The Glucocorticoid Antagonist RU-486 Suppresses HIV Infectivity and Replication

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The effects of RU-486, a glucocorticoid antagonist, on HIV infection and replication in depressed and nondepressed women were studied using ex vivo *models of HIV infection. RU-486 treatment of cells decreased HIV reverse transcriptase activity of monocyte-derived macrophages in a model of acute infectivity. RU-486 also decreased HIV viral replication in the chronically-infected T-cell line ACH-2, but not in the promonocyte cell line U1. No differences were associated with depression status. Thus, glucocorticoid antagonism may suppress HIV infectivity and replication* ex vivo. *Studies to determine the role of glucocorticoid antagonists in the host defense against HIV should be performed.*

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Glucocorticoids have been implicated in the pathophysiology of both depression and HIV. Depressed patients have elevated glucocorticoid levels in their plasma, urine, and cerebrospinal fluid.[1,2] Glucocorticoids play a central role in modulation of the immune response, exhibiting immunomodulatory effects upon T-lymphocytes, natural killer (NK) cells, and lymphocyte-derived soluble products, which may be important factors relevant to the progression of HIV/AIDS.[3–5] Phase II trials of RU-486, a functional antagonist of the glucocorticoid receptor, suggest efficacy in ameliorating symptoms of psychotic depression.[6–8] Also, several studies indicate that RU-486 may act to inhibit HIV replication.[9–13]

Depression, a frequent comorbidity of HIV, is associated with accelerated HIV progression.[14–18] The relationship between depression and HIV is complex and multifactorial.[19] Since glucocorticoids play a key role in

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the pathophysiology of both depression and HIV, glucocorticoid antagonism may be effective for the treatment of depression and HIV disease. We hypothesized that glucocorticoid antagonism may play a role in the treatment of HIV. In order to test this hypothesis, we investigated the effects of RU-486 on HIV infectivity in leukocytes obtained from HIV-positive women with and without depression.

In order to examine the effects of glucocorticoid antagonists on HIV infectivity, we developed *ex vivo* experimental models that allowed us to examine the effects of RU-486 on HIV infectivity in macrophages and T-cells. Macrophages and T-cells, the primary immune cells targeted by HIV, are reservoirs of HIV. Using *ex vivo* models for acute (direct) and chronic (indirect) infectivity, we investigated the pharmacologic effects of RU-486 on HIV infectivity.

As a model of acute HIV infection, isolated peripheral blood monocyte-derived macrophages (MDMs) obtained from depressed and nondepressed HIV-positive women were incubated with the Bal strain of HIV-1 and treated with RU-486.

As a model of chronic infectivity, supernatants obtained from peripheral blood mononuclear cells (PBMCs) were collected from depressed and nondepressed HIV-positive women and treated with RU-486, and the supernatants were added to latently infected promonocyte or T-lymphocyte cell lines.

We explored whether glucocorticoid antagonism inhibited HIV entry and replication of MDMs as a model of acute HIV infectivity. We assessed the effects of the supernatants on viral load, using two previously described models of chronic infectivity, a latently infected promonocyte cell line, U1, and a latently infected T lymphocyte cell line, ACH 2. Both cell lines survived acute infection with HIV 1, and the virus became latent.[20,21] Although killer lymphocytes are present in PBMCs, the supernatants derived from lymphocytes are cell-free. Thus, the absence of NK cells' cytolytic activities allowed us to measure the noncytolytic activity of the soluble mediators of killer lymphocyte function in our subjects.

METHODS

Subjects

The methods of this study have been described previously. In brief, 51 HIV-positive women age 18–60 years completed this study.[22,23] Seropositive women were recruited over a 29-month time-frame (December 2003–April 2006), from organizations focusing on HIV illness and clinical care through outreach presentations, clinical referrals, and word-of-mouth, as previously described.[22] HIV-seropositive status was determined by enzyme-linked immunosorbent assay and confirmed by Western blot analysis. Subjects were excluded if they had a significant chronic systemic illness other than HIV infection, had a history of schizophrenia or severe psychotic disorder, were pregnant or nursing, met DSM-IV criteria for current substance/alcohol abuse or dependence, or had used antipsychotic, antidepressant, or anxiolytic medications or mood stabilizers within the past 4 weeks. The protocol was approved by the Institutional Review Boards of the University of Pennsylvania and Children's Hospital of Philadelphia. All subjects provided written informed consent and were reimbursed for their time, travel expenses, and child care.

