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Unexpected Low-Level Viremia Among HIV-Infected Ugandan Adults With Untreated Active Tuberculosis

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To the Editor

Active tuberculosis (TB) has been associated with elevated HIV RNA levels.^{1,2} In vitro data indicate that *Mycobacterium tuberculosis* (MTb) triggers cellular immune activation via cytokines such as tumor necrosis factor- α , which, in turn, stimulates HIV replication leading to higher viral load (VL).³ We identified unexpectedly low HIV VLs among Ugandan adults with untreated HIV and smear-positive pulmonary TB and examined predictors of low VL and evolution of viremia during TB therapy.

Subjects were HIV-infected, antiretroviral therapy-naïve patients older than 18 years with acid-fast smear and culture-positive pulmonary TB enrolled in 2 different clinical trials at the Mulago TB Research Unit in Kampala, Uganda. Subjects had entry CD4 count >200 cells/mm³ (Trial 1; NCT00057421)⁴ and 350 cells/mm³ (Trial 2; NCT00078247), respectively.⁵ All subjects were treated with short-course antituberculous therapy.⁶ Baseline HIV serostatus was established by enzymelinked immunosorbent assay and confirmed in a random subset by Western blot for Trial 1 subjects and confirmed by Western blot for all Trial 2 subjects. HIV RNA testing was performed at baseline and 3 months for subjects in Trial 1 (Cambridge Bioscience Assay, Cambridge, United Kingdom), limit of detection 50 c/mL. Because there are no published performance data on this assay for non-B HIV subtypes, HIV RNA was confirmed on a subset of Trial 1 specimens using the Amplicor HIV-1 Monitor Test, v1.5 (Roche Molecular Systems, Branchburg, NJ; limit of detection 50 c/mL), which has been validated for subtypes A and D,⁷ the most common clades in Uganda.⁸ For Trial 2 subjects, baseline and 3-month HIV RNA testing was conducted with

the Amplicor HIV-1 Monitor Test, v1.5 (limit of detection 400 c/mL) at the TB Research Unit Laboratory. CD4 lymphocyte count (EPICS Profile 2 Flow Cytometry and Beckton Dickinson FACS Calibur, San Jose, CA) was performed on all subjects at entry and 3 months after initiation of TB therapy. HIV subtyping was performed on baseline plasma specimens for a random subset of patients from both trials using the Los Alamos National Laboratory subtyping tool RIP3 (<http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html>). Baseline chest radiography was performed on all patients and graded by blinded reviewers.⁹ Subjects with HIV RNA <4.0 log c/mL at study entry were classified as having “low baseline” VL and were compared with patients with baseline HIV RNA ≥4.0 log c/mL (high baseline VL).

Of 202 study subjects (Trial 1 n = 82; Trial 2 n = 120), the median baseline HIV RNA and CD4 count were 4.65 log c/mL (range 1.69–6.50) and 472 cells/mm³, respectively. There were 49 (24%) subjects with baseline HIV RNA <4.0 log c/mL (median 3.31 log c/mL, range 1.70–3.99) of whom 12 (6%) had HIV RNA <3.0 log c/mL at study entry. HIV subtyping conducted on 35 random baseline specimens indicated that 21 (60%) were subtype A, 11 (31%) were subtype D, 2 (6%) were A/D recombinants, and 1 (3%) was subtype C.

The 49 subjects with low baseline VL were compared with the 153 patients with baseline HIV RNA ≥4.0 log c/mL (median 4.86, range 4.00–6.50). Patients with low baseline VL exhibited no difference by median age (30 versus 30 years, $P = 0.89$), sex (57% versus 61% male, $P = 0.62$), median baseline CD4 (470 versus 474 cells/mm³, $P = 0.93$), or radiographic severity of TB (61% versus 61% severe disease, $P = 1.0$). Predictors of low-level viremia did not vary by trial. Of 12 subjects with baseline HIV RNA <1000 c/mL, the median HIV RNA and CD4 count at entry were 530 copies/mL and 383 cells/mm³, respectively (Table 1). Patients with baseline HIV RNA <1000 c/mL did not differ by baseline CD4 ($P = 0.28$), age ($P = 0.68$), sex ($P = 1.0$), or severity of TB disease ($P = 0.13$) when compared with patients with high baseline VL.

Among the 202 study subjects, 109 received standard TB therapy alone and 93 received antiretroviral therapy (n = 54) or prednisolone (n = 39) in addition to TB therapy. Of the 109 TB therapy-only subjects, baseline and 3-month HIV RNA data were available for 91 (84%). At entry, 72 (79%) of these 91 subjects had HIV RNA ≥4.0 log c/mL and 19 (21%) had HIV RNA <4.0 log c/mL (Table 2). A significantly higher proportion of patients with baseline VL ≥4.0 log c/mL had HIV RNA declines of >0.5 log c/mL after 3 months compared with patients with baseline VL <4.0 log c/mL ($P = 0.001$). Conversely, a higher proportion of patients with entry HIV RNA <4.0 log c/mL had HIV RNA increases of >0.5 log c/mL after 3 months of TB treatment compared with patients with entry HIV RNA ≥4.0 log c/mL ($P < 0.001$). These differences remained significant even after excluding 3 subjects in whom a 0.5 log HIV RNA decrease would not have been detectable due to assay sensitivity.

