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Selective Serotonin Reuptake Inhibitor Suppression of HIV Infectivity and Replication

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Abstract

Objective—To test the hypothesis that the selective serotonin reuptake inhibitor (SSRI) citalopram would down regulate HIV infectivity and that the greatest effects would be seen in people with depression. Depression is a risk factor for morbidity and mortality in HIV/AIDS. Serotonin (5-HT) neurotransmission has been implicated in the pathobiology of depression, and pharmacologic therapies for depression target this system. The 5-HT transporter and 5-HT receptors are widely distributed throughout the central nervous and immune systems. Depression has been associated with suppression of natural killer cells (NK) cells and CD8+ lymphocytes, key regulators of HIV infection.

Methods—*Ex-vivo* models for acute and chronic HIV infection were used to study the effects of citalopram on HIV viral infection and replication, in 48 depressed and non-depressed women. For both the acute and chronic infection models, HIV reverse transcriptase (RT) activity was measured in the citalopram treatment condition and the control condition.

Results—The SSRI significantly downregulated the RT response in both the acute and chronic infection models. Specifically, citalopram significantly decreased the acute HIV infectivity of macrophages. Citalopram also significantly decreased HIV viral replication in the latently infected T-cell line and in the latently infected macrophage cell line. There was no difference in down-regulation by depression status.

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Conflict of Interest Statement

Tami Benton, David R. Gettes, Nancy Tustin, Jian Ping Lai, declare no conflict of interest.

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Conclusions—These studies suggest that an SSRI enhances NK/CD8 non-cytolytic HIV suppression in HIV/AIDS and decreases HIV viral infectivity of macrophages, *ex vivo*, suggesting the need for *in vivo* studies to determine a potential role for agents targeting serotonin in the host defense against HIV.

Keywords

Serotonin; Immunity; Depression; HIV/AIDS

Introduction

The role of serotonin in the immune system has come under increasing study as growing evidence suggests that the 5-HT transporter and 5-HT receptors are not only widely distributed throughout the central nervous system, but also the immune system (1-5). The 5-HT transporter is responsible for the reuptake of 5-HT and present on macrophages (6) and mononuclear leukocytes (7), as well as human lymphocytes (8). 5-HT receptors are present on human mononuclear cells (9-11). Serotonergic pathways are involved in the pathogenesis of depression and drugs that block the reuptake of 5HT are the mainstay of pharmacological antidepressant treatment (12). Thus, serotonergic modulation of the immune system is a possible biological mediator between depression and HIV/AIDS disease progression.

Serotonergic pathways have important effects on killer lymphocytes (natural killer (NK) cells and CD8⁺ T cells) which are key cells in the immunopathogenesis of HIV/AIDS and which are affected in depression. NK cells and CD8⁺ cells exert anti-HIV effects by both direct killing of HIV-infected cells as well as through the production of HIV suppressive factors blocking viral entry and replication (13). HIV suppressive chemokines, cytokines, and HIV entry receptors and co-receptors play a key role in the host defense against HIV entry and replication (13-15). Serotonin treatment decreases HIV-I replication in human macrophages. 5-HT downregulated the β -chemokine receptor, CCR5, and upregulated the CCL5 chemokine, MIP-1 α , suggesting an effect of 5-HT on 5-HT_{1A} receptors on macrophages (16).

Depression is a risk factor for morbidity and mortality in HIV/AIDS (17-27). Depression has been associated with suppression of NK and CD8⁺ T cell immunity in clinically depressed individuals (28-32), including depressed HIV-infected individuals (20,33). In addition, several studies have found that antidepressants that block the re-uptake of serotonin (SSRIs) are associated with up-regulation of NK cells (4,34,35) in depressed individuals, including HIV-infected individuals (36).

