Association between Culturable Human Immunodeficiency Virus Type 1 (HIV-1) in Semen and HIV-1 RNA Levels in Semen and Blood: Evidence for Compartmentalization of HIV-1 between Semen and Blood

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Both qualitative and quantitative virologic measurements were compared between blood and genital compartments for 128 men infected with human immunodeficiency virus type 1 (HIV-1) to address several controversial issues concerning HIV-1 shedding in semen and to obtain further information about the distribution of virus between these two compartments. Evidence for viral compartmentalization was suggested by earlier studies that noted the poor correlation between blood and seminal virus load, phenotype, and genotype. Further support for this viral compartmentalization was based on the following observations between semen and blood: lack of association between culturability of virus in semen and viral RNA level in blood, discordant distribution of viral phenotypes, discordant viral RNA levels, a weak correlation between viral RNA level in semen and CD4 cell count in blood, differences in the biologic variability of viral RNA levels, and differences in the virus load response to antiretroviral therapy.

Worldwide, sexual contact is the most common mode of human immunodeficiency virus type 1 (HIV-1) transmission, and semen is the body fluid responsible for most instances of this transmission [1]. However, relatively little is known about the biology of HIV-1 within the male genital tract or the factors that determine either levels of virus expression and infectiousness of semen or the variation in these levels over time [2, 3]. HIV-1 nucleic acid (DNA or RNA) is detected in most semen specimens from infected men, but infectious virus is detected by coculture in fewer than half of specimens [4–10]. Because the male reproductive tract is a distinct immunologic compartment, the factors determining infectiousness of semen may differ substantially from the factors determining HIV-1 levels in the blood, lymphatic tissue, or central nervous system [2, 7, 11, 12]. Previous studies used a variety of techniques, such as cocultivation, electron microscopy, and molecular methods, to detect and quantify seminal levels of HIV-1 [4–9, 13–18]. These studies reported a number of contradictory findings, including intermittent HIV-1 shedding, varying culture-positivity rates, and discrepancies between detection of virus by use of mixed lymphocyte coculture or polymerase chain reaction (PCR) amplification methods.

We investigated the relationship between the HIV-1 RNA level in seminal plasma and presence of infectious virus in seminal cells and plasma and compared the variation over time in HIV-1 RNA levels in seminal and blood plasma. Defining these virus parameters would provide insight into the biologic factors responsible for the sexual transmission of HIV-1. Clinically, this information is important for epidemiologic studies, the design of therapeutic trials, and, possibly, reproductive management.

Materials and Methods

Subjects. A cohort of 149 HIV-1-seropositive men was enrolled between April 1994 and August 1995 through an advertisement placed in a local newspaper (50.3%), Seattle’s Harborview Medical Center’s outpatient HIV/AIDS clinic (36.2%), and other AIDS-related programs (13.5%). All subjects were believed to have acquired their HIV infection in the United States and were therefore likely infected with HIV-1 subtype B. Potential subjects had no symptoms of active sexually transmitted diseases other than HIV-1. Subjects either were not receiving or were receiving stable antiretroviral therapy for the duration of the study. Some subjects were enrolled in randomized blinded clinical studies and, therefore, the exact nature of their antiretroviral regimen was unknown. Each subject was asked to provide 3 semen specimens at
4-week intervals and to remain sexually abstinent for 2 days before providing each specimen.

**Semen processing.** Specimens were collected by masturbation at each subject’s home, then transported to the laboratory within 3.5 h for semen analyses (mean time ± SD, 72 ± 40 min; range, 20–210). Semen was diluted 1:1 with culture medium to reduce the viscosity before separation of the seminal plasma and seminal cell pellet by centrifugation at 2940 g for 2–4 min [5]. An aliquot of diluted seminal plasma was stored at −70°C for batched HIV-1 RNA analysis, and if sufficient specimen was available, each fraction was cocultured separately for HIV-1 [5].

**HIV-1 RNA quantitation in seminal plasma.** A reverse transcription (RT)–PCR amplification assay was used to quantify HIV-1 RNA in the cell-free seminal plasma (Amplicor HIV Monitor; Roche Molecular Systems, Branchburg, NJ) [19, 20]. Previous experiments had shown that some seminal plasma specimens inhibited amplification of the internal quantitative assay standard. To evaluate the possible presence of an inhibitor, 1 of 2 aliquots from each specimen was pretreated with silica gel (Sigma, St. Louis) to extract the HIV-1 RNA and remove the putative inhibitor before RT-PCR amplification [21]. Because of the dilution factor before the first centrifugation step, the assay sensitivity was 400 RNA copies/mL of seminal plasma. The HIV-1 RNA copy number per milliliter was reported for undiluted seminal plasma.

