV-1-LTR-luc reporter plasmid. As shown in figure 4A, progesterone did not inhibit

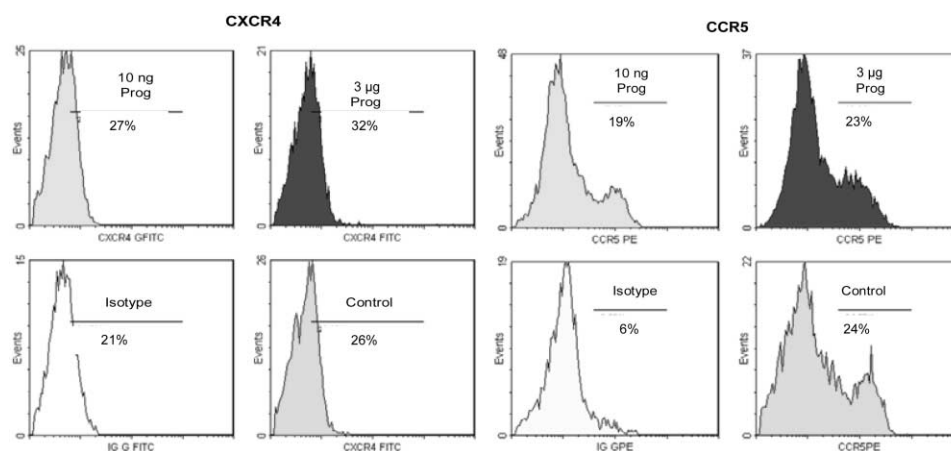


Figure 3. Effect of progesterone (Prog) on CCR5 and CXCR4 levels on trophoblast cells. JAR cells were cultured with the indicated doses of Prog for 48 h, and cell surface expression of CXCR4 and CCR5 was evaluated by flow cytometry. Values shown are the percentage of positive cells. The results shown are representative of the 4 experiments performed. Similar results were observed at 24 and 72 h of incubation (not shown). FITC, fluorescein isothiocyanate; IG G, immunoglobulin G; IG G PE, immunoglobulin type G in phycoerythrin; PE, phycoerythrin.

basal LTR transcription in any of the transfected single trophoblastic cell lines. Even though TNF was able to up-regulate LTR-driven transcription, progesterone did not inhibit this enhancing effect (figure 4B). LTR transcription is dependent on NF- κ B [20], and progesterone had no effect on basal transcription of a κ B-dependent luciferase reporter gene. On the other hand, TNF strongly induced this reporter, but this effect was not altered by progesterone (figure 4C).

Effect of TNF on HIV-1 replication in placental cells.

Recent results indicate that HIV-1 replication was strongly enhanced by TNF in placental cells and that progesterone can inhibit TNF synthesis by macrophages [28, 29]. Therefore, an attractive hypothesis was that HIV-1 induced TNF, which, in turn, increased HIV-1 replication, and that progesterone is able to break this cycle by inhibiting TNF synthesis. We first addressed whether “in vitro” HIV-1 infection of placental trophoblasts and placental cell lines could induce TNF expression. Trophoblast cells obtained from healthy pregnant women and JAR cells were cocultured or infected with HIV-1_{NL4.3}. Three days later, proviral DNA was detected to corroborate HIV-1 infection (not shown). TNF mRNA expression was observed by RT-PCR in primary trophoblasts and in JAR and JEG-3 cell lines infected by HIV-1_{NL4.3} in vitro but not in the uninfected cells (figure 5A). Similar results were observed for all trophoblast cell lines and primary trophoblasts, by use of either HIV-1_{NL4.3} or primary isolates HIV-1₄₉₈₁ and HIV-1₂₀₆₉₁ (data not shown). TNF expression could be detected in the JAR cell line as early as 4 and 8 h after infection with HIV-1₄₉₈₁, HIV-1_{NL4.3}, or HIV-1_{Bal} (figure 5B).

