Plasma Viral Loads During Early HIV-1 Infection Are Similar in Subtype C– and Non-Subtype C–Infected African Seroconverters

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Recent data suggest that infection with human immunodeficiency virus type 1 (HIV-1) subtype C results in prolonged high-level viremia (>5 log_{10} copies/mL) during early infection. We examined the relationship between HIV-1 subtype and plasma viremia among 153 African seroconverters. Mean setpoint viral loads were similar for C and non-C subtypes: 4.36 vs 4.42 log_{10} copies/mL (P = .61). The proportion of subtype C–infected participants with viral loads >5 log_{10} copies/mL was not greater than the proportion for those with non-C infection. Our data do not support the hypothesis that higher early viral load accounts for the rapid spread of HIV-1 subtype C in southern Africa.

Keywords. HIV-1; group M subtype; plasma viral load; early infection; Africa.

Human immunodeficiency virus type 1 (HIV-1) group M, which was introduced into the human population by a zoonotic transmission event, has diversified over time and currently clusters into 9 subtypes whose envelope sequences differ by 15%–20%. HIV-1 subtypes differ substantially in their geographic distributions, with subtype B predominant in the Americas, likely reflecting a founder event, and subtype C predominant in southern Africa, where HIV-1 prevalence is highest [1]. Data support a relationship between some HIV-1 subtypes and the rate of CD4 T-cell decline and clinical outcome, suggesting that subtype may be related to virulence [2].

The level of viremia in infected individuals is strongly associated with HIV-1 transmission [3] and disease progression [4]. Shortly after an individual acquires HIV-1, the number of viral copies in the bloodstream increases exponentially and then typically falls to a lower, relatively constant level where it remains or increases slowly until late in infection. The stable, lower concentration of virus in blood that follows peak viremia during acute infection is known as the viral setpoint. The impact of subtype on setpoint is unclear, with some studies showing equivalent viral loads across subtypes [5] and others showing intersubtype differences [6].

A recent analysis found that nearly one-fifth of subtype C–infected individuals had extended high viremia, with plasma viral loads >5 log_{10} copies/mL during the first 1.5 years after HIV-1 acquisition [7]. The authors interpreted those findings as potentially explaining the dominant and rapid spread of subtype C in southern Africa. We sought to understand the relationship between HIV-1 subtype and virulence using similar measures of plasma viremia. The goal of our study was to compare viremia during early infection in a multinational study of African HIV-1 seroconverters with different subtypes and to assess for the proportion with extended high viremia across subtypes. Participants were studied using identical methods in 2 regions with generalized HIV-1 epidemics, East and southern Africa, enabling comparisons of viremia in subtype C and other subtypes.

METHODS

Study Population

Participants included HIV-1 seroconverters from 2 prospective studies among African HIV-1 serodiscordant heterosexual couples. The Partners in Prevention HSV/HIV Transmission Study enrolled 3408 couples in a phase 3 trial of genital herpes suppression for HIV-1 prevention at sites in Botswana, Kenya, Rwanda, South Africa, Tanzania, Uganda, and Zambia [8]. An additional 485 HIV-1 serodiscordant couples were
enrolled in a parallel observational study in Kampala, Uganda, and Soweto, South Africa [9].

**Procedures**

Participants were followed quarterly for up to 24 months. HIV-1 seroconversion was determined by on-site dual rapid antibody testing and confirmed by enzyme-linked immuno-sorbent assay and Western blot at the University of Washington. HIV-1 RNA was measured in plasma, including from visits before seroconversion, to improve precision of the estimated date of infection. Viral load was quantified using the Roche COBAS Ampli-Prep/COBAS TaqMan 1.0 platform.

**Timing of HIV-1 Infection**

Fiebig staging, taking into account HIV-1 antibody and viral load measurements, was used to estimate the date of HIV-1 infection [10]. We defined participants with detectable viral load in blood plasma at preseroconversion visits as acutely infected, and the HIV-1 infection date was calculated as 17 days before the first positive plasma viral load measurement. In participants without detectable viremia before seroconversion, the HIV-1 infection date was calculated as either the midpoint between the date with undetectable antibodies and the seroconversion visit or 45 days before seroconversion, whichever was most recent.

**HIV-1 Subtyping**

We sequenced partial HIV-1 env and gag to determine whether seroconverters’ viruses were genetically linked to those of their enrolled partners [8]. Viral subtypes were determined with the REGA subtyping tool version 2.0 (http://dbpartners.stanford.edu/RegaSubtyping/).

