Plasma Viral Load in HIV-1 and HIV-2 Singly and Dually Infected Individuals in Guinea-Bissau, West Africa

Significantly Lower Plasma Virus Set Point in HIV-2 Infection Than in HIV-1 Infection

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Background: The intriguing differences in the natural course, transmissibility, and epidemiological characteristics of human immunodeficiency virus type 1 (HIV-1) and HIV-2 are still insufficiently explained. Differences in plasma viral load are an obvious possibility, but this has been difficult to investigate because of the lack of tests for HIV-2 RNA.

Objective: To compare plasma HIV RNA load between individuals infected with HIV-1 and HIV-2 in Guinea-Bissau, a West African country with high prevalence and incidence of HIV-1 and HIV-2 infection.

Methods: A total of 102 participants were recruited from ongoing prospective cohort studies. These included 19 HIV-1 and 29 HIV-2 seroincident cases tested at a median of less than 2 years after seroconversion as well as seroprevalent cases with single (9 HIV-1 cases and 31 HIV-2 cases) or dual (n=14) infections. Plasma HIV RNA levels were determined by a commercial HIV-1 assay and an experimental HIV-2 assay based on the same principles.

Results: The viral set point, ie, the semi-equilibrium reached after seroconversion, was 28-fold lower in recent HIV-2 seroconverters than in recent HIV-1 seroconverters (median, 2500 and 70,000 RNA copies per milliliter, respectively; P<.001). This difference appeared to persist to symptomatic stages of the diseases. Dually infected individuals had lower plasma HIV-1 RNA levels than singly infected individuals.

Conclusions: The differences between HIV-1 and HIV-2 infection are likely to be caused by differences in plasma viral set point and load, but the mechanisms through which HIV-2 infection is contained to a higher degree than HIV-1 remain to be identified.

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PATIENTS AND METHODS

PATIENTS AND SAMPLES

Blood samples in EDTA were obtained from 102 individuals participating in research cohorts in Guinea-Bissau: 33 from a tuberculosis cohort study (H.N., S.B., Z.D., S.A., Tuija Koivula, MPH, and G.B., unpublished data, 1997) and 69 from an occupational cohort. For the former cohort, patients with tuberculosis were recruited and followed up during the course of treatment for their tuberculosis infection, including an examination after completion of treatment. The occupational cohort was set up among the police officers in Guinea-Bissau for a study of natural course of HIV-1 and HIV-2 infections and determinations of basic epidemiological correlates of these infections.

Table 1 gives details regarding the samples included in the study presented herein. Briefly, our study included 28 HIV-1–infected individuals (19 of whom represented seroincident cases, ie, with known approximate dates of seroconversion), 60 HIV-2–infected individuals (29 seroincident cases, and 14 dually infected individuals. Follow-up samples were available from 6 HIV-1–infected persons (of whom 6 represented seroincident cases), 11 HIV-2–infected persons (of whom 6 represented HIV-2 seroincident cases), and 4 dually infected individuals. None of the patients was receiving antiretroviral therapy.

Plasma drawn in specimen tubes in EDTA was frozen at −20°C as soon as possible (no later than 6 hours) after collection. The samples were kept frozen during transport from Guinea-Bissau to Sweden for analysis. In Sweden all samples were kept at −70°C. All samples were treated similarly; there were no differences between HIV-1– and HIV-2–positive samples regarding the time they were kept at −20°C before transport to Sweden. Seroconversion date was estimated as the midpoint between the last HIV-negative and the first HIV-positive specimen.

Ethical clearance was obtained from the ethics committee of the Karolinska Institute, Stockholm, Sweden, and from the Ministry of Health, Guinea-Bissau.

ANTIBODY TESTING AND DIAGNOSTIC POLYMERASE CHAIN REACTION

All samples included in the study were screened by the routine diagnostic strategy at the National Public Health Laboratory, Bissau, Guinea-Bissau, as described.

