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Viral Load and CD4⁺ T-Cell Dynamics in Primary HIV-1 Subtype C Infection

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Abstract

Background—Most knowledge of primary HIV-1 infection is based on subtype B studies, whereas the evolution of viral parameters in the early phase of HIV-1 subtype C infection is not well characterized.

Methods—The kinetics of viral RNA, proviral DNA, CD4⁺ T-cell count, and subsets of CD4⁺ T cells expressing CCR5 or CXCR4 were characterized in 8 acute and 62 recent subtype C infections over the first year postseroconversion.

Results—The viral RNA peak was $6.25 \pm 0.92 \log_{10}$ copies per milliliter. After seroconversion, heterogeneity among acute cases was evident by patterns of change in viral load and CD4⁺ T-cell count over time. The patterns were supported by the rate of viral RNA decline from peak ($P = 0.022$), viral RNA means ($P = 0.005$), CD4 levels ($P < 0.001$), and CD4 decline to 350 ($P = 0.011$) or 200 ($P = 0.046$). Proviral DNA had no apparent peak and its mean was $2.59 \pm 0.69 \log_{10}$ per 106 peripheral blood mononuclear cell. In recent infections, viral RNA set point was $4.00 \pm 0.97 \log_{10}$ and viral RNA correlated inversely with CD4⁺ T cells ($P < 0.001$) and directly with proviral DNA ($P < 0.001$).

Conclusions—Distinct patterns of viral RNA evolution may exist shortly after seroconversion in HIV-1 subtype C infection. The study provides better understanding of the early phase of subtype C infection.

Keywords

acute HIV-1 infection; CD4 count; HIV-1 subtype C; primary HIV-1 infection; natural history; viral load

INTRODUCTION

The nonuniform distribution of HIV-1 subtypes in the worldwide AIDS epidemic has resulted in a gradual increase of HIV-1 subtype C,^{1,2} which became the predominant viral subtype causing the HIV/AIDS epidemic in countries with the highest HIV-1 prevalence.^{3,4} Southern

Africa is experiencing the most severe HIV/AIDS epidemic in the world. In 2007, this region accounted for 35% of all people living with HIV and 32% of all new HIV infections and AIDS deaths globally.⁵

Most studies on viral dynamics in primary HIV-1 infection have been performed in subtype B settings in cohorts of men having sex with men, injection drug users, or blood donors.^{6–14} It is known that the initial peak of plasma viremia reaches 10^5 – 10^7 copies per milliliter,^{15–17} lasts approximately 2–3 weeks,^{9,10,18} and then drops to a steady state level of viral replication, “viral set point” within 4–6 months after infection.^{7,13,14,19–21} The rate of viral RNA decline¹⁰ and the viral RNA in plasma¹³ vary among infected individuals in a wide range. The peak of viral RNA coincides with the time of seroconversion¹² and is accompanied by a profound depletion of CD4⁺ T cells expressing CCR5 at the mucosal effector site.^{22–25} In the peripheral blood, a fast initial decline of CD4⁺ T cells is followed by a slower rate after 5–6 months.¹³ Less is known about proviral DNA in primary HIV-1 infection, although it might be critical to HIV-1 pathogenesis because of its predictive value for disease progression.^{26–29}

The courses of natural HIV-1 subtype B infection and disease outcome are greatly affected by early events in primary HIV infection.^{8,11,20,21,30–35} The peak, nadir, and median of viral RNA load in early HIV-1 infection are associated with RNA load at 6–12 months, and nadir RNA load can predict the level of CD4⁺ T cells during the first year of infection.^{8,33,36}

In non-subtype B epidemics, both similarities and differences in relation to primary HIV-1 subtype B infection have been reported.^{37–42} In Kenya, where subtype A is a widespread HIV-1 subtype, viral load at the peak of acute infection was $4.7 \log_{10}$ ⁴³ to $5.1 \log_{10}$ ³⁹ whereas viral set point at 4 months was $4.6 \log_{10}$, and was associated with disease progression.³⁹ In Senegal, viral RNA in the early stage of CRF02_AG HIV-1 infection was found to be $4.5 \log_{10}$ ³⁸ but was about $0.7 \log_{10}$ lower in HIV-1 subtype A and subtype G infections.³⁸ In subtype C epidemic in Malawi, an RNA peak of $6.10 \log_{10}$ was reported.^{44,45}

Relatively little is known about the evolution and variance of virologic and immunologic parameters in primary HIV-1 subtype C infection. The rationale for the current study is the need to better understand the pathogenesis of the early phase of HIV-1 subtype C infection and to identify subtype-specific characteristics of viral dynamics, if any. We analyzed key viral parameters in a cohort with primary HIV-1 subtype C infection including 8 acute and 62 recent subjects and assessed potential associations between analyzed parameters.

METHODS

Definition of Terms

Acute HIV infection was defined as a preseroconversion phase that starts at the time when infection occurs and ends at detectable seroconversion. *Recent* HIV infection was defined as a phase that starts at seroconversion and lasts for approximately 1 year. A similar designation was described previously.⁴⁶ *Primary* HIV-1 infection was defined as the initial period of infection that includes the *acute* and *recent* phases. The viral RNA load *peak* was the observed highest level and may underestimate the *true peak*.

