Human Immunodeficiency Virus–1 RNA Levels and CD4 Lymphocyte Counts, during Treatment for Active Tuberculosis, in South African Patients


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During 6 months of treatment, we measured human immunodeficiency virus (HIV–1) virus loads, CD4 T cell counts, and immune activation markers, in 111 HIV–1–infected patients with active tuberculosis (TB). The median virus load (baseline, 5.58 log₁₀ copies/mL) significantly increased at 1 month (5.71 log₁₀ copies/mL), then returned to near-baseline levels at 3 months (5.40 log₁₀ copies/mL) and at 6 months (5.36 log₁₀ copies/mL). In contrast, the median CD4 counts increased at 1 month (186/mm³), at 3 months (238/mm³), and at 6 months (239/mm³). CD4 counts and virus loads did not change during therapy. Expression of CD38 and HLA-DR remained high throughout treatment, whereas plasma levels of interleukin-6 decreased over time.

A hallmark of advanced human immunodeficiency virus (HIV–1)–induced immunodeficiency is the development of opportunistic infections (OIs), which are associated with an increased risk of death [1, 2]. Infectious pathogens provoke the activation of immune-system cells, which may facilitate HIV–1 replication directly and indirectly, through the effects of cytokine networks. In one study, patients with a variety of HIV–associated acute OIs were found, at the time of diagnosis of the OIs, to have significant increases in HIV–1 levels in blood [3]. Patients with clinical recovery showed a reduction in HIV–1 levels within 2–4 weeks of treatment for the OIs. Other studies have yielded similar results [4–7].

In sub-Saharan Africa, tuberculosis (TB) is often the first OI to occur in patients with HIV–1 infection and is a leading cause of death in patients with AIDS [8]. HIV–1 infection increases the risk of Mycobacterium tuberculosis disease, and there is clinical and experimental evidence suggesting that TB accelerates the course of HIV–1 disease [9, 10]. Patients with both HIV–1 and TB show elevated levels of proinflammatory cytokines and increased expression of cellular activation markers [10], the latter of which correlates with the levels of HIV–1 plasma viremia in vivo [11]. Through interaction with the nuclear factor–κB binding sites in the HIV–1 genome, proinflammatory cytokines produced in response to TB [12] may be potent agents in the up-regulation of HIV–1 replication in vivo. Indeed, a small study has shown that active TB is associated with an increase in the levels of HIV–1 in plasma [7]. Furthermore, treatment of TB has been shown to be associated with a decrease in some markers of immune activation [10, 13]. To determine the effect that treatment for TB has on HIV–1 virus load, CD4 lymphocyte counts, and markers of immune activation, we prospectively analyzed a large cohort of HIV–1–infected patients in South Africa who had active TB and who were undergoing treatment for TB.

Patients, materials, and methods. Patients with newly diagnosed TB who had been admitted to either the Sizwe Infectious Diseases Hospital in Johannesburg or the Goldfields West Hospital in Westonaria were screened for entry into this study. Patients were generally referred for care to Sizwe Hospital because of known HIV infection, whereas gold miners with suspected tuberculosis were routinely admitted to Goldfields West Hospital for evaluation and initial treatment. HIV–1 and HIV–2 testing was done after pretest counselling had been administered and informed consent had been obtained, and those who were confirmed to be HIV–1 seropositive were invited to enter the study. Recruitment began in 1997 and was completed by the end of 1998. The diagnosis of TB was based on medical history and physical examination, sputum tests that were positive for acid-fast bacilli, and radiological features compatible with pulmonary TB. Patients who had evidence of extrapulmonary TB were also included. Mycobacterial cultures were performed in most cases, although the results were not availa-
ble at the time of recruitment. Patients received standard treatment for TB, which consisted of rifampin and isoniazid, for 6 months, plus pyrazinamide and ethambutol, for the first 2 months. Blood specimens were collected at enrollment and at 1, 3, and 6 months. Treatment with antiretroviral therapy was not available to any participants in the study.

Blood samples were collected in EDTA tubes, and plasma was separated and was frozen at −70°C, within 6 hours of collection. HIV-1 RNA levels in plasma were determined by use of a Monitor Version 1.0 kit (Roche), according to the manufacturer’s protocol. A single operator who had been trained and certified, by Roche Laboratories, to conduct this test performed all assays. Plasma samples obtained from a single patient at multiple times were batched and, in most instances, were run in the same assay.

CD4 T cell counts and expression of CD38 and HLA-DR on CD8 T cells was determined by dual-color flow cytometry. Fifty microliters of whole blood was stained by use of monoclonal antibody combinations CD45–fluorescein isothiocyanate (FITC)/CD14–phosphatidylethanolamine (PE), CD3–FITC/CD4–PE, CD3–FITC/CD8–PE, CD8–FITC/CD38–PE, and CD8–FITC/HLA-DR–PE (Simultest reagents; Becton Dickinson) and were analyzed on a FACSort analyzer by SimulSET software (version 1.1; Becton Dickinson). IgG1–FITC/IgG1–PE controls were used in each experiment. The levels of interleukin (IL)–6 in plasma were assessed by use of an ultrasensitive commercial assay with a limit of detection of 5 pg/mL (Amersham).