Given the potential regulatory role of serotonin on NK cell and CD8⁺ T cell immunity in HIV infection, we studied the effects of *ex vivo* treatment with an SSRI on HIV infectivity and viral replication. We have previously demonstrated that an SSRI increased natural killer cell cytotoxicity (NKCC) *ex vivo* in the cells of HIV positive women (36). In order to demonstrate that an SSRI has additional anti-HIV activity beyond its effect on NKCC, we have developed experimental protocols designed to detect decreases in viral load that cannot be attributed to enhanced NKCC. As a model of acute HIV infection, isolated monocyte-derived macrophages were incubated with citalopram, a representative SSRI, and then infected with a monocytopathic strain of HIV-1, Bal (37). Killer lymphocytes are not present in this system; therefore any effect of citalopram on acute infectivity would not be the result of enhanced killer lymphocyte activity and would represent an effect on HIV entry receptors and co-receptors.

We have also modified the chronic HIV infection systems used by several groups (38-41) in order to study the effects of an SSRI on HIV-related immunity, specifically the effects of citalopram on chronic HIV infection. In these two systems of chronic infectivity, we have

studied a latently infected macrophage cell line and a latently infected T-lymphocyte derived cell line. As we were interested in determining whether citalopram affected the increased release of anti-HIV soluble factors, we incubated peripheral blood mononuclear cells (PBMCs) taken from HIV-seropositive subjects with citalopram, then extracted the supernatant from the citalopram (or control) treated PBMCs and studied its effects on infectivity in our models of chronic infectivity. While NK cells and CD 8⁺ cells are present in the PBMCs of the subjects, “the conditioned supernatant” does not—by definition—contain any cells. Thus, any difference in the effects on infectivity between the supernatant of the citalopram treated PBMCs and the supernatant of the PBMCs without citalopram in our chronically infected cell lines must result from immune soluble factors such as non-cytolytic, anti-HIV chemokines and cytokines.

In order to demonstrate that an SSRI affects both acute and chronic HIV infectivity through means other than the enhancement of killer lymphocyte cytolysis, these experiments use three different cell lines in which viral load is assessed (macrophages, a promonocyte derived chronically infected cell line, and a T lymphocyte derived chronically infected cell line) and two different *ex vivo* interventions (direct incubation of macrophages with SSRI as a model of acute infectivity and treatment of each of the chronically infected cell lines with supernatant derived from subjects’ SSRI-treated PBMCs).

Thus, the present studies were designed to determine the potential role of serotonin in regulating non-cytolytic immune defenses in HIV/AIDS. We hypothesized that the SSRI citalopram would down regulate HIV infectivity, and that the effect would be greater in subjects with depression. We studied the effects of a serotonin reuptake inhibitor on HIV entry and replication in *ex vivo* models of acute and chronic HIV infection. These studies explore the potential role of serotonin and drugs that target serotonin in the host defenses against HIV infection, and set the stage for future mechanistic studies focusing on underlying relationships among serotonin and HIV-related immunity.

Methods and Materials

Subjects

Because HIV is a leading cause of morbidity and mortality among 25- to 44-year old US women, we studied HIV- seropositive women. 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 (36). Subjects aged 18 to 60 years were included, and HIV-seropositive status was determined by enzyme-linked immunosorbent assay and confirmed by Western blot analysis. Subjects were excluded if they had 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 anti-psychotic, anti-depressant, or anxiolytic medication or mood stabilizers within the past four 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

Each subject received a comprehensive medical and psychiatric assessment, including medical history, review of systems, and a physical examination. A menstrual history and status was obtained, and each subject was studied in the late follicular phase, day 6-14 of the menstrual cycle, in order to avoid potential effects of gonadotropic hormones on immunity. A modified Structured Clinical Interview for DSM-IV Axis I (42) was administered, and all psychiatric diagnoses were determined at a consensus conference. Depression severity was evaluated with

the 17-item Hamilton Depression Rating Scale. Subjects were referred for clinical care as indicated.

Subjects were studied at the same time of day in order to control for potential circadian effects on immunity (43). Subjects were recumbent for approximately 30 minutes following the placement of an intravenous line. The line was started at approximately 8:30 AM and blood was drawn at approximately 9:00 AM, following the 30 minute acclimation period (32).

Methods

Acute Infection Model—Monocyte-derived macrophages (MDM) preparation: Monocytes were isolated from peripheral whole blood of these subjects using our well established adherence to plastic technique (44) and the cells were maintained in 10% fetal calf serum (FCS) DMEM at 37°C in a CO₂ incubator for 7-10 days in order for the monocytes to differentiate into macrophages.

