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Depletion of CD4+ T cells in semen during HIV infection and their restoration following antiretroviral therapy

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Abstract

Background—Information concerning the effects of HIV-1 infection, disease progression and antiretroviral therapy (ART) on male genital white blood cell (WBC) profiles could provide important insight into genital immune defense in HIV-infected men and seminal HIV transmission mechanisms.

Objective—To compare concentrations of WBC populations in semen from HIV-1-seronegative (HIV^-) and seropositive (HIV^+) men, and determine whether HIV disease stage and ART are associated with alterations in seminal WBC profiles.

Subjects and Methods—Subjects were 102 HIV^- men, 98 ART-naive (ART^-) HIV^+ men, and 22 HIV^+ men on dual nucleoside ART, before and six months after addition of indinavir. Seminal WBCs, macrophages (MØ), and T lymphocyte subpopulations were enumerated by immunohistology technique.

Results—Seminal $CD4^+$ and $CD8^+$ T cell populations were severely depleted in ART^- HIV^+ men regardless of peripheral blood $CD4^+$ cell count; seminal MØ counts were also reduced. HIV^+ men on dual nucleoside ART had significantly higher seminal MØ, $CD4^+$ and $CD8^+$ T cell counts than ART^- HIV^+ men; addition of indinavir led to a dramatic (>25-fold, $p<0.001$) increase in seminal $CD4^+$ T cell counts which paralleled an increase in blood $CD4^+$ cell counts. Two ART^- HIV^+ men with notably elevated seminal WBC profiles ($>20 \times 10^6$ WBCs/ml) and infectious cell-associated HIV in semen are described.

Conclusions—HIV infection severely depletes $CD4^+$ T cells in the male genital tract as it does at other mucosal sites. This provides evidence that ART^- HIV^+ men have depressed T cell-dependent genital immune defense functions, and are vulnerable to other genital infections that could promote HIV transmission. Seminal $CD4^+$ T cell counts rebounded following treatment with a viral-suppressing ART regimen, indicating that ART may reverse HIV-associated genital immunosuppression. The relative abundance of seminal MØ in HIV^+ men suggests that these cells are important HIV host cells in the male genital tract and vectors of HIV transmission. A subgroup

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of HIV$^+$ men with exceptionally elevated seminal MØ and CD4$^+$ T cell counts and HIV titers may be highly infectious and contribute disproportionately to HIV transmission.

Keywords

Semen; HIV-1; antiretroviral therapy; protease inhibitor; white blood cells; CD4$^+$ lymphocytes; macrophages

INTRODUCTION

The Human Immunodeficiency Virus Type 1 (HIV-1) is transmitted primarily by sexual intercourse in the U.S.A. and worldwide [1-3]. Transmission of HIV is inefficient relative to many other sexually transmitted disease (STD) pathogens, and a number of covariates such as concomitant STDs and acute and advanced HIV disease stage, have been associated with elevated titers of HIV-1 in genital secretions and enhanced HIV transmission [4-6].

Male genital tract organs and secretions are populated with white blood cells (WBCs) which participate in immune defense functions (reviewed in Anderson and Pudney [7]). HIV-infected WBCs have been detected in genital organs of HIV$^+$ men [8]. HIV-infected CD4$^+$ T lymphocytes and macrophages migrate from male genital tissues into semen [9], and recent studies have implicated infected WBCs as vectors of HIV transmission [10-13]. Therefore, seminal WBC profiles provide important information concerning numbers and types of HIV-host cells in the male genital tract, potential cellular vectors of HIV transmission, and immune defense of the male genital tract.