This included screening with the Behring Enzygnost anti-HIV-1/2 enzyme-linked immunosorbent assay (Behring, Marburg, Germany; until 1995) or the Behring Enzygnost anti-HIV-1/2 Plus enzyme-linked immunosorbent assay (Behring; from 1995 until present). Findings were confirmed at the National Public Health Laboratory according to an alternative confirmatory strategy including a combination of rapid simple assays, However, for this study, all samples were also tested by Western blot for HIV-1 (Genelabs Diagnostics HIV blot 2.2; Genelabs Diagnostics, Science Park, Singapore) and HIV-2 (in house).

The HIV-1 and HIV-2 were further differentiated by testing with an enzyme-linked immunosorbent assay incorporating separate synthetic peptides for HIV-1 and HIV-2 (Pepti-LAV 1-2; Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) according to a strategy described previously. The criteria for Western blot interpretation recommended by the World Health Organization were applied, requiring reactivity by at least 2 cm-hands for a positive result. Dual serologic reactivity was determined by positive results for both HIV-1 and HIV-2 by Western blot as well as the enzyme-linked immunosorbent assay designed for differentiation between HIV-1 and HIV-2 (Pepti-LAV 1-2).

Results for both HIV-1 and HIV-2 by Western blot as well as the enzyme-linked immunosorbent assay designed for differentiation between HIV-1 and HIV-2 (Pepti-LAV 1-2),...
QUANTIFICATION OF PLASMA HIV-1 AND HIV-2 RNA LEVELS

Plasma HIV-1 RNA level was quantified with a commercial assay (Amplicor HIV-1 Monitor Assay, version 1.5; Roche Diagnostic Systems, Branchburg, NJ) according to the recommendations by the manufacturer. The assay was run according to the ultrasensitive protocol, but the sample volume was reduced from 500 μL of plasma to 200 μL because sample volumes were limited. When this setup is used, the assay is considered to have a lower limit of detection of 125 RNA copies per milliliter.

Plasma HIV-2 RNA levels were quantified by means of a new assay developed by Roche Diagnostics Systems according to the same principle as the HIV-1 assay. The HIV-2 assay used the primers LTR3 (upstream; 5'-GCTGGCAGAT-TAGGCCCCTGAGGAGTT-3') and RAR04 (downstream; 5'-GAAATCCACAGCAGGACTAGGAGAT-3'), which amplify a 202–base fragment in the HIV-2LTR region, and the capture probe RAR05 (5'-TGGCTGTTC-CCTGCTAGACTCTCACCAGTACT-3'). For reverse transcription the reaction mixes were incubated for 2 minutes at 50°C followed by 30 minutes at 60°C. The cycling profile was 10 seconds at 95°C, 10 seconds at 55°C, and 10 seconds at 72°C for 4 cycles, after which the annealing temperature was increased to 60°C for the remaining 30 cycles. All other aspects of the assay, including the lower limit of detection, were identical to those of the HIV-1 assay. The HIV-2 assay incorporates an internal quantitation standard that contains the HIV-2 primer binding sites but has a different intervening region that allows for differentiation from the target. The quantitation standard generates an amplified product that is of the same size and base composition as the HIV target.23 Negative and positive controls were included in each run. The positive control consisted of an HIV-2–seropositive plasma that had been aliquoted in small volumes and stored at −70°C. The log mean and SD of the positive control was 3.14 ± 0.06 HIV-2 RNA copies per milliliter in 9 consecutive analyses carried out during a period of more than 6 months. Negative controls (normal human plasma) were always below the detection limit of the assay.

STATISTICS

Statistical calculations were made with the Statistica software, version 3.0 (Statsoft Corp, Tulsa, Okla). Nonparametric tests (Mann-Whitney test, Kruskall-Wallis test, Wilcoxon matched-pairs test, and Spearman rank correlation) were used for most comparisons because several variables could not be considered to be normally distributed. However, the influence of covariates was investigated by means of analysis of covariance and multiple regression. In all of these analyses, RNA levels were log transformed.
HIV-1 AND HIV-2 PLASMA VIRAL LOAD IN DUALLY INFECTED INDIVIDUALS

Plasma samples from 14 individuals with HIV-1 and HIV-2 dual infection were analyzed for HIV-1 and HIV-2 RNA levels (Table 2). Ten of the 14 individuals were dually infected at the first contact with this project, ie, the seroconversion history was not known. Three persons were HIV-2 infected at the first visit and then became HIV-1 infected. One individual was initially HIV-1 infected and subsequently became HIV-2 infected. Nine of the dually infected individuals had clinical symptoms (tuberculosis), but the CD4+ lymphocyte counts were only moderately depressed (median, 295 \times 10^6/L).

