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Interaction of Pregnancy Steroid Hormones and Zidovudine in Inhibition of HIV Type 1 Replication in Monocytoid and Placental Hofbauer Cells: Implications for the Prevention of Maternal–Fetal Transmission of HIV

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ABSTRACT

Zidovudine (AZT) has been shown to reduce maternal–fetal transmission of HIV-1 by more than two-thirds in a variety of clinical settings. However, the mechanism of action of AZT in this setting is unclear. Suppression of vertical transmission has occurred in the absence of an impact on maternal plasma viremia and no lower threshold of viral load for such transmission has been identified. We hypothesized that augmentation of the anti-HIV effect of AZT may occur locally, at the maternal–fetal interface. We report that the pregnancy hormone progesterone at broad concentrations has little effect on acute HIV-1 infection of a monocytic cell line or primary peripheral blood cells. However, the combination of physiologic concentrations of progesterone (10^{-7} to 10^{-6} M) and low-dose AZT (10^{-8} to 10^{-9} M) produced markedly synergistic inhibition of HIV-1 replication within acutely infected monocytic cell lines (U937), and additive inhibition of HIV-1 growth within chronically infected monocytic cells (U1) and primary placental macrophages (Hofbauer cells). Anti-HIV effects were not seen with another pregnancy steroid hormone, estrogen. In terms of possible mechanisms of action for progesterone, we demonstrated that it incompletely suppressed *tat* activation of long terminal repeat (LTR)-driven gene expression in monocytic cells. However, the progesterone-mediated suppession of *tat* activation was not affected by mutation of the three consensus progesterone/androgen/glucocorticoid response elements within the HIV-1 LTR, previously shown by our group to be involved in glucocorticoid-mediated suppression of LTR-driven transcription. It is likely that progesterone suppresses LTR-driven gene expression through a nontranscriptional mechanism, and augments the efficacy of AZT through enhancement of its phosphorylation.

INTRODUCTION

ZIDOVUDINE (AZT) THERAPY has been shown to reduce the rate of maternal–infant transmission of HIV from 25.5 to 8.3% when given to HIV-infected mothers who were previously naive to antiretroviral therapy and had mild symptomatic disease (AIDS clinical trial group [ACTG] 076).[1] The explanation for this dramatic effect remains unclear. Some reports have found high maternal viremia levels to be predictive of vertical transmission of HIV, suggesting that AZT therapy exerts its protective effect through reducing maternal viral load prior to delivery.[2,3] However, other studies have found that mothers with low HIV copy numbers still transmit HIV to their children[4] and a viral load threshold above which transmission is likely does not exist.[5] In addition, reduction of transmission occurs among women receiving AZT who harbor AZT-resistant strains.[6] Finally, birth canal cleansing prior to delivery, despite its ability to lower levels of HIV and other pathogens in vaginal fluids, has no effect on rates of transmission.[7] This suggests that maternal–fetal transmission of HIV is not simply a function of plasma viral load.

Alterations in the ability of HIV to infect cells at the mater-

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nal–fetal interface may be more pertinent to the AZT effect. *In situ* hybridization and immunocytochemistry studies of both 8-week aborted and term placentas from HIV-infected mothers have shown that of infected infants, at least 38% are infected *in utero*.[8] HIV antigen localizes to the resident macrophage, known as the Hofbauer cell, within the placental chorionic villi.[9,10] Such cells may be more important than infected, circulating T lymphocytes in mediating maternal–fetal transmission. In addition, interactions between AZT and placental hormones may play a role. For example, it has been suggested that two steroid hormones elevated during pregnancy, estrogen and progesterone, can inhibit HIV growth in monocytic cells but not lymphocytic cells.[11] Our laboratory has identified and characterized several hormone receptor-binding sites within the HIV-1 long terminal repeat (LTR) promoter region.[12] These consensus sequences, homologous to progesterone/androgen/glucocorticoid response elements (PRE/ARE/GRE), appear responsible for glucocorticoid-mediated inhibition of HIV-1 LTR-driven transcription.[12,13]

We hypothesize that AZT interacts with progesterone to inhibit synergistically HIV growth within the placental milieu and thus prevents transplacental transmission of HIV, even in the absence of an effect on maternal viremia. This could occur either via a direct interaction with progesterone response elements within the LTR, or by way of an intracellular activation of AZT through enhancement of its phosphorylation, as has been shown for AZT and other hormones of immune cell and placental origin.[14,15]

