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Selective Serotonin Reuptake Inhibitor and Substance P Antagonist Enhancement of Natural Killer Cell Innate Immunity in Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome

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Abstract

Background—Natural killer (NK) cells play an important role in innate immunity and are involved in the host defense against human immunodeficiency virus (HIV) infection. This study examines the potential role of three underlying regulatory systems that have been under investigation in central nervous system research as well as immune and viral research: serotonin, neurokinin, and glucocorticoid systems.

Methods—Fifty-one HIV-seropositive subjects were recruited to achieve a representative sample of depressed and nondepressed women. The effects of a selective serotonin reuptake inhibitor (SSRI), a substance P (SP) antagonist, and a glucocorticoid antagonist on NK cell function were assessed in a series of ex vivo experiments of peripheral blood mononuclear cells from each HIV-seropositive subject.

Results—Natural killer cell cytolytic activity was significantly increased by the SSRI citalopram and by the substance P antagonist CP-96345 relative to control conditions; the glucocorticoid antagonist, RU486, showed no effect on NK cytotoxicity. Our results suggest that the effects of the three agents did not differ as a function of depression.

Conclusions—Our findings provide evidence that NK cell function in HIV infection may be enhanced by serotonin reuptake inhibition and by substance P antagonism. It remains to be determined if HIV-related impairment in not only NK cytolytic activity but also NK noncytolytic activity can be improved by an SSRI or an SP antagonist. Clinical studies are warranted to address these questions and the potential roles of serotonergic agents and SP antagonists in improving NK cell immunity, delaying HIV disease progression, and extending survival with HIV infection.

Keywords

Depression; innate immunity; natural killer cells; SSRI; substance P antagonists

Natural killer (NK) cells are a subset of lymphocytes that serve an important function in innate immunity, defending against viruses and tumors (1,2). Growing evidence suggests that NK cells play a key role in the host defense against human immunodeficiency virus (HIV) infection through direct killing of HIV-infected cells and through noncytolytic activity (2). Natural killer cells exert cytolytic activity against HIV-infected cells (3,4), and NK cells produce HIV suppressive factors blocking HIV entry into cells (5).

With HIV infection, however, there is a notable decrease in NK cell function early postinfection that persists and becomes more pronounced upon progression to acquired immunodeficiency syndrome (AIDS). Numerous experimental and clinical studies have documented this finding (6–8). The negative effects of HIV on NK cells are pervasive, involving both the quantity and functions of NK cells. These impairments of NK cells have been proposed to result in susceptibility to opportunistic infection and tumorigenesis, as well as decreased control of HIV replication. In general, highly active antiretroviral treatment (HAART) reverses the NK deficiencies in HIV-infected patients (9), but defects persist as long as viral replication can be measured (9).

Increasing evidence suggests that depression is a potential risk factor for the morbidity and mortality in other medical diseases, including HIV/AIDS (10–19). Depression has been associated with alterations in cellular immunity, including NK cell numbers and NK cell cytotoxicity (20–24) in individuals who are depressed but without other medical illnesses. In HIV-infected individuals, immune findings have been mixed (19), but several studies have found significant stress and depression effects on immunity (11,25), including NK cell number and activity (12,26,27). A small number of studies have examined the relationship between changes in depression and NK cell activity over time. Natural killer cell activity increased as depression decreased (28) and fluoxetine treatment of depression was associated with increased NK cell activity in subjects with initially low NK cell function (29). However, one study found no change in NK cell number or activity in antidepressant treated depressed subjects (30). In a recent study of HIV-seropositive women, we found that resolution of major depression was associated with increased NK cell activity (31). The study was an observational study, not an antidepressant treatment study; therefore, we cannot relate the increase in NK cell activity to specific antidepressant effects.

