Modifications of HIV-1 DNA and Provirus-Infected Cells During 24 Months of Intermittent Highly Active Antiretroviral Therapy

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Background: Few data have been reported on the dynamics of HIV-1 DNA during intermittent highly active antiretroviral therapy (HAART). In this study, we measured cell-associated HIV-1 DNA and provirus-infected cells during the Istituto Superiore di Sanità-Pulsed Antiretroviral Therapy (ISS-PART) clinical trial.

Methods: HIV-1 DNA was measured by real-time polymerase chain reaction (PCR) in the peripheral blood mononuclear cells (PBMCs) of 37 subjects enrolled in the ISS-PART, a randomized clinical trial comparing 24 months of intermittent (arm B) versus continuous (arm A) HAART in chronic HIV infection. In 14 subjects, the number of provirus-infected cells was also measured at baseline and at month 24.

Results: At baseline, the number of HIV-1 DNA copies/10^6 PBMCs was similar in arm B (mean ± SD: 121 ± 172, median = 35) and arm A (mean ± SD: 107 ± 153, median = 10) (P = not significant [n.s.]). No significant variations occurred over time; at 24 months, the HIV-1 DNA level was 77 ± 28 (median = 30) copies/10^6 PBMCs in arm B and 166 ± 321 copies/10^6 PBMCs (median = 10) in arm A (P = n.s.). At baseline, the provirus-infected cell counts were 85 ± 98 (median = 50) cells/10^6 PBMCs in arm B and 92 ± 113 (median = 50) cells/10^6 PBMCs in arm A (P = n.s.), with no variations at 24 months.

Conclusions: These findings suggest that the intermittent schedule of the ISS-PART has no major impact on viral reservoirs, at least in a midterm follow-up.

Key Words: highly active antiretroviral therapy, HIV-1 DNA, treatment interruptions

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The dynamics of HIV-1 RNA after highly active antiretroviral therapy (HAART) interruption have been widely described,[1,2] whereas fewer data exist on the evolution of cell-associated HIV-1 DNA in this setting.[3,4] In the present study, we measured the amount of HIV-1 DNA in the peripheral blood mononuclear cells (PBMCs) of 37 patients with chronic HIV-1 infection, who were randomized to 24 months of intermittent or continuous HAART. To evaluate the impact of HAART interruption on viral load further, in 14 subjects, we measured the number of provirus-infected cells in the peripheral blood.

METHODS

HIV-1 DNA was measured in 19 patients enrolled in the arm B of the Istituto Superiore di Sanità-Pulsed Antiretroviral Therapy (ISS-PART) trial,[5] who underwent 5 treatment interruptions of 1, 1.2, 2, and 3 months' duration, each separated by 3 months of HAART. Eight time points were considered, corresponding to different "on" and "off" therapy periods, with the last (24 months) representing the end of a treatment period. Results were compared with those obtained at 3 time points (months 0, 12, and 24) in 18 subjects randomized to the continuous HAART arm (group A) of the same trial.

HIV-1 DNA load in PBMCs was measured by a real-time polymerase chain reaction (PCR) assay with a limit of detection of 10 copies/10^6 cells.[6] In 14 patients (7 in arm B and 7 in arm A) of the same trial, the proportion of cells harboring HIV-1 DNA was quantified by PCR according to an established procedure,[7] with minor modifications. Briefly, cell suspensions were diluted 10-fold starting from 10^6 to 10 cells and reconstituted by adding the proper number of normal human PBMCs. Each dilution was then pelleted, lysed, and analyzed by real-time PCR concomitantly to serial dilutions of 8E5 LAV cells, containing 1 copy of HIV-1 DNA per cell. Results were expressed as values from 5 replicates, calculated according to the method of Reed and Muench.[8] The limit of sensitivity of this assay was 10 infected cells/10^6 PBMCs.

For statistical analysis on quantitative variables, we used parametric (Student t for independent groups and for paired observations) and nonparametric (Mann-Whitney U and Wilcoxon) tests. The latter were used to validate the results of parametric tests, in case of nonnormality and/or heteroscedasticity of the variable distributions, or in the presence of

cutoff values (eg, lower limit of detection for HIV-1 DNA, which was imputed for observations lower than this limit in the parametric analyses). Repeated measures analysis of variance was used to evaluate the change of HIV-1 DNA over time. Linear regression analysis was used to test the relation among quantitative variables. The Fisher exact probability test was used to assess the association between categoric variables (HIV-1 DNA values "detectable" vs. "at or below limit of detection") in 2-by-2 contingency tables. All P values were 2-tailed, and $P < 0.05$ was considered significant. Quantitative variables are presented as means ± SDs (median). Categoric variables are presented as percentages.

