Viral Dynamics of Primary HIV-1 Infection in Senegal, West Africa

Abdoulaye Dieng Sarr, Geoffrey Eisen, Aissatou Gueye-Ndiaye, Christopher Mullins, Ibrahima Traore, Mamadou Cire Dia, Jean-Louis Sankale, Diegane Faye, Souleymane Mboup, and Phyllis Kanki

1Department of Immunology and Infectious Diseases, Harvard AIDS Institute, Harvard School of Public Health, Boston, Massachusetts; 2Department of Bacteriology and Virology, Cheikh Anta Diop University, Dakar, Senegal

Background. Few studies have addressed primary human immunodeficiency virus (HIV) type 1 infection in sub-Saharan Africa, where the epidemic is of a predominantly heterosexual character and is caused by different subtypes. The present study examines the dynamics of viral replication in subjects infected with various HIV-1 subtypes.

Methods. Seven hundred fifty-two HIV-negative Senegalese women at high risk for infection were monitored every 3 months for acute/early HIV infection; 26 infections were identified (23 HIV-1 and 3 HIV-2), with an HIV-1 incidence rate of 3.23 cases/person-years observation. Multiple viral-load measurements were taken for all seroconverters.

Results. The mean ± standard deviation viral load for all subjects during the early stage of infection was $4.13 ± 0.66$ log$_{10}$ copies/mL, with an overall decrease of $0.22$ log$_{10}$ copies/mL after the early stage; the viral set point was reached after 12 months of infection. Most subjects had relatively low viral loads during the early stage of infection. HIV-1 CRF02_AG–infected women had a significantly higher mean viral load during the early stage of infection (mean ± SD, $4.45 ± 0.60$ log$_{10}$ copies/mL) than did non–HIV-1 CRF02_AG–infected women (mean ± SD, $3.78 ± 0.46$ log$_{10}$ copies/mL) ($P = .008$). None of the subjects reported symptoms consistent with primary HIV-1 infection.

Conclusion. Our findings in Senegalese women differ from what have been described for primary HIV-1 infection. Further investigations of primary infections with non-B subtypes are warranted, to better characterize their differences with primary infections with subtype B.

Primary HIV infection is defined as the period during which the naïve host immune system responds to its initial encounter with HIV; during this time, some 30%–70% of cases are associated with acute clinical manifestations that range from a mild viral syndrome to a severe systemic illness [1–5]. The study of primary HIV-1 infection has been important in understanding the viral-immunologic interactions involved in viral set point and rates of HIV disease progression. Primary HIV-1 infection has been well described in individuals living in the United States and Europe, but most of the studies have been conducted in cohorts of men who have sex with men or intravenous drug users who were predominantly infected with HIV-1 subtype B [3, 6, 7]. Current data suggest that the amount of circulating virus in the blood often peaks at a level in excess of 1 million RNA copies per milliliter of plasma [2, 7]. The incubation period ends and the self-limited illness resolves within 1–3 weeks [1, 3], and seroconversion to HIV major antigens occurs 14–21 days after the onset of symptoms, thus representing a window of 4–6 weeks [1, 3, 8]. During this critical period, a complex dynamic of infecting virus and responding host and immune factors leads to the establishment of a level of viremia—the viral set point—that appears to be predictive of subsequent rates of HIV disease progression and survival [9]. Existing studies of subtype B infection have shown that rates of disease progression can be predicted...
on the basis of the viral set point established at 1 year after seroconversion, with viral load inversely correlated with AIDS-free survival [9–11]. To date, few studies have examined the nature of primary HIV infection in sub-Saharan Africa, where most HIV infections occur [12–15].

The high mutation rate and the frequency of genetic recombination enable retroviruses to rapidly diversify and evolve at both host and population levels [16]. Although a variety of viral and host factors have been shown to influence pathogenesis, several studies have revealed a potential association between certain HIV-1 subtypes and higher rates of transmission and pathogenesis [17–21].

In previous studies conducted in Senegal, we and others have found that >50% of infections are associated with the circulating recombinant form HIV-1 CRF02_AG [19, 22, 23]. Because different HIV-1 subtypes have been shown to vary in terms of epidemiologic profile, pathogenicity, and transmission [17–21, 24], we hypothesized that the dynamics of primary HIV-1 infection might also differ by viral subtype. In the present study, we compare the dynamics of viral replication during primary infection among women infected with diverse HIV-1 subtypes in Dakar, Senegal.

