CORRESPONDENCE

Questions about Results Reported with Potent Antiretroviral Therapy for Human Immunodeficiency Virus Type 1 Infection

To the Editor—Zaunders et al. [1] report the clearance rates of plasma human immunodeficiency virus (HIV) type 1 RNA and peripheral blood HIV-1 DNA levels, the phenotypic profiles of CD4 and CD8 lymphocytes, and anti–HIV-1 antibody levels in patients treated for 52 weeks with antiretroviral therapy (combination of zidovudine, lamivudine, and indinovir). Therapy was begun during primary HIV-1 infection (PHI). Results were compared with results for HIV-1–uninfected subjects, untreated patients with PHI, and patients with established HIV-1 infection. The data reported raise several questions.

First, by week 8 a similar decrease in peripheral blood HIV DNA levels was observed in both treated and untreated patients. What was the reason for the decrease in untreated patients, and why was there no difference in the decreases in the 2 groups?

Second, reverse-transcriptase inhibitors prevent reverse transcription (RT) of HIV RNA into HIV DNA, whereas protease inhibitors render newly produced virions noninfectious. Furthermore, according to the most recent model of HIV-1 pathogenesis reported by Ho et al. [2] and Wei et al. [3], productively infected lymphocytes have a half-life of ∼1.6 days. However, Zaunders et al. [1] found continued expression of viral antigens. In this case, one would expect the HIV DNA to increase or at least to remain stable in the untreated patients with PHI and to decrease rapidly in treated patients. What is the explanation for their findings that treatment "had little direct effect on HIV-1 DNA burden" [1, p. 326] and that "no significant difference in the number of copies per microgram of PBMC [peripheral blood mononuclear cells] DNA was observed between treated and untreated PHI patients at baseline or at weeks 8, 24, or 52" [1, p. 322]?

Third, the antiretroviral drugs used by Zaunders et al. affect neither transcription of proviral DNA nor translation of HIV RNA into proteins (i.e., expression of viral RNA and proteins). In other words, they decrease HIV RNA indirectly by decreasing HIV-1 DNA viral burden. How then did treatment lead to a decline of HIV RNA from 6.0 $\log_{10}$ copies/mL at baseline to 0 copies/mL after week 36 while having "little" effect on HIV-1 DNA burden?

Fourth, when discussing their findings with regard to the phenotypic profiles of the CD8 lymphocytes, Zaunders et al. wrote, "The persistence of HIV-1 DNA together with increased CD8 T lymphocyte turnover and activation indicate continued expression of viral antigens [1, p. 320]." How is it possible to have continuous expression of HIV proteins in the absence of HIV RNA?

Fifth, if there was continuous expression of viral antigens, why did only 2 patients treated with highly active antiretroviral therapy (HAART) develop "typical antibody responses to HIV-1, as determined by serial Western blots" [1, p. 325]? Was there, at any stage of the study, a difference between the Western blot profiles of HAART-treated and -untreated patients with PHI?

Sixth, given that the aim of the study was to compare treatment between patients with PHI and "HIV-1–uninfected subjects, untreated PHI patients, and patients with established HIV-1 infection, [1, p. 320]" why, with the exception of the lymphocyte profiles, were no data presented on the HIV-1–uninfected subjects and the patients with established HIV-1 infection?

Finally, for the measurement of the HIV RNA, Zaunders et al. [1] used the Roche Amplicor quantitative RT polymerase chain reaction (PCR) kit. However, according to the manufacturer, "the Amplicor HIV-1 Monitor test is not intended to be used as a screening test for HIV or as a diagnostic test to confirm the presence of HIV infection" (Roche Diagnostic Systems, [Branchburg, NJ], 06/96, 13-08088-001). Researchers at the University of Massachusetts School of Medicine found that "plasma viral load tests were neither developed nor evaluated for the diagnosis of HIV infection. . . . Their performance in patients who are not infected with HIV is unknown," and their use leads to "misdiagnosis of HIV infection" [4, p. 37]. One British virologist noted, "Those laboratories which undertake HIV screening and confirmation assays understand fully the technical problems associated with PCR and other amplification assays and it is precisely for those reasons that PCR is NOT used as a confirmatory assay (as discussions with any competent virologist would have informed them)" [5, p. 38]. Thus, if it is possible that Zaunders et al. did not detect HIV RNA, could this explain the lack of correlation between HIV DNA and HIV RNA?

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Reply

To the Editor—Our results [1] showed that, although there was a 4–5 log decrease in plasma human immunodeficiency virus (HIV) type 1 RNA concentrations after the start of treatment for primary HIV-1 infection (PHI), there was a <1 log effect on the level of cell-associated HIV-1 DNA. The fall in HIV-1 DNA was not significantly different from that in untreated patients. Further experiments are required in order to investigate the dynamics of production and turnover of cells containing HIV-1 DNA in vivo before we can understand why the regimen used had little effect on this reservoir.

The probable reason for maintenance of the level of cell-associated HIV-1 DNA, despite large decreases in plasma HIV-1 RNA, is the generation of a large pool of long-lived, resting CD4 T lymphocytes containing integrated HIV-1 DNA at the very early stages of PHI [2]. The decay rate of this reservoir appears to be extremely slow, despite highly active antiretroviral therapy (HAART) [3–5].

Furthermore, activation of these latently infected cells leads to production of viral proteins [3–5]. Therefore, it is entirely feasible for low-level synthesis and presentation of viral antigens to occur, despite the presence of inhibitors of viral reverse transcriptase and protease. Also, the reduction of plasma HIV-1 RNA in the patients studied was not absolute; very low-level signals were observed in some samples from some patients but remained below the 50 copies/mL sensitivity of the polymerase chain reaction (PCR). Others have shown that, when such low-level RNA is present in the plasma, RNA can be detected in lymphoid tissue [6]. Retention of p24 in lymph nodes has been shown after HAART, even when RNA has been eliminated [7].

The reduction of antibody levels in some patients as a result of HAART has also been reported in other studies [8–10]. The reason for the fall in antibody concentrations is unknown but could be related to the lack of HIV-1–specific CD4 T lymphocytes needed to generate a normal immune response, or it may simply result from a decrease in the concentration of viral antigens. A similar decrease in antigen-specific CD8 T lymphocytes is also observed after the start of HAART [11]. We did not show our results for Western blot analysis and p24 antibody concentrations for untreated patients with PHI, because the development of reactivity is widely reported in the literature [12].

The measurement of HIV-1 RNA was applied to samples from patients who had been diagnosed as having HIV-1 infection on the basis of longitudinal, repeatedly reactive, licensed HIV-1 antibody tests and immunoblot reactivity typical of early seroconversion events. In the absence of HIV antibody reactivity, subjects were enrolled on the basis of reactivity determined by use of an EIA for HIV-1 p24 antigen, confirmed by neutralization, and a licensed qualitative HIV-1 DNA PCR, together with clinical assessment of exposure and symptoms. In each case, subsequent specimens were obtained and assessed for the further development of virus-specific antibodies.

HIV-1 RNA is never used as a screening test. Reported false-positive RNA test results for HIV-seronegative persons are generally at a low level and rarely >1000 copies/mL. HIV RNA measurements observed during PHI, however, often exceed the upper limit of quantitation of the assay, which was the case with all initial specimens assayed in our study. It therefore is highly unlikely that the HIV-1 RNA results obtained are due to false-positive reactions.

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