Some viral replication could be detected in the absence of TNF stimulation in HIV-1_{NL4.3}-infected trophoblast cells, but TNF had a strong dose-dependent stimulatory effect on HIV-

1 replication (figure 6A). We transfected single trophoblastic cell lines JEG-3 and JAR with the provirus pNL4.3, and p24 antigen levels were quantified. Higher viral replication was observed 1 and 2 days after TNF stimulation (figure 6B). The same experiment was performed by treating the placental cells with other cytokines (interleukin [IL]-4, IL-10, IL-6, interferon- γ , IL-2, and granulocyte-macrophage colony-stimulating factor) that are known to be present in the placental interface; however, no significant enhancing effect on HIV-1 replication was observed (data not shown). TNF induction by HIV-1 infection in trophoblasts seems to be important for HIV-1 replication, because neutralizing anti-TNF antibodies strongly decreased HIV-1 replication in HIV-1_{NL4.3}-infected JAR cells (figure 6C), as well as in other trophoblastic cell lines transfected or infected with various HIV-1 isolates (not shown).

TNF secretion by trophoblast cells decreased by progesterone.

To address the effect of progesterone on TNF, we performed quantitative PCR. As shown in figure 7A, HIV-1 infection induces TNF mRNA in a time-dependent manner. A significant amount of TNF mRNA was detected 4 h after infection, and the amount increased up to 72 h. Progesterone at a concentration of only 3 μ g/mL reduced this increase on the order of 2–3 logs, depending on the experiment and the time measured after infection (figure 7A). Interestingly, exogenous TNF strongly increased its own production (5–10-fold), and neutralizing anti-TNF antibodies reduced it by 70%.

The aforementioned results suggested that HIV-1 triggers TNF transcription. Therefore, we transfected single JAR cells with a full TNF-promoter construct [35] and cultured them in the presence of inactivated HIV-1. This was enough to induce a strong TNF promoter-dependent transcription (figure 7B). This effect can be mimicked by simply culturing the cells with

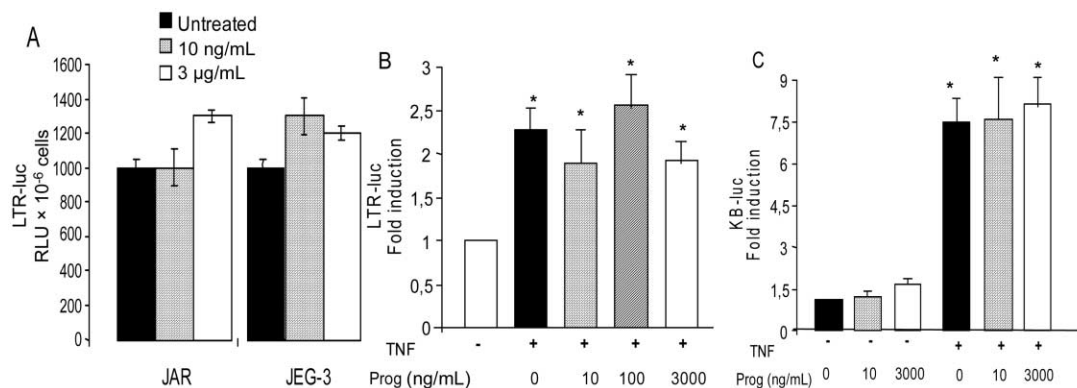


Figure 4. No inhibition of long terminal repeat (LTR)-dependent transcription by progesterone (Prog). *A*, LTR-driven transcription by JEG-3 and JAR cells. *B*, Tumor necrosis factor (TNF)-induced LTR-driven transcription in JAR cells. *C*, Basal and TNF-induced κ B-driven transcription by JAR cells. The cell line was transfected with the κ B-dependent luciferase reporter plasmid and was stimulated with different doses of Prog with and without TNF. Results are the mean \pm SD. * $P < .001$, from replicate assays of the 5 independent experiments performed respectively. RLU, relative luciferase units.