SUBJECTS, MATERIALS, AND METHODS

Study population. The present study draws its data from an ongoing prospective clinical cohort of registered female sex workers (FSWs) in Dakar, Senegal. We previously reported various epidemiologic and clinical aspects of this cohort [25–27]. Between October 1998 and April 2002, 752 HIV-negative women were monitored every 3 months for plasma HIV RNA, serum antibodies, and clinical signs of primary infection (fever, myalgias, rash, sore throat, headache, etc.); clinical signs were assessed by use of a questionnaire. Inclusion criteria for participation in this primary-infection protocol consisted of provision of written informed consent; Senegalese nationality; HIV seronegativity; and recent history of a sexually transmitted infection (STI). Our group of FSWs at high risk for HIV infection was selected from the main cohort by use of determinants previously shown to be associated with HIV incidence in Senegal [25, 28]. Subjects found to have primary infection and who were positive for plasma HIV RNA and/or HIV antibody reactivity were followed, and viral loads were sequentially evaluated.

Sample collection. Blood was collected in EDTA tubes; plasma and peripheral-blood mononuclear cells (PBMCs) were separated within 4 h by use of ficoll-hypaque (ICN Pharmaceutical). Plasma samples were frozen directly at −70°C, and PBMCs were frozen at −70°C overnight before being transferred to liquid nitrogen.

HIV antibody screening. All plasma samples were evaluated for antibody reactivity to the major viral antigens of HIV-1 and HIV-2 by immunoblot on disrupted whole viral lysates. Recombinant Env peptide and diagnostic DNA polymerase chain reaction (PCR) was employed for dual reactive samples, as described elsewhere [29, 30].

Reverse transcription (RT) PCR for HIV RNA screening. To detect HIV-1 and HIV-2 RNA, plasma was evaluated for all subjects the day after their clinical visit by use of a single-step RT-PCR in individual PCRs. Primers and PCR conditions have been described elsewhere; the limit of detection of the diagnostic RT-PCR technique was 100 copies/PCR for both HIV-1 and HIV-2 [31, 32].

Plasma HIV-1 load. Plasma HIV-1 load was measured by use of Quantiplex HIV RNA (version 3.0; Bayer); this assay quantifies accurately across the different HIV-1 subtypes [33–36]. All samples below the limit of detection were assigned a value of 50 copies/mL.

HIV-1 genotyping and sequence analysis. The HIV-1 C2-V3 envelope region was amplified by use of nested PCR. The conditions for PCR amplification and the cycle sequencing protocol have been described elsewhere [19]. Multiple alignments of all generated sequences and reference sequences (available at: http://www.lanl.gov) was performed by use of Clustal X, with minor manual adjustment when necessary [37]. Phylogenetic analyses were performed by the neighbor-joining method, and reliability was estimated by 100 bootstrap resamplings. Subtype assignment was established on the basis of phylogenetic clustering, with reference sequences supported by a bootstrap value of >75%.

Statistical analysis. The estimated date of infection was defined as the midpoint between the last negative antibody test and the first positive antibody test when seroconversion was documented; for subjects who had HIV-1 RNA detected at a visit before seroconversion, the estimated date of infection was set at 17 days before the detection of HIV-1 RNA, as described elsewhere [38]. Because the amount of time between sample collection for HIV-1 load measurements was not uniform among subjects, comparability was achieved by calculating the mean viral load for all measurements falling between specified time points for each subject’s set of observations. The first interval began at the estimated date of infection and ended at month 3; the second interval began at month 4 and ended at month 6; the third interval began at month 7 and ended at month 12; the fourth interval began at month 13 and ended at month 24 (the second year of observation); and the fifth interval consisted of any observations beyond 24 months.

Viral load data were log_{10} transformed, to achieve normality. Change in viral load over time and differences in viral load between subtypes at various time points were evaluated by Student’s t test. A multiple regression model was used to examine the association between viral load and (1) age at seroconversion...
and (2) years of registered prostitution at seroconversion. Statistical analyses were performed by use of Stata software (version 6.0; Stata Corporation). \( P < .05 \) was considered to be statistically significant.