Chronic Infection Model—The cellular and molecular mechanisms of activation of latently integrated HIV-1 provirus in promonocyte (U-1) and T-lymphocyte (ACH-2) cell lines have been well delineated and these cell lines are *in vitro* models for HIV viral latency (45). Studies of U-1 and ACH-2 have provided substantial information about the stimuli and regulatory pathways involved in the activation of HIV-1 replication *in vivo* (45). HIV-1 activation and replication in these cells are under the control of endogenous cytokine networks (38-41).

Effect of SSRI on Monocyte-derived macrophages (acute HIV infection)—

Following seven days in culture, MDM in 24-well plates (1×10^6 cells/well) were pre-incubated with SSRI (citalopram) (10^{-6} M) for 2 hours followed by HIV infection. The cells were infected with equal amounts of cell-free HIV-1 Bal strain based on p24 antigen content (20ng/ 10^6 cells) overnight, and then washed three times with DMEM in order to remove unbound virus. Fresh media with or without (control) SSRI (10^{-6} M) was added to the MDM. The culture supernatants were harvested after day 4 and 8 following HIV infection and HIV reverse transcriptase (RT) activity was measured (46). Fresh culture medium was added (without SSRI) when the supernatants were collected at day 4 and day 8. The M-tropic CCR5 prototype strain (Bal) of HIV-1 was obtained from the NIH AIDS Reagent Program and was used for all studies.

Effect of SSRI on latent HIV replication in U-1 and ACH-2 cell lines (chronic HIV infection)—PBMCs were isolated from heparinized whole blood of each subject by Ficoll centrifugation. Purified PBMCs were suspended in 10% FCS RPMI-1640 medium. PBMCs (1×10^6 /ml) were stimulated with phorbol myristate acetate (PMA), as a non-specific stimulus (1ng/ml) and anti-CD28 (1μg/ml) for 48 hours in the presence or absence (stimulation control) of SSRI (10^{-6} M). The supernatants obtained from PBMCs of each subject with 10% FCS RPMI-1640 medium only and PMA (1ng/ml) and anti-CD28 (1μg/ml) were used as medium only control and PMA only control. The cell-free supernatants were collected and stored at -70°C for subsequent incubation with either U-1 and ACH-2 cell lines (see below).

The HIV-1-latently infected U-1 and ACH-2 cell lines were obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, National Institutes of Health. U-1 is a cloned cell-line derived by limiting dilution cloning of U937 cells, a macrophage lineage cell line, which survived acute infection with HIV-1 (LAV-1 strain). U-1 cells are characterized by the presence of two copies of integrated HIV-1 proviral DNA per cell (47). ACH-2 is a cloned cell-line derived from a human T cell-line which survived acute HIV-1 infection. Restriction enzyme analyses demonstrated a single integrated copy of proviral DNA in the ACH-2 cell line (48). U-1 and ACH-2 cells were cultured in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml

streptomycin and 10% (v/v) heat-inactivated FCS (Hyclone Laboratories, Logan, UT). Cell viability was assessed using trypan blue dye exclusion. All media and reagents used were determined to be endotoxin-free by *Limulus* amoebocyte lysate assay.

SSRI-Conditioned Supernatant Treatment—The cells (U-1 and ACH-2) 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 ACH2). The cells were pretreated with TNF- α (2ng/ml) for 30 minutes followed by the addition of 100 μ l of “SSRI conditioned supernatant” (see above) into each well. TNF- α (2ng/ml) treatment of the cells served as a positive control; this treatment led to the activation of latent HIV replication in the U-1 and ACH2 cell lines. A supernatant with medium only was used as a “no treatment” (control).

The culture supernatants (100 μ l) were harvested at 24 hours and 48 hours post-stimulation (U-1 and ACH-2) for HIV-1 RT activity assay. The HIV RT activity assay was carried out based on the technique of Willey et al. (46) with modification (49). Briefly, 10 μ l of culture supernatant was added to a mixture containing poly (A), oligo (dT) (Pharmacia, Piscataway, NJ), $MgCl_2$ and ^{32}P dTTP (Amersham, Arlington Heights, IL) and incubated for 20 h at 37 ° C. Then 30 μ l of the mixture was spotted onto DE81 paper, dried and washed five times with 2 $\times$ saline-sodium citrate buffer (SSC) and once with 95% ethanol. The filter was then air-dried. Radioactivity was counted in a liquid scintillation counter (Packard Instrument, Palo Alto, CA).