Serotonergic mechanisms may have a direct activating effect on NK cell function. Hellstrand and Hermodsson (32) demonstrated that serotonin (5-HT) augments the cytolytic activity of NK cells and suggested that 5-HT_{1A} receptors on monocytes regulate the effects of serotonin on NK cell activity. Monocytes may exert suppressive effects on NK cell activity through their release of hydrogen peroxide. These suppressive effects are reversible by direct addition of serotonin (33–35) and may be therapeutically beneficial (34). Thus, the effects of 5-HT on the immune system have led to studies of selective serotonin reuptake inhibitors (SSRIs) and their effects on immunity. Faraj *et al.* (36) found fluoxetine blocked the reuptake of 5-HT to a similar degree in both leukocytes and 5-HT neurons, and Frank *et al.* (29) found fluoxetine and paroxetine augmented NK cell activity *ex vivo*. These findings have spurred recent studies suggesting potential clinical benefits of drugs affecting serotonin transmission, including SSRIs (37,38).

Modulation of substance P (SP) activity has been explored as a novel approach to the treatment of depression, anxiety, and stress (39). The SP antagonist MK-869 had significant antidepressant effects (39), supporting the concept that SP and its receptor (neurokinin-1R) are involved in the pathophysiology of depression (40), although MK-869 failed to demonstrate antidepressant effects in a subsequent trial (41). However, a different SP antagonist, L-759274, did demonstrate significant antidepressant effects (42). Similarly, elevated concentrations of cerebrospinal fluid (CSF) SP were found in both major depression and posttraumatic stress

disorder (PTSD) (43), and we demonstrated that anxiety (44) and physiologic stress (45) are associated with elevated plasma levels of SP. Substance P is present within nerves of the autonomic nervous system and within the immune system, and the SP preferring receptor neurokinin-1R is highly expressed in brain regions regulating affective behavior and neurochemical responses to stress. In individuals with HIV, we have observed increased plasma levels of SP, and thus SP may contribute to the immunopathogenesis of HIV disease (46,47).

Glucocorticoids (GC) of the hypothalamic-pituitary-adrenal (HPA) axis have widespread immunosuppressive effects relevant to HIV disease and have been shown to induce apoptosis in thymocytes and T cells. This is potentially important because virus-induced apoptosis of lymphocytes is implicated in T-cell depletion during HIV infection. Cortisol can inhibit NK cell activity in vitro (48), and the effects of glucocorticoids on NK cell function have been studied in the context of HIV infection. There is a synergistic effect of cortisol- and HIV-derived soluble products causing T-lymphocyte apoptosis (49), a finding consistent with the concept that distress and circulating human immunodeficiency virus type 1 (HIV-1) derived products may be involved in the pathogenesis of HIV infection. Cortisol synergizes with HIV peptides causing apoptosis of normal lymphocytes (50). Evidence suggests that inhibitors of the glucocorticoid receptor type II complex, such as RU486, may have relevance for the clinical management of HIV (51,52).

Thus, the aim of the present study was to determine the potential role of three underlying regulatory systems that have been extensively investigated in both stress and depression research as well as immune and viral research: serotonin, neurokinin, and glucocorticoid systems. In a series of ex vivo experiments, we studied the effects of a serotonin reuptake inhibitor, a substance P antagonist, and a glucocorticoid antagonist on natural killer cell activity in HIV-seropositive women.

Methods and Materials

Subjects

Human immunodeficiency virus-seropositive subjects were recruited to achieve a representative sample of depressed and nondepressed women. Subjects were recruited from organizations focusing on HIV illness and care through community outreach presentations, clinician referrals, and word of mouth to identify potential subjects. Subjects were included if they were female, 18 to 60 years of age, and able to communicate in English. Human immunodeficiency virus serostatus was determined by enzyme-linked immunoabsorbent assay and confirmed by Western blot analysis. Subjects were excluded if they 1) had significant chronic, systemic illness other than HIV infection; 2) had a significant neurologic disorder, including traumatic brain injury; 3) had a history of schizophrenia or severe psychotic disorder; 4) were pregnant or nursing; 5) met DSM-IV criteria for current substance/alcohol abuse or dependence; or 6) had used antipsychotic, antidepressant, or anxiolytic medication or mood stabilizers within the past 4 weeks (8 weeks for fluoxetine).