**HIV-1 cocultivation and MT-2 tropism in semen.** Our method for qualitative HIV-1 coculture is reported elsewhere [5]. The seminal plasma was filtered (0.45 μm), and the seminal cells were suspended in 2.0 mL of culture medium. Cellular and plasma fractions were cocultured separately with 10^3 to 4-day-old phytohemagglutinin (PHA)-stimulated donor cells in 15 mL of medium containing 5% nonrecombinant human interleukin-2 (Pharmacia, Piscataway, NJ). One-half of the culture medium was exchanged twice each week, and cultures were replenished with 3 × 10^6 new PHA-stimulated donor cells every 7 days for 28 days. HIV-1 production was assessed weekly by demonstrating a sustained HIV-1 p24 antigen increase to >30 pg/mL of culture medium (Abbott Laboratories, Abbott Park, IL). The syncytium-inducing (SI) or non–syncytium-inducing (NSI) phenotype of each virus isolate was assessed by inoculating 50 μL of an unfrozen, infected culture supernatant into duplicate microwells containing 50,000 MT-2 cells [22, 23].

**Blood processing and HIV-1 RNA quantitation in blood plasma.** Blood was collected into Vacutainer ACD (acid-citrate-dextrose) tubes (Becton Dickinson, Franklin Lakes, NJ), then separated into the plasma and peripheral blood mononuclear cell (PBMC) components by ficoll-hypaque gradient centrifugation within 4–6 h [23]. CD4 cell counts were determined by standard flow cytometric methods [24]. Blood plasma was stored at −70°C for batched HIV-1 RNA analyses.

The level of HIV-1 RNA in ACD-anticoagulated blood plasma was measured by the branched DNA (bDNA) assay (Quantiplex; Chiron, Emeryville, CA) [25]. The manufacturer’s standards were run with each assay, and results were reported as HIV-1 RNA copies per milliliter of blood plasma. Blood plasma HIV-1 RNA levels were not corrected for the dilution factor associated with ACD collection tubes.

**Statistical analyses.** Virologic and immunologic markers of disease activity were evaluated by nonparametric methods. Both HIV-1 RNA and CD4 cell counts were transformed to their base 10 logarithms to normalize the data and equalize the variances across the different concentrations of viral RNA. In all analyses except the random-effects models (see below), censoring of RT-PCR amplification values (<400 RNA copies/mL of seminal plasma) and bDNA values (<10,000 RNA copies/mL of blood plasma) was handled by assigning the values of one-half of the cutoff value, that is, 200 (2.30 log) RNA copies/mL and 5000 RNA (3.70 log) copies/mL, respectively. The Mann-Whitney U test, Wilcoxon signed rank test, Kolmogorov-Smirnov test, or Kruskal-Wallis test was used to assess potential associations of seminal HIV-1 RNA levels with categorical variables such as study visit or recovery of virus by coculture. Associations between independent categorical variables were assessed by the χ^2 or Fisher’s exact tests. Pearson product-moment correlation coefficients were used to assess trends in continuous data, Spearman rank correlation coefficients for ordinal data, and Kendall correlation coefficients for ordinal data from multiple independent variables such as antiretroviral therapy and HIV-1 coculture.

A random-effects model was used to estimate the variance components in the semen and blood (after controlling for therapy) and to establish the confidence intervals for the expected variation in HIV-1 RNA levels over the 2 months of observation. To account for the “left-censoring” of HIV-1 RNA levels in seminal and blood plasma, an adaptation of the method described by Pettit was used [26]. The person-to-person and within-person variances were used to calculate the sample size for a two-arm randomized control trial to detect 5-fold differences in seminal and blood plasma HIV-1 RNA levels due to a hypothetical treatment with 80% power and a two-tailed α = .05 [27]. A random effects model that controlled for the repeated measures on individual men was used to derive the P for the association between viral RNA level and recovery of virus by qualitative coculture and also the effect of antiretroviral therapy and blood CD4 cell count on viral RNA level in both semen and blood.

**Results**

**Subjects.** Baseline characteristics for the cohort are summarized in table 1. A total of 161 men were screened for the study. Of these, 12 (7.5%) were lost to follow-up before providing their first specimen. Of the remaining 149 subjects, 13 (8.7%) completed only one visit, 8 (5.4%) completed two visits, and 128 (85.9%) completed all three visits. The demographic characteristics were similar to those of patients with AIDS reported in our community [28]. No subjects reported signs or symptoms of urethritis during the study. To minimize selection bias, the analyses were restricted to the 128 men who completed all three study visits. Twelve (9.4%) of these men were enrolled in blinded clinical trials of combination nucleoside antiretroviral regimens. The baseline characteristics of the 21 men who were excluded from the final analysis were not significantly different from those of the analysis cohort (data not shown). For 91 evaluable men (71.1%), semen volume levels were not corrected for the dilution factor associated with ACD collection tubes.