MATERIALS AND METHODS

Cells

An immortalized, $CD4^+$ monocytic cell line (U937) and a human choriocarcinoma cell line (JEG3) were obtained from the American Type Culture Collection (Rockville, MD). U1 cells are a clone of U937 cells infected with the lymphadenopathy-associated virus (LAV) strain of HIV-1 and were obtained from T.M. Folks, then at the Centers for Disease Control. Each U1 cell contains two proviral copies of HIV-1.[12] U1 and U937 cells were cultured at a density of 5×10^5/ml in culture medium (RPMI 1640 supplemented with 10% fetal bovine serum [FBS], penicillin [100 U/ml], streptomycin [100 mg/ml], 2 mM glutamine). JEG3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented as described above.

Reagents

Progesterone (Sigma Chemical Co., St. Louis, MO) was used as a stock solution of 10 mg/ml in 95% ethanol and diluted in culture medium to give final concentrations of 10^{-5} to 10^{-10} M. Estrogen (Sigma) was kept in a stock solution of 1 mg/ml in 70% ethanol and diluted to final concentrations of 10^{-6} to 10^{-7} M. The dilutions were stored at −20°C in working aliquots in order to minimize freeze–thaw cycles.[16] These concentrations were chosen to encompass the physiologic ranges of progesterone and estrogen, which each achieve micromolar concentrations after pregnancy is established.[11] AZT (Sigma) was kept in a 5 mM stock solution and diluted in culture medium to final concentrations of 10^{-6} to 10^{-11} M.

Plasmid constructs

The reporter plasmid pU3R3 contains sequences for simian virus 40 (SV40) regulatory genes, bacterial chloramphenicol acetyltransferase (CAT), and the HIV-1 LTR. The *tat* plasmid pCV-1 contains a 1.8-kb fragment of HIV-1 cDNA encompassing the *tat* gene. They have been described previously.[12,13]

Mutant plasmid constructs

These mutants have been described previously.[12] The linker substitution mutant −273/−256, replacing 18 bp of wild-type sequence encompassing the first identified PRE/ARE/GRE consensus site, GRE I (nucleotides −264 to −259), with an *NdeI–XhoI–SalI* polylinker, was obtained from J. Alwine (University of Pennsylvania. Constructs containing clustered point mutations encompassing the second and third PRE/ARE/GRE-like sites in the HIV-1 LTR, −5/+1 (GRE II) and +15/+20 (GRE III), were provided by K. Jones (Salk Institute, La Jolla, CA).

Plasmid transfections and CAT assays

These experiments were performed as described previously,[12] with minor modifications. For U937 cells, 2.5×10^6 cells per condition were washed with serum-free (sf) RPMI 1640 and resuspended in 1 ml of sfRPMI containing DEAE-dextran (375 μg/ml; Sigma) in 50 mM Tris-HCl, pH 7.3, and 1 μg of plasmid DNA per condition. Cells were incubated at 37°C for 1 hr, washed with sfRPMI, resuspended in fresh culture medium, divided into aliquots of equal cell number, and reincubated at 37°C. Progesterone was added to selected aliquots 1 hr after washing. After incubation for 40–45 hr, cells were harvested, washed, and resuspended in 100 μl of 0.25 M Tris-HCl, pH 7.8, and cellular extracts were prepared by three cycles of freeze–thawing (in dry ice–ethanol and 37°C water bath, respectively). Extracts were centrifuged and protein content in supernatants estimated using the micro-BCA reagent kit (Pierce, Rockford, IL).

The JEG3 cells were transfected by the $CaPO_4$ precipitate method.[17] Cells were split 1:5 from a confluent 10-cm tissue culture plate the day before transfection and were fed with 9.0 ml of complete medium 3 hr before precipitation. A DNA–$CaCl_2$ solution was prepared by adding 5 μg of each plasmid to 0.5 ml of 0.25 M $CaCl_2$. The DNA–$CaCl_2$ solution was then added dropwise via pasteur pipette to 0.5 ml of HEPES-buffered saline (HeBS) in a 15-ml conical tube while bubbling the HeBS with a mechanical pipettor. The mixture was vortexed for 5 sec and then allowed to sit at room temperature for 20 min. The precipitate was then dropped over a selected 10-cm plate of cells. After 4 hr of incubation at 37°C, cells were washed twice with sfRPMI and 10 ml of complete medium was replaced. Progesterone was added to the culture medium 1 hr later and incubation proceeded at 37°C for 40–45 hr, after which the cells were harvested and cellular extracts were prepared as described above.