Interestingly, the difference in HIV-1 and HIV-2 plasma RNA levels was much less pronounced among dually infected individuals. Thus, the median plasma HIV-1 RNA level was merely 4-fold higher than the plasma HIV-2 RNA levels (19 000 vs 5100 copies per milliliter; \( P = .06 \), Wilcoxon matched-pairs test). Furthermore, there were considerable interindividual differences; some individuals even had higher HIV-2 than HIV-1 plasma RNA load (Table 2). There was no correlation between HIV-1 and HIV-2 plasma RNA levels (\( P = .40 \), Spearman rank correlation). Follow-up samples were available from 4 of these 14 study subjects, and they showed plasma RNA levels similar to those of the initial samples (not shown).

The median plasma HIV-1 RNA concentration was slightly lower among dually infected individuals than it was in recent HIV-1 seroconverters (median, 19 000 vs 70 000 copies per milliliter; \( P = .50 \)) and significantly lower than among HIV-1 seroprevalent cases (392 000 copies per milliliter; \( P = .01 \)). The median HIV-2 plasma RNA level was intermediate, ie, it was between that of the HIV-2 seroconverters and that of the HIV-2 seroprevalent group (median, 5100 copies per milliliter).

### Table 1. Characteristics and Main Findings of the Groups Analyzed for HIV-1 and HIV-2 Plasma RNA Level*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Individuals (F/M)</th>
<th>Age, y</th>
<th>CD4+ Lymphocytes, ( \times 10^3/L )</th>
<th>HIV-1 Plasma RNA Level, Copies/mL</th>
<th>HIV-2 Plasma RNA Level, Copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 seroincident</td>
<td>19 (3/16)</td>
<td>39 (33-41)</td>
<td>429 (246-564)</td>
<td>70 000 (40 000-150 000)</td>
<td></td>
</tr>
<tr>
<td>HIV-2 seroincident</td>
<td>29 (10/19)</td>
<td>36 (31-42)</td>
<td>525 (373-682)</td>
<td>2500 (410-15 500)</td>
<td></td>
</tr>
<tr>
<td>HIV-1 seroprevalent</td>
<td>9 (3/6)</td>
<td>40 (29-45)</td>
<td>80 (72-422)</td>
<td>392 000 (234 000-657 000)</td>
<td></td>
</tr>
<tr>
<td>HIV-2 seroprevalent</td>
<td>31 (9/22)</td>
<td>42 (33-52)</td>
<td>404 (271-743)</td>
<td>10 400 (370-49 000)</td>
<td></td>
</tr>
<tr>
<td>Dually infected</td>
<td>14 (9/5)</td>
<td>40 (33-45)</td>
<td>295 (158-595)</td>
<td>19 000 (2700-136 000)</td>
<td>5100 (680-12 600)</td>
</tr>
</tbody>
</table>

*Follow-up samples are excluded. Results are given as median values (25th to 75th percentile). HIV indicates human immunodeficiency virus.