**Data Analysis**

We assessed the relationship between viral subtype and several measures of early viremia to evaluate for consistency across measures and to compare our findings to other published analyses. First, we assessed viral setpoint, defined as viral load measured ≥4 months after the estimated date of HIV-1 infection because that definition has been used in previous publications [4, 11] and has been associated with the risk of subsequent HIV-1 disease progression. Second, to directly compare our results with those of Novitsky et al [7], we assessed for extended high viremia by evaluating viral loads 100–300 days postseroconversion. We calculated mean viral loads by dividing the sum of viral load measurements by the number of study visits, and we also determined the proportion of extended high viremics, including mean, first, and highest viral load >5 log10 copies/mL. We used logistic regression models adjusted for age and sex to compare viremia by subtype, performing each analysis separately for viral subtypes determined by env and gag sequencing. Finally, we did a sensitivity analysis to examine the impact of acute infection on the level of viremia at setpoint and at 100–300 days postseroconversion by env subtype. We included all available plasma viral load measurements in the analyses, except for those at visits with reported antiretroviral therapy use. Analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

**RESULTS**

Subtypes in env and/or gag were available from 153 seroconverters. Sixty-three percent were from East Africa (Kenya, Tanzania, and Uganda), and 37% were from southern Africa (Botswana, South Africa, and Zambia). The median age was 30 years (interquartile range [IQR], 25–38), and 57% were male. Seroconverters were followed for a median of 404 days (IQR, 312–454) after the estimated HIV-1 infection date. Most seroconverters had either subtype A (env/gag, 46%/45%) or C (env/gag, 36%/31%) viruses, with a minority having subtype D or other inter-subtype recombinant forms (D env/gag, 16%/10%; other env/gag, 3%/14%). Subtype C viruses were found in env in 5.6% and 78.8% of seroconverters in East and southern Africa, respectively. Eighty-one percent had agreement in subtype classification between genes.

Mean viral loads at setpoint and 100–300 days postseroconversion were 4.39 (IQR, 3.82–5.08) and 4.36 (IQR, 3.75–5.09) log10 copies/mL (Table 1). Based on env subtyping, seroconverters with mean, first, and highest setpoint viral load >5 log10 copies/mL comprised 25%, 29%, and 40% of the overall cohort, respectively. Approximately one-third had mean and highest viral load >5 log10 copies/mL 100–300 days postseroconversion.

Viral loads were similar for participants infected with subtype C vs non-C viruses. Mean setpoint viral load for subtype C– and non-C–infected participants classified by env subtype were 4.36 (IQR, 3.85–4.94) and 4.42 (IQR, 3.82–5.13) log10 copies/mL, respectively (Student’s t-test, 2-tailed, P = .61). Mean viral loads 100–300 days postseroconversion were 4.28 (IQR, 3.63–4.97) and 4.44 (IQR, 3.84–5.12) log10 copies/mL (Student’s t-test, 2-tailed, P = .26) in subtype C and non-subtype C infected participants.

Subtype C–infected participants were not statistically more likely to have mean, first, and highest setpoint viral load >5 log10 copies/mL compared with non-subtype C–infected participants (Table 2). The proportion with highest setpoint viral load >5 log10 copies/mL was higher for those with non-C infection than for those with subtype C infection (env: 46% vs 28%, P = .04; gag: 46% vs 23%, P = .01). Subtype C–infected participants were not more likely than those infected with non-C viruses to have mean and highest viral load >5 log10 copies/mL 100–300 days postseroconversion.

We performed a sensitivity analysis, limited to env subtype, for those seroconverters identified during acute infection (ie, viremic and antibody negative). Fifty (32.7%) seroconverters were identified during acute infection, 46 of whom had env subtyping and setpoint viral load. Mean viral setpoints were equal (4.66 log10 copies/mL) in subtype C– and non-subtype
C–infected participants identified during acute infection (Supplementary Table 1). At 100–300 days postseroconversion, mean viral loads were similar: 4.59 (IQR, 4.17–5.29) and 4.41 (IQR, 3.86–5.04) log_{10} copies/mL in those with C and non-C infection respectively.

Finally, we evaluated confounding and effect modification by viral genetic linkage to the enrolled partner to determine whether setpoint was influenced by HIV-1 acquisition from an enrolled partner versus an outside partner. We found no evidence that the level of plasma viremia was modified by transmission linkage status.

**DISCUSSION**

We evaluated plasma HIV-1 load in seroconverters from East and southern African countries. Among 153 seroconverters followed for a median of 1 year postseroconversion, we assessed the level of plasma viremia according to 2 criteria: (1) setpoint viral load, defined as any measurement ≥4 months after infection, and (2) viral load 100–300 days postseroconversion. In multiple analyses, we found no evidence that subtype C was associated with higher early plasma viral load, and thus our results do not support the hypothesis that higher or prolonged viremia in early infection in subtype C explains its dominance in southern Africa.

The levels of viremia in our cohort paralleled those in other African cohorts. Thirty-four percent of the subtype C–infected individuals in Botswana and South Africa described by Novitsky et al had plasma viral load >5 log_{10} copies/mL at 100–300 days postseroconversion [7]. Among subtype C–infected participants in the Center for HIV Vaccine Immunology acute infection cohort, 31% had setpoint plasma viral load >5 log_{10} copies/mL.
copies/mL (unpublished data described in [12]). We found that the mean setpoint viral load was 4.39 log_{10} copies/mL (IQR, 3.82–5.08) and that 25% of seroconverters had viral loads >5 log_{10} copies/mL. Thus, our data confirm the pattern of setpoint viral load in the 4–5 log_{10} copies/mL range, as observed in other African cohorts.