Procedures

As previously described, each subject received a comprehensive medical and psychiatric assessment, including medical history, review of systems, and a physical examination.[22,23] All women were assessed in the late follicular phase, between Days 6–14 of their menstrual cycles, to control for potential gonadotropic hormonal effects on immunity. A modified Structured Clinical Interview for DSM-IV Axis I was administered, and all diagnoses were determined at a consensus conference.[17] Depression severity was evaluated with the 17-item Hamilton Rating Scale for Depression (Ham-D). Subjects were referred for clinical care as indicated. All phlebotomies were performed at the same time of day, following 30 minutes of recumbency after the placement of an intravenous catheter, to avoid any diurnal effects on immunity.[24] The catheter insertion was started at approximately 8:30 A.M., and blood was drawn at approximately 9 A.M.[22]

Acute Infectivity Experiment

Monocyte-derived macrophages (MDMs) were prepared by isolating monocytes from peripheral whole blood of subjects by our established adherence method.[25] Cells were maintained in 10% fetal calf serum (FCS) Dulbecco's Modified Eagle Medium (DMEM) at 37°C in a CO_2 incubator for 7–10 days in order for the monocytes to differentiate into macrophages.[23] After 7 days in culture, MDMs in 24-well plates (1×10^6 cells/well)

were pre-incubated with or without (control) RU-486 (10^{-6}M) for 2 hours, followed by infection with equal amounts of cell-free HIV-1 Bal strain based on p24 antigen content (20ng/10^6 cells) overnight and then washed 3 times with DMEM to remove unbound virus. The culture supernatants were then harvested on Days 4 and 8 after HIV-1 infection, and HIV reverse transcriptase (RT) was measured.[26] On Day 4 post-HIV-1 infection, fresh media with or without RU-486 was added to samples collected on Day 8. The M-tropic CCR5 prototype strain (Bal) of HIV-1 was obtained from the National Institutes of Health (NIH) AIDS Reagent Program and was used for all studies.[23]

Chronic Infectivity Experiment

The mechanisms of activation of latently integrated HIV-1 provirus in promonocyte (U-1) and T-lymphocyte (ACH-2) cell lines have been well characterized.[27] These cell lines are *in vitro* models for chronic HIV infection.[23,27] PBMCs were isolated from heparinized whole blood of each subject by Ficoll centrifugation. Purified PBMCs were suspended in a 10% FCS RPMI-1640 medium. PBMCs (1×10^6/ml) from each subject were stimulated nonspecifically with phorbol myristate acetate (PMA, 1 ng/ml) and with anti-CD28 (1 μg/ml) for 48 hours in the presence or absence (stimulation control) of RU-486 (10^{-6} M). The supernatants obtained from PBMCs of each subject with 10% FCS RPMI-1640 medium were used as a medium-only control; those obtained with PMA and anti-CD28 were used as a PMA-only control. The cell-free supernatants were collected and stored at −70°C for subsequent incubation with either U-1 or ACH-2 cell lines.

U-1 and ACH-2 cells were cultured in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 2 mM/L glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% (v/v) heat-inactivated FCS (Hyclone Laboratories, Logan, UT). Cell viability was assessed with Trypan blue dye exclusion. All media and reagents used were endotoxin-free by *Limulus* amebocyte lysate assay.[20–23]

RU-486-Conditioned Supernatant Treatment

The cells were incubated in duplicate in 24-well culture plates (cell densities: 1×10^5 cells/ml/well for U-1 and 5×10^5 cells/ml/well for ACH-2). The cells were pretreated with TNF_α (2 ng/ml) for 30 minutes, followed by the addition of 100 μl of "RU-486 conditioned supernatant" into each well. TNF_α (2 ng/ml) served as a positive control; this treatment of the cells led to activation of latent HIV replication in both cell lines.