The protocol was approved by the Institutional Review Boards of the University of Pennsylvania and the 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 thorough outpatient assessment including a medical history, review of symptoms, a physical examination, and a structured psychiatric interview. Current and lifetime DSM-IV Axis I diagnoses were assessed by a research psychiatric clinician with a modified Structured Clinical Interview for DSM-IV (53). Consensus diagnoses were determined at

diagnostic conferences. Symptoms of depression were evaluated with the 17-item Hamilton Depression Rating Scale (54). Subjects were referred for care as indicated.

To control for potential circadian effects on immunity, subjects were studied at the same time of day, as in our previous studies (24). Subjects were placed in a recumbent position, an intravenous line was started at approximately 8:30 AM, and intravenous line patency was maintained via slow, saline drip. Blood was drawn at approximately 9:00 AM following a 30-minute acclimation period (55). To control for hormonal changes across the menstrual cycle, women were scheduled for assessment between days 6 to 14 of their menstrual cycle.

Immune Assessment

All HIV-seropositive women knew their HIV-1 status. Human immunodeficiency virus serostatus was confirmed by enzyme-linked immunosorbent assay with Western blot analysis for confirmation of anti-HIV-1 antibodies. Serum HIV RNA viral load was determined using the Amplicor Monitor assay (Roche Molecular Systems Inc., Branchburg, New Jersey), lowest limit of detection, 75 copies/mL blood.

Complete blood cell counts and flow cytometry panels were performed on peripheral blood samples from all subjects. For the flow cytometry panel, the specimens were collected in ethylenediaminetetraacetic acid (EDTA) tubes with a minimum of 2 mL of blood and the specimens were processed within 30 hours from collection time. Leukocyte viability was greater than 97%. Monoclonal antibodies (Becton Dickinson Immunocytometry System, San Jose, California) were used to measure the predominant reactivity to the following lymphocyte subsets: CD3+/CD4+; CD3+/CD8+; CD3-/CD56+/CD16+ (NK cells); and CD8+/CD38+/DR+ (activated CD8+) (56) (Table 1).

Peripheral blood mononuclear cells (PBMCs) were isolated by standard Ficoll-Paque (GE Healthcare, Piscataway, New Jersey) separation and studied as PBMCs as in our previous studies (56).

Natural killer cell activity was assessed using our standard techniques that have been well established and utilized repeatedly in our investigations (6), as well as in clinical investigation collaborative networks throughout the United States (Reaching for Excellence in Adolescent Care and Health). All subject samples were assayed within 30 hours of sampling. We have compared NK functional assays at multiple time points and found no significant difference in NK activity if the assay is performed within 30 hours from the time of phlebotomy. To determine the possible effects of SSRI (citalopram), SP antagonist CP-96345 (CP), or GC antagonist (RU486) on NK activity, each agent was incubated with PBMCs for 18 hours at 37°C, 5% carbon dioxide (CO₂) prior to NK assay. Pilot experiments were performed for PBMCs derived from 10 healthy adult donors, in which lytic units/10⁷ NK cells (LUNK) and concentrations of each pharmacological agent, ranging from 10⁻¹¹ to 10⁻³ mol/L, were evaluated. The optimal (maximum) concentration of SSRI (citalopram), SP antagonist (CP), and GC antagonist (RU486) was defined as that concentration which resulted in maximal natural killer activity per number of effector cells necessary to lyse 20% of the targets without reduction in PBMC viability (in all experiments cell viability was greater than 98% as determined by Trypan Blue exclusion [Invitrogen, Carlsbad, California]). At this concentration (10⁻⁶ mol/L), each pharmacologic agent (Atlanta Biologicals, Norcross, Georgia) was added to a separate aliquot of 4 × 10⁶ PBMCs/4 mL RPMI 1640 plus 10% fetal bovine serum (FBS). These ex vivo concentrations are consistent with steady state plasma concentrations in individuals taking citalopram and RU486, the two licensed drugs (57–60). The next morning following the 18 hours incubation and within our 30-hour window, the natural killer cell assay was performed.