Study Subjects

Acute and recent HIV-1 subtype C infections were identified in Botswana using a combination of a prospective cohort of postnatal HIV-negative women and a voluntary counseling and testing–based referral sample (Fig. 1). Participants in the prospective cohort were screened for HIV infection bimonthly. Referred subjects were screened for HIV infection cross sectionally.⁴⁷ Acute HIV-1 infections were defined by a negative HIV-1 serology using double enzyme-

linked immunosorbent assay (ELISA) tests combined with a positive HIV-1 reverse transcriptase–polymerase chain reaction test. The clinical status at first presentation for acutely infected subjects is shown in Table 1. Recent HIV-1 infections were identified by detuned ELISA testing according to the standardized algorithm for recent HIV-1 seroconversion algorithm, which was developed for HIV-1B infection.⁴⁸ Fiebig staging¹² was determined for each case of recent infection. Subjects identified with acute or recent HIV infection were enrolled and followed for 12 months. Acutely infected subjects had weekly visits for the first 2 months, biweekly visits for the second 2 months, and monthly visits for the following 8 months. Subjects with recent HIV infection had monthly visits. Individuals whose CD4⁺ T-cell count dropped below 200 cells per cubic millimeter or developed opportunistic infection had access to antiretroviral therapy (ART) [Combivir (azidothymidine/lamivudine) 300/150 mg twice a day plus nevirapine 200 mg twice a day if female, or efavirenz 600 mg every day if male] free of charge, in accordance with Botswana National Program guidelines.

The study was approved by Institutional Review Boards in Botswana and the United States. Written informed consent was obtained from each participant.

A total of 80 subjects including 9 acute and 71 recent cases were enrolled from April 2004 to December 2007 (Fig. 1). The 70 subjects who completed at least 4 study visits were included in the current analysis. The analysis cohort consisted of 8 acute and 62 recent cases. The distribution of 62 recent cases by Fiebig staging¹² was as follows: 10 (16.1%) cases were in stage IV; 18 (29.04%) cases were in stage V; 4 (6.5%) cases were on the edge of stages V and VI (extremely faint p31 band evident for a transition from stage V to stage VI); and 30 (48.4%) cases were in stage VI. There were 18 males (2 acute) and 52 females (6 acute). Analysis of virologic and immunologic parameters in recent infection was performed with adjustment for Fiebig stage.¹² The time since seroconversion was adjusted by 6 days for subjects in stage IV (3 days of phase 3 and 3 days to the midpoint of phase 4), by 44 days for subjects in stage V (9 days of phases 3 and 4 and 35 days to the midpoint of phase 5), by 79 days for stage V–VI (9 days of phases 3 and 4 and 70 days of phase 5), and by 117 days for subjects in stage VI (79 days for phases 3–5 and 38 days to the midpoint between the beginning of phase 6 and the mean of the estimated “recency window” of detuned ELISA). Participants’ ages ranged from 19 to 53 years. There were no significant age differences between the acute and recent infection groups or between male and female participants. The demographics of the study population have been described elsewhere.⁴⁷ All subjects were Botswana nationals.

Laboratory Methods

Antibodies to HIV-1/2 were tested by rapid ELISA Determine HIV-1/2 (Abbott Diagnostic Division, Belgium/Luxemburg) and Uni-Gold HIV Kit (Trinity Biotech, Wicklow Bay, Ireland); and/or regular ELISA Murex HIV 1.2.0 (Murex Biotech Ltd, Dartford, Kent, England) and Ortho HIV-1/2 Ab Capture (Ortho Diagnostics Systems Inc., Raritan, NJ). The ELISA kits used represented third-generation immunoassays that have an estimated “window period” of approximately 2 weeks.⁴⁹ The detuned ELISA was performed by Vironostika HIV-1 Plus O Microelisa System (BioMerieux Inc, Durham, NC) according to the protocol published elsewhere.^{50,51} Plasma RNA load was quantified by the COBAS Ampli-Prep/COBAS AMPLICOR HIV-1 MONITOR Test, version 1.5, according to the manufacturer’s instructions. The method of viral load quantification used in the study has been certified by the Virology Quality Assurance at Rush University, Chicago, IL, as a part of the laboratory proficiency testing. The level of detection was from 50 copies per milliliter for the ultrasensitive method and 400 copies per milliliter for the standard method to 750,000 copies per milliliter. Specimens exceeding the upper level of detection were retested by 10-fold dilutions. Quantification of total proviral DNA was performed by a previously published method⁵² that is used routinely in our lab and targets HIV-1 *gag* p24 using the TaqMan Universal PCR master