Mucosal epithelia are populated with memory CD4$^+$ CCR5$^+$ T cells which are prime targets of HIV infection [14]. Memory CD4$^+$ T cells in the gastrointestinal tract are dramatically depleted during the early stages of HIV-1 infection before effects are seen on CD4$^+$ T cells in the peripheral blood [15, 16]. Several theories have been presented to explain this effect: 1) memory CD4$^+$ T cells are preferentially infected and killed by HIV during acute infection because they express high levels of CCR5 (HIV co-receptor), 2) memory CD4$^+$ T cells are activated and their lifespan is shortened by HIV infection [17], 3) these cells are targeted by HIV through an interaction between gp120 and the integrin $\alpha_4\beta_7$, a mucosal homing receptor for peripheral blood T cells [18], and/or 4) the migration of memory T cells from peripheral blood to mucosal sites is disrupted [19]. CD4$^+$ T cells are also depleted in the female genital tract following HIV and SIV infection [20, 21]. However, the effects of HIV-infection, disease stage and antiretroviral therapy (ART) on WBC profiles in the male genital tract have not been described. The male genital tract is also populated with memory mucosal T cells [22, 23], and this cell population is targeted by SIV during acute infection in male macaques[24]. We therefore hypothesize that HIV infection leads to depletion of CD4$^+$ T lymphocytes in the male genital tract. To test this hypothesis, we compared concentrations of CD4$^+$ T cells, as well as other WBC populations, in archived semen samples from HIV$^-$ and untreated HIV$^+$ men. Since recent studies have shown that highly active antiretroviral therapy (HAART) partially reconstitutes peripheral blood and mucosal CD4$^+$ T cell populations and immune function in immunosuppressed HIV-infected subjects [25, 26], we also enumerated seminal CD4$^+$ T cells and other WBC populations in archived semen samples from HIV$^+$ men on dual nucleoside therapy, and in the same cohort 6 months after addition of a protease inhibitor (PI) (indinavir) to their ART regimen, to determine effects of these classes of antiretroviral drugs and HIV suppression on WBC profiles in semen.

MATERIALS AND METHODS

Patient Populations

HIV and ART-naive HIV$^+$ men—Subjects were 102 HIV-seronegative and 98 ART-naïve (ART$^-$) HIV-seropositive men who have sex with men (MSM), receiving medical care at Fenway Community Health in Boston, MA between 1988 and 1993, prior to the widespread use of ART. Fenway Community Health is the largest center caring for sexual and gender minority patients in New England [27]. Peripheral blood CD4$^+$ cell counts in the HIV$^+$ men ranged from undetectable to 1,290 cells/mm^3 (median=410).

ART-treated HIV$^+$ men—Twenty-two asymptomatic HIV$^+$ MSM attending Fenway Community Health (Boston, MA) for primary medical care at the beginning of the HAART era (1996−1997) provided semen and blood samples for this study after treatment for a minimum of 6 months with dual nucleoside ART, and then again 6 months after addition of a PI (indinavir) to their ART regimen. Details of the study population and effects of this treatment on seminal and blood HIV-1 levels are reported elsewhere [28]. At the start of the study, 15 participants were receiving zidovudine/lamivudine; 4 stavudine/lamivudine; and 3 zidovudine/didanosine. Following provision of blood and semen specimens for the first (pre-PI) time point, men received indinavir (800 mg three times a day) in conjunction with dual nucleoside analog therapy. Peripheral blood CD4$^+$ cell counts in these men prior to indinavir therapy ranged from 81 to 632 cells/mm^3 (median=238); six months after addition of indinavir, their CD4$^+$ cell counts ranged from 122 to 672 cells/mm^3 (median=314).

General Methods

Semen collection and preliminary analysis—This study was approved by the Institutional Review Board, and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. Men provided written informed consent for participation in the study. Semen was obtained after a minimum of 48 hours of abstinence by masturbation into sterile specimen containers. Samples were sent on ice packs immediately to the laboratory and processed within 2 hours. Semen volume was measured, and concentration of seminal “round cells” (a combination of WBCs and immature germ cells, indistinguishable by phase microscopy [29]) was assessed microscopically on a hemocytometer by a trained technician. Semen was diluted 1:1 in sterile phosphate-buffered saline (PBS), and semen cells were pelleted by centrifugation at 400 × g for 10 minutes. Semen cells were washed 2× in PBS prior to use in immunohistology assays.

Immunohistology Assay—The low numbers of WBC subpopulations in semen preclude routine quantitative analysis by flow cytometry [22]. WBCs in semen were enumerated by an immunohistology assay as previously described [30] with slight modifications. The following monoclonal antibodies (MAbs) were used: anti-HLe-1 (CD45) for simultaneous identification of all WBCs, anti-Leu 4 + 5b (CD3) for all T cells, anti-Leu 3a + 3b for detection of CD4$^+$ cells (including T helper/inducer lymphocytes, monocytes and macrophages), anti-Leu-2a for detection of CD8$^+$ T cytotoxic/suppressor lymphocytes (all from Becton Dickinson, Mountain View, CA), Dako IL-2R for detection of interleukin-2 receptor-α (CD25) on activated T lymphocytes, and Dako Macrophage for detection of CD68$^+$ monocytes/macrophages (both from Dako Corporation, Santa Barbara, CA).