Data were analyzed by the Stata statistical package (version 6.0; Stata). Virus-load levels were log-transformed for analysis. Baseline attributes were compared by Student’s t test. Changes in CD4 counts and cytokine levels were compared with Wilcoxon signed-rank test. Changes in log-transformed virus-load levels were compared by paired and unpaired Student’s t tests. Two-tailed P values were determined.

Results. We enrolled and collected samples from 111 HIV-1–infected patients who were hospitalized with active TB. Sixty-five percent of the patients were male, and 35% were female. The median age of men was 34 years, and the median age of women was 27.5 years (P < .05). Ninety-nine patients had pulmonary TB alone, and 12 patients had both pulmonary and extrapulmonary TB. Of the 111 patients originally enrolled, follow-up information and blood samples were available for 103 (93%) at 1 month, for 85 (77%) at 3 months, and for 57 (51%) at 6 months of treatment for TB. Many of these patients were migrant workers who left the area after being discharged and therefore were not available for continued participation. Clinical responses were favorable for the majority of patients, with the exception of 6 (5%) patients who were found to have multidrug-resistant (MDR) tuberculosis and 12 (11%) patients who died.

For the entire cohort, the median virus load at baseline was 5.58 log₁₀ copies/mL (range, 3.0–6.7-log₁₀ copies/mL) (figure 1A). The median CD4 count at enrollment was 169 cells/mm³ (range, 6–1,225 cells/mm³) (figure 1B). More than half (59%) of the patients had CD4 counts <200 cells/mm³, and 17 of them had CD4 counts <50 cells/mm³, whereas 13 (12%) patients had CD4 counts >500 cells/mm³. HIV-1 virus-load levels increased significantly after 1 month of treatment for TB, to a median of 5.71 log copies/mL (P = .02). At 3 months, median virus loads had significantly decreased, compared with the 1-month level, to 5.40 log copies/mL (P < .01), and this level was maintained at 6 months (5.36 log copies/mL). For the entire cohort, there was no significant difference between the median virus load at baseline and that at 6 months. The median CD4 count increased after 1 month (186 cells/mm³) and 3 months (238 cells/mm³), and this level was maintained at 6 months (239 cells/mm³) (P > .10). Compared with patients who survived infection, patients who died had significantly lower median baseline CD4 counts (85/mm³ vs. 173/mm³) and significantly higher virus loads (6.1 log copies/mL vs. 5.3 log copies/mL).
Figure 2. Changes in percentage of CD8 T cells expressing either CD38 (A) or HLA-DR (B), and levels of interleukin (IL)-6 in plasma (C), during 6 months of therapy for tuberculosis. In panels A and B, the boxes indicate the interquartile ranges, the horizontal lines transecting the boxes indicate the medians, and the whiskers indicate the highest and lowest values.

Analysis of virus RNA levels in individual patients showed that 12 patients had a >1-log increase in virus load while being treated for TB. This increase was maintained at 6 months in 5 of 8 patients from whom samples were available. One of these 5 patients had MDR TB, which may have facilitated viral replication; however, 5 other patients with MDR TB did not experience >1-log increases in virus levels, a finding suggesting that MDR TB was not the reason for high virus levels.

Additional analyses were performed for those patients for whom data from all 4 collection times were available. This subgroup excluded those patients who, before 6 months, either died or were lost to follow-up. The initial median virus level for these 57 patients was slightly lower (5.43 logs/mL), and the median CD4 count was higher (253/mm³), compared with the entire cohort. However, the virus levels were not significantly different from baseline to 6 months, a finding that is similar to what was seen in the entire cohort, and CD4 counts remained similar throughout treatment (6-month median, 239/mm³).

The percentage of CD8 T cells expressing CD38 was high and remained so throughout the 6 months of treatment. For the entire cohort, median levels at baseline and at 1, 3, and 6 months were 92%, 94%, 90%, and 90%, respectively (figure 2A). The proportion of CD8 T cells expressing HLA-DR was lower and remained unchanged for the duration of the study (55%, 53%, 53%, and 56%, at baseline and at 1, 3, and 6 months, respectively) (figure 2B).

Levels of IL-6 in plasma were measured in a subset of patients by an ultrasensitive ELISA assay. Results are shown in figure 2C, which indicates that many patients had measurable IL-6 levels that declined with treatment for TB. At 6 months, only 2 (11%) of 19 patients had detectable IL-6 in plasma, compared with 39 (67%) of 58 at enrollment (P < .001).