Statistical Analyses

Each experiment had a repeated measures design, with each patient providing a response under control and under SSRI treatment at each of two time points. We used mixed effects models to compare the within-subject effect of citalopram versus control. In addition to our primary models that focused on the citalopram effects, we also examined the effects of HIV disease status (measured by viral load dichotomized at detection threshold) and current use of antiretroviral medications (ART-use or ART-naïve). We also evaluated the effects of depression diagnosis and Hamilton depression score. For these repeated measures models, the responses were continuous, and the data were log-transformed (to base 10) in order to reduce skewness to appropriate levels for the models. Of the 48 women, 29 (60.42%) had undetectable viral loads, and we dichotomized the viral load variable, at the measurement threshold level ($</>+75$ RNA copies), for use in the analyses.

Results

As described by Evans et al. (36), the overall group studied was comprised of 51 women. Since some women did not provide sufficient volume of blood for the *ex vivo* studies, cells obtained from 48 women were available for the experiments reported in this paper. For the latent HIV replication experiments using U-1 or ACH-2 cell lines, 47 and 43 women were available, respectively. For the acute HIV infection experiments, 36 women were available. In order to study the effects of depression, the study recruited approximately equal numbers of depressed and non-depressed women. There were no significant differences between the depressed and non-depressed groups in ethnicity, age, or education. The characteristics of the subjects are presented in Table 1.

Effect of SSRI (citalopram) on acute HIV infectivity

In the acute HIV infection model (MDM), citalopram significantly downregulated the RT response at the first time point (day 4) ($t(35) = -2.39$, $p = 0.02$), and had no significant effects at the second time point (day 8) ($t(35) = -0.81$, $p = 0.42$). (Figure 1) In these experiments, the virus is replicating over time in both the control and drug (SSRI) conditions. Therefore, the control values are not stable over day 4 versus day 8 in the acute infectivity model. The mean

and standard errors for the absolute HIV-RT values for the acute experiments, control condition are day 4 = 25538 $\pm$ 5383; day 8 = 67112 $\pm$ 9722. The mean and standard errors for the absolute HIV-RT values for the acute experiments, drug condition are day 4 = 20094 $\pm$ 4756; day 8 = 62558 $\pm$ 10244. As the analyses were carried out on the log₁₀ scale, the corresponding numbers for that scale are as follows; the mean and standard errors for the log₁₀ HIV-RT values for the acute experiments, control condition are day 4 = 4.05 $\pm$ 0.10; day 8 = 4.57 $\pm$ 0.10, and the mean and standard errors for the log₁₀ HIV-RT values for the acute experiments, drug condition are day 4 = 3.89 $\pm$ 0.11; day 8 = 4.46 $\pm$ 0.11.

Effect of SSRI (citalopram) on chronic HIV Infection (latent HIV replication)

For the ACH-2 T-cell line, citalopram significantly downregulated the RT response ($t(43) = -2.69$, $p=0.01$) at the first timepoint (24 hours). The estimated effect at the second timepoint (48 hours) was in the same direction, but was not significantly different from zero ($t(43) = -1.09$, $p=0.28$). (Figure 2)

For the U-1 cell line, citalopram significantly downregulated the U-1 RT response at the second timepoint (48 hours) ($t(47) = -2.64$, $p=0.01$). The data at the first timepoint showed a trend in the same direction, but did not reach significance ($t(47) = -1.60$, $p=0.12$). (Figure 3) In these chronic models of HIV latency, viral replication increased over time, similar to the acute HIV infectivity model.

Effects of Depression

We analyzed these models, with the addition of terms for Hamilton assessment, and depression diagnosis. There was little evidence that the citalopram effects differed by either depression status or by Hamilton score. In particular, for the acute infectivity experiment's first timepoint, where a significant overall down-regulation was observed, there was no difference between depressed and non-depressed groups in the extent of down-regulation of the acute infectivity at the first timepoint ($p=0.86$). There were no differences between depressed and non-depressed groups at either timepoint for the chronic infectivity experiments.