CAT activity was determined by incubating aliquots of cell extracts with equal amounts of protein (10–25 μg) with [^{14}C]chloramphenicol (57.9 mCi/mmol; New England Nuclear, Boston, MA) and 2.5 mM acetyl coenzyme A (Pharmacia, Piscataway, NJ) at 37°C for 2 hr. Incubation was followed by extraction with ethyl acetate and ascending thin-layer chro-

matography (TLC). Chromatograms were autoradiographed and areas of radioactivity cut out and counted in scintillation fluid. All groups were compared statistically by paired Student's *t* tests.

Infectivity assays

The effects of progesterone and estrogen on HIV-1 synthesis in U1 cells were determined using assays standard in our laboratory.[13,18] U1 cells (10^4 in 0.2 ml of culture medium per flat-bottom microwell) were incubated at 37°C, with varying concentrations of progesterone, estrogen, AZT, and combinations of AZT and hormone in selected aliquots for 48 hr. After incubation, the cellular supernatant was assayed for HIV-1 p24 using extremely sensitive p24 enzyme-linked immunosorbent assay (ELISA) antigen capture kits (Dupont; analytical sensitivity $\geq$ 5 pg/ml), according to manufacturer instructions.

To infect the U937 cells, 100 μl of culture medium was removed and 100 μl of stock HIV-1(IIIB) per 1×10^6 cells (MOI $\cong$ 0.1) added for 2 hr and then washed twice with PBS. The cells were then resuspended in complete medium and divided into selected flat-bottom microwells (10^5/0.2 ml of medium/well) containing varying concentrations of progesterone, AZT, and combinations of the two. The cells were then cultured, with media (containing hormone and/or drug) being replaced every 3 or 4 days. The cellular supernatant was assayed for viral p24 every 7 days. Additional microwells of cells were not infected with HIV but exposed to the same concentrations of hormone and/or drug, followed by trypan blue staining after 14 days of culture to determine cell viability.

Isolation, culture, and identification of primary placental macrophages

Placental macrophages were isolated by a modification of the method reported by Kesson *et al.*[19] Human placentas were obtained from anonymous, normal term deliveries within 2 hr of birth. About 30 g was removed from the chorionic villous tissue and finely minced while in culture medium, over ice, and with a minimum of manipulation so as to minimize tissue protease release and activation. The tissue was washed three times in PBS to remove blood. The washed minced tissue was then transferred to a sterile bottle, and the cells were released from the tissue by digestion in 100 ml of RPMI containing Dispase II (3 units/ml; Boehringer Mannheim, Indianapolis, IN), DNase (30 units/ml; Sigma), penicillin G (100 units/ml), and streptomycin (100 μg/ml; GIBCO-BRL, Gaithersburg, MD). The mixture was placed in a 37°C shaker for 2 hr, and the released cells were separated from the undigested tissue by careful pipetting and decanting. Erythrocytes and dead cells were removed by centrifugation over Ficoll (Pharmacia). The interface cells were washed twice with PBS and enriched for macrophages/Hofbauer cells over a discontinous Percoll gradient (Pharmacia), as calibrated by density marker beads (Pharmacia) run in parallel. The cells at the 1.045- to 1.065-g/ml interface were collected and washed twice with PBS. They had the morphology of dendritic-like cells after overnight culture. They were resuspended at 5×10^5/ml in culture medium (RPMI 1640 supplemented with 10% fetal bovine serum [FBS], penicillin [100 U/ml], and streptomycin [100 mg/ml], 2 mM glutamine) for culture in 24-well plates (Falcon). Some cultures were also supplemented with macrophage colony-stimulating factor (M-CSF) (1 ng/ml; Sigma). The cells were cultured at a concentration of 5×10^5/ml/well at 37°C in an atmosphere of 5% CO_2 in air and washed on days 2, 5, and 8 with PBS to remove nonadherent cells.