An aliquot of the washed semen cell fraction (about 1/5 of the original sample) was adjusted to a concentration of 10^6 round cells/ml saline, and five microliters of washed semen cells or PBMCs (positive control) were applied to individual spots of Teflon-coated multiwell microscope slides (Roboz Surgical Instruments, Washington, DC), dried, fixed in absolute acetone and stored at $^-$70° C. For use in the immunohistology assay, slides were thawed and

rehydrated in TRIS buffer (0.05 TRIS, 0.15M NaCl, pH 7.6). MAbs from the panel were applied to individual spots on the multiwell slides, incubated at 37° C for 30 minutes, and rinsed in TRIS buffer. Antibody-positive cells were visualized using an alkaline phosphatase anti-alkaline phosphatase kit (Dako Corporation, Santa Barbara, CA). All cells reactive with a MAb developed a red precipitate, whereas immature germ cells, spermatozoa and other MAb-negative cells appeared blue due to the hematoxylin counterstain. After microscopically counting both antibody-positive and -negative round (non-sperm) cells in a minimum of 10 reticle fields, the cell number was calculated based on the known round cell count determined previously from the fresh sample. Testing of semen samples was conducted without knowledge of serostatus, peripheral blood $CD4^+$ cell count or therapy status.

For HIV-1-infected men, the concentration of peripheral blood $CD4^+$ cells was determined by flow cytometry at an off-site clinical laboratory certified by the AIDS Clinical Trial Group.

Statistical Analysis—StatView (version 5.0.1, SAS Institute, Cary, NC, USA) statistical software was utilized to perform the statistical computations. The various WBC measures did not satisfy the assumptions of normal distribution and/or homogeneity of variance. Therefore, the non-parametric Mann-Whitney U test was performed to determine differences between two independent samples, whereas the nonparametric Wilcoxon signed ranks test was used for comparing two related samples. For three group comparisons, one factor analysis of variance (ANOVA) was performed on log-transformed data. Statistically significant ANOVA ($p<0.05$) was followed by Fisher's protected least significant difference (PLSD) post hoc tests for pairwise comparison of groups. Correlations between variables were determined by the Spearman rank-order correlation coefficient. [31].

RESULTS

Comparison of seminal WBC subpopulations in HIV^- and ART-naive HIV^+ men

ART^- HIV^+ men had significantly lower concentrations of total WBCs ($p=0.0008$), macrophages ($p=0.0026$), total T lymphocytes ($p=0.0001$), $CD4^+$ cells ($p=0.0001$), $CD8^+$ T lymphocytes ($p=0.0063$) and activated (IL-2 receptor-α^+) T lymphocytes ($p=0.0001$) in semen compared to HIV^- men (Table 1). Because the anti-CD4 monoclonal antibody recognizes $CD4^+$ monocytes/macrophages [32, 33], seminal $CD4^+$ T lymphocyte counts were determined by subtracting the number of $CD8^+$ lymphocytes from the total number of T lymphocytes. The seminal $CD4^+$ cell count was highly correlated with the seminal $CD4^+$ T lymphocytes count obtained using this subtraction method ($rho=^+0.50$, $p<0.0001$); as was the case with the total $CD4^+$ cell count, $CD4^+$ T lymphocyte counts were also dramatically reduced in ART^- HIV-1-infected men (median counts: 5,700/ml in HIV^- men vs. 0/ml in ART^- HIV^+ men, $p=0.0001$).

Peripheral blood $CD4^+$ cell counts did not significantly correlate with seminal $CD4^+$ T cell counts or any of the other seminal WBC measures in ART^- HIV^+ men. To further elucidate the relationship between peripheral blood $CD4^+$ cell and seminal WBC counts, ART^- HIV^+ men were stratified into high ($\geq 500/mm^3$) and low ($<500/mm^3$) peripheral blood $CD4^+$ cell groups. ANOVA indicated that both ART^- HIV^+ groups had significantly lower concentrations of all seminal WBC variables, except for $CD8^+$ T lymphocytes, than HIV^- men (p's<0.01, Fisher's PLSD tests), and did not differ from each other (p's>0.10). The results for seminal $CD4^+$ T lymphocytes are shown in Figure 1. The majority of HIV^+ men in both groups had undetectable seminal $CD4^+$ T cell counts. Although we did not study men during acute HIV infection, six out of seven subjects with the highest peripheral blood $CD4^+$ cell counts ($>1,000/mm^3$) had undetectable seminal $CD4^+$ T cells, providing further evidence that genital T cell depletion occurs early in HIV disease, before profound reduction in peripheral $CD4^+$ cell counts. For $CD8^+$ T lymphocytes, although both ART^- HIV^+ groups had a median of 0, only

ART$^-$ HIV$^+$ men with peripheral blood CD4$^+$ counts <500/mm^3 showed a significantly lower concentration compared to HIV$^-$ men (p=0.0004).