### Table 2. Main Characteristics of the HIV-1 and HIV-2 Dually Infected Individuals*

<table>
<thead>
<tr>
<th>Patient/Sex/Age, y</th>
<th>CD4+ Lymphocytes, ( \times 10^3/L )</th>
<th>HIV-1 Plasma RNA Level, Copies/mL</th>
<th>HIV-2 Plasma RNA Level, Copies/mL</th>
<th>Clinical and Epidemiological Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>P109/M/44</td>
<td>462</td>
<td>231 000</td>
<td>5900</td>
<td>PCS; sample 1 HIV-2; sample 2 dual</td>
</tr>
<tr>
<td>P138/F/46</td>
<td>948</td>
<td>2700</td>
<td>(&lt;125)</td>
<td>PCS; samples 1 and 2 HIV-2; sample 3 dual</td>
</tr>
<tr>
<td>P225/M/40</td>
<td>235</td>
<td>2700</td>
<td>3600</td>
<td>PCS; samples 1 and 2 HIV-2; samples 3 and 4 dual</td>
</tr>
<tr>
<td>P1137/F/22</td>
<td>330</td>
<td>3600</td>
<td>(&lt;125)</td>
<td>PCS; sample 1 HIV-1; sample 2 dual</td>
</tr>
<tr>
<td>P1315/M/31</td>
<td>77</td>
<td>17 000</td>
<td>250</td>
<td>PCS; sample 1 dual</td>
</tr>
<tr>
<td>TB26/F/60</td>
<td>295</td>
<td>100</td>
<td>4300</td>
<td>Tuberculosis; sample 1 dual</td>
</tr>
<tr>
<td>TB228/F/43</td>
<td>390</td>
<td>136 000</td>
<td>680</td>
<td>Tuberculosis; sample 1 dual</td>
</tr>
<tr>
<td>TB60/F/32</td>
<td>276</td>
<td>25 000</td>
<td>12 600</td>
<td>Tuberculosis; sample 1 dual</td>
</tr>
<tr>
<td>TB94/F/38</td>
<td>807</td>
<td>697 000</td>
<td>8700</td>
<td>Tuberculosis; sample 1 dual</td>
</tr>
<tr>
<td>TB100/F/42</td>
<td>201</td>
<td>11 000</td>
<td>13 000</td>
<td>Tuberculosis; sample 1 dual</td>
</tr>
<tr>
<td>TB127/F/40</td>
<td>23</td>
<td>435 000</td>
<td>6300</td>
<td>Tuberculosis; sample 1 dual</td>
</tr>
<tr>
<td>TB176/F/50</td>
<td>114</td>
<td>1600</td>
<td>43 000</td>
<td>Tuberculosis; sample 1 dual</td>
</tr>
<tr>
<td>TB185/M/40</td>
<td>158</td>
<td>96 000</td>
<td>300 000</td>
<td>Tuberculosis; sample 1 dual</td>
</tr>
</tbody>
</table>

*HIV indicates human immunodeficiency virus; P and PCS, prospective cohort study; and TB, tuberculosis.
Table 3. Plasma HIV RNA Levels, CD4+ Lymphocyte Counts, and Age
Among Patients With Tuberculosis Infected With HIV-1 and HIV-2

<table>
<thead>
<tr>
<th>Group of Patients With Tuberculosis</th>
<th>No. of Individuals (F/M)</th>
<th>Age, y</th>
<th>CD4+ Lymphocytes, $\times 10^6/L$</th>
<th>HIV-1 Plasma RNA Level, Copies/mL</th>
<th>HIV-2 Plasma RNA Level, Copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>8 (3/5)</td>
<td>41 (29-46)</td>
<td>80 (70-516)</td>
<td>331 000 (207 000-580 000)</td>
<td>4500 (300-61 000)</td>
</tr>
<tr>
<td>HIV-2</td>
<td>16 (6/10)</td>
<td>51 (41-58)</td>
<td>394 (210-871)</td>
<td>25 000 (11 000-136 000)</td>
<td>8700 (4300-13 000)</td>
</tr>
<tr>
<td>Dual</td>
<td>9 (7/2)</td>
<td>40 (39-43)</td>
<td>201 (115-295)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*R Results are given as median values (25th to 75th percentile). HIV indicates human immunodeficiency virus.