**HIV-1 RNA levels in seminal plasma by RT-PCR analysis.** Amplification of the internal quantitative assay standard was
Table 1. Characteristics of 149 HIV-1–seropositive men who enrolled in study of HIV-1 compartmentalization.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years (SD)</td>
<td>36.2 (7.0)</td>
</tr>
<tr>
<td>White</td>
<td>81</td>
</tr>
<tr>
<td>Risk factors for HIV infection</td>
<td></td>
</tr>
<tr>
<td>Male-to-male sex only</td>
<td>81</td>
</tr>
<tr>
<td>Injection drug use only</td>
<td>5</td>
</tr>
<tr>
<td>Both of above</td>
<td>11</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
<tr>
<td>CDC clinical stage</td>
<td></td>
</tr>
<tr>
<td>A (asymptomatic)</td>
<td>33</td>
</tr>
<tr>
<td>B (symptomatic)</td>
<td>42</td>
</tr>
<tr>
<td>C (AIDS)</td>
<td>25</td>
</tr>
<tr>
<td>Baseline CD4 T cell count, median cells/μL (range)</td>
<td>338 (0–1034)</td>
</tr>
<tr>
<td>≥500</td>
<td>32</td>
</tr>
<tr>
<td>200–499</td>
<td>39</td>
</tr>
<tr>
<td>&lt;200</td>
<td>29</td>
</tr>
<tr>
<td>Baseline HIV-1 RNA median log copies/mL (range)</td>
<td></td>
</tr>
<tr>
<td>Blood plasma (n = 124; bDNA assay)</td>
<td>4.434 (4.0–5.722)</td>
</tr>
<tr>
<td>Seminal plasma (n = 117; RT-PCR assay)</td>
<td>3.287 (2.602–6.855)</td>
</tr>
<tr>
<td>Antiretroviral (nucleoside) use at entry</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>63</td>
</tr>
<tr>
<td>1 agent</td>
<td>26</td>
</tr>
<tr>
<td>2 or more agents</td>
<td>11</td>
</tr>
<tr>
<td>Ejaculations before semen specimen collection, median no. (range)</td>
<td></td>
</tr>
<tr>
<td>In last 24 hours</td>
<td>0.0 (0–6)</td>
</tr>
<tr>
<td>In last week</td>
<td>3.0 (0–45)</td>
</tr>
<tr>
<td>Semen volume, median mL (range)</td>
<td>1.7 (0.1–4.9)</td>
</tr>
<tr>
<td>Sex partners in 30 days before providing any semen specimen, median no. (range)</td>
<td>1.0 (1–27)</td>
</tr>
</tbody>
</table>

NOTE. Unless indicated, data are % of subjects. bDNA, branched-chain DNA assay; RT-PCR, reverse transcriptase–polymerase chain reaction amplification assay; CDC, Centers for Disease Control and Prevention.

inhibited in 149 (41.4%) of 360 available specimens across the full range of HIV-1 RNA levels (data not shown). Of these 149 inhibited specimens, 127 (85.2%) had sufficient volumes to permit pretreatment with silica gel to remove the putative inhibitor and to be reassayed, thus giving a total of 338 evaluable specimens with amplification of the internal quantitative standard. Of 128 men, 104 (81.3%) had each of their 3 semen specimens assessed for HIV-1 RNA without silica gel extraction of the viral RNA before RT-PCR amplification. The semen from 17 (16.3%) of these men consistently inhibited the internal assay standard, the semen from 29 (27.9%) men inhibited inconsistently, and the semen from the remaining 58 (55.8%) men never inhibited the internal quantitative assay standard.

For untreated semen specimens with amplification of the internal quantitative assay standard, silica gel extraction did not affect the quantitation of HIV-1 RNA (data not shown).

The median (range) HIV-1 RNA level was 3.189 (2.602–7.205) log copies/mL for 338 seminal plasma specimens from 128 men. HIV-1 RNA was detected at ≥400 (or 2.602 log) copies/mL of seminal plasma in 204 (60.4%) of 338 semen specimens with internal quantitative assay standard amplification and at least once in 101 (78.9%) of the 128 men. The median (range) HIV-1 RNA level for the 204 specimens was 3.762 (2.617–7.205) log copies/mL. Among the 84 men who provided 3 semen specimens with a suitable volume for viral RNA analysis, HIV-1 RNA was never detected in 20 (23.8%), detected only once in 16 (19.1%), detected twice in 17 (20.2%), and detected all three times in 31 (36.9%) subjects. Among the 37 men who provided 2 suitable specimens, HIV-1 RNA was not detected in 6 (16.2%), detected once in 10 (27.0%), and detected twice in 21 (56.8%) subjects. Among the 7 (5.5%) men who provided only 1 suitable specimen, HIV-1 RNA was detected in 6 (85.7%).