HIV-1 gp120. Progesterone inhibited virus or gp120-mediated TNF transcription, especially at a concentration of 3 μ g/mL. The effect of TNF on the induction of its own promoter was mostly unaffected by progesterone, indicating that the effect of progesterone on TNF induction was specific for virus-induced TNF transcription.

DISCUSSION

Transplacental transmission of HIV-1 is the result of a complex interplay of maternal, fetal, and viral factors. Progesterone is a hormone essential for the maintenance of pregnancy [21, 40]. However, its influence on HIV-1 replication, especially in the placental cells, had not been studied. In the present study, we showed that progesterone limits HIV-1 replication in primary trophoblasts and cell lines. Progesterone does not inhibit HIV replication by acting on NF- κ B or LTR-dependent transcription but, likely, through an indirect mechanism involving the inhibition of TNF synthesis in infected placental cells. This is suggested by the fact that HIV-1 infection induces TNF in placental cells and that progesterone inhibits this induction. Neutralization of endogenous TNF by anti-TNF antibodies has an effect similar to that of progesterone on viral replication. Of interest, inactivated virus or gp120 is sufficient to induce TNF mRNA transcription in placental cells, effects that are blocked by progesterone.

Some reports have suggested that HIV-1 infects placental cells via a coreceptor-dependent and CD4-independent mechanism [37] and that an up-regulation of CCR5 in the placenta has been associated with vertical transmission [38]. Progesterone inhibited CXCR4 and CCR5 expression on PBMCs, thereby reducing HIV-1 infectivity [39]. However, we found no significant alteration in coreceptor expression in placental cells by

progesterone treatment administered at any dose tested, thus discarding this mechanism as an explanation of the anti-HIV-1 activity of progesterone in placental cells.

Because HIV-1 may enter by endocytosis or by cell-to-cell contact [6–8], we tested the effect of progesterone on single placental cells transfected with an HIV-1 plasmid that mimics the effect of entering the cells through a receptor-independent entry [36]. Even in single cells transfected with the entire provirus, progesterone strongly inhibits HIV-1 replication, which is more pronounced in primary trophoblasts than in established cell lines.

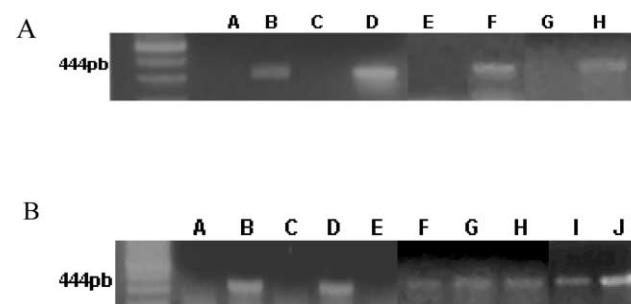


Figure 5. *A*, Primary trophoblasts, cell line JAR, and cell line JEG-3 three days after infection with HIV-1_{NL4.3}. *Lane A*, no cells; *lane B*, HIV-1-infected primary trophoblast; *lane C*, mock-infected primary trophoblast; *lane D*, positive control; *lane E*, mock-infected cell line JAR; *lane F*, HIV-1-infected cell line JAR; *lane G*, mock-infected cell line JEG-3; and *lane H*, HIV-1-infected cell line JEG-3. *B*, JAR cells after infection with HIV-1_{NL4.3}, HIV-1_{NL4.3}, and HIV-1_{Bal}. *Lane A*, no cells; *lane B*, 4 h after infection with HIV-1_{NL4.3}; *lane C*, 4 h after mock infection; *lane D*, 8 h after infection with HIV-1_{NL4.3}; *lane E*, 8 h after mock infection; *lane F*, 4 h after infection with HIV-1_{NL4.3}; *lane G*, 4 h after infection with HIV-1_{Bal}; *lane H*, 8 h after infection with HIV-1_{NL4.3}; *lane I*, 8 h after infection with HIV-1_{Bal}; and *lane J*, positive control.