Several of the individuals in both the seroprevalent and the dually infected groups had tuberculosis. As they represented a defined advanced stage of HIV disease, ie, an acquired immunodeficiency syndrome (AIDS)—defining condition, we performed separate analyses for these individuals. The HIV-2–infected patients with tuberculosis were older, had higher CD4+ lymphocyte counts, and had more than 70 times lower levels of HIV plasma RNA than the HIV-1–associated tuberculosis cases (Table 3). The patients with tuberculosis who had dual infection had lower HIV-1 plasma load than the HIV-1 singly infected patients with tuberculosis ($P=.04$, Mann-Whitney test), while the HIV-2 load was similar in patients with tuberculosis who had single and dual infection. The mean plasma HIV RNA level was 4.7 times higher in the HIV-1–infected patients with tuberculosis than in the HIV-1 seroincident group (331 000/70 000; $P=.03$, Mann-Whitney test), while the corresponding ratio for HIV-2 was 1.8 (4500/2500; $P=.60$, Mann-Whitney test).

CORRELATION BETWEEN PLASMA HIV RNA LEVELS AND CD4+ LYMPHOCYTE COUNTS

The Figure shows the relationship between CD4+ lymphocyte counts and plasma HIV-1 and HIV-2 levels for singly infected individuals. There was a statistically significant inverse correlation between CD4+ lymphocyte counts and plasma HIV-1 RNA levels ($P<.001$; Spearman rank $r=-0.51$) as well as plasma HIV-2 RNA levels ($P<.001$; Spearman rank $r=-0.47$). Linear regression analysis showed that plasma HIV-1 levels were approximately 10 times higher than plasma HIV-2 RNA levels throughout the comparable span of CD4+ lymphocyte counts, and that the HIV-1 and HIV-2 regression lines had very similar slopes (Figure). No trend in residual values was observed. Furthermore, multivariate analysis indicated that plasma HIV RNA levels were independently influenced by HIV type ($P<.001$) and CD4+ lymphocyte count ($P<.001$), but not by sex or age. Thus, the difference in plasma RNA levels between HIV-1– and HIV-2–infected individuals does not appear to be simply an effect of differences in CD4+ lymphocyte counts.

Among dually infected individuals included in this study, there was no correlation between CD4+ lymphocyte counts and either plasma HIV-1 or HIV-2 levels (not shown). Because HIV-1 and HIV-2 are competing for the same target cells, we also calculated the total plasma RNA level (HIV-1+HIV-2) for the dually infected individuals to establish whether there was a relationship between this value and the CD4+ lymphocyte counts. However, no such relationship was evident in this material (not shown).

PATIENTS WITH TUBERCULOSIS

In this study we have shown that plasma viral levels are significantly lower in HIV-2–infected individuals than in HIV-1–infected individuals. The study included patients across a spectrum of disease stages, from recent seroconversion, through asymptomatic phases, to late stages (patients with tuberculosis). Follow-up samples obtained 1 to 2 years after the first sample were included when available. A cross-sectional set of samples from HIV-1 and HIV-2 dually infected individuals was also analyzed. By using a similar method for HIV-1 and HIV-2, we could directly compare the plasma HIV RNA levels in individuals infected with the 2 viruses.

As early as 1993, De Kock et al proposed that the differences in the epidemiological and biological characteristics of HIV-1 and HIV-2 could be due to differences in viral load. Although some experimental support for this hypothesis has been published previously, the present study documents that plasma viral levels are lower in individuals infected with HIV-2 than in individuals infected with HIV-1. Our study is the first, to our knowledge, that provides a direct comparison of plasma viral levels in HIV-1– and HIV-2–infected individuals less than 2 years after seroconversion, thus allowing for an estimation of the plasma virus set point. The only pre-
Previous studies on plasma HIV-2 levels were recently published and included only a cross-sectional sample of patients with unknown seroconversion dates or seroincident cases tested at a median of approximately 5 years after seroconversion. Popper et al found a consistently lower HIV-2 than HIV-1 plasma viral load, with a median difference of about 1.5 log_{10}. Berry et al reported that the main differences in plasma viral levels between HIV-1- and HIV-2-infected individuals were found in those with high CD4+ lymphocyte levels. In the patients with low CD4+ lymphocyte levels, the plasma viral levels were similar for HIV-1 and HIV-2. In our study there was a more linear relationship between the HIV-1 and HIV-2 RNA levels, the latter being approximately 1 log lower throughout the comparable spectrum of CD4 counts. If these data are interpreted according to the often cited "locomotive model," it is possible that HIV-2 infection also progresses more slowly than HIV-1 infection in individuals with low CD4+ lymphocyte counts.