RESULTS

Over a 4-year period, we screened \( >4862 \) sequential plasma samples corresponding to 752 women at high risk for HIV infection. On the basis of a combination of HIV RNA and antibody testing, a total of 26 HIV infections were identified (23 HIV-1 and 3 HIV-2). In the present study, we addressed only the HIV-1–infected subjects; those with primary HIV-2 infection were excluded because the number was too small to support any meaningful statistical analysis. One subject withdrew from the study and was not included. Between 1998 and 2002, the overall HIV-1 incidence rate in the main cohort was 1.86 cases/100 person-years observation (PYO). In the high-risk FSW group we screened for the present study, we found an HIV-1 incidence rate of 3.23 cases/100 PYO. Six subjects were enrolled while HIV-1 antibody negative and HIV-1 RNA positive, and 8 subjects were enrolled while HIV-1 RNA positive with antibodies to HIV-1 p24 as the only sign of HIV infection. The remaining 9 women were enrolled while HIV-1 RNA positive and had HIV-1 Western-blot profiles. The incidence of HIV-2 during the same screening period was 0.0027 cases/100 PYO. The mean age of HIV-1–infected subjects was 35.2 years (SD, 6.16 years; range, 23–44 years). A logistic regression model was used to assess potential risk factors associated with HIV infection; no significant differences were found in number of sex partners per week, condom use, STIs at visit date, age at registration, and years of registered prostitution between the subjects who seroconverted and those who remained seronegative throughout the course of the study. None of the 23 HIV-1–infected subjects reported the flu-like symptoms that are associated with primary HIV-1 infection. The median duration of time between the last negative test and the first positive test for either HIV-1 RNA or antibodies was 92 days (interquartile range, 57–132 days). For all subjects, plasma HIV-1 load measurements from at least 4 time points were available. Follow-up ranged from 12.9 to 51.4 months. Phylogenetic analyses revealed that 13 subjects were infected with HIV-1 CRF02_AG, 6 were infected with subtype A (including 4 infected with sub-subtype A3 [39]), 2 were infected with subtype G, 1 was infected with subtype C, and 1 was infected with sub-subtype F1 (figure 1).

To assess the longitudinal trend of the viral dynamics of HIV-1 infection, we compared plasma HIV-1 loads from the early stage of infection (defined as the time within 3 months of the estimated infection date) with those from late time points during follow-up. The mean \( \pm SD \) plasma viral load for all subjects during the early stage of infection was \( 4.13 \pm 0.66 \log_{10} \) copies/mL. The mean difference in plasma viral load between the early stage of infection and during months 4–12 was \(-0.22 \log_{10} \) copies/mL, but this decrease was not statistically significant. The decrease in plasma viral load during the early stage of infection reached significance when compared with the mean plasma viral load during the second year of infection, with a mean \( \pm SD \) reduction of \( 0.36 \pm 0.60 \log_{10} \) copies/mL \( (P = .012) \). During the second year of infection, plasma viral load reached a viral set point of \( 3.76 \log_{10} \) copies/mL and remained relatively stable for nearly all subjects during the follow-up period of \( >24 \) months (figure 2).

A multiple regression analysis was conducted to examine the association between plasma viral load and (1) age at seroconversion and (2) years of registered prostitution at seroconversion for both the early stage of infection and the period representing the viral set point. We did not find a significant association between mean plasma viral load during the early stage of infection or at viral set point and either age at seroconversion or years of registered prostitution at seroconversion. There was a trend toward an association between older age at seroconversion and lower mean viral load during the early stage of infection; however, the association fell short of statistical significance \( (P = .076) \).

We investigated whether patterns of viral dynamics differed between the HIV-1 CRF02_AG–infected subjects and the non–HIV-1 CRF02_AG–infected subjects in our study. The HIV-1 CRF02_AG–infected subjects had a higher mean plasma viral load during the early stage of infection \( (\text{mean} \pm SD, 4.45 \pm 0.60 \log_{10} \) copies/mL) than did the non–HIV-1 CRF02_AG–infected subjects \( (\text{mean} \pm SD, 3.78 \pm 0.46 \log_{10} \) copies/mL) \( (P = .008) \). The mean plasma viral load remained higher in the HIV-1 CRF02_AG–infected subjects than in the non–HIV-1 CRF02_AG–infected subjects throughout the additional periods of observation, although the differences were not significant during months 4–12 or during the second year of infection. However, for observations beyond 24 months, the mean plasma viral load was significantly higher in the HIV-1 CRF02_AG–infected subjects \( (\text{mean} \pm SD, 4.06 \pm 0.64 \log_{10} \) copies/mL) than in the non–HIV-1 CRF02_AG–infected subjects \( (\text{mean} \pm SD, 3.29 \pm 0.57 \log_{10} \) copies/mL) \( (P = .02) \).