A portion of the cells were analyzed on day 2 prior to infection with HIV by fluorescence-activated cell sorting. The adherent cells were detached from culture wells by removal of culture medium followed by incubation with 0.25% trypsin at 37°C and rinsing with PBS. Cells resuspended in culture medium were then analyzed by fluorescence-activated cell sorting and flow cytometry by standard methods with labeling of CD45, CD11, and CD4.

Infection of primary placental macrophages

The Hofbauer cells were infected on day 2 of culture by adding 100 μl of undiluted stock HIV-1 (IIIB strain) per 10^6 cells to each well and incubating at 37°C for 3 hr. The BAL strain of HIV-1 was also used (data not shown). The culture medium was removed, the cells washed twice with PBS, and fresh medium containing M-CSF, AZT, and/or progesterone was replaced. The cells were then fed every 3 days with fresh medium being replaced.

DNA extraction and polymerase chain reaction

The cells were detached with 0.25% trypsin in 1 mM EDTA (GIBCO-BRL) followed by rinsing with PBS. Total cellular DNA was isolated by the Chelex-100 (Bio-Rad, Richmond, CA) method.[20] The cells were resuspended in PBS, pelleted, and vortexed. Seventy-five microliters of a 50% suspension of Chelex-100 in H_2O was added for every 2×10^5 cells and the mixture was boiled for 5 min, then chilled on ice or stored at 4°C. The suspension was microcentrifuged for 30 sec and the supernatant used for polymerase chain reaction (PCR). Aliquots of the DNA were then amplified by PCR, using *gag* (Perkin-Elmer [Norwalk, CT] SK145 and SK431) or β-globin (Perkin-Elmer, PC04 and GH20) primers. Fifty microliters of reaction volume, including 1$\times$ reaction buffer (Perkin-Elmer), optimized concentrations of $MgCl_2$, dNTP, primers, and 2 units of *Taq* polymerase (Perkin-Elmer), were used. The DNAs were denatured for 2 min at 97°C before 35 runs in a thermal cycler, with denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min in each cycle. A final extension at 72°C for 5 min was included. The PCR products were separated by electrophoresis in a 1.5% agarose gel, visualized by ethidium bromide staining under ultraviolet illumination, and photographed. The expected sizes of the amplicons were as follows: *gag*, 142 nucleotides; β-globin, 268 nucleotides. The gels were scanned by a Logitech Scanman Easytouch scanner (Logitech Inc., Fremont, CA) and analyzed using Sigmagel software (Jandel Scientific, San Rafael, CA).

RESULTS

The effect of progesterone and AZT on HIV-1 replication within U937 cells

The ability of progesterone to affect HIV-1 growth within U937 cells was investigated, with progesterone alone, AZT

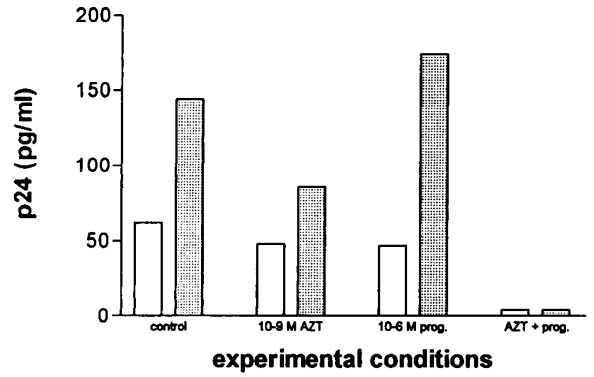

FIG. 1. Effect of progesterone, AZT, and progesterone + AZT combinations on HIV-1 p24 levels within acutely infected U937 cells on day 14 after infection. The U937 cells were infected by addition of 100 μl of the IIIB strain of HIV-1 per 1×10^6 cells (MOI ~0.1) to the cells in suspension for 2 hr, followed by washing and resuspension in complete medium. The cells were plated at 1×10^5/microwell in 0.2 ml of medium containing appropriate concentrations of progesterone and/or AZT. The cells were cultured for up to 3 weeks, with medium replaced every 3 or 4 days and supernatants harvested and assayed for HIV-1 p24 antigen via antigen capture ELISA (sensitivity $\geq$ 5 pg/ml) every 7 days. Gray bars, experiment 2; white bars, experiment 1.

