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Comparison of Human Immunodeficiency Virus Type 1 Viral Loads in Kenyan Women, Men, and Infants during Primary and Early Infection

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Steady-state levels of human immunodeficiency virus type 1 (HIV-1) RNA in plasma reached at approximately 4 months postinfection are highly predictive of disease progression. Several studies have investigated viral levels in adults or infants during primary and early infection. However, no studies have directly compared these groups. We compared differences in peak and set point plasma HIV-1 RNA viral loads among antiretrovirus-naive Kenyan infants and adults for whom the timing of infection was well defined. Peak and set point viral loads were significantly higher in infants than in adults. We did not observe any gender-specific differences in viral set point in either adults or infants. However, infants who acquired HIV-1 in the first 2 months of life, either in utero, intrapartum, or through early breast milk transmission, had significantly higher set point HIV-1 RNA levels than infants who were infected after 2 months of age through late breast milk transmission or adults who were infected through heterosexual transmission.

Primary human immunodeficiency virus type 1 (HIV-1) infection in adults is characterized by high levels of virus replication. This is manifested as a steep rise in plasma HIV-1 RNA levels that reach a peak of between 10^5 and 10^6 copies/ml approximately 2 weeks after infection. Once the host defenses are mobilized against the virus, there is a slow decline to a steady-state viral load, or set point, of between 10^4 and 10^5 copies/ml at approximately 4 months postinfection (9, 21, 23). Peak viral levels in adults are not predictive of the rate of disease progression. However, the viral set point, which is most likely a measure of the dynamics between the virulence of the infecting virus strain and the ability of the host immune system to contain the virus, is highly predictive of disease progression. This has been exhibited in studies in which high steady-state levels of HIV-1 RNA in plasma at 4- to 12-months postinfection translated to significantly faster progression to AIDS (21, 23).

The levels of viral replication during primary and early infection in infants seem to be more variable than those seen in adults. Most infants experience a peak HIV-1 RNA viral load in the first few weeks after infection (2, 5, 22). However, after this peak, some infants exhibit a decrease to a viral set point like that seen in adults while others exhibit a continued gradual decrease in viral load for several months or years (2, 5, 7, 14, 22). Because very few studies of HIV-1 RNA viral loads in infected infants have been done in the absence of antiretroviral treatment, some of the gradual decrease with increasing age reported from these studies could have been due to the effects of treatment. Finally, unlike those found in adult infections, high HIV-1 RNA viral-load levels very early in infant infections are highly predictive of disease progression (1, 4, 5, 16, 19, 20, 22, 24).

The reported levels of HIV-1 RNA viral loads in infants during primary and early infection appear to be higher than those seen in adults (2, 22). However, there are no published results directly comparing HIV-1 RNA viral-load levels during early infections in adult and infant populations for whom the timing of infection is well defined. Thus, there are no data that can be used to precisely compare the differences in peak viral-load levels and viral-load set points between these groups. To address this, we undertook a study to compare HIV-1 RNA viral loads in antiretrovirus-naive adults and infants during primary and early infection by using data obtained from studies of HIV-1 acquisition in Kenya. In addition, because the age at acquisition or the route of infection may influence viral-load peak and set point levels, we compared infants infected at different times during infancy (i.e., during the first 2 months of life due to in utero, intrapartum, or early breast milk infection or after the second month of life due to late breast milk transmission). All of the studies from which data were used for these analyses were approved by the University of Nairobi, University of Washington, and Fred Hutchinson Cancer Research Center institutional review boards, and all participants provided informed consent. The human experimentation guidelines of these institutions were followed in conducting the clinical research.