Of note, some subjects had very low plasma viral loads during the early stage of infection. Two subjects infected with HIV-1 subtype G never had a viral load \( >6370 \) \( (3.8 \log_{10}) \) copies/mL during a follow-up period of \( >17 \) months. One of these subjects was enrolled while positive for HIV-1 RNA but negative for antibodies; her highest viral load was \( 6370 \) \( (3.8 \log_{10}) \) copies/mL, at 5.2 months after infection. The second subject infected with HIV-1 subtype G was enrolled while positive for HIV-1 RNA and p24 antibodies. She was followed for 13 months, and her highest plasma viral load was \( 1522 \) \( (3.18 \log_{10}) \) copies/mL, at 6 months after infection. During the early stage of infection,
Figure 1. Neighbor-joining trees of C2-V3 envelope sequences from 23 HIV-1–infected subjects (denoted as p1–p23, in boldface). Alignment was subjected to 100 bootstrap resamplings; the scale represents 5% nucleotide-sequence divergence.

their plasma viral loads were 3254 (3.5 log_{10}) copies/mL and 760 (2.8 log_{10}) copies/mL, respectively (figure 3A). To determine whether the low plasma viral loads found in the subjects infected with HIV-1 subtype G was due to a lack of specificity of the Quantiplex assay, we evaluated all of the sequential samples from these 2 subjects by use of the Amplicor HIV-1 Ultrasensitive Monitor Assay (version 1.5; Roche Diagnostic Systems). We found that there was no statistically significant difference between the results from the 2 quantification assays.

A subject infected with HIV-1 CRF02_AG was enrolled within 1 month of the estimated date of infection, with HIV-1 RNA and p24 antibody positivity being the sole markers of her HIV-1 infection. She presented with a low plasma viral load of 4811 (3.7 log_{10}) copies/mL 1 month after infection, after which her plasma viral load became undetectable without treatment for almost 20 months. After 30.1 months of infection, her plasma viral load remained very low and stable at <500 (2.6 log_{10}) copies/mL (figure 3B).

One subject infected with HIV-1 subtype A presented with the typical profile of primary HIV-1 infection, as described in the literature. This subject was also enrolled while she was antibody negative but HIV-1 RNA positive. She was the only subject
screened with a high plasma viral load during the early stage of infection—$1.68 \times 10^6$ (6.3 log$_{10}$) copies/mL. Her plasma viral load decreased to $8660$ (3.9 log$_{10}$) copies/mL 3 months after infection; after $>36.3$ months of infection, her plasma viral load was stable at $659$ (2.8 log$_{10}$) copies/mL (figure 3C).

Over the course of the present study, only 2 subjects demonstrated difficulty in containing viral replication and had viral set points of $>5$ log$_{10}$ copies/mL after 12 months of infection. In accordance with the Senegalese selection committee’s criteria for initiation of highly active antiretroviral therapy, they were given therapy [40].

**DISCUSSION**

The present study describes the results of a 4-year screening and follow-up of a group of women with primary HIV-1 infection from a cohort of 752 FSWs in Dakar, Senegal. In the substudy group, we found an HIV-1 incidence of 3.23 cases/100 PYO, significantly higher than the incidence in the main cohort (1.86 cases/100 PYO) ($P<.05$). We did not observe a difference in number of sex partners per week, condom use, STIs at visit date, age at registration, and years of registered prostitution between the subjects who seroconverted and those who remained seronegative throughout the course of the study. This observation suggests that, in this population of Senegalese FSWs, there might be other important risk factors associated with HIV transmission that need to be investigated in order to devise better prevention strategies.

The identification of individuals with primary HIV infection represents an opportunity to reduce HIV transmission at a stage when these individuals have high levels of viral replication, are still developing immune responses, and are relatively more infectious [1, 41]. Moreover, such individuals are probably unaware of their HIV infection status and are less likely to engage in safe-sex practices. Although infection without seroconversion has been reported [42], all of the subjects in the present study who were HIV-1 RNA positive while antibody negative seroconverted to a full Western-blot serologic profile for HIV-1 infection in subsequent samples.