Lytic units/ 10^7 peripheral blood mononuclear cells (LU20PBM) and lytic units/ 10^7 NK cells were calculated for the expression of NK cytotoxicity (61,62). By measuring the percentage of CD16+/CD56+ cells in PBMCs, we determined the lytic units of NK activity per NK cell. Four effector-to-target (E:T) ratios were used. Two and a half million PBMCs were used for the starting E:T ratio of 50:1 and then diluted to 25:1, 12.5:1, and 6.25:1. Lytic units are used for data presentation, which is less subject to the problems of nonlinearity of response, as discussed by Pollock *et al.* (63) and Sephton *et al.* (64).

Statistical Analyses

We used repeated measures models to compare the within-subject effects of three agents (citalopram, CP, and RU486) versus control conditions. These models controlled for disease status and use of antiretroviral medication by including binary indicators of detectable viral load and for current use of any antiretroviral medication, respectively. The models were also used to assess the effects of depression status and Hamilton Depression Rating Scale score on the NK responses and on the effects of the agents on these responses. For these repeated measures models, the responses were all continuous and were log-transformed (to base 10) to reduce skewness to appropriate levels for the models. About 60% of the subjects had nondetectable viral load, and we dichotomized the viral load variable, at the level of detection ($\leq$ 75 copies/mL), for use in the analyses.

Analyses were performed on 51 women. The sample was predominantly African American (80.4%). The median age was 40.5 years, with a mean of 40.64 and standard deviation of 6.16. Of the 51 women, 14 (27.5%) did not have a high school diploma or equivalent, 23 (45.1%) had completed high school, and 14 (27.5%) had some college experience.

As intended, the sample was evenly divided between depressed and nondepressed women. The rate of current depression was 52.94% ($n = 27$ of 51). Of the women with depression, 14 (27.5% of 51) had major depression and 13 (25.5% of 51) had dysthymia, minor depression, or other nonmajor depression. The mean level of depressive symptoms, as measured by the 17-item Hamilton Depression Rating Scale, was 10.85 with standard deviation of 7.55. The mean level of depressive symptoms for the depressed group was 15.3 with standard deviation of 6.95; the nondepressed group mean was 5.82 with standard deviation of 4.43. Four women (7.8%) did not provide a Hamilton Depression Rating Scale at assessment. Their values were imputed using a regression model utilizing their screening Hamilton Depression Rating Scale score, and the screening and assessment Hamilton Depression Rating Scale scores were used for the remaining women. There were no significant differences between the depressed and nondepressed groups on age, ethnicity, or education.

The majority of the sample (78.0%, $n = 39$) was using antiretroviral therapy (ART). Thirty-one (60.8%) of the 51 women had nondetectable viral loads (<75 copies/mL), 5 (9.8%) had viral loads between 80 and 400 copies/mL, 14 (27.5%) had viral loads between 400 and 10,000 copies/mL, and 1 (2.0%) had a viral load above 10,000 copies/mL (190,000 copies/mL). More than 75% of the women on ART had viral loads below detectable limit, while at least 75% of the non-ART group were above detectable limits.

Results

Effects of SSRI (Citalopram), SP Antagonist (CP), and GC Antagonist (RU486) on NK Cytotoxicity (LUNK and LU20PBM)

The responses for these experiments were LUNK and LU20PBM scores, measured for each woman under four settings: control, control + citalopram, control + CP, and control + RU486.

Responses were log10-transformed to reduce skewness (as skewness coefficients exceeded 2 for each measure under most treatments).

For the LUNK (LU20 NK) response, there was a significant overall effect of agent [$F(3,48) = 8.59, p < 0.001$]. Contrasts between each of the three agents and the control condition showed that citalopram ($t = 3.62, df = 48, p < .001$) and CP ($t = 2.53, df = 48, p = .01$) both significantly upregulated LUNK relative to control condition, while RU486 ($t = .41, df = 48, p = .69$) showed no effect. CP-96345 and citalopram were not significantly different from each other ($t = -.66, df = 48, p = .51$) (Figure 1).