Although ART$^-$ HIV$^+$ men as a group had lower seminal WBC counts than HIV$^-$ men, two ART$^-$ HIV$^+$ men with advanced disease stage had extremely high seminal WBC concentrations (Table 2). One ART$^-$ HIV$^+$ man with a peripheral blood CD4$^+$ count of 40/mm^3 had a seminal WBC concentration of 55.68 × 10^6/ml, with 15.5 × 10^6/ml macrophages and 6.2 × 10^6/ml CD4$^+$ T lymphocytes. Another ART$^-$ HIV$^+$ subject with a peripheral blood CD4$^+$ T lymphocyte count of 23/mm^3 had a seminal WBC concentration of 29.00 × 10^6/ml, with 24.35 × 10^6/ml macrophages and 2.2 × 10^6/ml CD4$^+$ T lymphocytes. In both of these cases, seminal WBC concentrations were >100-fold higher than the median values for HIV$^-$ and ART$^-$ HIV$^+$ groups. When assessed for HIV-1 using a microculture technique [34], both samples had high titers of infectious HIV-1 in the cellular fraction, but not in the cell-free seminal plasma fraction. Neither of these men had recent or concurrent symptomatic STDs as assessed by clinical history and physical exam.

The effect of ART on seminal WBC profiles in HIV$^+$ men

HIV$^+$ men receiving dual nucleoside ART had significantly higher concentrations of seminal WBCs including total, CD8$^+$ and activated T lymphocytes, and macrophages than did ART$^-$ HIV$^+$ men (p=0.0003, 0.0001, 0.0001 and 0.03, respectively). HIV$^+$ men on dual nucleoside ART also had modestly higher concentrations of seminal CD4$^+$ cells (p=0.056) and seminal CD4$^+$ T lymphocytes (p=0.03) than ART$^-$ HIV$^+$ men. Six months following the addition of indinavir to the ART regimen, substantial increases in CD4$^+$ cell counts were observed in both semen and blood [seminal CD4$^+$ cells, p=0.03; seminal CD4$^+$ T lymphocytes, p=0.001; peripheral blood CD4$^+$ cells, p=0.05 (Table 3)]. As was the case with ART$^-$ HIV$^+$ men, none of the seminal WBC measures was significantly correlated with peripheral blood CD4$^+$ T lymphocyte count either before or after addition of indinavir therapy in the ART cohort.

DISCUSSION

Results from this study indicate that CD4$^+$ T cells are depleted in the male genital tract during HIV infection. Seminal CD4$^+$ T cell counts were significantly decreased in ART$^-$ HIV$^+$ men, but were restored following treatment with combination ART. These findings are consistent with clinical studies that have documented depletion of CD4$^+$ T cells at other mucosal sites during early HIV infection, and reconstitution of both peripheral blood and mucosal CD4$^+$ T cells in HIV$^+$ subjects receiving HAART. A number of reports have documented depletion of CD4$^+$ T lymphocytes in the gastrointestinal mucosa of HIV-infected individuals [15, 35], and their partial restoration following HAART [25, 36, 37]. An earlier study from our group also documented depletion of WBCs and CD4$^+$ T lymphocytes in the endocervix of HIV$^+$ women in comparison to uninfected women [20], and Veasey and coworkers reported a similar effect following SIV infection of macaques [21]. Because CD4$^+$ T cells play an important helper role in both cellular and humoral acquired immune defense functions, the results of these earlier studies suggest that HIV infection has a broad suppressive effect on mucosal immune defense functions at various mucosal sites. A small study performed by Denny and associates [22] showing a 50% reduction in the proportion of CD4$^+$ T cells in semen from HIV$^+$ men provided the first evidence that seminal CD4$^+$ T lymphocytes may be depleted following HIV infection. The results of the present study showing decreased concentrations of CD4$^+$ T cells in semen from HIV$^+$ men with high peripheral CD4$^+$ cell counts provide evidence that selective CD4$^+$ T cell depletion also occurs in the male genital tract. These findings suggest that genital T-cell dependent immune defense functions may be impaired in HIV-infected men. If so, HIV$^+$ men may be more vulnerable to genital infections, some of which are co-factors for HIV transmission [5]. Our observation that seminal CD4$^+$ T cell counts rebound in HIV$^+$ men

following treatment with a viral suppressing ART regimen provides evidence that genital $CD4^+$ T cell populations are restored following suppression of viral replication.

