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Evidence of Persistent Low-level Viremia in Long-term HAART-suppressed, HIV-Infected Individuals

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Abstract

Background—Highly active antiretroviral therapy (HAART) can effectively reduce plasma HIV RNA levels to below the level of detection in most HIV-infected patients. The degree to which residual low-level viremia persists during HAART remains unclear.

Methods—We identified 180 subjects (median duration of HIV infection 12 years) who had ≥ 2 consecutive plasma HIV-1 RNA levels below the level of detection (<50 -75 copies/mL) while taking antiretroviral drugs; 36/180 had been virologically suppressed for >5 years. Longitudinal plasma samples that were taken from these subjects during periods of viral load suppression were selected and analyzed. The isothermal Transcription Mediated Amplification (TMA) (limit of detection <3.5 copies RNA/mL) assay was used to measure persistent viremia. A “detuned” EIA assay was used to obtain quantitative HIV antibody levels.

Results—A total of 1606 TMA assays were performed on 438 specimens in 180 HAART-suppressed subjects (median 3 replicates per specimen). In the first year of viral suppression, plasma RNA levels declined significantly ($p=0.001$), but after month 12 there was no evidence for a continued decline ($p=0.383$). In the first year of viral suppression, HIV antibody levels also declined ($p=0.054$), but after month 12 there was no evidence for a continued decline ($p=0.988$).

Conclusions—Viremia continued to decline during the first 12 months after viremia became undetectable using conventional methods, and then remained stable. HIV antibody levels also decreased in the first year of viral suppression and then remained stable. Viremia and the HIV-associated host response appear to achieve a steady-state “set-point” during long-term combination therapy.

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Keywords

Residual viremia; HAART-suppressed; HIV antibody levels

INTRODUCTION

Highly active antiretroviral therapy (HAART) can effectively reduce plasma HIV RNA levels to below the level of detection using conventional assays in the majority of HIV-infected patients who have access to antiretroviral drugs. This has led to significant gains in HIV-related morbidity and mortality. However, the degree to which residual low-level viremia persists during long-term virologic suppression with HAART remains unclear, as most studies using highly sensitive assays have had followed a small number of patients or had limited follow-up times. The extent of residual viremia is central to studies of persistent inflammation during therapy and critical to the design of studies aimed at viral eradication.

We conducted a longitudinal study evaluating the levels of low-level viremia in a large cohort of virologically-suppressed subjects treated with HAART. We also measured longitudinal HIV antibody levels to assess whether the host response to the virus waned over time.

MATERIALS AND METHODS

Study Participants

All subjects were enrolled in SCOPE, an ongoing prospective cohort study based at the University of California, San Francisco. All subjects in SCOPE are seen every four months, at which time they are interviewed and plasma and peripheral blood mononuclear cells are stored. From this cohort, we identified a cohort of subjects who had at least 2 consecutive plasma HIV RNA levels below the level of detection (<50 -75 copies/mL) while taking antiretroviral drugs. Subjects were required to remain virologically suppressed during the period of analysis with isolated “blips” of <1000 copies/mL allowed, as long as they were preceded and followed by a viral load below the level of detection. Subjects were also required to remain on antiretroviral therapy during the period of analysis; antiretroviral changes were allowed as long as plasma HIV RNA levels remained below the level of conventional detection. Plasma samples from these subjects that were obtained during periods of viral load suppression were selected and analyzed. All subjects provided written informed consent. This study was approved by the University of California San Francisco Committee on Human Research.

Ultra-sensitive Plasma HIV RNA Levels

Longitudinal plasma HIV RNA levels were measured using the ultrasensitive isothermal Transcription Mediated Amplification (TMA) assay (Aptima®, Gen-Probe, San Diego, CA). This is a nucleic acid-amplification test that has been FDA-approved for the early detection of HIV infection in blood donors and validated for clinical use [1-3]. It is a highly specific and sensitive assay, with a 50% detection limit of 3.6-14 copies RNA/mL when performed in singlicate [4,5]; the sensitivity of the assay is <3.5 copies RNA/mL when 4 replicates are performed (0.5 mL of plasma per replicate). The output is a signal:cutoff (S/Co) ratio (range 0-30), with $S/Co < 1.0$ considered HIV RNA “negative” and $S/Co \geq 1.0$ considered HIV RNA “positive.”