The results were similar for the LU20 per PBMC response: there was a significant overall effect of agent [$F(3,48) = 13.21, p < .001$]. Contrasts between each of the three agents and the control condition showed that citalopram ($t = 3.89, df = 48, p < .001$) and CP ($t = 3.72, df = 48, p < .001$) both significantly upregulated LU20 per PBMC relative to control condition, while RU486 ($t = .19, df = 48, p = .85$) showed no effect. CP-96345 and citalopram were not significantly different from each other ($t = -.57, df = 48, p = .57$).

Effects of Depression

The omnibus test of whether the effects of the agents on LUNK differed significantly across levels of depression diagnosis was not significant: $F(3,47) = .36, p = .78$. Individual comparisons, however, showed that citalopram increased LUNK significantly in the depressed group (effect = .07, $t = 3.20, df = 47, p = .002$) and showed a similar trend in the nondepressed group (effect = .04, $t = 1.86, df = 47, p = .07$). Similarly, CP increased LUNK significantly in the depressed group (effect = .06, $t = 2.19, df = 47, p = .03$) and showed a similar, but nonsignificant effect, in the nondepressed group (effect = .04, $t = 1.50, df = 47, p = .14$). Similar analyses showed no interaction [$F(3,47) = .51, p = .68$] or main effects [$F(1,47) = .07, p = .69$] of the Hamilton Depression Rating Scale. Similar results were found for the LU20 per PBMC response.

Effects of (Detectable) Viral Load and Use of ART

The above analyses controlled for viral load and use of ART. We also determined whether these factors had an effect on NK levels or on the effects of the agents. Neither detectable viral load [$F(3,48) = .22, p = .88$] nor ART use [$F(3,48) = .65, p = .58$] had a significant influence on the effects of the agents on the LUNK response. Neither showed any significant effect on the levels of LUNK: for viral load, $F(1,48) = 1.12, p = .29$; for ART, $F(1,48) = .42, p = .52$. Again, similar results were found for the LU20 per PBMC response.

Discussion

Our results indicate that an SSRI and an SP antagonist enhance NK cell innate immunity in HIV/AIDS ex vivo. Specifically, the SSRI citalopram and the SP antagonist CP-96345 each produced a significant upregulation of NK cell activity in peripheral blood mononuclear cells of HIV-seropositive women. We found no significant effect of a glucocorticoid antagonist, RU486, on NK cell function. These findings, coupled with our recent findings that resolution of depression in HIV is associated with increased NK cell activity (31), suggest that antidepressant treatment may reverse impairments in innate immunity potentially relevant to delaying the progression of HIV/AIDS in vivo.

Serotonin neuronal pathways are involved in the mechanism of action of antidepressant medications and there is considerable evidence that they are involved in the pathogenesis of depression (65–67). The functional role of 5-HT in the immune system has come under increasing investigation as neuroscientists and immunologists have begun to study the

underlying biology that is common to the central nervous system and the immune system (68,69). The 5-HT transporter and 5-HT receptors are widely distributed throughout immune cells. The 5-HT transporter, which is responsible for the reuptake of 5-HT, is present on both macrophages (70) and mononuclear leukocytes (71), as well as human lymphocytes (36). In addition, 5-HT receptors have been demonstrated on human mononuclear cells (72,73). Serotonin augmentation of NK cytotoxicity (32) and serotonin protection of NK cells (33–35), coupled with studies of SSRIs and their potential beneficial effects on immunity (29,36–38), led us to postulate that the SSRI citalopram would block 5-HT transporters, leading to an increase in extracellular 5-HT, producing upregulation of NK cytotoxicity.