ART^- HIV^+ men in this study also had reduced seminal $CD8^+$ T lymphocyte concentrations, suggesting that HIV infection impairs anti-viral cellular immune defense mechanisms in the male genital tract. This differs from earlier reports that showed increased concentrations of $CD8^+$ T cells in the endocervix [20] and gastrointestinal mucosa [15] following HIV infection. This discrepancy may be related to the time course of HIV disease and treatment, or to differential effects of HIV infection on the various compartments of the body. The men enrolled in this study tended to have highly advanced HIV disease, and may have initially manifested increases in mucosal $CD8^+$ cells that declined over time as they became more immunocompromised. This is supported by data from the present study indicating that seminal $CD8^+$ T cells were significantly reduced only in men with peripheral blood $CD4^+$ cell counts less than 500/mm^3. Lim et al. [16] found that gut mucosal $CD8^+$ T lymphocyte counts initially increased following HIV infection, but decreased following depletion of $CD4^+$ blood counts. $CD8^+$ T cell counts in semen were dramatically increased following administration of combination ART suggesting that ART also reconstitutes CD8-mediated cellular immune functions in the male genital tract.

The effect of ART on semen WBC populations was most pronounced after the addition of the PI, indinavir, to combination therapy. Since PIs suppress HIV viral load in blood and semen [28, 38-40], and indinavir penetrates the male genital tract very effectively [41-43], this observation provides evidence that male genital tract immune reconstitution occurs when HIV replication is suppressed, and suggests that genital immune depletion is directly related to HIV viral load. The results of this study are limited by the small sample size, the short duration of therapy, and the use of a narrow panel of ART drugs. The effects of longer periods of HAART, as well as other combinations of antiretroviral drugs on seminal WBC populations, should be studied.

Peripheral blood $CD4^+$ cell counts in HIV^+ men were not associated with $CD4^+$ T lymphocyte or other WBC concentrations in semen. Although this study did not include men with acute HIV infection, decreased numbers of seminal $CD4^+$ T cells in men with high peripheral blood $CD4^+$ cell counts suggest that this cell population is depleted during early stages of HIV disease, and remains so until viral replication is fully suppressed by ART. Normally, HIV^- and HIV^+ men have much higher concentrations of $CD4^+$ cells in blood than semen [44, 45]. However, two men in the ART^- HIV^+ cohort were extreme outliers. These men, who had very low peripheral blood $CD4^+$ cell counts (40 and 23 $CD4^+$ cells/mm^3 blood), had extraordinarily high concentrations of seminal $CD4^+$ T cells (6.2×10^6 and 2.1×10^6/ml) and macrophages (15.5×10^6 and 24.4×10^6/ml). This dramatic dissociation between $CD4^+$ cell concentrations in blood and genital secretions can occur because mucosal tissues and especially the male genital tract are compartments with distinct immunological microenvironments. Regions of the male genital tract are immunologically privileged sites due to immunological barriers and high concentrations of immunosuppressive factors [7], and immune cell numbers and their activation are usually tightly controlled. However, as the two outlier subjects demonstrate, normal immune regulation in the male genital tract can be disrupted. A number of studies have documented discordance between blood and semen viral load in some subjects [46-48]. Elevated seminal WBC counts are associated with high seminal HIV viral loads [34, 49, 50], and the two individuals in this study with dramatically elevated seminal WBC counts were no exception. It is possible that such men are highly infectious. Factors that have been associated with elevated concentrations of seminal WBCs and HIV include genital infections with organisms such as cytomegalovirus [50] and *Neisseria gonorrhea* [49]. The men in this study with highly elevated seminal WBC counts did not have a documented symptomatic STD, but it is possible that they had an asymptomatic genital infection.

