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HIV RNA level in early infection is predicted by viral load in the transmission source

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Abstract

Objective—HIV-1 viral load in early infection predicts the risk of subsequent disease progression but the factors responsible for the differences between individuals in viral load during this period have not been fully identified. We sought to determine the relationship between HIV-1 RNA levels in the source partner and recently infected recipient partners within transmission pairs.

Methods—We recruited donor partners of persons who presented with acute or recent (< 6 months) HIV infection. Transmission was confirmed by phylogenetic comparison of virus sequence in the donor and recipient partners. We compared viral load in the donor partner and the recipient in the first 6 months of HIV infection.

Results—We identified 24 transmission pairs. The median estimated time from infection to evaluation in acutely/recently infected recipient individuals was 72 days. The viral load in the donor was closely associated with viral load at presentation in the recipient case ($r=0.55$, $P=0.006$).

Conclusion—The strong correlation between HIV-1 RNA levels within HIV transmission pairs indicates that virus characteristics are an important determinant of viral load in early HIV infection.

Keywords

HIV-1 RNA; acute HIV-1 infection; HIV-1 transmission; viral load set-point; HIV-1 pathogenesis

INTRODUCTION

HIV-1 plasma RNA in early infection is a key prognostic indicator of the risk of subsequent disease progression. In a cohort study with measures within 6 months of seroconversion, an HIV-1 RNA level above 10,000 copies/ml at the initial visit was associated with an odds ratio of 10.8 for progression to AIDS during follow-up compared to those below this threshold [1]. HIV-1 RNA level during early HIV-1 infection also correlates with the number of symptoms

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during acute retroviral syndrome [2,3], which in turn has been associated with subsequent risk of progression to AIDS [4].

Although the importance of viral load in predicting subsequent disease progression is well established, the factors that influence the level of viremia during early infection remain less well defined. Much research on this topic has focused on host immune and genetic factors such as HLA [5], which have been presumed to be key factors explaining these differences. Prior reports have suggested a significant association between *in vitro* measures of viral fitness and plasma HIV-1 RNA levels in chronic HIV-1 infection [6,7]. It is unclear, however, to what degree viral characteristics influence HIV-1 RNA level *in vivo* following transmission. To better address this issue, we examined the association between HIV-1 RNA level in recently infected individuals and the HIV-1 RNA level in source partners from whom the virus was transmitted. In this model, associations between HIV-1 RNA level in the source and recipient partners, in whom host genetic factors that influence viral control presumably differ, provide evidence of viral genetic influences on the concentration of HIV-1 RNA in the blood. We focused on HIV-1 RNA level in the early post-seroconversion period because viral load during this period has been associated with risk of disease progression [1]. Because HIV-1 RNA levels tend to increase during the course of disease, studying HIV-1 RNA level soon after seroconversion also provides an important anchor in the timeline of disease, which insures that HIV-1 RNA levels are not being compared in widely differing phases of infection. HIV-1 RNA levels very early in infection are also likely to better reflect the effects of the fitness of the virus inherited from the prior host on HIV-1 RNA level, before evolution of the virus in the new host may alter virus fitness in substantial ways.

METHODS

The UCSF Options Project recruited subjects with possible primary HIV infection who met one of two criteria: potential acute retroviral syndrome or potential recent HIV antibody seroconversion. Detailed referral methods have been reported elsewhere [8]. Participants were classified as preseroconversion HIV infected if antibody testing was negative or indeterminate and an HIV-1 RNA test showed > 2,000 copies/ml on two or more tests. Participants were classified as post-seroconversion recently infected if they were HIV-1 antibody positive and had either: (1) a documented negative HIV-1 antibody test within 6 months of enrollment, or (2) a history consistent with recent HIV-1 infection, including prior negative HIV-1 antibody tests and recent HIV exposure, and a less-sensitive EIA (LS-EIA) Vironostika based HIV-1 antibody test that was not reactive using a cut-off of 0.75 standardized optical density [9].

We interviewed newly HIV-1 infected cases to determine which sexual or drug use partners might have transmitted HIV-1 to the participant during the likely exposure period, and sought to enroll potential source partners for studies of HIV transmission. Written informed consent was obtained from all potential source and recipient partners using protocols that were reviewed and approved by the UCSF Institutional Review Board.

Phylogenetic analysis was performed using *pol* and *env* nucleotide sequences of all suspected partner pairs to determine whether there was viral genetic evidence that virus in a suspected source partner was closely related to virus in a recipient partner. Two individuals were considered to have phylogenetic evidence that the two sequence pairs were linked if they clustered on the same branch with a bootstrap value ≥ 700 (70%) and a difference in branch length representing less than approximately 6% of genetic difference. Phylogenetic analysis was performed by an analyst who was blinded to the suspected partner pairing. Specimens used for the recipient partner results described in this report came from those obtained following the screening visit, at which recent infection had been confirmed; at this date specimens were stored and were available for additional testing, including the phylogenetic analysis. This

second source visit typically occurred 1-2 weeks after screening, and frequently coincided with partner enrollment. HIV-1 RNA in plasma was quantitated using the bDNA assay 2.0 or 3.0 (Siemens Healthcare Diagnostics, Deerfield, IL). In cases in which there were HIV-1 RNA and CD4+ T-cell count measures available at multiple time points in the source partner, we used the value closest to the estimated date of transmission.