There are relatively few double-blind, randomized, and placebo-controlled trials of antidepressant medication among HIV-seropositive individuals (74–77). Overall, these trials document the positive effect of antidepressants on mood among depressed HIV-seropositive individuals, but none have examined killer lymphocyte function or antiviral activity. There is little information regarding the reversibility of depression-associated alterations of immunity in individuals who are depressed but otherwise medically healthy (28–30). One antidepressant treatment study failed to demonstrate an effect of antidepressant treatment on natural killer cell activity (30), while several others have been positive. Frank *et al.* (29) found that fluoxetine treatment was associated with augmented NK cell activity among depressed individuals who exhibited low NK activity at baseline. Irwin *et al.* (28) found NK activity to be temporarily associated with the state of acute depression, suggesting clinical improvements in depression may be associated with a normalization of depression-associated alterations in NK cell activity. Kook *et al.* (78) and Mizruchin *et al.* (68) found significant increases in NK activity following SSRI treatment of depressed individuals.

Substance P has been studied for its role in depression and in HIV/AIDS. Cerebrospinal fluid SP is elevated in depression, suggesting a pathophysiological role in depression (43), and plasma SP is elevated in HIV/AIDS, suggesting an immunophysiological role for SP in HIV/AIDS (46). Substance P released from infected immune cells may upregulate HIV by directly facilitating HIV replication within macrophages and T cells and/or by indirectly affecting HIV proliferation through alteration of expression of β -chemokines and their receptors, which may reciprocally enhance HIV replication in these cells (79). The SP antagonist CP-96345 inhibits HIV infection of macrophages (79). These data suggest that the interaction between SP and HIV in these immune cells may have an important role in the immunopathogenesis of HIV infection and AIDS (80), and SP antagonism may upregulate NK cell immunity and enhance HIV inhibition.

Natural killer cells may play an important role in regulating HIV infection. Natural killer cell immune-mediated control of HIV is believed to be the result of NK cell killing of HIV-infected cells (3,4), as well as NK cell production of HIV suppressive factors. Specifically, NK cells secrete soluble factors, CC chemokines (CCL3, CCL4, CCL5), which inhibit CC-chemokine receptor 5 (CCR5) viral entry (5). In addition to producing chemokines, NK cells also secrete cytokines, including interferon γ (INF- γ), tumor necrosis factor (TNF), and granulocyte/macrophage colony-stimulating factor (GM-CSF), which may produce further inhibition of HIV replication (81,82). Natural killer cells may have a key regulatory role in controlling HIV infection, and there are adverse effects of HIV on NK cell function, including reduced lysis of HIV-infected cells, reduced production of CC chemokines, and reduced secretion of cytokines (5,83–85). Thus, specific impairments in NK cell function may compromise the host's natural resistance against HIV. These HIV effects on NK cell function, coupled with the reports of reduced NK cell activity in depression, including depressed HIV-infected individuals, could underlie the findings from studies suggesting that depression adversely affects HIV disease progression in prospective clinical investigations of HIV-seropositive individuals (11,13,14,17,18,86–88).

Several strengths of the present studies should be noted. We studied HIV-seropositive women, largely of African American representation, who underwent comprehensive standardized interviews and clinical assessments, including psychiatric and medical characterization. We excluded subjects with current alcohol or substance abuse or dependence to avoid the potential confounding effects on immune function. We focused specifically on NK cell cytolytic activity, given the key role that NK activity is believed to play in the host defense against HIV infection. Our immune assessment was 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 nonspecific methodological factors (26,27,55). To avoid potential confounding effects of gonadotrophic hormones on immunity, we studied subjects during the late follicular phase, days 6 to 14 of the menstrual cycle. In all statistical analyses, we controlled for HIV disease status and stage by controlling for viral load and antiretroviral use.

Some possible limitations of the study should be noted. The study focused on HIV infection and did not assess these agents in non-HIV infected subjects. Because HIV is a leading cause of morbidity and mortality among US women between the ages of 25 and 44, we focused on seropositive women. We recruited women of any race or ethnic background, and African American and Caucasian subjects comprised the majority of our study population. These findings, while representative of HIV infection in US women, may not be generalizable to women of other race/ethnicity representations or to men. Since PBMCs and not purified NK cells were used, this study did not address whether these compounds directly affect NK cell activity or if the effects are mediated via other cell types. Also, the use of washed PBMCs may have limited the potential effects of the GC antagonist on NK activity, and we will modify our ex vivo model in future studies of GC antagonists. We had hypothesized that depression would have a significant negative effect on NK cell function and that the three agents would have the greatest effects on NK upregulation in depressed subjects. No significant depression effects were seen on NK cell function, and the effects of the three agents did not differ significantly as a function of depression in this cohort.