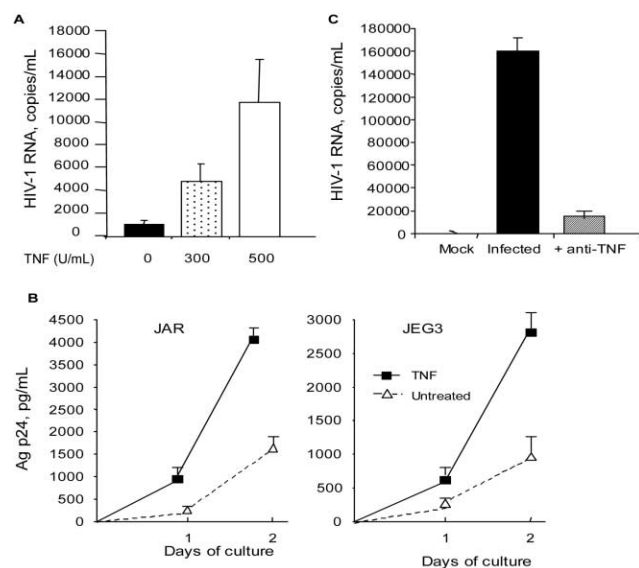


Figure 6. Induction of HIV-1 replication in placental cells by autocrine tumor necrosis factor (TNF). TNF-treated placental cells enhanced HIV-1 replication. *A*, Infected JAR cells infected with HIV-1_{NL4.3} (MOI, 1) and treated with TNF, 300 or 500 U/mL. Twenty-four hours later, viral production was determined by viral load measurement. Results are the mean \pm SD from replicate assays of the 4 independent experiments performed. *B*, JAR and JEG-3 cell lines transfected with pNL4.3 and cultured with or without TNF, 300 U/mL. Ag p24 was measured 1 or 2 days after infection. *C*, Anti-TNF blocks HIV-1 replication. Cells were infected (as described in panel *A*) in the presence of neutralizing anti-TNF antibodies. Results are the mean \pm SD from replicate assays of the 3 independent experiments performed.

Previous studies have shown that the susceptibility of trophoblast cells to HIV-1 infection was enhanced by TNF. We found that replication of HIV-1 in placental cells, either by direct infection of cells or transfection of single cells with provirus, is strongly enhanced by the addition of TNF. To our knowledge, we show, for the first time, that neutralizing anti-TNF antibodies are able to significantly and severely reduce HIV-1 replication in infected cells, indicating an autocrine role of this cytokine in placental HIV-1 replication.

Inflammatory cytokines, such as TNF, have been more often produced both by the PBMCs and, in vivo, by the placental trophoblast cells of HIV-1-infected women [13, 15]. TNF levels may be a determining factor in the probability of vertical transmission. In this regard, a recent study indicates that some of the effects of zidovudine in preventing vertical transmission may be caused by decreasing TNF expression in the placenta [41]. TNF induced by HIV-1 infection, in turn, increases viral replication in placental cells that are infected either directly, through transfection (as in the present study), or through endocytosis [7].

Our results suggest that virus released from the first rounds of infection could continuously stimulate TNF, which, in turn,

induces virus release, thus perpetuating the infection. This TNF could act at various levels. First, it could activate viral replication in the same cells in which it is produced (as in the present study), and this increase in trophoblast cells increases the likelihood of new infections from the infected maternal PBMCs [7, 8]. These newly released virions could then infect underlying susceptible fetal cells, including Hofbauer cells [42] or PBMCs of the fetus. TNF would also contribute to placental damage and would favor the entry of either HIV-1 or the maternal infected cells through placental tissues from maternal to fetal blood [43]. Progesterone may act by breaking this cycle. Progesterone blocks TNF production in macrophages [28, 29] and placental cells (as in the present study) and diminishes HIV-1 replication in PBMCs [39] and placental cells. Thus, high progesterone levels during gestation could prevent vertical HIV-1 transmission by inhibiting TNF and viral replication in trophoblast cells and in PBMCs in contact with the placental interface. Preliminary results from our laboratory indicate that the progesterone level is higher in HIV-1-infected mothers who