Our findings are also in line with those of Simon et al, who reported that isolation of HIV-2 from plasma was less efficient than isolation of HIV-1. Interestingly, previous studies have indicated that the HIV proviral (DNA) load is similar for HIV-1 and HIV-2. No previous data exist on plasma viral load for HIV-2 at such an early stage of the infection. Information on HIV-1 RNA load shortly after primary infection and with longitudinal follow-up in persons living in Africa is also very limited. It may be argued that the HIV-1-infected persons included in this study represent a subgroup with particularly high progression rate. However, HIV-1 was recently introduced in Guinea-Bissau, and the persons included here constituted a majority of the then-existing HIV-1-infected individuals in this particular cohort. A molecular characterization of the HIV-1 variants prevailing in Guinea-Bissau in various population groups have demonstrated only 1 subtype, an A/G recombinant variant recently described. Continued follow-up of these individuals with known seroconversion times will provide information about not only the natural course of HIV-1 and HIV-2, but also the predictive power of early plasma RNA measurements in this particular setting.

For HIV-1 it has been clearly demonstrated that the viral set point, ie, the plasma HIV-1 RNA level reached after the initial peak of viremia, is a very good predictor of disease outcome. It is interesting to speculate whether the difference between HIV-1 and HIV-2 in the natural course, transmissibility, and epidemiological features is due entirely to lower viral levels in plasma. Among HIV-1-infected individuals with plasma viral levels similar to those of our recent HIV-2 seroconverters, ie, 2500 RNA copies per milliliter of plasma, only approximately 17% progress to AIDS within 6 years and 35% in 9 years. Correspondingly, the groups with plasma HIV-1 RNA levels above 30000 copies per milliliter were reported to progress to AIDS at a rate of 67% to 98% within 6 years and 76% to 100% in 9 years (the intervals depending on their CD4+ lymphocyte counts). Furthermore, the median plasma HIV-2 RNA levels detected in this study are comparable with those of many HIV-1-infected individuals with long-term nonprogression. In that sense, HIV-2 infection may resemble a slowly progressive or nonprogressive HIV-1 infection. Thus, although it remains to be proved, it is likely that HIV-2 infection progresses more slowly than HIV-1 infection simply because plasma viral load is lower. However, the mechanisms through which HIV-2 infection is contained to a higher degree than HIV-1 remain to be identified. Differences in the interaction between the human immune system and the 2 HIV types is a tempting explanation. Inherent differences in the biological characteristics of the virus is yet another possibility. Autologous neutralizing antibodies have been found more frequently in HIV-2-infected than in HIV-1-infected individuals, and it has been suggested that this difference in virus neutralizing activity may contribute to the slower disease progression in HIV-2. Studies on the cellular immune response to HIV-2 are in progress in Guinea-Bissau but have been delayed by the recent civil war.