The cohorts for this study consisted of initially HIV-1-uninfected high-risk adults in Mombasa, Kenya, who were monitored through HIV-1 seroconversion, (13) and infants of HIV-1-infected mothers in Nairobi, Kenya, who were monitored from birth (15). The adult cohorts were initiated in 1993, and the infant cohort was initiated in 1992. In all of the cohorts, the participants were tested at frequent intervals for HIV-1 infection; the infants were tested by using HIV-1 DNA PCR (17), and the adults were tested by using a combination of serology

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and a plasma HIV-1 RNA test (11). After infection, the viral-load levels in the plasma of all the cohorts were determined by using the Gen-Probe HIV-1 RNA assay as previously described (6). The peak viral load was defined as the maximum viral load in the first 2 months after the estimated infection time. The set point viral load was defined as the first viral load measured between 4 and 12 months after the estimated infection time. Less than 1% of the subjects had viral loads below the limit of detection of the assay. For these few observations, the value for viral load was set at the midpoint between zero and the limit of detection of the assay.

The estimated time of infection in the infants was defined as the midpoint between the last negative and the first positive HIV-1 DNA PCR test for children who were uninfected at birth or had an unknown infection status at birth. The estimated time of infection for infants that tested HIV-1 DNA PCR positive at birth was set at birth. Infants that first tested HIV positive prior to 2 months of age were defined as having early infections. HIV-1 RNA viral-load assays were run on samples at and after the visit for which a child was first HIV-1 DNA PCR positive. Of the 38 HIV-infected infants available for analysis, 22 (60%) were female. Twenty-six (68%) were infected prior to 2 months of age, and 12 (32%) were infected at or after 2 months of age. Early infection occurred either in utero, intrapartum, or by breast milk transmission, while late infection was by breast milk transmission.

For adults who first tested HIV-1 RNA positive at the visit when seroconversion was first documented, the estimated time of infection was defined as the midpoint between that visit and the previous visit. The estimated time of infection for adults who had HIV-1 RNA detected at a visit prior to seroconversion was set to be 17 days prior to the detection of HIV-1 RNA, as described previously (3, 11). Of the 181 adults available for analysis, 150 (83%) were women and the remaining 31 (17%) were men. Based on the results of an independent samples *t* test, the average age of the men at their estimated time of infection was not significantly different from that of the women {29.6 years for the men (95% confidence interval [CI], 26.8 to 32.4 years) versus 29.9 years for the women (95% CI, 28.9 to 31.0); $P = 0.8$}. HIV-1 infection in the adults was assumed to be acquired through heterosexual contact, since none of the men or women reported a history of injection drug use and none of the men reported ever having had sex with a male partner.

For the infants and adults for whom there were both peak and set point viral-load measurements, these measurements were taken at very similar intervals after their estimated dates of infection (peak, a mean of 30 days for adults versus a mean of 27 days for infants; $P = 0.3$; set point, a mean of 209 days for adults versus a mean of 196 days for infants; $P = 0.3$; all means were determined by independent samples *t* tests). The mean peak viral load in infants was significantly higher than in adults (6.06 versus 5.07 $\log_{10}$ copies/ml, $P < 0.001$, determined by independent samples *t* tests), as was the mean set point plasma viral load (5.84 versus 4.60 $\log_{10}$ copies/ml, $P < 0.001$, determined by independent samples *t* tests) (Fig. 1). The average change in plasma HIV-1 RNA viral load per month between the peak and set points was -0.087 $\log_{10}$ copies/ml in adults compared to -0.042 $\log_{10}$ copies/ml in infants ($P = 0.4$; determined by independent samples *t* tests). In addition, there