RESULTS

At baseline, patients in the 2 groups (intermittent therapy = arm B and continuous therapy = arm A) were balanced for their main characteristics. The CD4 count was 743 ± 294 (median = 712) cells/mm^3 in arm B and 725 ± 308 (median = 675) cells/mm^3 in arm A, and the pre-HAART CD4

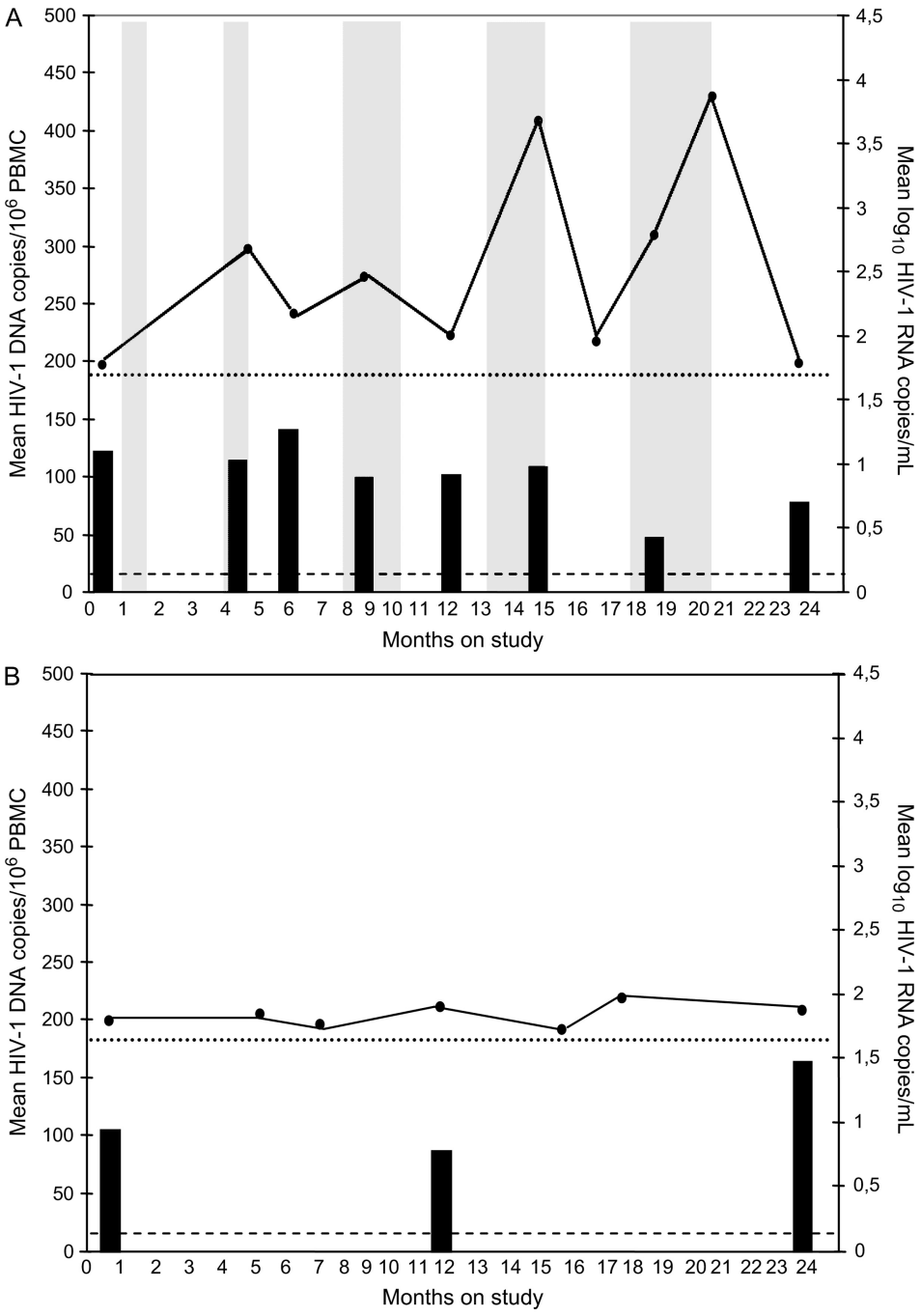

FIGURE 1. Modifications of HIV-1 DNA and RNA during 24 months of intermittent (A) or continuous (B) HAART. The bars represent mean HIV-1 DNA values (copies/10^6 PBMCs) at different time points. The continuous line represents mean HIV-1 RNA plasma viremia ($\log_{10}$ copies/mL). The dotted horizontal line represents the limit of detection (LOD) of the assay for HIV-1 RNA, and the dashed horizontal line represents the LOD of the assay for HIV-1 DNA. A, Shaded areas indicate periods ''off'' therapy.