Dual infection of HIV-1 and HIV-2 appears to constitute a special situation where the interplay between the 2 viruses and the host defense mechanisms is less predictable. Dieng-Sarr et al recently showed that the proviral DNA levels of HIV-2 were significantly lower among dually infected patients than among HIV-2 singly infected individuals. Their dually infected cases also displayed an inverse relationship between levels of CD4+ lymphocytes and proviral HIV-2 DNA as compared with singly infected individuals, ie, decreasing proviral DNA load was correlated with decreasing CD4+ lymphocyte counts. However, their study did not include measurements of HIV-1 DNA and RNA levels or plasma HIV-2 RNA levels. In the study presented herein, which is the first, to our knowledge, to include plasma RNA measurements of HIV-1 and HIV-2 in dually infected individuals, albeit in a limited sample, we did not find any correlation between levels of CD4+ lymphocytes and plasma RNA. Nor was there any correlation between HIV-1 and HIV-2 RNA load. The CD4+ lymphocyte levels were moderately depressed in this group, indicating that these patients were at later stages of HIV disease, where the correlations may not be as strong. On the other hand, the viral loads were remarkably low in view of the CD4+ lymphocyte levels. It has been suggested that the number of available target cells is a limiting factor for viral replication (the host-parasite hypothesis), and Dieng-Sarr et al proposed that HIV-1 may outgrow HIV-2 in dually infected individuals. A study in Senegal has shown a protection of HIV-2 infection against subsequent HIV-1 infection. However, it has not been possible to confirm these findings in other settings. Interestingly, the total plasma viral load (HIV-1 + HIV-2) in our study was lower than the load detected in HIV-1 singly infected individuals. This reduction in total viral burden could result from a partially protective immune response of one virus against HIV-2.
the other, or it may be that HIV-2 competes for the same target cells but produces fewer new virus particles per cell and time unit. However, the long-term dynamics of viral load in dually infected individuals require further investigation.

It is also interesting to reflect on the evolutionary implications of our findings, because the HIV-1 and HIV-2 epidemics are both believed to be the result of relatively recent cross-species transmission of simian immunodeficiency virus from chimpanzees and sooty mangabeys monkeys, respectively.37,38 Interestingly, these viruses, ie, SIVcpz and SIVsmm, appear to be nonpathogenic in their monkey hosts despite the fact that virus replicates to high titers in vivo.39,40 Perhaps HIV-2 infection in humans should be considered to resemble more closely than HIV-1 the “natural” infection in the nonhuman primate host because it is less pathogenic. However, an alternative interpretation is that HIV-2 is less well adapted to replication in the new human host, because it replicates to lower titers, ie, HIV-2 is not “humanized” to the same extent as HIV-1.

We found that HIV-2–infected patients with tuberculosis had only moderately depressed CD4+ lymphocyte levels, whereas most HIV-1–infected patients with tuberculosis had severe immunodeficiency. Tuberculosis is one of the conditions included in the Centers for Disease Control and Prevention and World Health Organization AIDS case definitions,41,42 but the large differences between HIV-1- and HIV-2–infected patients with tuberculosis may indicate that tuberculosis should not be an AIDS-defining condition in HIV-2 infection. However, to address this question, more in-depth studies including longitudinal cohorts are required.

An important methodological aspect of this study is the relative and absolute precision of the tests for HIV-1 and HIV-2 RNA quantification. The HIV-1 RNA measurements were done with a widely used and highly standardized method, ie, the Amplicor HIV-1 Monitor Assay. It is important to stress that we used version 1.5, which, in contrast to version 1.0, appears to reliably quantify all known genetic subtypes of HIV-1 group M.43-45 For HIV-2 quantification we used a novel assay developed by Roche that is based on the same principles as the HIV-1 assay. In pilot tests, the HIV-2 RNA quantification assay has been found to accurately quantify a majority of HIV-2 subtype A samples46 (Ronald A. Otten, PhD, oral and e-mail communication, May 1999), the prevailing subtype in Guinea-Bissau.12,23 Furthermore, Berry et al14 and Popper et al,13 who each used different quantification assays, reported HIV-2 plasma RNA levels that were similar to those reported in the present study. This suggests that both their and our assays correctly quantify plasma HIV-2 RNA levels.

Taken together, this study has shown that HIV-2 infection is characterized by a significantly lower plasma viral load set point than HIV-1 infection. The difference in plasma HIV RNA levels appears to persist into late stages of the disease when immunodeficiency is becoming severe. The HIV-2 plasma viral load early after seroconversion was similar to the levels observed in other studies among HIV-1–infected persons with long-term nonprogression. It also appears that dual infection has unpredictable consequences for HIV-1 and HIV-2 plasma viral load. These data provide a compelling explanation for the differences in the natural course, transmissibility, and epidemiological characteristics of HIV-1 and HIV-2, as well as new insights into the biological interplay between the 2 viruses in individuals exposed to both.

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REFERENCES