Because there was insufficient seminal plasma volume to use in the bDNA assay, we compared the two assay methods using HIV-1–spiked pooled seronegative seminal plasma provided by the National Institute of Allergy and Infectious Diseases Division of AIDS–sponsored Virology Quality Assurance Program. When nominal 10-fold concentrations of viral RNA from 3 to 6 log copies/mL were used, the measured viral RNA levels in seminal plasma were ~2.5-fold higher by the RT-PCR assay and 2.0-fold lower by bDNA assay. The intraassay variation (SD) for HIV-1 RNA measurement by use of the RT-PCR assay was 0.16 log copies/mL of seminal plasma (data not shown). The relationship between the two assays for detecting viral RNA in seminal plasma was described by the following equation: RT-PCR log copy number/mL = 0.987(bDNA log copy number/mL) + 0.716 (Pearson correlation coefficient, r² = .986).

HIV-1 RNA level in blood plasma by bDNA analysis. The median (range) HIV-1 RNA level for 338 plasma specimens was 4.436 (4.0–5.967) log copies/mL by use of the bDNA assay. HIV-1 RNA was detected at ≥10,000 (or 4.0 log) copies/mL of blood plasma in 251 (74.3%) of 338 specimens for which the median (range) HIV-1 RNA level was 4.627 (4.017–5.967) log copies/mL.

HIV-1 RNA variation in seminal and blood plasma. The within-person variation for HIV-1 RNA level in either seminal or blood plasma was determined by plotting the difference between versus the average of the viral RNA measurements for the first and second visits (figure 1). The relationship between the two variables was not significant for either semen or blood (P = .73 vs. P = .51), and the median differences were centered around zero, indicating stability of viral RNA variability over the range of viral RNA concentrations. The within-person viral RNA variation (SD) was 0.812 log copies/mL of seminal plasma versus 0.368 log copies/mL of blood plasma. We expected these values to underestimate the true variation because of the left-censoring that artificially reduced differences associated with viral RNA measurement near the lower boundary of detection for each assay. This effect accounted for the arrowhead shape in the difference plots. Therefore, we used random effects modeling (see Materials and Methods), controlling for antiretroviral therapy, to account for
Table 2. Random-effects modeling of expected within-person HIV-1 RNA variation in seminal and blood plasma.

<table>
<thead>
<tr>
<th>No. of times sampled</th>
<th>95% confidence interval for expected within-person HIV-1 RNA variation (log copies/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminal plasma</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.24</td>
</tr>
<tr>
<td>2</td>
<td>1.58</td>
</tr>
<tr>
<td>3</td>
<td>1.29</td>
</tr>
<tr>
<td>4</td>
<td>1.12</td>
</tr>
<tr>
<td>Blood plasma</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.70</td>
</tr>
<tr>
<td>2</td>
<td>0.49</td>
</tr>
<tr>
<td>3</td>
<td>0.40</td>
</tr>
<tr>
<td>4</td>
<td>0.35</td>
</tr>
</tbody>
</table>

NOTE. 95% confidence interval was calculated as $\pm 1.96 \times \sqrt{\text{variance}/\text{no. of samples}}$. Data analysis was based on 338 specimen pairs of semen and blood obtained from 128 men over 2-month interval. Within-person HIV-1 RNA variance (log copies/mL) was 0.6513 for semen and 0.0632 for blood.

The expected within-person variation in seminal and blood plasma, after controlling for therapy, is shown in table 2. To show the utility of these variation measures for assessing the effect of a hypothetical therapy on HIV-1 RNA level, we determined the sample size required to detect a 5-fold treatment difference in either seminal or blood plasma HIV-1 RNA levels (table 3).

Table 3. Sample size calculations for hypothetical, 2-arm, randomized, controlled treatment trial designed to detect 5-fold difference in seminal or blood plasma HIV-1 RNA levels, with 80% power and 2-tailed $\alpha = .05$.

<table>
<thead>
<tr>
<th>No. of samples per person</th>
<th>No. of persons per study arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminal plasma</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>67</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
</tr>
<tr>
<td>Blood plasma</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
</tr>
</tbody>
</table>

NOTE. Sample size was calculated as follows: HIV-1 RNA level person-to-person variance (log copies/mL) was 1.4429 for semen and 0.3329 for blood and within-person variance was 0.6513 for semen and 0.0632 for blood; $\rho = (\text{between person variance}/\text{total variance})$; variance of mean of $k$ specimens collected from each person $= (\text{total variance}/k) \times [1 + (\rho \times (k - 1))]$; sample size $= [(1.96 + 0.84)^2 \times (2 \times \text{variance})]/(\text{log 5})$, where 0.84 is normal deviate corresponding to 80% power and 1.96 is 0.975 percentile of standard normal distribution corresponding to 2-tailed $\alpha = .05$ [27].
**HIV-1 coculture of semen.** HIV-1 was cultured from the seminal cell pellet at least once from 20 (17.4%) of 115 evaluable men and from 27 (10.8%) of 251 evaluable semen specimens. HIV was isolated by coculture from the seminal plasma fraction from 5 (2.0%) of 249 evaluable specimens. The virus was cultured equally from either the cell pellet or seminal plasma from subjects with or without the seminal plasma inhibitor (data not shown). Three specimens were concordant and 26 were discordant for recovery of virus from both seminal pellet and plasma. 220 specimens were discordant for no recovery of virus. Because of limited specimen volumes, 87 (25.7%) of 338 specimens were assessed only for HIV-1 RNA copy number. The distribution of HIV-1 RNA copy numbers for the specimens without coculture was similar to the distribution of HIV-1 RNA copy numbers for the 251 semen specimens submitted for coculture (data not shown).