RESULTS

We identified suspected source partners with viral phylogenetic evidence of linkage in 24 individuals who presented with acute or recent HIV-1 infection. One participant was the source of infection for two recently infected partners; thus there were 23 unique sources among the 24 transmission pairs. On phylogenetic analysis with all the 47 individuals, each source-recipient pair clustered on the same branch with a bootstrap value 1000, except for the cluster of two recipients with the same source, who had a bootstrap value of 992 for the cluster (Figure 1). The greatest branch length represented less than 3% genetic difference. In each pair, transmission was sexual and between two male partners. Of the 24 recently infected recipient cases, three were enrolled before antibody seroconversion, while the remainder were enrolled post-seroconversion. The estimated median duration of infection in the recipient cases at the specimen collection date was 72 days (IQR 72 – 77) and the median number of days between the date that source specimens used in this study were obtained and recipient specimen collection was performed 0 days (IQR 0 to 20 days). In the recipient partners, the median initial CD4+ T cell count was 528 cells/ μ l (IQR 480 - 768) and the median initial plasma HIV-1 RNA level was 86,332 copies/ml (IQR 18, 396 – 344,149).

The source subjects had a median CD4+ T cell count of 372 cells/ μ l (IQR 230 - 600) and a median plasma HIV RNA level of 23,951 copies/ml (IQR 1,647-78,921). Nine of the sources had evidence of recent infection, based on a LS-EIA value ≤ 1.0 OD without receiving antiretroviral therapy. The remaining 14 sources had an LS-EIA > 1.0 and no history of recent HIV antibody seroconversion, suggesting they were in chronic infection. Of these 14 sources with presumed chronic HIV infection, four had previously received antiretroviral therapy. One had received treatment for only 12 weeks, the remaining 3 had received 6 months or more of treatment. One of the four sources who had received antiretrovirals had stopped medications one week before the study visit, while the other three remained on treatment; all four had detectable viremia. The three sources who were still on treatment had HIV-1 RNA levels between 6,776 and 137,022 copies/ml, suggesting only limited effects of treatment on viremia. Two of the previously treated sources (the one who had recently stopped treatment and the one who had only received 12 weeks of therapy) had no resistance mutations, while both the remaining two sources had extensive, multi-class drug resistance.

The HIV-1 RNA level in the source and the initial HIV-1 RNA level in the newly infected recipient case were closely associated, with a Pearson correlation coefficient of 0.55 (95% CI 0.19 – 0.78 $P = 0.006$), and a Spearman Rank correlation coefficient of 0.52 (95% CI 0.14-0.76, $p = 0.0096$). Recipient viral load was estimated to increase by 0.43 $\log_{10}$ (95% CI 0.16-0.70) for each $\log_{10}$ increase in donor viral load. (Figure 2). The viral load in the recipient cases was a median of 0.56 $\log_{10}$ higher in the recipient than in the donor cases, consistent with the recipient cases being early in HIV infection, before viral load set-point had been fully achieved. Excluding pairs in which the source was receiving antiretroviral therapy did not appreciably affect the correlation between source and spread partner HIV-1 RNA level (correlation coefficient = 0.55)

To further assess the relationship between HIV-1 RNA levels in the source and recipient partners over time, we tested the persistence of the correlation between source and recipient

viral load in nine pairs in which the recipient partner did not initiate antiretroviral therapy during the first year of follow-up. In these nine pairs, the Pearson correlation between original donor viral load and recipient viral load after 24 weeks ($\pm$ 5 weeks) of observation in the recipient was $r = 0.71$ (95% CI 0.09-0.94, $p=0.031$), and the Spearman Rank correlation was 0.45 (95% CI -0.31 - 0.86, $p=0.22$). At 48 weeks of follow-up ($\pm$ 7 weeks) in the recipient, the Pearson correlation between viral load in these 9 pairs was $r = 0.71$ (95% CI 0.09-0.93, $p=0.031$), and the Spearman Rank correlation was 0.68 (95% CI 0.04-0.93, $p=0.042$).

To assess whether clustering of host characteristics between source and recipient partners might account in part for the association we observed of HIV-1 RNA levels within transmission pairs, we tested additional models that controlled for host characteristics that might conceivably influence viral load and be associated within partner pairs, including age, race, and favorable or unfavorable HLA class I B alleles based on the scoring system developed by Saah et al [5]. The association between source and recipient partners HIV-1 RNA remained statistically significant, and none of these other factors had a statistically significant association with recipient HIV-1 RNA or appreciably altered the correlation coefficient for source and recipient HIV-1 RNA (data not shown).