In conclusion, the findings from the present study provide evidence that NK cell function in HIV infection may be enhanced by selective serotonin reuptake inhibition and also by substance P antagonism. Specifically, NK cell cytolytic activity was increased by citalopram and CP-96345 in ex vivo experiments of PBMCs from seropositive women. These findings represent an initial step in identifying serotonin and substance P regulation of innate immunity in HIV infection. Subsequent studies are indicated to determine if these findings apply to other SSRIs and SP antagonists and to elucidate more precise mechanisms and specific regulatory pathways. While the current study was not large enough to have adequate statistical power for definitive tests of whether agent effects differed across levels of depression diagnosis, it is noteworthy that the upregulation of NK cytolytic activity was observed in both groups. For each of citalopram and CP, the upregulation was numerically greater in the depressed group than in the nondepressed group, but further experiments would be needed to determine whether this is a true effect. Further studies are required to determine if these ex vivo findings can be extended into the in vivo clinical setting in the treatment of seropositive men and women with and without depression. It remains to be determined if HIV-related impairments in not only NK cytolytic activity but also NK noncytolytic activity can be improved by an SSRI or an SP antagonist and if depressed HIV-infected individuals have the greatest immune impairment in NK cell killer activity and HIV suppressive activity. If so, this population may be most responsive to treatment with an SSRI or an SP antagonist. Clinical studies are warranted to address these questions and the potential role of serotonergic agents and SP antagonists in improving NK cell immunity, delaying HIV disease progression, and extending survival with HIV infection.

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Figure 1.

Ex vivo increase in log₁₀ NK cell activity (lytic units) in PBMCs from 51 HIV-seropositive women treated with SSRI, citalopram (10^{-6} mol/L); SP-A, CP-96345 (10^{-6} mol/L); GC-A, RU486 (10^{-6} mol/L). SSRI: $p < .001$; SP-A: $p = .01$; GC-A: $p = .69$. GC-A, glucocorticoid antagonist; HIV, human immunodeficiency virus; NK, natural killer; PBMC, peripheral blood mononuclear cell; SP-A, substance P antagonist; SSRI, selective serotonin reuptake inhibitor.

Table 1**Immune Variables for 51 women with HIV Infection**

	Median	Mean (SD)
Characteristic		
NK activity (lytic units/ 10^7 NK cells) LUNK	807.90	972.10 (708.30)
NK activity (lytic units/ 10^7 peripheral blood mononuclear cells) LU20PBMC	47.22	58.96 (44.41)
Cell Counts (cell/ mm^3)		
Peripheral blood mononuclear cells (PBMC)	5.70	6.63 (3.71)
Total helper T cells (CD4)	482.00	484.38 (240.98)
Cytotoxic/suppressor T cells (CD8)	818.00	868.66 (429.46)
Absolute natural killer cells (CD3 ⁻ /CD16 ⁺ /CD56 ⁺)	79.00	101.23 (63.16)
Absolute count cytotoxic T cells with low and high lytic activity (CD8/CD38)	702	705.36 (362.80)
Average number CD38 molecules per CD3 ⁺ /CD8 ⁺ /CD38 ⁺ cytotoxic T cell	1647.00	2052.96 (1170.21)
Absolute count cytotoxic T cells with high lytic activity (CD8 ⁺ /CD38 ⁺ /DR ⁺ , activated CD8 ⁺)	29.00	99.83 (245.99)

LU20PBMC, lytic units/ 10^7 peripheral blood mononuclear cells; LUNK, lytic units/ 10^7 NK cells; NK, natural killer; PBMC, peripheral blood mononuclear cells; SD, standard deviation.