HIV-1 was cocultured from the seminal cell pellet or plasma once from 17 (14.8%) of 115 men with 1 or more cultures, twice from 3 (5.6%) of 54 men with 2 cultures each, and three times from 2 (4.9%) of 41 men who had 3 cultures each. The median HIV-1 RNA level in seminal plasma was significantly greater for culture-positive compared with culture-negative semen specimens (figure 2). At the first study visit, 10 men were HIV-1 coculture-positive from either the seminal cell pellet (9 men) or plasma (1 man), and 73 men were negative; the corresponding median (interquartile range) HIV-1 RNA log copies/mL of seminal plasma was 5.315 (4.121–6.06) and 3.144 (2.301–3.749), respectively (Mann-Whitney U test, \( P < .001 \))

**Figure 2.** HIV-1 RNA level in seminal plasma compared with detection of HIV-1 by mixed lymphocyte coculture of seminal cell pellet for 24 culture-positive and 208 culture-negative semen specimens from 113 evaluable men. Median HIV-1 RNA level was 5.05 log copies/mL (interquartile range, 4.10–6.03) for coculture-positive versus 2.93 log copies/mL (2.30–3.71) for coculture-negative specimens (random effects model, \( P < .001 \)). Data are median and interquartile range; bars, 10th and 90th percentiles; ○, outlying values; dotted line, lower limit of detection at 400 (or 2.60 log) RNA copies/mL, below which values were censored and assigned arbitrary value of 200 (or 2.30 log) HIV-1 RNA copies/mL for analysis.

Four (4.2%) of the 95 men with 2 or more specimens had HIV-1 cultured from all 10 of their seminal cell pellets (termed persistent shedders) and 14 (14.7%) men had virus cocultured only once from 38 cell pellet specimens (termed intermittent shedders). At study entry, the median (interquartile range) HIV-1 RNA log copies/mL for the 4 men with persistent shedding was significantly greater than that for the 14 men who had intermittent shedding (5.653 [4.83–6.131] vs. 3.745 [2.848–4.155]; Mann-Whitney U test, \( P < .001 \)).

For the 82 subjects with both coculture and seminal plasma HIV-1 RNA results at the first study visit, 8 (88.9%) of 9 coculture-positive men and 44 (60.3%) of 73 coculture-negative men had HIV-1 RNA detected in their seminal plasma (Fisher’s exact test, \( P = .14 \)). The recovery of HIV-1 from semen by coculture at study entry was associated with the seminal plasma HIV-1 RNA level (Kolmogorov-Smirnov test, \( P = .025 \)). To investigate this association further, the seminal plasma HIV-1 RNA levels for 232 specimens from 113 men with matching viral coculture results were divided into deciles for the coculture-positive and -negative specimens. Only 1 (4.2%) of 24 coculture-positive specimens was associated with a seminal plasma HIV-1 RNA level <2.80 log RNA copies/mL. Ten (71.4%) of 14 specimens with a seminal plasma HIV-1 RNA level >5.30 log RNA copies/mL and 8 (88.9%) of 9 specimens with >5.80 log RNA copies/mL were coculture-positive.

MT-2 cell tropism was determined for 25 of 27 virus isolates from semen. Twenty-one isolates were from the seminal cell pellet from 16 men and 4 isolates were from the seminal plasma of 3 men, 2 of whom also had virus isolated from the seminal cell pellet. For the seminal cell pellet fractions, 7 (33%) contained SI and 14 (67%) contained NSI isolates. For the 4 virus isolates from seminal plasma, 2 were SI and 2 were NSI phenotypes, and the phenotypes were concordant for seminal cell pellet- and plasma-associated virus. For 22 seminal virus isolates, the corresponding virus from PBMC was also characterized for SI or NSI phenotype. The viral phenotypes from semen and blood were concordant for 16, or 73% (4 SI and 12 NSI), and discordant for 6, or 27% (3 SI and 3 NSI). The seminal plasma HIV-1 RNA levels (median and interquartile range) were not different for either SI (\( n = 7 \); 6.060 [3.893–6.237] log copies/mL) or NSI (\( n = 13 \); 5.176 [4.130–5.995] log copies/mL) phenotypes (Mann-Whitney U test, \( P = .78 \)). A similar result was observed for the blood-derived HIV-1 RNA levels and virus phenotype (data not shown).