count was 499 ± 177 (median = 497) cells/mm^3 in arm B and 437 ± 207 (median = 391) cells/mm^3 in arm A. Patients had been on their first-line HAART for 26.7 ± 9.7 (median = 25) months (arm B) and 24.7 ± 9 (median = 20.5) months (arm A). In arm B, 73.6% were treated with nonnucleoside reverse transcriptase inhibitor (NNRTI)–based HAART and 10.5% were treated with protease inhibitor (PI; mainly unboosted)–based HAART; in arm A, these proportions were 52.6% and 15.7%, respectively. All subjects had HIV-1 plasma viremia <2.6 $\log_{10}$ copies/mL: 1.8 ± 0.24 (median = 1.7) $\log_{10}$ copies in arm B and 1.7 ± 0.21 (median = 1.7) in arm A.

At baseline, HIV-1 DNA value, expressed as copies/10^6 PBMCs, was 121 ± 172 (median = 35) cells/mm^3 in arm B and 107 ± 153 (median = 10) cells/mm^3 in arm A (*P* = not significant [n.s.]). Figure 1 shows the pattern of HIV-1 DNA over time in the 2 groups, along with HIV-1 RNA results. Repeated measures analysis of variance failed to detect statistically significant variations of HIV-1 DNA in both groups, and values measured at 24 months (arm B: 77 ± 98 [median = 30] cells/mm^3; arm A: 166 ± 321 [median = 10] cells/mm^3) were not different from baseline and between each other. As expected, in arm B, HIV-1 RNA rebounded at each treatment interruption and returned to baseline values at the end of the last treatment period (month 24), whereas in arm A, its values remained consistently <2.6 $\log_{10}$ copies/mL.

Baseline and 24-month HIV-1 DNA values in arm A and arm B were also compared using a binary variable of "detectable" versus "at or below limit of detection": the 2 arms did not differ with respect to baseline and 24-month DNA ("detectable" values for arm A vs. arm B, respectively: 41% vs. 59% at baseline [*P* = 0.325] and 46% vs. 54% at 24 months [*P* = n.s.]).

The number of provirus-infected cells/10^6 PBMCs was 85 ± 98 (median = 50) in arm B and 92 ± 113 (median = 50) in arm A (*P* = n.s.) at baseline; at 24 months, these values were 42 ± 34 (median = 50) cells/10^6 PBMCs in arm B and 121 ± 140 (median = 100) cells/10^6 PBMCs in arm A, which were not statistically different from baseline and between each other. At month 24, the CD4 count was 647 ± 183 (median = 613) cells/mm^3 in arm B and 741 ± 301 (median = 605) cells/mm^3 (*P* = n.s.). Normalizing HIV-1 DNA values to CD4 percentage (CD4%) yielded similar results to those obtained with PBMC HIV-1 DNA (data not shown). Linear regression analysis failed to show significant relations between HIV-1 DNA or provirus-infected cells and other clinical, immunologic, and virologic variables.

DISCUSSION

Total (integrated and unintegrated) HIV-1 DNA is one of the most accessible and easy markers of viral burden in HIV-1–infected subjects,[9] which gradually declines during successful HAART.[10] Because reseeding of viral reservoirs has been hypothesized after HAART cessation,[11] monitoring HIV-1 DNA may provide some information on the consequences of HAART interruption on viral reservoirs. Overall, our findings suggest that the fixed-time treatment interruption protocol of the ISS-PART study, which was conducted in patients who had presumably achieved their steady-state level of HIV-1 DNA, has a negligible effect on total HIV-1 DNA burden and on the number of provirus-infected cells. Some peculiar features of the study, including the patients' low HIV-1 DNA burden at baseline, the relatively short duration of interruptions (all but the last not exceeding 2 months), and the relatively long treatment periods between them, may have contributed to these results; indeed, a previous study in subjects undergoing a long-term treatment interruption showed a high variability of HIV-1 DNA pattern, with an average doubling time of 2 months.[3]

Our study has several limitations. The relatively small sample size and unique features of the cohort (low baseline HIV-1 DNA and short interruptions) argue for caution in generalizing to other patient populations undergoing treatment interruption under different circumstances. In addition, the assay used to quantify provirus-infected cells does not discriminate between replication-competent and replication-defective virus. Conversely, considering the repeated measures of HIV-1 DNA, the homogeneity of the studied population, and the presence of a control group, one may cautiously conclude that a fixed-time treatment interruption strategy such as that adopted in the ISS-PART does not significantly affect viral reservoirs in the medium term. Moreover, because the modifications in HIV-1 DNA basically parallel those in provirus-infected cells, one might speculate that the number of HIV-1 DNA copies roughly corresponds to the number of total (resting and activated) infected cells, thus strengthening the value of HIV-1 DNA as a marker of viral burden.

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