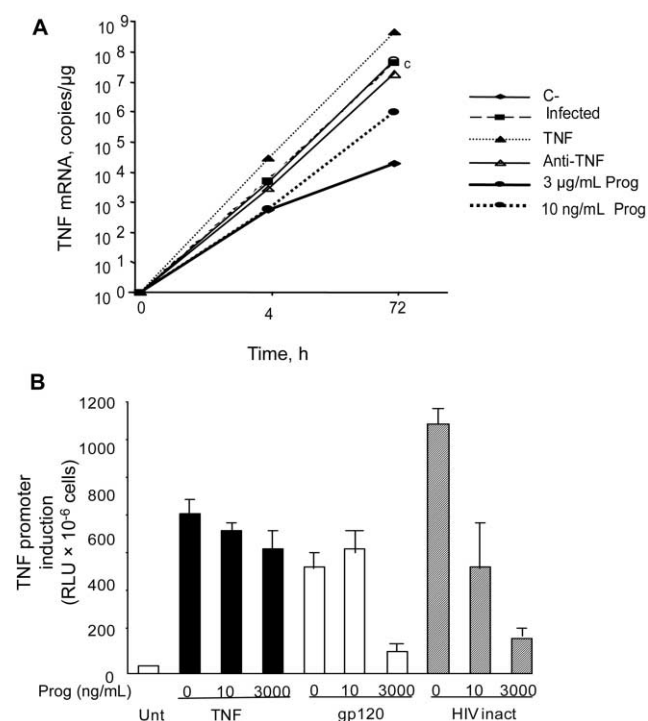


Figure 7. Tumor necrosis factor (TNF) transcription induced by HIV-1 or gp120. *A*, JAR cells infected with HIV-1_{NL4.3} (MOI, 1) and cultured in the presence of the indicated doses of progesterone (Prog) or neutralizing anti-TNF antibodies or TNF (300 U/mL). After the times indicated, TNF mRNA was evaluated by quantitative reverse-transcription polymerase chain reaction. *B*, JAR cells transfected with TNF-luc and treated with inactivated virus (MOI, 10) or soluble NL4.3 gp120 (7 μ g/mL) in the presence of the indicated doses of Prog. Luciferase activity was recorded 16 h later. Results are the mean \pm SD from replicate assays of the 3 independent experiments performed. C-, unstimulated or negative control; Inact, inactivated; Unt, unstimulated.

do not transmit HIV-1 than in HIV-1-infected mothers who do transmit HIV-1. On the contrary, progesterone treatment seems to enhance vaginal transmission of simian immunodeficiency virus in rhesus macaques, although this is likely the result of thinning of the vaginal barrier [44].

Progesterone enhances IL-10 [45], which is known to suppress TNF induction in some cell types [46]. In the presence of progesterone, activated human lymphocytes, especially γ/δ T cells, synthesize a 34-kDa molecule (progesterone-induced blocking factor [PGIF]) that suppresses TNF production by NK cells [47]. We do not know whether the release of similar factor(s) has taken place in our system. However, we think that this is unlikely, because the effects of progesterone on TNF are observed from very early in the infection, which is not entirely compatible with the need for newly synthesized proteins, either IL-10 or PGIF. The mechanism by which progesterone affects HIV-1 or gp120-induced TNF transcription is not known and is currently under investigation. TNF transcription is dependent of NF- κ B, although many different transcription factors may play a role, depending on the cell and the stimulus [48, 49]. A progesterone-mediated increase in I κ B (a protein that blocks NF- κ B) in macrophages [28, 29] or myometrial cells [50] and the eventual reduction in TNF mRNA have been reported. However, this hormone was unable to inhibit basal or TNF-induced activation of NF- κ B in our trophoblast cells. This will fit with the fact that progesterone did not affect TNF-dependent LTR transcription, which is dependent on NF- κ B activation. Progesterone does not affect TNF induction of its own promoter. Thus, it is likely that progesterone may affect transcription factors specifically induced by gp120.

Acknowledgment

We thank Dolores García for her excellent technical assistance.

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