**CD4 cell count in blood and virologic comparisons between blood and semen.** The CD4 cell count in peripheral blood at study entry showed a weak inverse correlation with the seminal plasma HIV-1 RNA level (Spearman rank correlation coefficient, \( \rho = - .246 \); \( P = .024 \)) (figure 3A). There was also a weak correlation between HIV-1 RNA levels in blood plasma and HIV-1 RNA levels in the seminal plasma for matched specimens from 114 men at visit one (Spearman rank correlation coefficient, \( \rho = .265 \); \( P = .005 \)) (figure 3B). These findings
Figure 3. Correlation between seminal plasma HIV-1 RNA level, peripheral blood CD4 cell count, and blood plasma HIV-1 RNA level at study entry. A, Seminal HIV-1 RNA level and blood CD4 cell count for 85 men (Spearman rank correlation coefficient, \( \rho = -0.246; P = 0.024 \)). B, HIV-1 RNA level in seminal plasma compared with blood plasma for 114 men (Spearman rank correlation coefficient, \( \rho = 0.265; P = 0.005 \)). Left-censored branched-chain DNA-derived values for blood plasma HIV-1 RNA (<10,000 copies/mL) were assigned value of 5000 copies/mL (3.70 log) and reverse transcription–polymerase chain reaction amplification–derived values for seminal plasma (<400 copies/mL) were assigned value of 200 copies/mL (2.30 log) for analysis.

Contrasted with the stronger inverse correlation between CD4 cell count and blood plasma HIV-1 RNA level (Spearman rank correlation coefficient, \( \rho = -0.447; P < 0.001 \)). In contrast to the seminal plasma HIV-1 RNA level, the recovery of HIV-1 from the semen by coculture was not associated with either the blood plasma HIV-1 RNA level or the CD4 cell count (Kolmogorov-Smirnov test, \( P = 0.36 \) and \( P = 0.39 \), respectively).

Effect of antiretroviral therapy. There was a trend for men who reported receiving combination antiretroviral therapy at the first visit to have lower HIV-1 RNA levels in their seminal plasma but not in blood plasma (figure 4). The random-effects model was used to evaluate the treatment effect for all 338 paired specimens of semen and blood. The therapy comparisons for seminal plasma HIV-1 RNA levels (mean difference RNA log copies per milliliter) were as follows: monotherapy less than no therapy (−0.18 log; \( P = 0.64 \)); combination therapy less than no therapy (−0.57 log; \( P = 0.05 \)); combination therapy less than monotherapy (−0.39 log; \( P = 0.18 \)). The therapy comparisons for blood plasma HIV-1 RNA levels were as follows: monotherapy greater than no therapy (0.12 log; \( P = 0.10 \)); combination therapy less than no therapy (−0.13 log; \( P = 0.26 \)); combination therapy less than monotherapy (−0.25 log; \( P = 0.023 \)). HIV-1 was cultured from the seminal cell pellet at the first study visit from 7 (11.7%) of 60 subjects who received no therapy, 2 (9.5%) of 21 subjects who received monotherapy, and none of 7 subjects who received combination therapy (\( \chi^2; P = 0.62 \)). The mean CD4 cell counts per microliter for evaluable subjects in each therapy group at the first study visit were as follows: no therapy, 389 (SD, 260; \( n = 64 \)); monotherapy, 302 (SD, 199; \( n = 24 \)); and combination therapy, 286 (SD, 125; \( n = 7 \)) (Kendall correlation coefficient, \( \tau = 1.677; P = 0.094 \)).

A multivariate analysis using the random effects model was done to assess the independent effects of therapy and CD4 cell count on seminal and blood plasma HIV-1 RNA levels. For
semenal plasma ($n = 276$ specimens with matched viral RNA and CD4 values), antiretroviral therapy (after adjusting for CD4 count) was associated with a 0.49 log decline in RNA copies/mL ($P = .04$), and a 100-cell increase in blood CD4 cell count (after adjusting for therapy) was associated with a 0.16 log decline in viral RNA log copies/mL ($P < .001$). After similar adjustments for blood ($n = 282$ specimens with matched values), antiretroviral therapy was associated with a 0.038 log decline ($P = .68$), and a 100-cell increase in CD4 cell count was associated with a 0.093 log decline in RNA copies/mL ($P < .001$). After adjusting for both antiretroviral therapy and CD4 cell count, the within-person variation in viral RNA log copies/mL was 0.84 for seminal plasma and 0.25 for blood plasma. These numbers are consistent with the within-person HIV-1 RNA level variation obtained using data from all 338 pairs of seminal and blood plasma specimens.