Multiple clinical and in vitro studies have shown that active TB during HIV infection is associated with significantly elevated HIV RNA levels.^{1,2,10,11} Contrary to these observations, we detected low-level HIV viremia (<4.0 log c/mL) in almost 25% of Ugandan patients with untreated HIV-TB coinfection in 2 different clinical trials and found

that low-level viremia was not related to baseline CD4 or severity of TB. Notably, 6% of study subjects with acute TB had baseline HIV RNA <3.0 log c/mL. In contrast, among a cohort of HIV-infected Malawian adults (CD4 count >300 cells/mm³) assessed during an episode of malaria, no subject had HIV RNA <3.0 log c/mL.¹²

Treatment of TB in HIV-infected patients has also been reported to result in a reduction of HIV VL.^{1,2} Interestingly, we observed paradoxical increases in HIV RNA after 3 months of TB treatment among some of the subjects with low baseline HIV RNA. When controlling for the baseline value of HIV RNA, an analysis of covariance indicated that this observation did not simply reflect regression to the mean.¹³ Furthermore, we expected that TB therapy would result in a decrease, not an increase, in HIV RNA toward a lower viral set point.

Several potential explanations exist for both the low baseline HIV RNA and paradoxical increase in VL after TB therapy observed in this setting. There are emerging data to suggest that certain pathogens, including measles virus and *Orientia tsutsugamushi*, may have the ability to attenuate HIV infection.¹⁴ Although most data indicate a potentiating effect of *MTb* on HIV replication, recent in vitro data suggest that *MTb* can also inhibit HIV replication in monocyte-derived macrophages.¹⁵ Although it is felt that most plasma virus comes from infected activated CD4 T cells, one study suggests that in some patients HIV-1 may preferentially replicate in tissue macrophages.¹⁶ Thus, the modulating effects of *MTb* on HIV infection of macrophages may differ between patients. Finally, the upregulation of T-regulatory cells during active TB may also result in fewer target cells for productive HIV viremia.¹⁷

Differing HIV responses to *MTb* coinfection may therefore reflect important but as yet unexplored variables that dictate the magnitude of viral replication in individual patients. These include the relative contribution of macrophage infection, T-cell activation, stimulating and inhibitory cytokines, viral tropism, and viral subtype. Investigation of these host and viral factors may shed further light on potential causes of low-level HIV viremia in the setting of active TB and provide additional insights into HIV and TB pathogenesis.

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TABLE 1

CD4 and HIV RNA Measurements Among HIV-Infected Patients With Smear-Positive TB and Baseline HIV RNA <3.0 Log c/mL

Patient	Age/Sex	CD4 Count Pre-TB Rx	HIV RNA Pre-TB Rx Kampala [*]	HIV RNA Pre-TB Rx UCSF [†]
1	31/male	305	<50 [‡]	<50
2	25/female	1024	<400 [‡]	NA
3	36/male	361	<400 [‡]	NA
4	18/male	696	206	<50
5	34/male	431	305	556
6	26/male	474	423	530
7	26/male	390	636	423
8	31/male	218	670	1330
9	38/male	271	712	83
10	36/female	808	764	NA
11	33/male	332	835	418
12	32/male	376	934	NA

Age in years. CD4 count in cells/mm³.

Rx, Treatment. NA, not available; LOD, limit of detection.

^{*} HIV RNA (in c/mL) performed at TB Research Unit Laboratory in Kampala, Uganda (Cambridge Bioscience, LOD 50 c/mL and Roche Amplicor Monitor 1.5 Assays, LOD 400 c/mL).

[†] HIV RNA (in c/mL) confirmatory testing performed at AIDS Research Institute-University of California, San Francisco, Laboratory of Clinical Virology (Amplicor HIV-1 Monitor Test, v1.5; LOD 50 c/mL).

[‡] HIV serostatus confirmed by Western blot.

TABLE 2

HIV RNA Measurements Among HIV-Infected Patients With Smear-Positive TB by Baseline HIV RNA Level (N = 91)

Baseline HIV RNA (log c/mL)	n	Median Baseline HIV RNA (c/mL)	HIV RNA p 3mo TB Rx	HIV RNA p 3 mo TB Rx (log c/mL)	HIV RNA > +0.5 log c/mL p 3 mo TB Rx (%)	HIV RNA > -0.5 log c/mL p 3 mo TB Rx (%)
<4.0 log c/mL	19	2239	8252	+0.66	12 (63)	0 (0)
>4.0 log c/mL	72	70,664	56,384	-0.28	9 (13)	26 (36)

All subjects received standard TB therapy.

Rx, treatment.