Our study was conducted in women infected with non–subtype B viruses, a group for whom information regarding primary infection is currently scarce [12–14, 38]. Although reports of primary HIV infection in US and European studies of subtype B–infected individuals have indicated that 30%–70% develop symptoms and that the duration and severity of symptoms appear to be related to the prognosis [3, 5], we failed to find women who reported clinical signs or symptoms typical of primary infection. Lavreys et al. reported an association between high plasma viral load and the length and severity of primary HIV-1 illness, observing an increase in plasma HIV-1 load of $0.4 \log_{10}$ copies/mL with each additional symptom during primary infection [12]. This may be one explanation for the lack of symptoms associated with primary HIV infection in the present study, in which the subjects manifested a very low plasma viral load at this stage of infection. Similar to our findings, Morgan et al., in their study in a rural Ugandan cohort of men and women, found no association between reporting possible seroconversion illness and infection with HIV-1 subtypes A or D [43]. In addition, a high prevalence of malaria and other infectious diseases is common in this region of West Africa, and the presence of these diseases could make it difficult to distinguish the early symptoms of HIV disease. Nevertheless, we cannot dismiss the possibility that the expected clinical signs associated with primary HIV in-
Most of the subjects in the present study had relatively low viral loads during the early stage of infection. This was unexpected and in sharp contrast to what has been described in the literature [3, 6, 7, 44]. It is unclear to what extent the route of transmission and subtype variation affect the course of HIV infection. We cannot disregard the possibility that the peak viral replication associated with primary HIV infection was missed in some of our subjects. Nonetheless, 60.9% (14/23) of the women in our study were enrolled very early during the course of their infections, while they were HIV-1 antibody negative and HIV-1 RNA positive (6 subjects) or a combination of HIV-1 RNA and p24 antibody positive (8 subjects). It is also possible that these women, infected through heterosexual exposure, might have received a smaller inoculum of HIV-1. Alternatively, it is possible that the sex of the infected person may play a role. It has been reported that HIV-1–infected women tend to have lower viral loads than do men because of the effect of estrogens that down-regulate tumor necrosis factor–α, which, in turn, has a negative regulatory effect on HIV replication [45]; these issues are worthy of further study.

Because the immune response may be less capable of controlling replication during primary infection, subtype variability with respect to replication capacity or virulence may explain our results for HIV-1 subtypes that are common in West Africa relative to the results of other studies that focused on subtypes B and C [46, 47]. However, the possibility of an early and effective HIV-specific cell-mediated immune response cannot be dismissed. Several studies have reported a rapid induction of a weak and narrowly focused HIV-specific cytotoxic T lymphocyte (CTL) response that occurs at the same time as the decrease in viral load during primary infection [48, 49]. It is possible that, for heterosexual transmission, the induction and maturation of HIV-specific T cell responses could be faster, more potent, and more effective because of priming through the genital mucosa.

It has been reported that HIV-1–infected individuals in Africa tend to have higher viral loads and more-rapid disease progression because of a state of hyperimmunity that results from activation associated with the high frequency of other infectious diseases [50]. This higher activation state could increase the number of activated CD4+ target cells that are susceptible to HIV-1 infection. We found that the plasma viral loads of the HIV-1–infected subjects in the present study were very low, compared with published data from other studies [3, 4, 46, 47]. This difference may be attributed to the prospective design of our study and its focus on the early stage of infection.

HIV-1 CRF02_AG is the leading cause of the AIDS epidemic in West Africa [19, 22, 23, 51, 52]. In the present study, we found that, during the early stage of infection, the HIV-1 CRF02_AG–infected subjects had higher viral loads than did...
the non–HIV-1 CRF02_AG–infected subjects. However, once their viral set points were reached, there were no significant differences in mean viral load between these 2 groups of subjects until after 24 months of infection, when the mean viral load in the HIV-1 CRF02_AG–infected subjects was statistically significantly higher ($P = 0.02$). Interestingly, some of the subjects in our study—2 infected with subtype G and 1 infected with CRF02_AG—demonstrated very low initial viral loads and viral set points. These results could be interpreted in at least 2 ways: first, that the natural history of subtype G might differ from those of other subtypes; and second, that these subjects might have immunologic or genetic correlates that helped them to control HIV-1 replication.

We noted a trend toward an association, which fell short of statistical significance ($P = 0.076$), between older age at seroconversion and lower mean viral load during the early stage of infection. In a group of highly exposed but persistently seroconversion and lower mean viral load during the early stage of infection. N Engl J Med 1994;324:961–4.


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References