**Discussion**

We compared both qualitative and quantitative virologic measurements between the blood and genital compartments to address several controversial issues concerning HIV-1 shedding in semen and to obtain further information about the distribution of virus between these two compartments. Evidence for viral compartmentalization was suggested by earlier studies that noted the poor correlation between blood and seminal virus load, phenotype, and genotype [8, 10, 12, 29–31]. We found further support for this viral compartmentalization based on...
the following observations between semen and blood: lack of association between culturability of virus in semen and viral RNA level in blood, discordant distribution of viral phenotypes, discordant viral RNA levels, a weak correlation between viral RNA level in semen and CD4 cell count in blood, differences in the biologic variability of viral RNA levels, and differences in the viral load response to antiretroviral therapy.

Culturable virus in semen was strongly associated with the viral RNA level in semen. As such, the HIV-1 RNA level in the seminal plasma might be used to estimate potential infectiousness of semen. For example, men with persistently culturable HIV-1 in their semen had the highest seminal plasma virus levels, with a median of 1,023,000 (6.01 log) RNA copies/mL. However, the broad range of HIV-1 RNA levels associated with the recovery of culturable virus from either the seminal cell pellet or seminal plasma (or both) underscores the importance of other virologic and host factors that determine detection of virus by mixed lymphocyte coculture and, by inference, virus that is replications “fit” for sexual transmission [10].

In this regard, the male reproductive tract is similar to the blood compartment, in which higher HIV-1 RNA levels are associated with increased rates of HIV-1 culture positivity in the blood plasma [32] and a greater risk for percutaneous transmission [33]. However, unlike the situation in the blood compartment, the vast majority of infectious virus in semen is associated with the cell pellet fraction and only rarely with the seminal plasma despite very high cell-free viral RNA levels. Although we have no direct evidence regarding the influence of seminal HIV levels on sexual transmission, there is indirect evidence to support such a relationship from studies correlating viral RNA levels in blood with the likelihood of sexual transmission [34, 35].

Our study was hampered somewhat by the largely unknown aspects of the biology of semen from HIV-infected men that affect the semen volume and the detection of both viral nucleic acid by RT-PCR amplification and infectious virus by coculture [29, 36–38]. We found an inhibitor of the RT-PCR amplification assay in the semen of 44% of men; this extends a similar observation by Dyer et al. [30]. Although the mechanism for this inhibition is unknown, semen contains a number of inhibitory antimicrobial compounds, such as exceptionally high levels of zinc, that specifically inhibit some viral enzymes [39]. Despite the use of silica gel to remove viral RNA from this inhibition, viral RNA was not detected (at ≥400 RNA copies/mL) in ~20% of men studied. Importantly, the seminal plasma inhibitor of the RT-PCR amplification assay did not appear to influence the detection of HIV by coculture. Therefore, the failure to detect virus from the semen by coculture despite high levels of seminal plasma HIV-1 RNA in some men may represent the presence of defective virus, effect of therapy on virus infectivity, insensitivity of the coculture assay, effect of the genital tract immune response, semen-specific toxicity to or inhibition of either the virus or the mixed-lymphocyte coculture (or both), or compartmentalization of infectious virus within the cell or cell-free compartments of the genital tract [7]. The mechanism for this discordance between viral RNA levels and recovery of infectious virus by coculture requires further study.

There was no apparent association between the SI or NSI viral phenotypes and seminal plasma HIV-1 RNA levels. This complements the work of Vernazza et al. [10] and further supports the lack of association between the virus’s MT-2 tropism and viral RNA levels reported for blood [40], and the 27% discordance in phenotype between blood and semen offers additional support for the compartmentalization of virus [12].

Although there was a weak correlation between HIV-1 RNA levels in the blood plasma measured by the bDNA assay and HIV-1 RNA levels in the seminal plasma measured by the RT-PCR assay, neither the blood levels of HIV-1 RNA nor the CD4 cell count reliably predicted either shedding of culturable virus or detection of viral RNA in semen. These observations fit with our earlier work as well as data from some, but not all, other reports [2, 15, 30, 41]. The clinical implication of these findings is that markers of systemic HIV infection, such as CD4 cell count or HIV-1 RNA level in the blood plasma, cannot be used alone to accurately predict HIV-1 RNA levels in the semen. As such, these findings support the current recommendations to use condoms for all sexual exposures despite low viral RNA levels in either blood or semen. The discordance between very low seminal HIV-1 RNA levels and much higher blood plasma HIV-1 RNA levels in some men is further evidence for the virologic separation between the genital and blood compartments [7, 8, 42]. This discordance was not explained by differences between the two viral RNA assays used in our study, given the high correlation between the RT-PCR and bDNA assays and the similar intraassay variabilities between blood and seminal plasma noted for RT-PCR [40, 43, 44].