mix on the Applied Biosystems 7500 Real-Time PCR system. Quantification of CD4⁺ and CD8⁺ T cells was combined with CCR5 and CXCR4 enumeration and was performed by flow cytometry using the 4-color FACSCalibur. The TriTest kit (BD Biosciences, San Diego, CA) that includes a mixture of CD4-FITC/CD3-PE/CD8-PerCP monoclonal antibodies (mAb) was complemented by the CCR5-APC (clone 2D7, Pharmingen) or CXCR4-APC (clone 12G5, Pharmingen) mAb. To determine the HIV-1 subtype of the acute and recent infections studied, HIV-1 subtyping was performed. The V1C5 region of gp120 was amplified and sequenced in 8 of 8 acute cases and in a subset of 41 of 62 recent infections (the subset of recent infections includes those enrolled by December 2006; 21 cases enrolled in 2007 have not been sequenced). Three sequences per patient from the earliest available time points were aligned with 37 reference sequences from the Los Alamos HIV Sequence Database (<http://www.hiv.lanl.gov/>) and analyzed by the maximum likelihood (ML) and neighbor-joining (NJ) methods. The ML phylogeny was inferred by PhyML⁵³ using the HKY model of nucleotide substitution and the tree was visualized by FigTree v.1.1.2.⁵⁴ Analysis by the NJ method was performed with the MEGA4 program using the Kimura 2-parameter model of nucleotide substitution.⁵⁵

Statistical Analysis

Data were summarized with means $\pm$ SDs. Comparisons between groups were based on *t* tests and χ^2 tests for continuous and binary outcomes, respectively. Linear regression was used to assess time trends and correlations among CD4⁺ T-cell count, viral RNA, and proviral DNA. All reported *P* values are 2 sided.

RESULTS

Subjects are infected with HIV-1 subtype C. The branching topology of the ML phylogenetic tree demonstrated that *env* sequences spanning the V1C5 region from all genotyped subjects clustered with subtype C references. The ML results were comparable to the NJ analysis by MEGA4. Clustering of analyzed *env* sequences with subtype C references in the NJ tree was supported by a high bootstrap value of 99 of 100 replicates (data not shown). Therefore, the phylogenetic analysis indicated that subjects in this study were infected with HIV-1 subtype C (Fig. 2).

Viral RNA in Acute HIV-1 Subtype C Infection

The preseroconversion RNA⁺Ab⁻ status of 8 acutely infected subjects was confirmed by laboratory results of positive reverse transcriptase–polymerase chain reaction and negative double ELISA tests. Individual trajectories of viral RNA, proviral DNA, and CD4⁺ T cells are shown in Figure 3. A summary of viral RNA kinetics is presented in Table 2. The first viral RNA load corresponds to preseroconversion time point for all acutely infected subjects and is presented in Table 2. For our analyses, we assigned the peak of viral load to the higher value of viral RNA either before or at sero-conversion.¹² As evident from Figure 3 and Table 2, a high peak of viral RNA was evident in all acute cases, with a mean value of 6.25 ± 0.92 log₁₀ copies per milliliter.

After seroconversion, heterogeneity was evident among acute cases with respect to patterns of change over time in viral load and CD4⁺ T-cell count. To assess this heterogeneity, we categorized acute cases into 1 of 2 categories. The patterns were apparent from differences in decline of viral RNA from peak, differences in levels of viral RNA within the first 2 months and from 50 to 200 days postseroconversion, and differences in levels and decline of CD4⁺ T cells to the clinically important thresholds of 350 and 200 (Fig. 4). We tested whether the parameters analyzed differ between groups with apparently different patterns, assuming that biological significance of the patterns can be confirmed or rejected based on statistical analyses

between groups. Four of 8 subjects—1811, 2865, 3312, and 5018—showed *slow decline* of viral RNA load from peak and had high levels of viral RNA accompanied by low levels and faster decline of CD4⁺ T cells. In contrast, subjects 3430, 3505, 3603, and 5582 experienced a relatively *fast decline* of viral RNA from peak, lower levels of viral RNA, and higher levels of CD4⁺ T-cell count. Therefore we refer to the observed patterns as groups with *slow* and *fast* decline of viral RNA as the first indicator of potential differences between groups.

The peak values of viral RNA did not significantly differ between the defined groups [$6.79 \pm 0.97 \log_{10}$ vs. $5.72 \pm 0.53 \log_{10}$ in groups with slow and fast decline of viral RNA, respectively; 95% confidence interval (CI) for difference of means: -0.28 to 2.41 ; $P = 0.10$]. No difference was found between groups in the rate of viral RNA decline within the first month after seroconversion, (mean slopes -0.046 ± 0.028 vs. -0.043 ± 0.028 , $P = 0.87$). However, by 2 months after seroconversion, the rate of viral RNA decline from peak differed between groups, which was evident from a difference in RNA slopes, $-0.014 \pm 0.005 \log_{10}$ copies per milliliter per day in subjects with slow decline of viral RNA vs. $-0.028 \pm 0.008 \log_{10}$ copies per milliliter per day in subjects with fast decline ($P = 0.022$; 95% CI for difference of means 0.003 to 0.026). The rate of viral RNA decline from peak also differed between groups at later time points (mean slopes -0.008 ± 0.006 vs. -0.016 ± 0.002 at 4 months after seroconversion, $P = 0.029$; 95% CI for difference of means 0.001 to 0.015). The levels of viral RNA differed between groups at 2 months (Fig. 4B; 5.47 ± 0.46 vs. $3.72 \pm 0.65 \log_{10}$ copies/mL; $P = 0.005$) and at 6 months (Fig. 4C; 5.21 ± 0.21 vs. $3.71 \pm 0.32 \log_{10}$ copies/mL; $P < 0.001$). Differences in CD4⁺ T-cell count between groups were significant and are shown in the results section “Trajectories of CD4⁺ T-Cell Count in Acute Cases” below. In 3 of 4 subjects with slow decline of viral RNA—1811, 2865, and 3312—initiation of ART within the first year of infection was triggered by a drop of their CD4⁺ T-cell counts below 200 cells per cubic millimeter and occurred at 6, 7, and 12 months, respectively. As expected, ART resulted in reduction of viral RNA to below the detectable level within 1 month.