In vitro spiking experiments were conducted to validate the specificity of the TMA assay and its use for ultrasensitive, semiquantitative measurement of HIV RNA levels (Figure 1).

Samples of known HIV viral load copy number (0, 1, 3, 10, 30, 100, and 300 copies/mL) were tested with the TMA assay 20 times each by 4 different laboratory technicians (Jeff Linnen, personal communication). A random selection of 3 replicates (the same number of replicates performed on the study subjects) for each of the technicians showed excellent correlation between HIV viral load copy number and S/Co ratio.

HIV Antibody Levels

A “de-tuned” or less-sensitive enzyme immunoassay (Organon Tecnika Vironostika [OTV], BioMerieux) was used to measure longitudinal, semiquantitative HIV antibody levels. The OTV is a second-generation ELISA that detects both IgG and IgM antibodies to HIV-1 and is FDA-approved for diagnostic testing. The less-sensitive modification involves testing 1:20,000 dilutions of plasma under abbreviated incubation conditions and calculating a standardized optical density (SOD) for each sample. Previously, the less sensitive-EIA has been used to identify patients with early HIV infection, with such patients exhibiting a positive result on the standard EIA and a negative result on the less sensitive-EIA [6]. In this study, we used the less sensitive-EIA to evaluate change in HIV antibody levels over time.

Statistical Methods

Spline regression was performed using mixed effect linear models to examine change in plasma HIV RNA and HIV antibody levels over time. Residuals were checked to verify that the data conformed with model assumptions. All statistical analyses were conducted with the Stata version 9.0 software program (Stata Corp, College Station, TX).

RESULTS

180 HAART-suppressed subjects with at least 2 consecutive plasma HIV RNA levels below the level of detection (<50-75 copies/mL) while taking antiretroviral drugs were identified from the SCOPE cohort. Only plasma samples that were taken during periods of viral load suppression were selected and analyzed.

The 180 subjects were 84% male, 15% female, and 1% male-to-female. At baseline (defined as the time of the first viral load measurement performed for this analysis), the median age was 46 (interquartile range [IQR] 40-52) years and self-reported duration of HIV infection was 12 (8-16) years. The baseline median CD4+ T cell count was 423 (IQR 258-620) cells/mm³. The median self-reported pre-HAART CD4+ T cell nadir count was 100 (IQR 40-188) cells/mm³. At baseline, the median duration of suppressive HAART was 13 (IQR 3.9-46) months. A total of 36 subjects had been virologically suppressed for more than five years. Subjects were taking various different antiretroviral regimens: 93 subjects were taking protease inhibitor (PI)-based HAART (62 “boosted” PI, 31 “unboosted” PI), 31 subjects were taking non-nucleoside reverse transcriptase inhibitor (NNRTI)-based HAART, 50 subjects were taking both a PI and NNRTI as part of their HAART regimen, and 3 subjects were taking a nucleoside reverse transcriptase inhibitor (NRTI)-only regimen.

A total of 1606 TMA assays were performed on 438 specimens (median 3 replicates per specimen) from these 180 subjects. During the first year of viral suppression (defined as the first 12 months after plasma HIV RNA levels became undetectable using conventional methods), plasma HIV RNA levels declined at a rate of -0.25 S/Co per month ($p=0.001$) (Figure 2A). There was no evidence for decline in low level viremia after month 12 of viral suppression ($p=0.38$). This initial decline, then subsequent stability in plasma RNA levels occurred regardless of class of HAART regimen.

A total of 189 semiquantitative EIAs were performed on 98 of the 180 HAART-suppressed subjects. In the first year of viral suppression, HIV antibody levels declined at a rate of -0.10

SOD per month ($p=0.05$), but after month 12 there was no evidence for a continued decline ($p=0.99$) (Figure 2B).

DISCUSSION

In this study, we confirmed that residual, low-level viremia continued to decline during the first year after plasma HIV RNA levels had become undetectable using conventional assays (< 50 to 75 copies RNA/mL). Moreover, the host response to the virus—as defined by HIV specific antibody levels—also decreased during the first year of effective viral suppression, and then subsequently remained stable. The observed initial decrease, then stability, of residual viremia may represent residual virus arising from different cellular compartments. The observed low-level viremia in these HAART-suppressed subjects may reflect ongoing viral replication or release of RNA from a long-lived latent reservoir (which can theoretically persist indefinitely in the absence of active viral turnover). This remains an ongoing debate [7-11]. These data collectively suggest that virus production (or replication) will likely persist indefinitely during long-term therapy, and that a steady-state viral load and host response “set-point” may exist during therapy.