As a measure of the effect of the Hamilton assessment scale on the citalopram effect, we estimated the citalopram effect at each timepoint, for subjects with a Hamilton score of 5, 10, and 15, and compared these effects. There were no significant differences in down-regulation at the first timepoints of the acute infection and ACH-2 chronic infection experiments, nor at the second timepoint of the U-1 chronic infection experiment. There were no significant effects, at any of the three Hamilton levels, at the other timepoint in any of the three experiments.

Effects of viral load and the use of ART

We also analyzed the primary models with the addition of terms for current use of antiretroviral medication and detectable viral load. No significant effects of viral load or current use of antiretroviral medication on citalopram effects were found on any of the responses, at any of the timepoints.

Discussion

These findings suggest that an SSRI has positive effects on HIV-related immunity. Specifically, these results suggest that citalopram enhances killer lymphocyte (NK/CD8) non-cytolytic HIV suppression in HIV/AIDS, and also inhibits HIV infection of macrophages in HIV/AIDS *ex vivo*. The SSRI citalopram significantly decreased HIV viral replication in the latently infected ACH-2 T-cell line, and also the U-1 cell line *ex vivo*. In addition, the SSRI citalopram significantly decreased HIV viral infectivity of macrophages. These *ex vivo* findings, coupled with our findings that resolution of depression in HIV is associated with increased NK cytolytic

activity (50), as well as our recent findings that NK cell cytolytic function may be enhanced by serotonin reuptake inhibition *ex vivo* (36), suggest a potential role for drugs that target serotonin in the host defense against HIV *in vivo*. These findings also warrant mechanistic studies to determine the effects of serotonin and drugs that target serotonin on anti-HIV suppressive factors and HIV receptors and co-receptors.

There are several studies which have implicated stress and depression as risk factors in the morbidity and mortality of a wide range of human diseases (18,51). Growing evidence suggests that stress and depression may impair key components of cell-mediated immunity (15,22, 34-36,52-56), and heighten susceptibility to infectious diseases (57-59) including HIV infection (17-27,32). Although the specific immune mechanisms by which depression may influence immunity and HIV disease progression and mortality remain to be understood, increasing evidence suggests that killer lymphocytes may play a key role. Clinical studies of depression in subjects without other medical illnesses (29,30,32) have demonstrated significant alterations in cellular immunity, NK cells as well as CD8⁺ cells, two immune populations that may play key roles in regulating HIV infection. NK and CD8 T-lymphocytes may be involved in the natural resistance against HIV infection through both cytolytic and non-cytolytic activity. NK and CD8 cells have the capacity to lyse HIV-1 infected cells, and also produce HIV suppressive factors which include chemokines (CCL3, CCL4, and CCL5) and cytokines (INF γ and TNF α , and GMCSF) that inhibit HIV-1 by suppressing viral entry and replication (13,20,38,60-65).

Serotonin neurotransmission is involved in the regulation of mood and is a pharmacologic target in the treatment of depression (12,66). A considerable number of studies have documented the widespread distribution of 5-HT receptors and the 5-HT transporter on monocytes, macrophages, T-cells, and possibly NK cells (1-11). Serotonin may have a direct modulating effect on both NK cell function and T-lymphocyte function. Serotonin enhances the cytolytic activity of NK cells, possibly through 5-HT 1A receptors on monocytes (3,67), and may protect the function of NK cells (67). Serotonin also activates 5-HT receptors on T-cells and inhibits serotonin synthesis. Antagonists of 5HT 1A receptors inhibit T-cell function *in vitro* and cell mediated immunity *in vivo* in human and murine T-cells; this inhibition is reversed by serotonin and by a 5HT 1A receptor agonist (68). The findings from the present *ex vivo* study extend these data and suggest that an SSRI may enhance killer lymphocyte non-cytolytic HIV suppression in HIV/AIDS.