Proviral DNA Load in Acute HIV-1 Subtype C Infection

No peak or obvious time trends in the kinetics of cell-associated proviral DNA load over the 12 months after seroconversion were observed (Fig. 3). The mean of proviral DNA was $2.59 \pm 0.69 \log_{10}$. The range of proviral DNA varied from $0.58 \log_{10}$ to $1.73 \log_{10}$, suggesting a substantial intersubject variation. In contrast to viral RNA in plasma, no immediate effect of ART on the level of proviral DNA load was observed, although the follow-up time was only up to 12 months after seroconversion. No difference in proviral DNA means or range was evident between groups of subjects with fast and slow decline of viral RNA.

Trajectories of CD4⁺ T-Cell Count in Acute Infection

Subjects in the groups with slow and fast decline of viral RNA seemed to experience different CD4⁺ T-cell count trajectories during the first 6 months (Table 3). Subjects with slow decline of viral RNA exhibited lower means of CD4⁺ T cells, whereas subjects with fast decline of viral load had more stable CD4 trajectories (Fig. 3). For the periods 0–2 and 2–6 months, the CD4⁺ T-cell counts of the 2 groups were significantly different (Table 3; $P < 0.001$ and $P = 0.002$, respectively). The Kaplan–Meier plots showed significant differences between groups with slow and fast decline of viral RNA over time in reaching the clinically important thresholds of 350 (Fig. 4D; $P = 0.011$) and 200 (Fig. 4E; $P = 0.046$) CD4⁺ T-cell count per cubic millimeter. The CD4⁺ T-cell counts varied more in subjects with fast decline of viral RNA but remained relatively stable in subjects with slow decline ($P = 0.014$ and $P = 0.037$ for comparison of CD4 variance between groups during the periods 0–2 and 2–6 months, respectively).

The percentage of CCR5⁺CD4⁺ and CXCR4⁺CD4⁺ T cells fluctuated in a wide range between patients over the first year of HIV-1 subtype C infection (Fig. 3; pink and yellow trajectories

in the CD4⁺ T cells graphs). The average of CCR5⁺CD4⁺ T cells over the first year of infection was $14.3\% \pm 5.5\%$. The overall mean value of CXCR4⁺CD4⁺ T cells was $57.6\% \pm 11.7\%$. The average level of CCR5⁺CD4⁺ T cells remained relatively stable during the first year after seroconversion. By contrast, relatively high fluctuations in the percentage of CXCR4⁺CD4⁺ T cells between patients were accompanied by a gradual decrease over the first year of infection.

Evolution of Viral Load and CD4⁺ T-Cell Count in Recent HIV-1 Subtype C Infection

The linear regression curve of viral RNA quantified in 62 patients with recent HIV-1 subtype C infection over 1 year summarizes and averages the viral set point at the level of about 4.0 log₁₀ copies per milliliter (Fig. 5A). Most subjects had established viral RNA set points by the end of the first year of infection, which is evident from the shape of individual regression curves (Fig. 5B). Thirty-three subjects had shown an increase in viral RNA (mean rate of 0.0020 ± 0.0021 log₁₀ copies/mL/day) and 29 had shown a decrease of viral RNA (mean rate of -0.0028 ± 0.0024 log₁₀ copies/mL/day) over 1 year. The mean value of viral RNA in recent HIV-1 subtype C infection was 4.00 ± 0.97 log₁₀ copies per milliliter and the median was 4.10 log₁₀ copies per milliliter (Fig. 5C). The distribution of viral RNA means (Fig. 5D) was close to normal.

The average proviral DNA set point was at the level of 2.3 log₁₀ copies per 10⁶ peripheral blood mononuclear cell (PBMC) over 1 year of HIV-1 subtype C infection (Fig. 5E). Individual fluctuations of proviral DNA resulted in heterogeneous proviral set points (Fig. 5F), suggesting that more than 1 year may be required to establish a stable proviral DNA set point.