Our analysis had several strengths. Our study protocol was implemented consistently in 2 high HIV-1 prevalence regions with diverse strains of HIV-1, allowing comparisons of viral load between subtypes. Few prior studies have been able to directly compare the relationship between subtype and HIV-1 viremia because many subtypes are geographically isolated. Quarterly HIV-1 serologic testing to identify seroconverters increased precision in estimated dates of HIV-1 infection and permitted viral dynamics to be monitored from soon after infection through >1 year after infection. Finally, seroconverters’ viral subtypes were determined by sequencing env and gag, with analyses of each gene showing similar results.

Some limitations should be considered. First, the majority of seroconverters had subtype A and C infections, with few cases of subtype D, G, or recombinant forms. Although the study was not powered to assess differences in viral setpoint in each subtype, the mean and IQR for viral load overlapped in all subtypes, suggesting that substantial differences are unlikely. Because host genetics affects viral setpoint [13], it would have been ideal to compare viral load by HIV-1 subtype in the same host genetic background, but this was not feasible because of the geographic distribution of subtypes in Africa. Thus, host genetic effects on viral load may confound our results. Furthermore, several studies have found correlations in setpoint viral load among transmission pairs [14]. Accordingly, our study may be biased toward lower levels of viremia because HIV-1–infected partners who have not transmitted over several years may have lower viral loads and may not fully reflect transmissions in other seroconverter populations. However, the similar plasma viral load distribution and viral setpoints in our cohort compared with other studies suggest that our study design did not substantially confound our results.

In conclusion, we compared levels of plasma viremia during early HIV-1 infection in East and southern Africa and found that seroconverters infected with subtypes A, C, D, and other subtypes had similar viral loads at setpoint and 100–300 days postseroconversion. Our analyses indicate that high viral loads (>5 log_{10} copies/mL) are not more common in subtype C than in non-subtype C HIV-1 infection. Thus, our data do not support the hypothesis that higher early viremia explains the rapid and substantial spread of subtype C in southern Africa.

### Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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### Table 2. Associations Between HIV-1 Subtype C vs Non-Subtype C Infection and Plasma Viral Load Measurements

<table>
<thead>
<tr>
<th>Plasma Viral Load Measurement</th>
<th>Subtype C,No. (%)</th>
<th>Non-Subtype C,No. (%)</th>
<th>Adjusted Odds Ratioa</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. with setpoint viral load measurements</td>
<td>53</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean setpoint viral load &gt;5 log_{10} copies/mL</td>
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<td></td>
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</tr>
<tr>
<td>env</td>
<td>10 (19%)</td>
<td>27 (28%)</td>
<td>0.60</td>
<td>.26–1.39</td>
<td>.20</td>
</tr>
<tr>
<td>gag</td>
<td>7 (16%)</td>
<td>29 (30%)</td>
<td>1.02</td>
<td>.98–1.06</td>
<td>.10</td>
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<tr>
<td>First setpoint viral load &gt;5 log_{10} copies/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>env</td>
<td>11 (21%)</td>
<td>32 (34%)</td>
<td>0.58</td>
<td>.26–1.30</td>
<td>.20</td>
</tr>
<tr>
<td>gag</td>
<td>6 (14%)</td>
<td>32 (33%)</td>
<td>0.33</td>
<td>.13–0.88</td>
<td>.03</td>
</tr>
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<td>Highest setpoint viral load &gt;5 log_{10} copies/mL</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>env</td>
<td>15 (28%)</td>
<td>44 (46%)</td>
<td>0.47</td>
<td>.23–.98</td>
<td>.04</td>
</tr>
<tr>
<td>gag</td>
<td>10 (23%)</td>
<td>45 (46%)</td>
<td>0.35</td>
<td>.15–.79</td>
<td>.01</td>
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<tr>
<td>No. with viral load 100–300 days postseroconversion measurements</td>
<td>46</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean &gt;5 log_{10} copies/mL</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>env</td>
<td>11 (24%)</td>
<td>27 (32%)</td>
<td>0.69</td>
<td>.30–1.59</td>
<td>.40</td>
</tr>
<tr>
<td>gag</td>
<td>7 (19%)</td>
<td>30 (34%)</td>
<td>0.48</td>
<td>.19–1.25</td>
<td>.10</td>
</tr>
<tr>
<td>Highest &gt;5 log_{10} copies/mL</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>env</td>
<td>12 (26%)</td>
<td>32 (38%)</td>
<td>0.58</td>
<td>.26–1.31</td>
<td>.20</td>
</tr>
<tr>
<td>gag</td>
<td>8 (22%)</td>
<td>34 (39%)</td>
<td>0.44</td>
<td>.18–1.11</td>
<td>.08</td>
</tr>
</tbody>
</table>

*a Odds ratio adjusted for age and gender. Data were not corrected for multiple comparisons.*
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