alone, and the combination of progesterone and AZT. U937 cells were also acutely infected with the IIIB strain of HIV-1. It was found that physiologic concentrations of progesterone (10^{-6} M) were incapable of inhibition of HIV-1 replication (Fig. 1). However, combinations of AZT below the 50% inhibitory combinations (IC_{50}) for HIV-1 IIIB (10^{-9} M) and progesterone were markedly synergistic in their antiviral effect, inhibiting HIV-1 p24 levels to below detectable levels after 14 days (Fig. 1, two representative experiments). Equivalent cell viability was demonstrated by trypan blue staining after 14 days of cell culture under control conditions (range, 49–53%) and exposure to the AZT and progesterone combination (range, 45–50%).

In contrast, phytohemagglutinin-activated peripheral blood cells, when acutely infected with the same strains of HIV and then incubated under similar experimental conditions, were not susceptible to either progesterone-mediated inhibition of HIV or to a synergistic interaction between progesterone and AZT (data not shown).

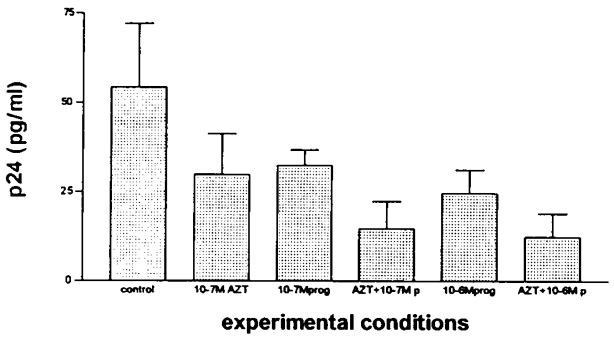

FIG. 2. Effect of progesterone, AZT, and progesterone + AZT combinations on HIV-1 p24 within chronically infected U1 cells. Cells were plated at 1×10^4/microwell in 0.2 ml of culture medium with the addition of the indicated concentrations of AZT and/or progesterone. Supernatants were harvested 48 hr later and HIV-1 p24 Ag concentration was measured via antigen capture ELISA. The data represent the mean of three experiments.

The effect of progesterone and AZT on HIV-1 replication within U1 cells

In addition to the U937 cell experiments, which modeled acute infection and replication within monocytic cells, we also studied chronic infection of monocytic cells, using the U1 cells. These experiments showed that progesterone (10^{-6} to 10^{-7} M) and AZT (10^{-7} to 10^{-8} M) alone were each capable of only minor inhibition of HIV levels. Combinations of these agents in this case were additive in their effects (Fig. 2, Table 1).

Control experiments investigated the effect of estrogen on HIV levels within U1 cells. Estrogen (10^{-6} M) alone increased HIV levels, so that combinations of AZT and estrogen resulted in a diminished antiviral effect compared to AZT alone (Table 2). This is consistent with other reports that activation of a thyroid hormone/estrogen receptor consensus binding region in the HIV LTR upregulates LTR-mediated gene expression.[21]

The effect of progesterone and AZT on HIV-1 replication within Hofbauer cells

Further infectivity assays were performed on primary placental macrophages (Hofbauer cells). The monocytic lineage of the isolated cells was established by flow cytometry experiments. The cells were first labeled with a monoclonal antibody against a pan-leukocytic marker, CD45. The cells were then further characterized by labeling with monoclonal antibodies against a macrophage cell surface marker, CD11 (anti-Leu-M5), as well as lymphocytic cell surface marker, CD4. Of the cells expressing CD45, 67% were found to express CD11. In contrast, only 8% of the cells expressed CD4. These results are in accordance with previous reports that placental macrophages express monocytic surface markers at relatively high levels but CD4 at very low but detectable levels.[19]

Because of the limited replicative capacity of HIV within these cells,[19] levels of HIV were determined by PCR performed on total DNA preparations from Hofbauer cells present in single tissue culture wells (9 days after HIV infection). Relative levels of HIV infection within the Hofbauer cells were therefore estimated as a ratio of *gag* signal over β-globin signal for each group of cells within a single culture well. Comparisons were made with varying numbers of U1 cells, which contain two stably integrated copies of HIV-1. The signal intensity of both the *gag* and β-globin amplicons, as detected and quantified by the gel scanning program, increased in a nearly linear fashion when plotted over a 2-log increase in the number of U1 cells (data not shown).