The stability in plasma viremia after the first 12 months of viral load suppression is consistent with data from other groups [12]. In one study, residual viremia below the level of conventional detection was assessed in HAART-suppressed patients using a single copy assay [12]. Longitudinal assessments of low-level residual viremia revealed no significant decrease in the level of plasma viremia between the first and second years of suppressive therapy. Another study by the same group measured residual viremia in patients with a longer duration (7 years) of HAART suppression using the same single copy assay [13]. In contrast to our study, the authors observed a third and fourth phase of viral decay, occurring beyond the first year of viral suppression. This difference may be explained by the fact that subjects were required to have a detectable plasma HIV RNA level at baseline using the single copy assay. In other words, this study may have selected for patients with a higher level of residual viremia at baseline compared to our study. Both studies, however, reached the same central finding that during very long-term HAART (up to 6.2 years in our study), HIV persists at a relatively stable level.

In this present study of long-term HAART-suppressed subjects, we did not observe a direct relationship between baseline plasma HIV RNA levels and baseline HIV antibody levels. This is in contrast to so-called “elite controllers” (HIV-infected individuals who are able to maintain undetectable viral loads in the absence of antiretroviral therapy), in whom we have previously shown a direct correlation between plasma HIV RNA levels and HIV antibody levels [14]. The lack of association in HAART-suppressed individuals may point to different mechanisms of viral control in these two patient populations.

Several limitations of our study deserve comment. The TMA assay provides a semiquantitative (although highly specific [14]) measurement of ultrasensitive plasma HIV RNA and confirmatory studies utilizing a more quantitative measurement of very low levels of viremia are warranted. Similarly, the less sensitive-EIA is a semiquantitative measure of total antibody reactivity, although it has been studied in patients treated with HAART during acute [15] and chronic [16] infection and appears to correlate directly with level of plasma viremia.

The clinical consequences of very low-level viremia during HAART are not known. Low-level viremia (below the conventional level of detection) has been associated with increased immune activation in HAART-suppressed subjects [7,17] and may contribute to poor immunologic recovery during long-term HAART [17]. It is possible that oscillating, very

low levels of viremia lead to increased levels of immune activation, which may lead to AIDS- and non-AIDS defining events. The steady-state levels of HIV antibodies during long-term therapy clearly indicate that the virus is generating some persistent inflammatory response. This response may in theory be contributing to the inflammation-associated, non-AIDS events now being observed in treated patients [18,19].

In conclusion, residual, low-level viremia is commonly observed in long-term HAART-suppressed patients, and appears to remain stable after one year of viral load suppression. HIV antibody levels also decline initially but stabilize during long-term HAART, suggesting a steady-state relationship between virus and the host response. Therefore, it appears that HAART alone will not be sufficient in eradicating HIV.

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Figure 1. In vitro spiking experiments showing relationship between HIV RNA level and TMA assay

Spiking experiments showing relationship between HIV RNA level and signal:cutoff (S/Co) ratio using the Transcription Mediated Amplification (TMA) assay. Each dot represents the mean of three replicates (randomly selected from 20 replicates) performed by 4 different laboratory technicians; the lines represent the mean S/Co for each viral load copy number (0, 1, 3, 10, 30, 100, and 300 copies/mL).

Figure 2.

“Month 0 of viral suppression” refers to the first timepoint at which subjects had the first of at least 2 consecutive plasma HIV RNA levels below the level of conventional detection (<50-75 copies/mL) while taking antiretroviral drugs.

A. Plasma HIV-1 RNA levels. Ultrasensitive plasma HIV RNA levels were measured using the Transcription Mediated Amplification assay in 180 HAART-suppressed subjects. Each dot represents the mean signal:cutoff (S/Co) of all replicates for each timepoint (median 3 replicates per specimen). The thick line indicates estimated change in plasma HIV RNA over time using mixed effect linear models.

B. HIV-1 antibody levels. HIV antibody levels were measured using a “detuned” or less-sensitive EIA on 98/180 HAART-suppressed subjects. SOD=standardized optical density using the less-sensitive EIA. The thick line indicates estimated change in HIV antibody levels over time using mixed effect linear models.