In summary, data from this study demonstrate that $CD4^+$ T cell counts decline in the male genital tract before they decrease in the peripheral circulation of HIV-infected men. This provides evidence that genital tract T cell-dependent acquired immune functions are impaired in HIV-infected men, perhaps rendering them more susceptible to concomitant STD infections that can increase HIV transmission rates. Data from this study also show that treatment with a viral suppressive ART regimen is associated with significant restoration of $CD4^+$ T cells in the genital tract, and thus could improve acquired immune function in the genital tract. Because the relative concentration of seminal macrophages compared to $CD4^+$ T lymphocytes was considerably higher in HIV^+ men regardless of disease stage, macrophages are likely primary HIV host cells in the male genital tract and vectors of HIV transmission. The numbers of WBCs capable of transmitting HIV (macrophages and $CD4^+$ T cells) were reduced overall in semen from HIV^+ men, but were strikingly elevated in a small subset (2 out of 98 ART-naive HIV^+ men in this study). It is possible that men such as these with apparent genital immune activation and elevated HIV titers in semen are highly infectious, and may contribute disproportionately to HIV transmission.

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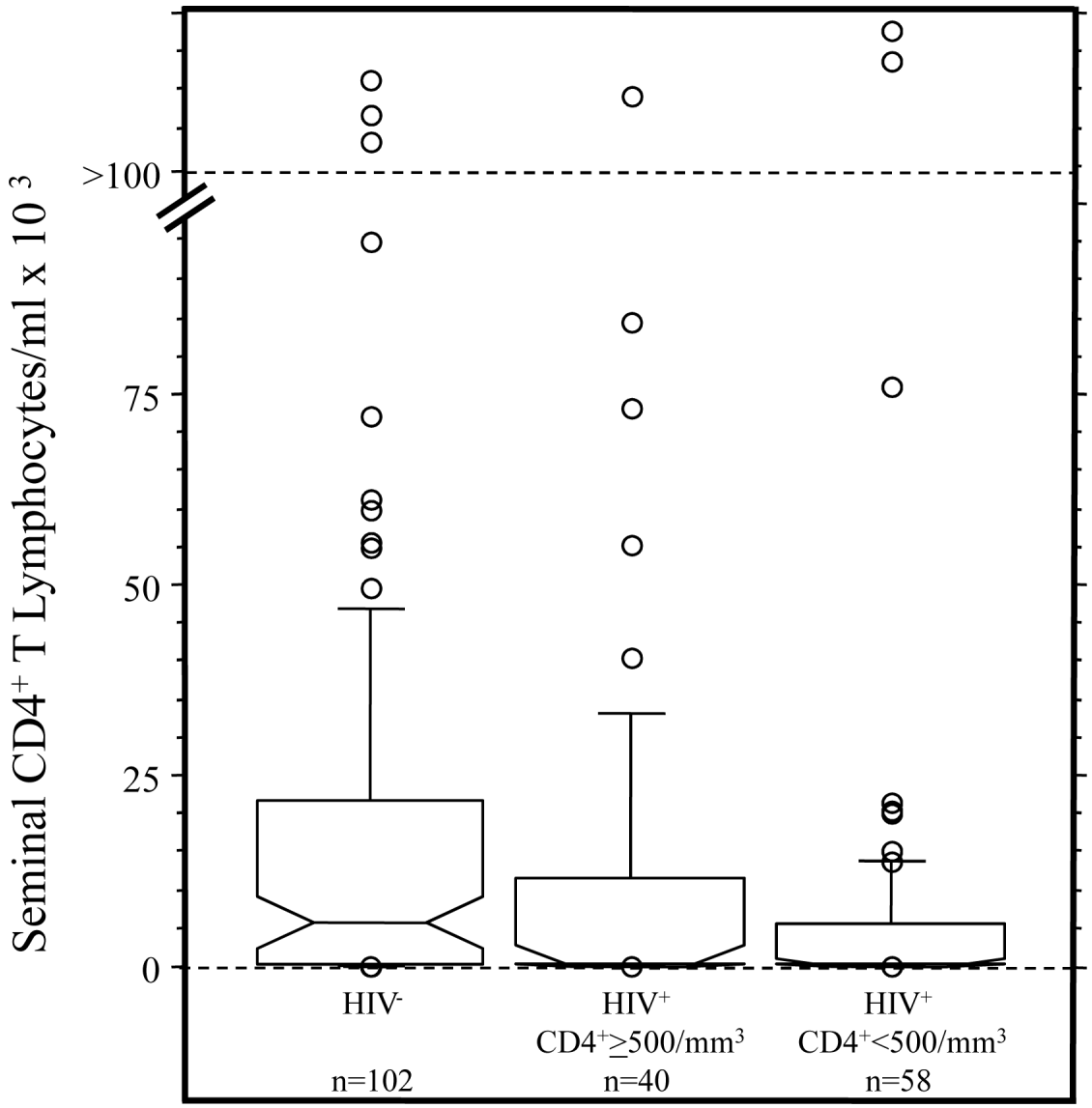

Figure 1.