Approximately half of the patients (29/62 patients; 46.8%) showed an increase in proviral load at the rate of 0.0029 ± 0.0032 log₁₀ copies per 10⁶ PBMC per day with a relatively high interpatient variability evident from the SD value. Patients with negative slopes of proviral DNA (33/62 measured; 53.2%) demonstrated decline of DNA load at the rate of -0.0030 ± 0.0057 log₁₀ copies per 10⁶ PBMC per day with high interpatient variation. The average of proviral DNA load was 2.30 ± 0.64 log₁₀ copies per 10⁶ PBMC, whereas the median was 2.21 log₁₀ copies per 10⁶ PBMC (Fig. 5G), suggesting a relatively normal distribution of means (Fig. 5H).

The kinetics of CD4⁺ T cells in recent HIV-1 subtype C infection was relatively stable over 1 year (Fig. 5I). The CD4 curves of most subjects demonstrated little change over time (Fig. 5J), although a majority of patients (44/62; 71.0%) had negative slopes with declines of CD4⁺ T cells at -0.48 ± 0.47 cells per day. The average (median) of CD4⁺ T-cell counts in recent infections was 472 ± 225 (449) (Fig. 5K). The means of CD4⁺ T cells varied from 145 to 1437 between patients. The distribution of CD4⁺ T-cell means was unimodal and skewed to the right (Fig. 5L). The kinetics of CCR5⁺CD4⁺ and CXCR4⁺CD4⁺ T cells was similar to acutely infected cases. The CCR5⁺CD4⁺ T-cell mean was $16.3\% \pm 6.5\%$ ranging from 5.0% to 31.8%, whereas the CXCR4⁺CD4⁺ T-cell mean was $59.6\% \pm 13.3\%$ fluctuating widely from 25.3% to 85.4%.

Associations in Recent HIV-1 Subtype C Infection

As expected, a positive correlation was evident between mean viral RNA and mean proviral DNA (Fig. 6A, $R = 0.68$, $P < 0.001$). Both mean plasma RNA and mean proviral DNA inversely correlated with mean CD4⁺ T-cell count (Fig. 6B, $R = -0.57$, $P < 0.001$; and Fig. 6C, $R = -0.51$, $P < 0.001$; respectively). There was a direct association between mean and range of CD4⁺ T cells (Fig. 6D, $R = 0.78$, $P < 0.001$), suggesting higher fluctuations of CD4⁺ T cells in patients with higher CD4 counts and a weak inverse association between mean and range of viral RNA ($R = -0.253$, $P = 0.048$). In contrast, no correlation between mean and range was found for viral RNA or proviral DNA. Mean viral RNA load seemed to be higher in younger participants.

Regression analysis revealed a weak but statistically significant inverse association between mean plasma RNA and age (Fig. 6E, $R = -0.36$, $P = 0.004$).

Analysis of proviral DNA and CD4⁺ T-cell counts in viral RNA quartiles provide an alternative look at potential associations between virologic and immunologic markers in primary HIV-1 subtype C infection (Fig. 7). Lower CD4⁺ T-cell counts with median below 400 cells per cubic millimeter were associated with higher viral RNA quartiles, whereas subjects with median CD4⁺ T-cell counts above 400 cells per cubic millimeter had lower levels of viral RNA (Fig. 7A). Similarly, high proviral DNA was associated with high viral RNA (Fig. 7B).

DISCUSSION

Despite the predominance of HIV-1 subtype C worldwide, the evolution of virologic and immunologic parameters in primary subtype C infection have not been well characterized. Better understanding of early events in HIV-1 subtype C infection could guide prevention strategies, facilitate proper initiation of antiretroviral treatment, and possibly help in the design of a more efficient vaccine. The current prospective follow-up study of 8 (62) subjects identified with acute (recent) HIV-1 subtype C infection addresses the magnitude and kinetics of viral RNA, proviral DNA, CD4⁺ T cells, and their potential associations.

Our data might suggest differential although transient evolution of viral RNA after seroconversion. We did not mean to suggest that all acute HIV infections are of 2 distinct types, but rather to provide evidence of heterogeneity among acute cases with respect to patterns of change over time in viral load and CD4⁺ T-cell count. Based on substantial heterogeneity in viral RNA among subjects, we categorized acutely infected subjects into 1 of 2 categories. The patterns were apparent from differences in decline of viral RNA from peak ($P = 0.022$), in levels of viral RNA within the first 2 months ($P = 0.005$) and from 50 to 200 days ($P < 0.001$) postseroconversion and in levels ($P < 0.001$ and $P = 0.002$ for 0–2 and 2–6 months, respectively), and decline of CD4⁺ T cells to the clinically important thresholds of 350 ($P = 0.011$) and 200 ($P = 0.046$). Taken together, the differences between subjects described here might represent a composite pattern of viral dynamics in the early phase of HIV-1 subtype C infection that requires further analysis using a larger sample.

It is possible that reduction of viral RNA load in subjects with fast decline of viral RNA may be a result of successful innate and efficient adaptive immune responses at the very early stage of infection. If this assumption can be confirmed, a detailed analysis of immune responses in patients with fast decline of viral RNA may provide important insights for HIV vaccine design. A fraction of HIV-1 subtype C–infected subjects with slow decline of viral RNA may contribute disproportionately to the transmission of virus during the early stage of infection, and therefore, their identification might be of high public health priority.