A supernatant with medium-only was used as a "no-treatment" control. The culture supernatants (100 μl) were harvested at 24 and 48 hours post-stimulation (U-1 and ACH-2) for HIV-1 RT activity assay, which was carried out based on modification[25] of Willey's method.[26,28]

Statistical Analyses

Each experiment had a repeated-measures design; each patient provided a response under control and under RU-486 treatment at each of two time points. We used mixed-effects models to compare the within-subject effect of RU-486 versus control. In addition to our primary models that focused on the RU-486 effects, we examined the effects of HIV disease status by viral load and current use of antiretroviral therapy (ART) medications. We also evaluated the effects of depression diagnosis and Ham-D Score. For these repeated-measures models, the responses were all continuous and log–transformed (to base 10) to reduce skewness to appropriate levels for the models.

RESULTS

As previously described, the overall sample for this study comprised 51 women.[22,23] Because some women did not provide sufficient volume of blood and because some experiments failed, only 49 women contributed to these experiments: 35 were available for the acute infectivity experiment, and 43 and 47 for the chronic infectivity experiment using either ACH-2 or U1 cell lines, respectively.

The sample was predominantly African American (N=38; 77.5%). The median age was 41.0 years (mean: 40.83 [standard deviation {SD} 6.24] years). Of the 49 women, 21 (42.9%) had completed high school; 15 (30.6%) did not have a high school diploma or equivalent; and 13 (26.5%) had some college experience. Most women were using ART therapy (N=37; 75.5%). By design, the study recruited approximately equal numbers of depressed and nondepressed women. The rate of current depression was 51.0% (N=25). Of the women with depression, 13 (26.5%) had major depression, and 12 (24.5%) had dysthymia, minor depression, or other non-major depression. The mean Ham-D score for the depressed group was 15.01 (SD: 6.66); the nondepressed group mean was 5.80 (SD: 4.43). There were no significant

TABLE 1. The Effects of RU-486 on Acute HIV Infectivity and Chronic HIV Infectivity

	Acute HIV Infectivity		Chronic HIV Infectivity			
	Bal[a] (N=35)		ACH-2[b] (N=43)		U-1[c] (N=47)	
	4 Days	8 Days	24 Hours	48 Hours	24 Hours	48 Hours
Control[d]	4.05 (0.10)	4.55 (0.11)	3.85 (0.03)	4.26 (0.04)	3.37 (0.05)	3.86 (0.04)
RU-486 Treatment[e]	3.89 (0.11)	4.45 (0.11)	3.83 (0.03)	4.26 (0.04)	3.37 (0.05)	3.85 (0.05)
Within-subject RU-486 effect	0.16 (0.07)*	0.10 (0.12)	0.02 (0.01)*	0.00 (0.01)	0.01 (0.01)	0.01 (0.01)

Values are means (standard errors) of $\log_{10}$HIV RT measurements in control and RU-486 treated cells.
[a]Primary monocyte-derived macrophages cells infected with HIV-Bal strain.
[b]ACH-2 T lymphocyte chronically (latently)-infected cell line.
[c]U-1 promonocyte chronically (latently)-infected cell line.
[d]Media control.
[e]RU-486 pretreatment with 100μl of 10^{-6} M RU-486.
*Significant at 0.05 level.

differences between the depressed and nondepressed groups in ethnicity, age, or education.

RU-486 Effects on Acute HIV Infectivity

Treatment of MDMs with RU-486 significantly downregulated HIV RT activity at the first time-point (Day 4; $t[35] = -2.35$, $p=0.02$) but not at the second time-point (Day 8; $t[35] = -0.82$; NS; Table 1).

RU-486 Effects on Chronic HIV Infectivity

Treatment with RU-486 significantly downregulated the HIV RT response ($t[43] = -2.07$; $p=0.04$) at the first time-point (Hour 24) in the T-cell line ACH-2; however, there was no significant effect at the second time-point (Hour 48). There were no significant effects of RU-486 treatment at either time-point in the promonocyte cell line U1($t[47] = -0.90$; NS, at Hour 24, and $t[47] = -0.97$; NS, at Hour 48; Table 1).