Notched box plots representing $CD4^+$ T lymphocyte concentrations in semen of HIV^- men and HIV^+ men with peripheral blood $CD4^+$ cell counts ≥ and < 500/mm^3. For each box, the *horizontal lines*, from bottom to top, represent 25th, 50th (median) and 75th percentiles, the *whiskers* delineate the 10th and 90th percentiles, the *notch* defines the 95% confidence interval around the median and the *open circles* identify outlying values. Statistically significant group comparisons: HIV^- vs. $HIV^+ \geq 500/mm^3$ (p=0.0017) and HIV^- vs. $HIV^+ < 500/mm^3$ (p=0.0001).

Table 1

Total White Blood Cells, Macrophages and T Lymphocyte Subpopulations in Semen from HIV-1 Seronegative and ART-naive Seropositive Men

Variable[1]	HIV$^-$ (N=102)	ART$^-$ HIV$^+$ (N=98)	Significance[2]
Total WBCs	243,600 (80,960−612,000)[3]	104,286 (30,000−328,960)	0.0008
Macrophages	53,077 (21,780−152,288)	22,162 (2,284−120,606)	0.0026
T Lymphocytes	11,566 (2,713−27,827)	0 (0−13,815)	0.0001
CD4$^+$ Cells	10,492 (0−27,576)	0 (0−11,250)	0.0001
CD4$^+$ T Lymphocytes[4]	5,700 (0−21,851)	0 (0−5,600)	0.0001
CD8$^+$ T Lymphocytes	1,889 (0−9,261)	0 (0−5,889)	0.0063
IL-2 Receptor-α$^+$ T Lymphocytes	5,855 (0−22,192)	0 (0−122)	0.0001

[1] Per ml semen

[2] Difference between HIV$^-$ and HIV$^+$ groups; Mann-Whitney U test

[3] Median (Interquartile Range)

[4] Number of total T cells minus the number of CD8$^+$ T cells

Table 2

Elevated Concentrations of White Blood Cells in Semen from Two ART^{-} HIV^{+} Men with AIDS

Subject	Total WBCs[1]	Macrophages	CD4^{+} T Lymphocytes[2]	CD8^{+} T Lymphocytes	IL-2 Receptor-α^{+} T Lymphocytes
D096	55,680,000	15,467,000	6,186,665	1,546,666	9,666,663
19950	29,000,000	24,349,055	2,188,676	0	0

[1] Semen variables per ml semen

[2] All T cells minus CD8^{+} cells

Table 3

Effect of Dual Nucleoside and Protease Inhibitor (PI) (Indinavir) Antiretroviral Therapy on WBC Populations in Semen and Blood (n=20)

Variable	Body Fluid	Dual Nucleoside Therapy, Pre PI ART	Dual Nucleoside + Indinavir (6 Mos.)	Significance[1]
Total WBCs	Semen[2]	213,440 (69,008−295,653)[3]	312,000 139,808−567,410)	NS
Macrophages	Semen	77,510 15,518−158,615)	73,470 13,823−307,758)	NS
T Lymphocytes	Semen	12,180 4,955−34,715)	110,000 35,968−200,213)	0.0004
CD4$^+$ T Lymphocytes	Semen	2,610 0−15,060)	67,435 1,640−111,850)	0.001
CD8$^+$ T Lymphocytes	Semen	5,340 700−5,340)	12,000 5,150−45,000)	NS
IL-2 Receptor-α$^+$ T Lymphocytes	Semen	1,380 450−30,485)	10,000 0−43,113)	NS
CD4$^+$ Cells[4]	Peripheral Blood	238 157−425)	314 241−441)	0.05

[1] Difference between pre- and post-PI ART values; Wilcoxon signed ranks test

[2] Semen WBC variables per ml semen

[3] Median (Interquartile Range)

[4] Per mm^3