HIV-1 *gag* signal was detected in all Hofbauer cells infected with HIV under experimental conditions and was not seen in any of the uninfected controls (Figs. 3 and 4). The relative HIV level within the Hofbauer cells incubated under various conditions, based on PCR product analysis, with the pos-

TABLE 1. THE EFFECT OF AZT AND PROGESTERONE ON HIV-1 p24 ANTIGEN LEVELS IN U1 CELLS[a]

Conditions		*p24 levels (pg/ml)*			
AZT (M)	*Progesterone (M)*	*Exp. 1*	*Exp. 2*	*Exp. 3*	*Average ± SEM*
—	—	79.6	63.7	19.5	54.3 ± 18.0
10^{-7}	—	52.0	13.4	23.5	29.6 ± 11.6
—	10^{-7}	36.8	23.5	36.6	32.3 ± 4.4
10^{-7}	10^{-7}	25.5	18.1	0.0	14.5 ± 7.6
—	10^{-6}	13.0	35.4	25.3	24.6 ± 6.5
10^{-7}	10^{-6}	22.7	14.2	0.0	12.3 ± 6.6

[a]Cells were plated at 1×10^4/microwell in 0.2 ml of culture medium with the addition of the indicated concentrations of AZT and/or progesterone. Supernatants were harvested 48 hr later and p24 antigen concentrations measured via antigen capture ELISA kits (sensitivity ≥ 5 pg/ml).

itive control set to = 1, were as follows: 10^{-9} M AZT = 0.346; 10^{-7} M progesterone = 0.609; 10^{-9} M AZT + 10^{-7} M progestrone = 0.309. This result confirms the fact that low-dose AZT and physiologic concentrations of progesterone each possess anti-HIV activity and that their antiviral effects are complementary within primary cells such as the placental macrophage.

The effect of progesterone on HIV-1 LTR-driven gene expression

The ability of progesterone to influence HIV-1 LTR-driven expression of a reporter gene, CAT, was studied in two cell types, U937 and JEG3 cells, of monocytic and trophoblastic lineages, respectively. Progesterone was added in 10^{-6} M concentrations.

In U937 cells, basal levels of LTR-driven CAT expression routinely ranged below 5% acetylation and were unaffected by incubation with progesterone. Cotransfection of the HIV-1 LTR and the transcriptional activator *tat* gene resulted in much higher levels of CAT expression (Table 3). *tat* activation of CAT expression in U937 cells was suppressed by 10^{-7} M progesterone by a mean of 38% (range, 14–79%; Table 3), with little increase in suppression at 10^{-6} M (not shown). In the same system but using T cell lines, glucocorticoid (10^{-6} M) leads to complete suppression of LTR-driven CAT expression while U937 cells are unaffected.[12,13]

Further experiments with mutant plasmids revealed that the progesterone-mediated suppression of *tat* activation was unaffected by mutations within each of the various hormone response elements. As described previously by this laboratory, the hormone response elements present in the HIV-1 LTR include GRE I, a consensus glucocorticoid/progesterone/androgen response element (−264 to −259: AGAACA), and two GRE-like sites, GRE II (−5 to +1: TGTACT) and GRE III (+15 to +20: AGACCA). Experiments with cotransfections with *tat* and wild-type LTRs, conducted in parallel with those of all three GRE mutant LTRs, showed no significant effect on the action of progesterone. This was true even with low basal levels of *tat*-induced LTR activity as, in agreement with other reports, mutations within the GRE III itself reduced the magnitude of *tat* activation.[22] Six corresponding experiments on JEG3 cells also showed that progesterone had no effect on *tat* activation (data not shown).