Effects of Depression

We analyzed these models, with the addition of terms for Ham-D score and depression diagnosis. The RU-486 effects were demonstrated; there was little evidence that the RU-486 effects differed by either depression status or Ham-D Score. For the chronic infection experiments, the *F*-tests for the ACH-2 cell line and for the U1-cell line, were $F[1,42]=0.93$; NS, and $F[1,46]=0.03$; NS, respectively. For the acute infectivity experiment, the *F*-test for a diagnosis interaction was $F[1,34]=2.99$; $p=0.09$). There were no significant effects of the Ham-D Score on changes in HIV RT response.

Effects of Viral Load and Use of ART

We also studied the primary models with the addition of terms for current use of ART and detectable viral load. We dichotomized the viral load variable at the measurement threshold level ($</\geq 75$) in the analyses. No significant effects of viral load or current use of ART on RU-486 efficacy were observed at any of the time-points.

DISCUSSION

Our findings suggest that a glucocorticoid antagonist has positive effects on HIV-related immunity. The glucocorticoid antagonist RU-486 significantly decreased acute HIV viral infectivity in macrophages. Also, RU-486 significantly decreased HIV viral replication in the latently infected ACH-2 T-cell line but not in the U-1 cell line *ex vivo*. These findings, together with the evidence implicating glucocorticoids in the immune dysregulation found in HIV disease, suggest a potential role for the development of therapeutic approaches toward targeting glucocorticoid receptors in the host response to HIV *in vivo*. Studies examining the mechanisms of interactions between glucocorticoids, glucocorticoid antagonists, HIV receptors and co-receptors, and anti HIV-suppressive factors are warranted.

Longitudinal studies conducted with HIV+ cohorts before the introduction of highly active antiretroviral therapies (HAART) and after HAART became widely available implicated depression as a risk factor for morbidity and mortality in HIV/AIDS.[14,16,29–34] We hypothesized that depression would have a negative effect on *ex vivo* models of both acute and chronic HIV infection and that the glucocorticoid antagonist, RU-486, would have the greatest effect on depressed subjects. The aim of this study was to examine the effects of RU-486 on HIV infectivity in blood obtained from HIV-positive women with and without depression. We also

examined whether the effects of glucocorticoid antagonism on chronic HIV infectivity were due to HIV-suppressive chemokines and cytokines. We found that RU-486 attenuates acute infectivity in MDMs *ex vivo* and reduces infectivity in a chronically-infected T-cell line; these effects may be attributable to HIV entry receptors and co-receptors, in addition to altered secretion of HIV-suppressive factors. Similar to previous findings with this cohort in relationship to SSRI antidepressants, the effects of RU-486 did not differ significantly as a function of depression in this cohort as hypothesized.[23]

Our findings were significant in two of the three conditions at the first time-point. In the chronic model, we observed significant downregulation in the T-cell line at the first time-point (24 hours) but not in the monocyte cell line. Significant downregulation was noted at the first time-point (4 days) in the acute infectivity model, but not at the second time-point (8 days). Although our findings suggest significant downregulation at the first time-point in the acute infectivity model and in the chronically infected T-cell line, there were not similar findings in the U1 cell line. The differences in response of the chronically-infected T-cell line in comparison to the chronically-infected U1 cell line may be explained by the different characteristics of these two cell lines. U1, a promonocyte cell line, contains two integrated copies of proviral HIV DNA, whereas the T-lymphocyte cell line ACH-2 has a single copy of proviral DNA per cell, potentially resulting in different responses to RU-486.

The mechanisms underlying the relationship between depression and morbidity/mortality in HIV/AIDS are poorly understood.[19] Glucocorticoids have been implicated in the pathophysiology of depression-related immune dysregulation. Evidence derived from mechanistic studies suggests that glucocorticoids bind to cytosolic glucocorticoid receptors, which translocate into the cell nucleus. In the nucleus, they bind to glucocorticoid-sensitive DNA regions of lymphocytes, leading to upregulation or downregulation of glucocorticoid-regulated genes.[35–37] Glucocorticoid-induced apoptosis of thymocytes, T-lymphocytes, and B-lymphocytes, and a shift from a T-helper cell 1 (Th1) to a T-helper cell 2 (Th2) immune profile results in T-cell depletion during HIV infection and the progression of disease.[4,38–40] Glucocorticoids play an important role in innate immunity; previous studies have shown that cortisol inhibits NK cell activity *in vitro*.[4,38,41] Although this finding has not been consistent, several studies suggest that the percentage and/or absolute numbers of circulating CD4+ T-lymphocytes are increased with higher levels of serum DHEA and decreased with higher levels of serum cortisol.[42–45]