DISCUSSION

We found a strong correlation between HIV-1 RNA levels in source and recipient partners in HIV-1 transmission pairs. We also found a clear correlation between the source HIV-1 RNA level and HIV-1 RNA level measures taken over the first year of observation in a subset of recipient partners who did not initiate antiretroviral therapy.

Although prior studies have shown that host immune factors such as HLA type predict HIV-1 RNA level and risk of HIV-1 disease progression, the correlation we observed between HIV-1 RNA levels in genetically unrelated hosts infected with very similar virus suggests a strong influence of viral genetic factors on plasma HIV-1 RNA levels. Similar conclusions have been made in the SIV infected macaque model [10]. Although we did not directly test this hypothesis, we believe our data suggest that host-selected immunologic escape mutations or other genetic factors that affect viral fitness were retained during transmission and influenced viremia in both the source and recipient partners. Along these lines, it has recently been shown that strong cytotoxic T cell responses can select for escape mutations within *gag* that dramatically affect fitness and viremia [11,12]. These mutations can persist after transmission and influence the level of viremia during early HIV infection [13,14].

There are several limitations of study. First, our sample size was relatively small. It will be important to see our results confirmed in other cohorts. Second, while we believe this is unlikely, it is possible that unmeasured host factors that might influence HIV-1 RNA levels and be associated within transmission pairs might account, at least in part, for the association of HIV-1 RNA within transmission pairs. We tested models controlling for age, race, and HLA. These factors did not appear to explain the observed association, but did not have genetic data on all the factors that have been shown to influence HIV-1 RNA levels. Third, if we recruited transmission pairs in which acutely infected sources were linked to acutely infected recipients, while more chronically infected sources were linked to more chronically infected recipients, then our observations could have been spurious. This seems unlikely, however, as our analysis only focused on those recipients who were observed during very early HIV infection. Fourth, the duration of HIV-1 infection in the recipient partner at the time of the initial HIV-1 RNA level varied. This variation in the timing of the initial HIV-1 RNA level in the recipient, however, would tend to attenuate the true correlation between HIV-1 RNA level in source and recipient partners. Fifth, three of our source patients reported being on antiretrovirals at the time of their evaluation visit, which may have influenced the viral load we measured. From

the substantial HIV-1 RNA levels we obtained in these subjects, however, we believe that the treatment effects on the viral load had important limitations; particularly in the two sources with multi-drug resistant HIV, effects of on-going treatment on HIV-1 RNA levels may have been mediated largely through maintaining drug resistance mutations, which were in turn transmitted to the recipient partners. Excluding the sources who were on treatment did not change the correlation between source and spread HIV-1 RNA levels appreciably.

Our results are consistent with earlier reports finding an association between HIV-1 plasma RNA levels and viral replication rates *in vitro*, as well as with replication capacity measured in recombinant virus single-cycle infection assays utilizing patient reverse transcriptase and protease genes [6,7]. These studies, while important, may be subject to confounding as they have studied subjects at a variety of stages of chronic infection. It is possible that factors such as duration of infection, which might allow virus in a new host to evolve in ways that increase replication fitness over time, while simultaneously leading to declines in immune function that permit higher viral loads, may exaggerate the observed association between *in vivo* replication rate assays and plasma HIV-1 RNA. Our data address this issue by showing that closely related viruses result in similar levels of viremia in two different hosts, and that the relationship is evident when one of the hosts is consistently in early stage infection (the recipient in our partner pairs). Our results reinforce an earlier study of HIV-1 transmission within couples in Zambia [15]. This study of 115 initially serodiscordant couples found a correlation between HIV-1 RNA level in the source and recipient partner ($r = 0.21$) that was somewhat weaker than the correlation we found. The mean duration of infection when the HIV-1 RNA level was measured in recipient partners in this study was 9.5 months in this Zambian study compared with 2.5 months in our study. The longer duration of infection and potentially greater variation in the duration of infection in the serodiscordant couple study compared to ours may be one factor explaining the weaker correlation.

In summary, our observations suggest a strong influence of viral genetic factors on HIV-1 RNA level during early HIV infection. Subsequent research is needed to better identify the viral genetic characteristics associated with higher or lower HIV-1 RNA level, and to further understand host immune responses that shape viral replication fitness over time.

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Figure 1. Phylogenetic tree of partner pairs

Partner pairs were closely associated on phylogenetic analysis using *pol* sequences. Note that sequences from subjects 15, 16, and 17 show a cluster of three closely related sequences, in which one subject was the source partner for two new infection cases.

Figure 2. Relationship of source to recipient HIV-1 RNA level

HIV-1 RNA levels in the source subject (x-axis) were associated with initial HIV-1 RNA levels obtained on enrollment of recently infected recipient partners (y-axis).