Several clinical studies have demonstrated a positive effect of SSRIs on NK cytolytic activity in depression (4,34,35,69). In HIV infection, we found a similar increase in NK cytolytic activity associated with the resolution of depression in a naturalistic study of HIV-seropositive women (50). Several studies have also suggested similar serotonin up-regulation of T-cell function in HIV infection (70-72). Thus, serotonin and drugs affecting serotonin transmission, including SSRIs, may play important regulatory roles in immune system dysregulation suggesting potential clinical benefits in HIV infection (73,74).

Further supporting these clinical findings on the effects of serotonin on HIV-related immunity are recent findings from *in vitro* experiments indicating that 5-HT decreases acute HIV replication by down-regulating the CCR5 receptor as well as by increasing the secretion of the HIV suppressive chemokine MIP-1 α (16). These findings on the direct effects of serotonin are consistent with our finding of the direct effects of the SSRI citalopram on decreasing HIV viral infectivity of human macrophages in an acute *ex vivo* model. In our chronic model, the anti-HIV time effect difference between the U-1 and ACH-2 cell lines may be explained by the properties of these two cell lines (47,48). U-1 is a promonocyte cell line, while ACH-2 is T-lymphocyte (ACH-2) cell line. U-1 cells have two copies of integrated HIV-1 proviral DNA per cell (45), while ACH-2 cells have single copy of the proviral DNA per cell (48). The latent

HIV replication in these two cell lines in response to “SSRI-conditioned supernatant” may also be different. The anti-HIV effect was significant at time point 1 in the ACH-2 cell line and time point 2 in the U-1 cell line; the effects at the other time points were in the same direction, but were not significant for either cell line.

We have studied three systems for HIV infection, an acute model and two chronic models and demonstrated effects of SSRI in all three models. As noted previously, these cell systems are valuable tools to demonstrate potential efficacy of antiviral agents. It must be recognized, however, that these *ex vivo* cell systems do not completely simulate *in vivo* conditions. These issues have recently been reviewed in context of monocyte-derived macrophages and latently infected cell lines by Cassol, et al. (39). Our observations in these *ex vivo* systems demonstrate that the SSRI citalopram suppresses HIV infectivity.

Several strengths and limitations of the present studies should be noted. Because HIV is a leading cause of morbidity and mortality among 25- to 44-year-old US women, we focused on seropositive women. We recruited women of any race or ethnicity, and the study sample was largely African-American and white. Thus, the sample is representative of HIV infection in US women, but may not be generalizable to women of other races/ethnicities nor to men. In order to avoid potential confounding effects on immune function, we excluded subjects with current alcohol or substance abuse or dependence. As in previous investigations, immune assessments were standardized by performing all blood draws at the same time of day, following 30 minutes of recumbency to avoid diurnal effects on immunity and possible non-specific methodological factors (32,43). We also studied subjects during the late follicular phase, day 6-14 of the menstrual cycle, in order to avoid potential confounding effects of gonadotropic hormones on immunity. The majority of the subjects were taking antiviral medication, and we observed no significant effects of the subjects' HIV viral load or antiviral therapy on the observed *ex vivo* citalopram effects. We controlled for HIV disease status and stage by controlling for viral load and anti-retroviral use in all statistical analyses. We had hypothesized that depression would have a significant negative effect on these *ex vivo* models of acute and chronic HIV infection and that the SSRI would have the greatest HIV suppressant effect on depressed subjects. However the effects of SSRI did not differ significantly as a function of depression in this cohort, perhaps because the subjects were characterized by mild to moderate depression and not severe to very severe depression.