DISCUSSION

We examined the effects of the placental steroid hormone progesterone on HIV replication within monocytic cells, on HIV growth in combination with the antiretroviral zidovudine (AZT), and on HIV-1 LTR-driven transcription. Our data show that progesterone, at concentrations within the range of those normally achieved during pregnancy, has no effect on acute HIV-1 infection of a monocytic cell line or primary peripheral blood mononuclear cells, and a modest inhibitory effect in Hofbauer cells and a chronically infected monocytic cell line. In

TABLE 2. THE EFFECT OF AZT AND ESTROGEN ON HIV-1 p24 ANTIGEN LEVELS IN U1 CELLS[a]

Conditions		*p24 levels (pg/ml)*			
		Exp. 1		*Exp. 2*	
AZT (M)	*Estrogen (M)*	*p24 (pg/ml)*	*% change*	*p24 (pg/ml)*	*% change*
—	—	246.0	—	432.0	—
10^{-7}	—	199.0	−19.1	271.0	−37.3
—	10^{-6}	539.0	+119	651.0	+50.7
10^{-7}	10^{-6}	224.0	−8.94	467.0	+8.11

[a]Cells were plated at 1×10^4/microwell in 0.2 ml of culture medium with the addition of the indicated concentrations of AZT and/or progesterone. Supernatants were harvested 48 hr later and p24 antigen concentrations measured via antigen capture ELISA kits (sensitivity ≥ 5 pg/ml).

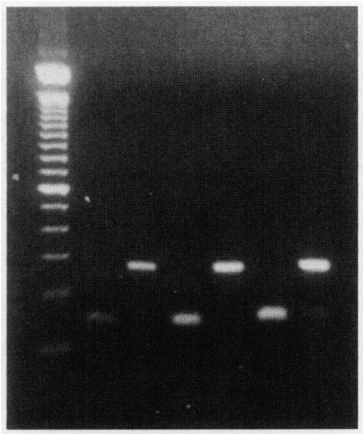

FIG. 3. Cells: lanes 1 and 2, 10^3 U1 cells; lanes 3 and 4, 10^4 U1 cells; lanes 5 and 6, 10^5 U1 cells; M, markers. Lanes 1, 3, and 5: *gag* amplification (142 nucleotides); lanes 2, 4, and 6: β-globin amplification (268 nucleotides).

TABLE 3. THE EFFECT OF PROGESTERONE ON *tat* ACTIVATION OF HIV LTR-DRIVEN CAT EXPRESSION IN U937 CELLS[a]

	Experimental conditions		
		LTR-CAT + tat + 10^{-7} M progesterone	
Experiment No.	*LTR-CAT + tat (% acetylation)*	*% acetylation*	*% inhibition*
1	41.3	30.5	26.2
2	20.3	4.3	78.8
3	37.0	32.0	13.5
4	76.3	52	31.8
Average:	43.7	29.7	37.6

[a]*Note*: The percentage of CAT acetylation in the presence and absence of progesterone (10^{-7}, 10^{-6} M), with percentage of inhibition, is shown for transient transfection assays in U937 cells. All experiments involved cotransfection of LTR-CAT + *tat* plasmids. Cells cultures were maintained for 42–45 hr.

addition, estrogen caused an increase in HIV infection in monocytic cells. The latter is in contrast to a previous report that showed that estrogen could inhibit HIV replication within chronically infected monocytic cells but not in lymphocytic cells.[11] Our results are consistent with the presence of a thyroid/estrogen superfamily consensus response element (TRE/ERE) at −347/−328 within the LTR that stimulates HIV gene expression, as well as the ability of tamoxifen, an estrogen receptor antagonist, to block upregulation of HIV-1 LTR-mediated gene expression.[18] The fact that progesterone is capable of inhibiting HIV growth within the cell type that has been most strongly implicated in transplacental transmission of HIV may help to explain the naturally low vertical transmission rate of HIV.

We sought to extend the scope of these results and investigate whether the antiviral effect of progesterone demonstrated in the above experiments could be useful in combination with other antiviral agents, namely the antiretroviral drug AZT. Experiments on acute HIV infection of monocytic U937 cells, as well as on HIV levels within U1 cells, the chronically infected promonocytic cell line, illustrated that combinations of progesterone and low doses of AZT (10^{-7}–10^{-9} M) exhibited synergistic (U937) or additive (U1) antiviral effects, as compared to their activity when used alone.