There was a larger variation in seminal plasma HIV-1 RNA levels between men than there was within individual men. This finding offers an explanation for the lack of association between the number of unprotected sexual contacts and the probability of infection [45, 46]. However, even though HIV-1 RNA levels in seminal plasma were relatively stable in individuals throughout the 2-month study period, there was 4-fold more variation in the HIV-1 RNA levels in seminal plasma than in blood plasma. Therefore, for the individual man, multiple semen specimens are required to obtain a reliable estimate of the seminal plasma RNA copy number. Longer-term studies are needed to determine if seminal plasma viral RNA levels are stable over many months, or perhaps years, in individual men and the factors that influence these levels: for example, systemic illness, antiretroviral therapy, male genital tract inflammation, reactivation of genital herpes virus infections, and seminal shedding of cytomegalovirus or other viruses [2, 15, 41, 47–51]. With these caveats, one potential implication of our study is that measuring the seminal plasma HIV-1 RNA level may prove useful in counseling couples (who wish to have children) about the likelihood of HIV-1 transmission to the
female when the male is seropositive and the female is seronegative.

Our data suggest that variation in virus load may be responsible for the intermittent shedding of culturable HIV-1 from semen [2]. In our earlier studies, infectious virus was recovered from 11 (32%) of 34 men [5], while another study reported recovery of virus in 10 (55%) of 18 men [10]. We have shown previously that concurrent cytomegalovirus shedding was associated with higher shedding rates of culturable HIV-1 in semen, again suggesting that factors other than HIV-1 RNA level determine the presence of culturable HIV-1 in semen and, indirectly, the likelihood of HIV-1 sexual transmission [2, 34, 48, 52, 53].

This study has important implications for the design of clinical studies to evaluate new antiretroviral therapies. For men with 3 semen samples taken before and after therapy, only changes in HIV-1 RNA level of > 1.29 log (19-fold) could be measured reliably over the short term; this contrasts with a 0.40-log (2.5-fold) change for blood plasma [54–57]. Estimates of sample sizes necessary to assess the effect of antiviral therapy on the seminal plasma HIV-1 RNA level could be determined by use of the person-to-person HIV-1 RNA variation and the within-person HIV-1 RNA variation. For example, in our sample-size calculations for a hypothetical two-arm, randomized study, a 5-fold-larger sample size was required to demonstrate 0.7-log (5-fold) changes in HIV-1 RNA level in seminal plasma compared with blood plasma. Because the person-to-person variation was large relative to the within-person variation, taking more than 2 blood or semen samples per subject did not reduce the sample-size estimate by very much. Such estimates of the total (assay plus biologic) variation in HIV-1 RNA will also prove useful for the design of epidemiologic studies of the sexual transmission of HIV-1 [46].

Our findings strongly support a more comprehensive approach to assessing the impact of antiretroviral therapy on the different HIV-1–infected compartments (i.e., blood, genital tract, and possibly lymphoid and central nervous system), as long-term changes in virus load in one compartment may not indicate comparable changes in the others [42, 58]. The reported use of combination antiretroviral therapy compared with monotherapy was associated with significantly lower viral RNA levels in seminal plasma and contrasted with nonsignificant changes in blood plasma viral RNA levels. However, the culturability of HIV-1 from the seminal cell pellet was not decreased significantly by antiretroviral therapy. These data extend and support our earlier report about the limited effectiveness of monotherapy to reduce HIV-1 shedding from the genital tract [29] but contrast with the results of Anderson et al. [15]. Knowledge of these compartmentalized responses to antiretroviral therapy is important for understanding the pathogenesis of HIV-1 in the male genital tract and, together with complementary studies in the female [42, 59], will provide a better understanding of the determinants of HIV-1 sexual transmission [46].

There were specific limitations to our study. First, our observational period of 2 months was short, and conclusions about the relationship between HIV-1 RNA levels and infectiousness (culturability) cannot be generalized for longer time intervals. Second, other than the direct measurement of culturable virus from the semen, we can only infer the impact of viral RNA level on the potential for sexual transmission of HIV-1; furthermore, the insensitivity of coculture may have limited our power to detect associations between culturability, viral RNA levels, and our inferences about sexual transmissibility. Third, the effect of antiretroviral therapy on virus levels in semen and blood at study entry was observational, relied on the self-reporting of therapy by the subjects, and clearly illustrates the need for prospective studies to address this important issue [14, 60, 61].

In conclusion, there was only a weak correlation between viral RNA levels in seminal and blood plasma. Viral RNA levels in seminal plasma were more variable than were those in blood plasma. Detection of culturable HIV in semen correlated strongly with the HIV-1 RNA levels in cell-free seminal plasma but only weakly with the blood plasma HIV-1 RNA level and CD4 cell count. These observations help resolve some important controversies concerning HIV-1 shedding in semen, confirm the compartmentalization of HIV-1 between the blood and the genital tract, and hold important implications for patient counseling and the design of clinical trials and epidemiologic studies of HIV-1 sexual transmission.

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