Similarly to our findings, patterns of differential viral evolution have been described at the early phase of HIV-1 subtype B infection.^{7,11} Viral kinetics differed between patients with rapid and slow viral load drop during the first 3 months of infection,⁷ but not after 12 months. In contrast to our findings, no association with CD4⁺ T-cell dynamics, even transient, was found.⁷ Blattner et al¹¹ reported that patients with rapid clearance of viral RNA had lower set points, whereas patients with less-rapid clearance had high set points.

In this study, the peak observed viral RNA before seroconversion, which might underestimate the true peak value, was high in all subjects with a mean value of $6.25 \pm 0.92 \log_{10}$ copies per milliliter, which was close to the RNA peak of $6.35 \pm 0.71 \log_{10}$ reported in subtype B in the Sydney cohort.⁷ In southern Africa, viral set point in subtype C infection has been reported to be similar to that of HIV-1 subtype B,³⁷ which is consistent with our data. In Kenya, a viral RNA set point of $4.60 \log_{10}$ was found in the adult population.³⁹ In Senegal, a plasma RNA

set point of $3.76 \log_{10}$ ³⁸ was reported in HIV-1 CRF02_AG infection, whereas lower levels of set point were observed in other non-CRF02_AG HIV-1 subtypes.³⁸

The viral RNA set point in our study was at the level of $4.0 \log_{10}$, although the mean in the higher RNA quartile was $5.21 \pm 0.23 \log_{10}$, suggesting that a fraction of HIV-1 subtype C–infected participants maintain high viral set points. In fact, 3 of 8 acutely infected subjects in this study dropped their CD4⁺ T cells below 200 and initiated ART within the first year postseroconversion. Potential reasons for such a rapid progression could include contributions from both host and virus. The host could be responsible for an altered frequency, copy number, or expression of AIDS-related genes, which could account for impaired immune response (eg, homozygosity of major histocompatibility complex class I HLA alleles). Viral infection could be caused by multiple viral variants (eg, dual or superinfection), and the transmitted population of viral quasispecies might have increased evolutionary rates or higher levels of transcription and/or replication. Interestingly, the initial fast decline of viral RNA in patients 3430 and 3505 was followed by fluctuations and increase of viral RNA at about 6 months, which might suggest a potential viral escape from immune recognition by 6 months after seroconversion. The kinetics of viral load and CD4⁺ T-cell count in acutely infected subjects in this study suggest that monitoring of viral RNA during the first few months postseroconversion may guide treatment strategies including selective early initiation of ART.

The importance of cell-associated proviral DNA load in the course of HIV-1 infection has been well documented.^{26–28,56–61} Although the evolution of proviral DNA in primary HIV-1 subtype C infection has not been described previously, levels of subtype C proviral DNA were associated with mother-to-child transmission⁶² and HIV transmission by breast feeding has been linked to proviral DNA in breast milk.⁶³ We found no peak of cell-associated proviral DNA in acute HIV-1 subtype C infection. The levels of proviral DNA varied within and between patients, suggesting that no stable set point of proviral DNA has been reached by the end of the first year of infection. Subjects in the higher RNA quartile had high levels of proviral DNA. Variations in the provirus DNA testing cannot be ruled out as a potential cause of proviral DNA fluctuations. In contrast to the RNA trajectories, no immediate reduction of proviral DNA was observed in patients who started ART.

A gradual decrease of the CD4⁺ T-cell count was observed on a background of seemingly random fluctuations in both acute and recent infection. The level of CCR5⁺CD4⁺ T cells was relatively stable over the first year of HIV-1 subtype C infection with no evidence for depletion of CCR5⁺CD4⁺ T cells after seroconversion. In contrast, CXCR4⁺CD4⁺ T cells varied in a wide range both within and between subjects and demonstrated a trend toward reduction by the end of the first year. The mechanistic reason for the relative stability of CCR5⁺CD4⁺ T cells and declining CXCR4⁺CD4⁺ T cells is not clear at the moment and requires further studies.

Markers of viral replication, viral RNA, and proviral DNA correlated with one another, and were inversely related to CD4⁺ T cells, resembling a pattern described in subtype B studies.⁶⁴ No significant gender differences in the relationships among viral RNA, proviral DNA, and CD4⁺ T cells were found during primary HIV-1 subtype C infection, which is consistent with previous reports on other non-B subtypes from Kenya³⁹ and Uganda,⁶⁵ but differs from subtype B studies that reported higher viral RNA load in men.^{14,66,67} The association between mean viral RNA and age is of interest and highlights the importance of HIV-1 prevention efforts which target a predominantly younger population. This finding is in agreement with the study from Kenya reporting a higher plasma RNA load in infants as compared with RNA load in adults³⁹ and the age-dependent trend for RNA load in primary HIV-1 infection in the cohort of commercial sex worker from Senegal.³⁸

In summary, we characterized viral dynamics in primary HIV-1 subtype C infection. The patterns of viral load and CD4⁺ T-cell evolution in acute and recent HIV-1 subtype C infection share many common characteristics with HIV-1 subtype B infection. It is likely that the peak of viral RNA in subtype C infection is higher than in other non-B subtype settings. Our results suggest that distinct patterns of viral RNA evolution may exist shortly after seroconversion in HIV-1 subtype C infection. Monitoring of key viral parameters including viral RNA in plasma and cell-associated proviral DNA load in the early phase of infection might be useful for timely initiation of ART.