Discussion. In this prospective cohort study of HIV-related TB, we have shown that virus load is high at the time of diagnosis and remains elevated during treatment for TB. Virus loads increase significantly during the first month of treatment for TB and then return to baseline levels. Because virus loads were not determined before the diagnosis of TB, it is not possible to tell whether active TB results in a sustained increase in virus load, compared with the premorbid state. Day et al,
in a recent prospective study of HIV-infected South African miners, compared individuals who, during follow-up, developed TB and CD4-matched control subjects, who did not develop TB [14]; their data showed that baseline virus levels were significantly higher in the individuals who subsequently developed TB, but that virus load increased further after the development of TB, whereas it remained stable in the control subjects. As has the present study, Lawn et al. showed persistent elevation of virus levels during treatment for TB in a small cohort in Ghana [13].

In contrast to virus levels, CD4 cell counts, which were low at diagnosis of TB, rose slightly throughout treatment, although these increases were not statistically significant. In addition, there was essentially no change in the CD4 counts of those patients for whom data from all collection times were available. A previous study by several of the authors of the present study showed that, in both HIV-positive and HIV-negative patients with TB, the number of CD4 T cells increases after 3 months of treatment for TB [15]. In the present study, we have extended that observation, to show that CD4 cell increases are sustained through 6 months of treatment for TB. The factors that are responsible for this increase in CD4 cells, particularly during the first month of therapy, at the same time that virus loads increase, are unknown. This increase in CD4 cells suggests that, in patients with HIV infection, CD4 suppression at the onset of TB is the direct result of M. tuberculosis growth and inflammation, as well as the result of HIV-1. Several studies have shown that CD4 lymphopenia in HIV-uninfected patients with TB resolves during treatment for TB [16–17].

Markers of immune activation were elevated throughout follow-up, with CD38 and HLA-DR present on >90% and ~50% of CD8 cells, respectively. IL-6, a proinflammatory cytokine produced by monocytes and macrophages, was initially detectable in >66% of patients but, at the end of treatment for TB, was found in only 11% of patients. This finding is consistent with other reports of transient elevation of IL-6 in patients with TB who are not infected with HIV [18]. TB is associated with immune activation including increased expression of (1) cellular-activation markers, such as CD38 and HLA-DR, (2) cytokines, and (3) other inflammatory molecules, such as neopterin and β₂-microglobulin [10, 19, 20]. In some cases, treatment is associated with a reduction of these cellular-activation markers [10, 13, 19]. HIV infection is also associated with activation of CD8 cells, and CD38 expression is seen in patients with advanced HIV infection who do not have TB and is a marker of higher risk of disease progression [21]. In the present study, there was some indication that the immune activation caused by TB was reduced, as evidenced by plasma IL-6 levels, but high levels of CD38 persisted for 6 months. Sustained expression of CD38 could be related to immune activation caused by advanced HIV disease. Alternatively, TB is a chronic disease, and its effects on the immune system may be long lasting and perhaps may be permanent. It is also possible that other factors may be involved in the maintenance of high virus levels: for example, TB is associated with elevated expression of CCR5 [22], which may encourage the replication of R5 variants, which are preferentially recovered from patients with active TB [23].

The effect that TB could have on the natural history of HIV infection in developing countries is substantial. For the many people with HIV who live in countries that cannot afford antiretroviral therapy, treatment of HIV-1–related OIs is often the only way to slow the progression of HIV disease. Indeed, a number of studies have shown that treating certain OIs, such as bacterial pneumonia and herpes simplex virus infections, leads to a reduction in the plasma levels of HIV-1 RNA, presumably by reducing inflammation [3–7]. Here, we have shown that a similar scenario does not hold true for TB, as virus loads first increased during treatment and never fell to levels that were significantly lower than baseline, throughout 6 months of treatment for TB.

TB is both the most common cause of death in autopsied African patients with AIDS and one of the leading opportunistic diseases that is diagnosed in patients with AIDS while they are alive. Unlike most other OIs associated with AIDS, TB can occur at relatively high CD4 counts. The association of TB and high virus loads, however, portends a more rapid course of HIV disease, even in those patients whose TB is treated and cured. Thus, TB can contribute to higher mortality in patients with HIV infection, particularly when patients have limited access to antiretroviral therapy. In such settings, prevention of TB, by treatment of latent TB infection, is an important priority [24].

In developed countries, the use of antiretroviral therapy has had a major influence on the incidence of OIs and AIDS-related deaths [25]. In recent months, there has been increasing attention paid to providing antiretroviral therapy to patients with HIV-1 disease in resource-poor settings, despite the many economic, political, health-system, and behavioral barriers to doing so. The World Health Organization has recently promulgated guidelines for treatment of HIV-1 infection in developing countries. One target population of patients who may be readily identified and likely to benefit from antiretroviral treatment is patients with TB. The identification of such target groups may be important in resource-poor settings, where treatment may have to be prioritized for economic reasons. Although controversy on the optimal timing of antiretroviral treatment in patients with HIV and TB continues, our data provide an additional impetus for the implementation of programs to provide antiretroviral therapy to patients with TB, many of whom have extremely high virus loads, low CD4 counts, and an unnecessarily high risk of imminent death.
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References