Glucocorticoids also modulate host response to HIV. Penton Rol et al.[46] examined the effects of glucocorticoids on human monocyte chemokine receptor expression and found that the synthetic glucocorticoid, dexamethasone, upregulated the mRNA expression of the receptor for monocyte chemotactic protein (MCP) CCR2, which is known to be involved in HIV entry of monocytes, and the glucocorticoid antagonist RU-486 inhibited this upregulation.[46] Dexamethasone also induced replication of the HIV strain 89.6, which uses the CCR2 receptor, in freshly isolated monocytes.[46] In the acute model, we observed significant downregulation at the first time-point (24 hours). This finding could be consistent with evidence suggesting that synthetic glucocorticoids mediate MCP upregulation by interacting with glucocorticoid receptors and prolonging the half-life of its transcripts; dexamethasone upregulates expression of CCR2 in monocytes and less so in MDMs.[46]

Glucocorticoids also interact with circulating HIV-1 derived products.[46] The gene product vpr is involved in the regulation of HIV replication in T-lymphocytes and monocytes *in vitro* by directly interacting with proteins associated with the glucocorticoid transcriptional complex. Glucocorticoid antagonists may reverse this process.[9,10] Thus, vpr may enable HIV to evade the immune system by inhibiting the production of co-stimulating molecules and cytokines responsible for immune activation.[47]

Efforts were made to avoid confounding factors in the analysis of data presented here. We excluded subjects with current alcohol or substance abuse or dependence. As in previous investigations, immune assessments were standardized.[23] Blood was drawn under controlled conditions at the same time of day, after 30 minutes of recumbency to avoid diurnal effects on immunity.[24] We studied subjects during the late follicular phase, Days 6 to 14 of the menstrual cycle, to avoid potential confounding effects of gonadotropic hormones on immunity. All psychiatric and medical assessments were standardized. Although, the majority of the subjects were taking ART, we observed no significant effects of subjects' HIV viral load or ART on the RU-486 effects. We controlled for HIV disease severity by controlling for viral load and ART use in all analyses.

Some potential limitations of our study should be noted. Our *ex vivo* models for acute and chronic HIV infection were designed specifically to study the effects of RU-486 on HIV infectivity of macrophages and

T-cells, the primary immune cells targeted by HIV. Thus, our *ex vivo* models do not account for other cellular elements and co-factors involved in the HIV immune response, including innate immunity (B-cell responses) and *in vivo* co-factors. Some of these *in vivo* modulators include the polymorphisms in MHC-class alleles that may alter the immune response of T-cells, the complement system, and other cellular elements that exist *in vivo*.[48,49] Therefore, these *ex vivo* findings suggest the need for further studies using *in vivo* systems.

Our study focused on HIV-positive women because HIV is a leading cause of morbidity and mortality among young women in the United States. Although we recruited women of all backgrounds, the study sample was largely African American. As a result, further study is required to determine whether our findings are generalizable to women of different backgrounds and to men. Our inability to detect differences between depressed and nondepressed subjects may be limited by sample size, the mild-to-moderate depression severity of our sample, or the ability of an immune system impaired by HIV to produce a detectable response.[23] The present study did not have adequate statistical power to address the effects of depression, but the HIV-suppressive effects of RU-486 were observed in all subjects.[23] Future research might benefit from investigating a more severely depressed group because these participants might produce a more easily detectable immune response.[19]

In conclusion, these findings provide additional support for the role of glucocorticoids and glucocorticoid antagonists in the regulation of immunity in HIV. Specifically, these results suggest that glucocorticoid antagonism may suppress HIV infectivity and replication, possibly through the secretion of HIV-suppressive factors as well as a direct downregulation effect. Studies of glucocorticoids in the pathogenesis of HIV/AIDS are needed. Further mechanistic studies are needed to determine whether depression impairs killer lymphocyte noncytolytic activity and heightens susceptibility to HIV infectivity and replication; and further clinical investigations of glucocorticoid antagonists are needed to determine their potential role as adjunctive therapy for HIV infection.

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