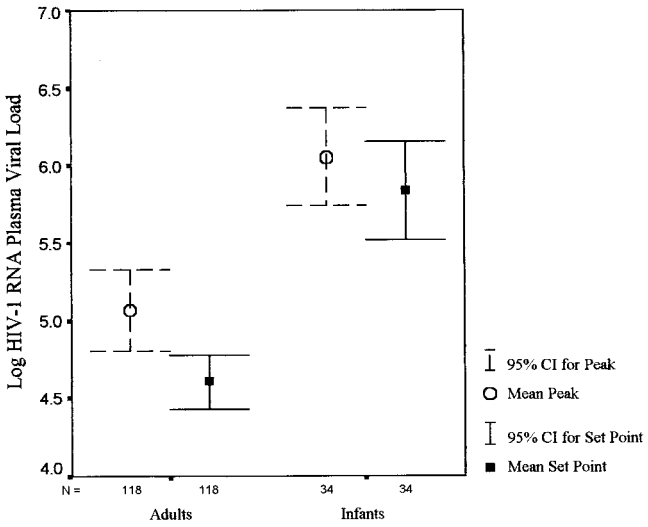

FIG. 1. Peak and set point HIV-1 RNA viral loads for adults versus infants. The mean peak viral load was significantly higher for infants than for adults (6.06 versus 5.07 $\log_{10}$ copies/ml, determined by independent samples *t* test; $P < 0.001$). The mean set point viral load was significantly higher for infants than for adults (5.84 versus 4.60 $\log_{10}$ copies/ml, determined by independent samples *t* test; $P < 0.001$).

was not a significant difference in the average change in $\log_{10}$ HIV-1 RNA viral load per month between the viral-load set point and the first measurement 12 to 24 months after infection for adults versus that for infants ($P = 0.1$, data not shown; determined by independent-sample *t* tests). Because infants were monitored only to a maximum age of 24 months, we were unable to look at viral loads beyond 2 years postinfection. The continued high viral levels following primary HIV-1 infection for infants compared to those for adults may explain why viral loads soon after infection have been found to be highly predictive of disease progression in infants (1, 4, 5, 16, 19, 20, 21, 24).

We further subdivided the infant cohort based on the timing of transmission. We also divided the adult cohort based on gender. We were unable to compare peak viral levels among these groups because of significant differences in the times of collection of the first sample after infection among the groups. However, we were able to examine viral levels at the set point for all participants with set point measurements, because there were no significant differences in the times between infection and the times at which the sample for the viral set point was collected for any of the four groups (Table 1).

The effects of gender on the viral set point were examined because some previous studies have reported differences in viral levels between adult men and women (23), although this observation is not universal (8). In our study, there was not a statistically significant difference in the mean viral set point for men versus that for women (Fig. 2) ($P = 0.4$; determined by independent samples *t* tests) or for infants (data not shown). In addition, we did not see a significant difference in the average levels of change in viral loads between the set point and 12 to 24 months postinfection for female versus male adults (data not shown). Comparisons of median viral loads among women and men gave similar results. This is one of the few longitudi-

TABLE 1. Number of days between estimated HIV infection date and collection of set point plasma HIV-1 RNA viral-load samples

Group	Mean no. of days (95% CIs)	*P* value for difference in results for[a]:		
		Women	Men	Late-infection infants
Women	205 (195,216)	NA	0.5	0.9
Men	215 (187,243)	0.5	NA	0.7
Late-infection infants	205 (159,251)	0.9	0.7	NA
Early-infection infants	187 (170,203)	0.2	0.1	0.3

[a] All values were determined by independent sample *t* test. NA, not applicable.

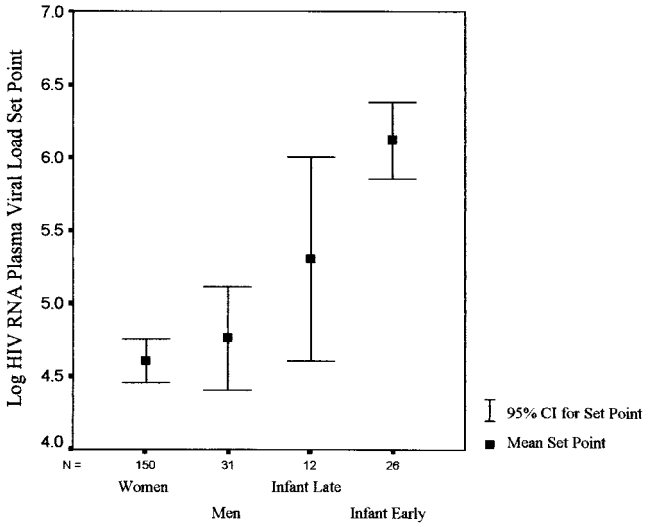

FIG. 2. Set point HIV-1 RNA viral loads in women, men, late-infection infants (≥2 months old), and early-infection infants (<2 months old). There was no significant difference in the viral set points for women and men (determined by independent samples *t* test; $P = 0.4$). The mean set point viral load was significantly higher for infants infected early than for women ($P < 0.001$), men ($P < 0.001$), and infants infected late ($P = 0.03$). The mean set point viral load for infants infected late was significantly higher than that observed for adult women ($P = 0.01$).