In conclusion, the findings from the present study suggest that an SSRI has positive effects on HIV-related immunity. These results provide initial evidence that killer lymphocyte (NK-CD8) non-cytolytic HIV suppression in HIV/AIDS may be enhanced by an SSRI, and an SSRI may also inhibit acute HIV infection of macrophages in HIV/AIDS. Specifically, HIV replication in latently infected cell lines was significantly decreased by the SSRI citalopram, and HIV viral infectivity of macrophages also was significantly decreased by the SSRI citalopram. These data extend previous findings suggesting a role for serotonin in the regulation of immunity in HIV infection, and suggests that serotonin reuptake inhibition may increase extracellular concentrations of serotonin and thereby suppress HIV infectivity and replication, possibly through the secretion of HIV suppressive factors as well as HIV receptor and co-receptor down-regulation. Additional study is needed to determine whether these findings pertain to other SSRIs. These findings set the stage for mechanistic studies to determine if SSRIs enhance the release of anti-HIV chemokines and cytokines, as well as down-regulate macrophage receptor HIV binding sites. It is also important to determine if SSRIs have similar effects on acute HIV infectivity of T-cells. Further studies are also needed to determine if these *ex vivo* effects on HIV infectivity can be extended to the *in vivo* clinical setting. The present study did not have adequate statistical power to address the effects of depression, and the cohort was characterized by mild to moderate depression, not more severe depression. Thus, the HIV suppressive effects of the SSRI were observed in both the depressed and non-depressed subjects. Further studies

are required to determine if depression, particularly more severe depression, impairs killer lymphocyte non-cytolytic activity and heightens susceptibility to HIV infectivity and replication, as well as whether an SSRI might normalize this impairment and up-regulate HIV suppressive activity, as suggested in the present study. The findings from the present *ex vivo* study provide additional evidence for the role of serotonin in the regulation of immunity in HIV/AIDS, and suggest the need for *in vivo* studies to determine a potential role for agents that affect serotonin transmission in the treatment of HIV/AIDS.

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Abbreviations

SSRI	selective serotonin reuptake inhibitor
NK	natural killer
RT	reverse transcriptase
NKCC	natural killer cell cytotoxicity
PBMC	peripheral blood mononuclear cells
MDM	monocyte-derived macrophages
FCS	fetal calf serum
PMA	phorbol myristate acetate
ART	antiretroviral

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Figure 1.

Effect of SSRI on acute HIV infectivity. For 7-10 days monocyte-derived macrophages (MDM) from HIV-positive women (N=36) were incubated in the presence or absence of the SSRI, citalopram (10^{-6} mol/L), for 2 h followed by infection with HIV-Bal strain for 4 and 8 days. HIV reverse transcriptase (RT) activity (viral load) was determined. The vertical bars represent the distributions of within subject *differences* in HIV RT activity (log 10 scale) at both 4 and 8 days between SSRI-treated MDM culture and untreated cultures.

Figure 2.

Effect of SSRI on replication of latent HIV in a chronically infected T-cell line. HIV RT activity (viral load) was determined in chronically infected T-cell cell cultures (24 and 48 hours post-treatment)(N=43) treated with supernatant from PBMCs that in turn were treated with SSRI (treatment) and compared to viral load in chronically infected T-cells treated with supernatant from PBMCs that were not treated with SSRI(control). The vertical bars represent the distributions of within subject *differences* in HIV RT activity (log 10 scale) at both 24 and 48 hours between cells in the treatment and control arms.

Figure 3.

Effect of SSRI on replication of latent HIV in a chronically infected macrophage cell line. HIV RT activity (viral load) was determined in chronically infected macrophage cell cultures (24 and 48 hours post-treatment)(N=47) treated with supernatant from PBMCs that in turn were treated with SSRI's (treatment arm) and compared to viral load in chronically infected T-cells treated with supernatant from PBMCs that were not treated with SSRI(control). The vertical bars represent the distributions of within subject *differences* in HIV RT activity (log 10 scale) at both 24 and 48 hours between cells in the treatment and control arms.

Table 1

Characteristics of Study Subjects

Characteristics	N=48
Age (years) Mean (SD)	40.75 (6.28)
Education (years)	
Grade 7-12 (did not graduate)	15 (31.2%)
Graduated High School or equivalent	20 (41.7 %)
Some college experience	13 (27.1 %)
Ethnicity	
African American	37 (77.1 %)
Caucasion	11 (22.9%)
Diagnosis:	
Depressed	25 (52.1%)
Major Depression	13 (27.1%)
Non-major depression	12 (25%)
Non-Depressed	23 (47.9%)
Hamilton Scores, Mean (SD)	
Depressed	15.01 (6.66)
Non-Depressed	5.53 (4.32)
Established Antiretroviral Therapy	36 (75%)

* Non-major depression includes dysthymia, minor depression or other non-major depression