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FIGURE 1.

Primary HIV-1 infection cohort. Two sources for identification of acute and recent HIV-1 infections are presented: a postnatal cohort and voluntary counseling and testing (VCT)-based referrals. A total of 324 HIV-negative postnatal women were enrolled in the prospective cohort in Botswana and were followed for up to 12 months; 2 cases were enrolled in the primary HIV-1 infection cohort (1 acute and 1 recent infection) from March 2004 to March 2005. The VCT-based referrals have been described elsewhere.⁴⁷ A total of 8 acute and 70 recent HIV-1 infections were enrolled by VCT-based referrals from November 2004 to December 2007. One acutely and 9 recently infected enrollees completed fewer than 4 study visits and were excluded from analysis. Therefore, a total of 8 acute and 62 recent HIV-1 infections including 1 acute and 1 recent case enrolled in the postnatal cohort were analyzed in this study.

FIGURE 2.

ML phylogenetic tree provides evidence that all subjects genotyped in this study were infected with HIV-1 subtype C. Eight of 8 acutely infected subjects and 41 of 62 recent infections are presented. Three sequences per subject spanning the V1C5 region of *env* were included in the analysis. The tree was generated by PhyML⁵³ using the HKY model, and visualized in FigTree v.1.1.2.⁵⁴ HIV-1 subtype C reference sequences are highlighted by circles. The HIV-1 M group non-subtype C references are shown at the bottom.

FIGURE 3.

Intrapatient evolution of viral RNA, proviral DNA, and CD4⁺ T cells in acute HIV-1 subtype C infection. The timeline shows days from detected seroconversion. Time 0 corresponds to the first seropositive test. Plasma viral RNA load is expressed as log₁₀ copies per milliliter of plasma. Measurements of viral RNA before time 0 are preseroconversion. Cell-associated proviral DNA load is expressed as log₁₀ copies per million of PBMC. T cells expressing CCR5 and CXCR4 coreceptors are shown as percentage of CD4⁺ T cells. The percentage of CCR5 and CXCR4 on the surface of CD4⁺ T cells do not necessarily add up to 100%, reflecting a continuum of fluorescent intensity of CCR5⁺CD4⁺ and CXCR4⁺CD4⁺ T cells. Numbers at top correspond to patient cases. Dotted lines with arrows indicate initiation of ART.

FIGURE 4.

Summary of viral RNA evolution in acute HIV-1 subtype C infection. The timeline shows days from detected seroconversion. Time 0 corresponds to the first seropositive test. Plasma viral RNA load is expressed as $\log_{10}$ copies per milliliter of plasma. Post-ART data were excluded. A, viral RNA measurements in subjects with slow decline of viral RNA—1811, 2865, 3312, and 5018—are shown with solid circles. Measurements in subjects with fast decline of viral RNA—3430, 3505, 3603, and 5582—are shown with open circles. B, Summary of viral RNA levels per subject within the first 2 months postseroconversion. C, Summary of viral RNA levels per subject from 2 to 6 months postseroconversion. D, Kaplan-Meier curve of CD4⁺ T cells decline to 350 between groups with slow (red) and fast (green) RNA decline. E, Kaplan-Meier

curve of CD4⁺ T cells decline to 200 between groups with slow (red) and fast (green) RNA decline.

FIGURE 5.

Viral RNA (A–D), proviral DNA (E–H), and CD4⁺ T cells (I–L) in recent HIV-1 subtype C infection, $n = 62$. The timeline shows days from detected seropositivity after adjusting for Fiebig stage. Plasma viral RNA load is expressed as log₁₀ copies per milliliter of plasma. Proviral DNA load is expressed as log₁₀ copies per million of PBMC. In the box plots (C, G, and K), the boundary of the box closest to zero indicates the 25th percentile, a solid line within the box marks the mean value, a dashed line within the box shows the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate outliers outside the 10th and 90th percentiles. A, Viral RNA. Regression curve represents summarized viral set point. The distribution of dots at the bottom of the graph reflects the threshold for detection of viral RNA by standard reverse transcriptase–polymerase chain reaction (400 copies/mL) and ultrasensitive reverse transcriptase–polymerase chain reaction (50 copies/mL). B, Individual regression curves of viral RNA over follow-up. C, Viral RNA means box plot. D, Distribution of viral RNA means. E, Proviral DNA. Regression curve represents summarized proviral DNA set point. F, Individual regression curves of proviral DNA over follow-up. G, Proviral DNA means box plot. H, Distribution of proviral DNA means. I, CD4⁺ T-cell counts. Regression curve represents summarized CD4⁺ T-cell counts. J, Individual regression curves of CD4⁺ T-cell counts over follow-up. K, CD4⁺ T-cell means box plot. L, Distribution of CD4⁺ T-cell count means.