nal studies that have used the same HIV-1 RNA viral-load assay to compare viral RNA loads in parallel cohorts of antiretrovirus-naive adult males and females from the same city who were infected with similar HIV-1 subtypes and whose only risk for HIV-1 infection was through heterosexual contact. However, our study is limited by a rather small number of male subjects ($n = 31$). It is possible that our observation of similar viral-load set points among women and men in this study reflects the high number of women with viral diversity at the time of infection in this cohort. Recent studies suggest that the acquisition of multiple variants is associated with an increased viral-load set point (M. Sagar, L. Lavreys, J. M. Baeten, B. A. Richardson, K. Mandaliya, J. Ndinya-Achola, J. K. Kreiss, and J. Overbaugh, submitted for publication), while in previous studies the diversity of the infecting virus population was not known. Thus, our results support the need for additional studies comparing similar male and female cohorts with known times of infection to clarify whether observed differences in viral loads between genders can be assumed for all populations.

Based on the results of independent samples *t* tests, the mean set point viral load was significantly higher for infants infected early either in utero, intrapartum, or through early breast milk transmission (6.12 $\log_{10}$ copies/ml) than those for women (4.61 $\log_{10}$ copies/ml, $P < 0.001$), men (4.76 $\log_{10}$ copies/ml, $P < 0.001$) and infants infected through breastfeeding at or after 2 months of age (5.31 $\log_{10}$ copies/ml, $P = 0.03$) (Fig. 2). In addition, the average set point viral load for infants infected through breastfeeding at or after 2 months of age was significantly higher than that for adult women and trended towards being higher than that for adult men (*P* was 0.01 when the viral load for infants was compared to that for women; *P* was 0.1 when the viral load for infants was compared to that for men; determined by independent samples *t* tests). There was not a significant difference in the average changes in $\log_{10}$ HIV-1 RNA viral loads per month between the viral set point and the first measurement 12 to 24 months after infection for any of the groups in comparison to the others ($P = 0.2$, data not shown; determined by one-way analysis of variance). Thus, the magnitude of the viral-load set point was inversely related to the age of the host at the time of infection, with infants infected in the first 2 months of life having the highest set point, followed by infants infected at or after 2 months of age through breast milk transmission and then by adults. In addition, these differences in plasma HIV-1 RNA viral loads were sustained through at least the first 2 years after infection. The finding of a high viral-load set point among infants infected in the first 2 months of life compared to that of those infected later complements and extends previous findings for non-breastfeeding cohorts that infants infected in utero have a more rapid disease progression than those infected intrapartum (5).

The fact that average peak plasma HIV-1 viral loads are 1 $\log_{10}$ higher in infants than in adults indicates that the level of viral replication is higher in infants, starting at the earliest stages of infection. This difference may be a result of infants having a high concentration of circulating $CD4^+$ T lymphocytes, which are the major target cells for HIV-1 replication, compared to that of adults (10). Alternatively, it may reflect a delayed and less robust early immune response to infection in the infants. Differences in the abilities of younger versus older infants to mount a strong immune response and/or the presence of maternal antibodies in breastfed infants may also explain the high viral set point among infants infected in the first 2 months of life compared to that of those infected later (12, 18). It is also possible that the differences in viral set points between younger and older infants is due to differences in the type of virus transmitted via breast milk versus the type of virus transmitted in utero or intrapartum. Detailed studies of both cell-free and cell-associated viral levels, as well as host immune responses in cohorts with frequent follow-ups starting at or before infection, will be useful in further dissecting the mechanisms underlying the differences in early virus replication in these groups.

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