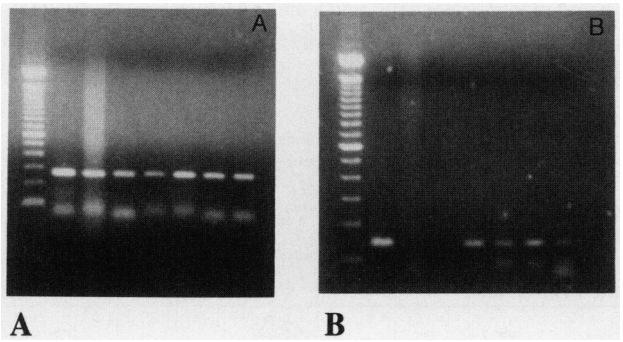

FIG. 4. (A) β-Globin amplification; (B) *gag* amplification. Lane 1, U1 cells; lane 2, Raji cells; lane 3: Hofbauer cells (−HIV); lane 4, Hofbauer cells (+HIV); lane 5, Hofbauer cells (+ HIV, + 10^{-9} M AZT); lane 6, Hofbauer cells (+ HIV, + 10^{-7} M progesterone); lane 7, Hofbauer cells (+ HIV, + 10^{-9} M AZT, + 10^{-7} M progesterone).

To offer a more realistic experimental model for the transplacental transmission of HIV, we examined primary placental macrophage Hofbauer cells. The Hofbauer cell has been identified as an important cell type for transplacental transmission of HIV as a result of *in situ* hybridization and immunocytochemistry studies, which found that HIV antigen localized to these cells in both 8-week and term placentas from HIV-infected pregnancies.[9,10] The Hofbauer cell is therefore likely to be one of the first primary cell types to become infected with HIV in vertical transmission, at least that occurrring *in utero*, with subsequent cell–cell transmission to trophoblastic and other placental cell types, and finally to fetal peripheral blood cells. The infectivity assays performed on the Hobauer cells confirmed that these cells are indeed infectable with HIV in cell culture, and that the antiviral effects of AZT and progesterone are complementary within these cells.

In exploring possible mechanisms for the antiviral effect of progesterone, we then examined the effects of progesterone on HIV-1 LTR-driven transcription. We found that progesterone, in concentrations normally achieved during pregnancy, results in an approximately 40% suppression of *tat* activation of LTR-driven gene expression. Basal levels of LTR-driven transcription in the absence of *tat* were unaffected. Cell lineage restriction of the progesterone effect was confirmed by experiments on a trophoblastic cell line, JEG3 cells.

In terms of possible mechanisms of actions of progesterone, experiments with mutant plasmid constructs showed that this modest inhibition of LTR-directed gene expression was not affected by mutations within the various hormone response elements, described in previous work done in our laboratory.[12]

They include GRE I (−273/−256), a consensus GRE/PRE/ARE, and two GRE-like half-sites, GRE II (−5/+1), and GRE III (+15/+20). GRE III was previously found to be important for dexamethasone-mediated suppression of HIV-1 LTR-driven transcription,[12] and, presumably, the anti-HIV effect of glucocorticoids *in vitro*[13] and in HIV$^+$ patients.[23]

The mechanism by which progesterone and AZT synergistically inhibit HIV replication within monocytic cells remains undetermined. Although we have shown that progesterone partially suppresses *tat* activation of LTR-driven gene expression, this modest effect is not likely to explain potentiation of the antiviral effect of AZT and may be contributing only a partial effect. AZT must be triply phosphorylated by cellular kinases such as thymidine kinase in order to be active. It has been shown that micromolar concentrations of progesterone stimulate the activity of thymidine kinase within breast cancer cell lines.[24] Furthermore, the rate-limiting step for conversion of AZT to AZT triphosphate (AZTTP) within Hofbauer cells is the thymidine kinase-catalyzed conversion of AZTDP to AZTTP.[25] Thus it is possible that progesterone is increasing the concentration of activated AZT within Hofbauer cells, accounting for the potentiation of the anti-HIV effect of AZT by progesterone that we have demonstrated in our experiments. Future experiments will explore this possibility by measuring intracellular concentrations of the various phosphorylated forms of AZT within various cell types exposed to a range of concentrations of progesterone. Additional effects of progesterone have come to light, including the thinning of vaginal mucous layer and mucosa, resulting in a higher rate of SIV transmission vaginally in macaques.[26] As ongoing clinical studies refine our approach to the prevention of maternal–infant transmission of HIV, the elaboration of the interplay between the antiretroviral AZT and the pregnancy hormone progesterone may offer some insight to the demonstrated success of maternal AZT therapy in preventing the vertical transmission of HIV.

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