FIGURE 6.

Associations in recent HIV-1 subtype C infection, $n = 62$ (linear regression analyses). Data is adjusted for Fiebig stage. A, Direct correlation between means of viral RNA and proviral DNA. B, Inverse correlation between means of CD4⁺ T-cell counts and viral RNA. C, Inverse correlation between means of CD4⁺ T-cell counts and proviral DNA. D, Direct correlation between means and range of CD4⁺ T-cell counts. E, Inverse correlation between age and viral RNA means.

FIGURE 7.

CD4⁺ T-cell counts and proviral DNA within viral RNA quartiles, $n = 62$. Data is adjusted for Fiebig stage. Boundaries of viral RNA quartiles are shown at the bottom of each box plot: the first quartile included patients with mean RNA load of more than 4.74 log₁₀ copies per milliliter, the second quartile was >4.10 log₁₀ and ≤4.74 log₁₀, the third quartile was >3.36 log₁₀ and ≤4.10 log₁₀, and the fourth quartile was ≤3.36 log₁₀. In the box plots, the boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate outliers outside the 10th and 90th percentiles. Comparisons between groups are based on t tests or the Mann–Whitney sum rank test if the normality test failed within the group. The P values obtained by Mann–Whitney sum rank test are shown with asterisks. A, CD4⁺ T-cell counts. 95% CIs for difference of means are –474 to –151 between the first and fourth quartiles. B, Proviral DNA. 95% CIs for difference of means are 0.38 to 1.19 between the first and third quartiles, 0.72–1.47 between the first and fourth quartiles, 0.02–0.75 between the second and third quartiles, 0.39–1.01 between the second and fourth quartiles, and 0.01–0.61 between the third and fourth quartiles.

TABLE 1**Presenting Symptoms in Acute HIV-1 Subtype C Infection**

Subject	Gender	Age	Risk Factor	Clinical Summary at Presentation
1811	F	31	Spouse never tested for HIV and refused HIV testing	No symptoms reported
2865	F	20	New partner; unprotected sex	No symptoms reported
3312	F	32	NA	Persistent headache and fever
5018	M	27	NA	Joint pain, fever, fatigue, fine rash, dizziness, sore throat, and loss of appetite. Physical finding: cervical lymphadenopathy
3430	F	35	Unprotected sex	No symptoms reported
3505	F	53	Unprotected sex	Fever, fatigue, and mild upper respiratory tract symptoms including rhinorrhea and cough
3603	M	34	HIV-positive partner; unprotected sex	No symptoms reported
5582	F	26	New partner; unprotected sex	No symptoms reported

TABLE 2

Viral RNA in Acute HIV-1 Subtype C Infection

Viral RNA Load in Plasma, log ₁₀ Copies per Milliliter, Mean Value ± SD									
Seroconversion	At Seroconversion	Postseroconversion							
		0-3 Months	3-6 Months	6-9 Months	9-12 Months	Slope 0-2 Months	Range	Mean	Change From Peak
5.06	6.09	4.85 ± 0.15	5.19 ± 0.13	NA	NA	-0.013	0.75	4.96 ± 0.21	1.13
5.88	5.74	5.36 ± 0.20	5.05 ± 0.03	NA	NA	-0.008	0.72	5.29 ± 0.22	0.59
7.28	6.56	5.71 ± 0.25	5.52 ± 0.19	5.32 ± 0.35	4.77 ± 0.39	-0.014	1.71	5.49 ± 0.39	1.79
7.90	6.32	5.70 ± 0.45	5.12 ± 0.27	4.92 ± 0.21	5.10 ± 0.22	-0.021	1.51	5.39 ± 0.49	2.50
5.38	2.60	2.85 ± 0.38	3.53 ± 1.03	3.88 ± 0.49	3.65 ± 0.46	-0.018	2.03	3.27 ± 0.67	2.11
5.54	5.57	4.36 ± 0.53	4.10 ± 0.96	4.52 ± 0.80	4.75 ± 0.06	-0.030	2.20	4.37 ± 0.64	1.20
6.50	5.76	3.54 ± 0.33	3.96 ± 0.42	3.90 ± 0.10	4.20 ± 0.45	-0.037	1.43	3.75 ± 0.40	2.75
5.43	5.18	4.00 ± 0.44	3.64 ± 1.32	4.24 ± 0.28	4.36 ± 0.20	-0.029	2.92	4.02 ± 0.67	1.41

antiretroviral therapy; NA, not available.

TABLE 3Evolution of CD4⁺ T-Cell Count in Patients With Slow and Fast Decline of Viral RNA in Plasma After Seroconversion

Time After Seroconversion in Months	CD4 ⁺ T-Cell Count, Mean Value $\pm$ SD		<i>P</i>	95% CI for Difference of Means
	Slow Decline of Viral RNA	Fast Decline of Viral RNA		
0–2	271 $\pm$ 59	491 $\pm$ 38	< 0.001	–305 to –134
2–6	250 $\pm$ 71	482 $\pm$ 57	0.002	–344 to –121