Effects of Antiretroviral Therapy on Immune Function of HIV-infected Adults with Pulmonary Tuberculosis and CD4+ >350 Cells/mm³

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Background. Human immunodeficiency virus (HIV)–tuberculosis coinfection is associated with heightened immune activation, viral replication, and T cell dysfunction. We compared changes in T cell activation and function between patients receiving concurrent treatment for HIV-tuberculosis coinfection and those receiving treatment for tuberculosis alone.

Methods. HIV-infected adults with tuberculosis and CD4+ T cell counts >350 cells/mm³ were randomized to receive tuberculosis treatment alone (control arm; n = 36) or 6 months of antiretroviral therapy (ART) concurrent with tuberculosis treatment (intervention arm; n = 38). HIV viral load, T cell subsets, T cell activation, and cytokine production were measured at enrollment and every 3 months for 12 months.

Results. Differences in absolute CD4+ and CD8+ T cell counts were not observed between arms. Viral load was reduced while participants received ART; control patients maintained viral load at baseline levels. Both arms had significant reductions in T cell expression of CD38 and HLA-DR. Interferon-γ production in response to mitogen increased significantly in the intervention arm.

Conclusions. In HIV-infected adults with tuberculosis and CD4+ T cell counts >350 cells/mm³, both tuberculosis treatment and concurrent HIV-tuberculosis treatment reduce T cell activation and stabilize T cell counts. Concurrent ART with tuberculosis treatment does not provide additional, sustained reductions in T cell activation among individuals with preserved immunologic function.

Coinfection with Mycobacterium tuberculosis remains a leading cause of morbidity and mortality among human immunodeficiency virus (HIV)–infected individuals in the developing world. HIV-infected individuals are more likely to develop tuberculosis disease following M. tuberculosis exposure, are at risk for severe, disseminated forms of tuberculosis, and have higher case fatality rates and rates of recurrent tuberculosis than individuals without HIV infection [1–5]. Tuberculosis accelerates the progression of HIV infection, especially among individuals who develop tuberculosis with preserved CD4+ T cell counts [6–10]. Uganda, where at least 39% of incident tuberculosis cases are complicated by HIV infection, is particularly affected by the overlapping epidemics of HIV and tuberculosis [11]. Tuberculosis has multiple deleterious effects on host immunity, including increased immune activation, apoptosis of CD4+ T cells, loss of both M. tuberculosis–specific and naive CD4+ T cells, and defective cytokine

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production [12–15]. Immune activation and erosion of the naive T cell pool are strongly associated with HIV infection. T cell activation, specifically CD38 expression on CD8\(^+\) T cells, is a marker for AIDS progression and death among untreated HIV-infected individuals and is predictive of poor CD4\(^+\) T cell recovery among patients receiving antiretroviral therapy (ART) [16–21]. Although the pathogenesis of HIV-tuberculosis coinfection is not well understood, the immunologic environment during \(M. \text{tuberculosis}\) infection is characterized by cytokine and chemokine irregularities that are believed to increase T cell activation, enhance HIV replication, and result in a dysfunctional immune response [22–28]. Thus, heightened immune activation as a result of both HIV and tuberculosis leads to multiple perturbations of the immune system that likely contribute to disease severity and progression.

The immunologic benefits of ART for HIV-infected patients, including reduction in viral load, immune activation, and restoration of CD4\(^+\) T cells, are well recognized [29, 30]. The effect of ART on T cell activation and function among HIV-infected adults with tuberculosis, however, is poorly characterized. In our earlier study, we reported that tuberculosis treatment alone significantly reduced T cell activation among HIV-infected adults with tuberculosis [31]. Therefore, we hypothesized that combination HIV-tuberculosis treatment would permit greater reduction in HIV replication than tuberculosis therapy alone, leading to decreased T cell activation and restoration of CD4\(^+\) and CD8\(^+\) T cell subsets and function. To test this hypothesis, we examined HIV-infected Ugandan adults with pulmonary tuberculosis and baseline CD4\(^+\) counts >350 cells/mm\(^3\) who were participants in a randomized clinical trial of ART during tuberculosis treatment. During this trial, which was initiated prior to recognition of the risks of both episodic and delayed ART, patients were treated with either a 6-month course of tuberculosis therapy (\(n = 36\)) or tuberculosis treatment combined with a punctuated 6-month course of ART (\(n = 38\)) [32, 33]. Using flow cytometry and a whole-blood enzyme-linked immunosorbent assay (ELISA), we compared markers of T cell activation, changes in T cell subsets, and production of interferon-\(\gamma\) (IFN-\(\gamma\)) in response to mitogen and \(M. \text{tuberculosis}\)- and HIV-specific antigens between study cohorts during the 6-month period of assigned therapy and for an additional 6-month period of observation.

**METHODS**

**Participants**

We present analysis of a subset of subjects enrolled in a larger phase 3 open-label randomized clinical trial entitled “Randomized clinical trial of a 6-month punctuated course of anti-retroviral therapy in Ugandan HIV+ adults with pulmonary tuberculosis and CD4\(^+\) >350 cells/mm\(^3\) (#NCT00782474/PART).” PART’s objective was to compare HIV disease progression and clinical outcomes of HIV-infected adults with tuberculosis and high CD4\(^+\) T cell counts treated with standard tuberculosis therapy (control arm) and those treated with tuberculosis therapy plus a limited 6-month course of ART (intervention arm). Participants aged between 13 and 60 were recruited from the Uganda–Case Western Reserve University Research Collaboration within the Mulago Hospital Complex in Kampala, Uganda. All participants had HIV type 1 (HIV-1) infection and smear- and/or culture-confirmed acute pulmonary tuberculosis. HIV-1 infection was diagnosed by means of ELISA and Western blot test and confirmed with HIV RNA copy levels. Only HIV-1–infected participants who were ART naive and had CD4\(^+\) counts >350 cells/mm\(^3\) were eligible for study participation. Data for this substudy were accrued during the period March 2006 through February 2009. Participants were selected for substudy enrollment if results of immunologic assessments (flow cytometry and whole-blood ELISA) were available from study enrollment and at least 2 additional time points during a 12-month period. Written informed consent was obtained from participants prior to enrollment, and the study protocol was approved by the institutional review boards of Case Western Reserve University/University Hospitals, University of California at San Francisco, the Joint Clinical Research Center in Kampala, Uganda, and the Ugandan National Council for Science and Technology.

Participants initially received a standard regimen of directly observed tuberculosis therapy: 2 months of isoniazid, rifampin, pyrazinamide, and ethambutol, followed by 4 months of daily isoniazid and rifampin. Two weeks after study enrollment, participants were randomized to receive either 6 months of standard tuberculosis therapy (control arm) or tuberculosis therapy plus a punctuated 6-month course of ART (trizivir, a combination of abacavir, lamivudine, and zidovudine) (intervention arm). Daily cotrimoxazole was provided to participants in both arms after they had completed 2 months of tuberculosis or HIV-tuberculosis therapy.

**Measurements**

Data regarding demographic characteristics and symptoms of pulmonary tuberculosis were collected on standardized forms. Participants underwent a physical exam, baseline chest radiograph, and collection of sputum samples for acid-fast bacilli (AFB) smear and culture at enrollment. Additional sputum specimens for AFB smear and culture were obtained at 2 and 5 months after initiation of tuberculosis treatment.

Median viral load was measured using Amplicor quantitative restriction transcriptase-polymerase chain reaction assay (Roche Amplicor 1.5) at enrollment (baseline) and every 3 months during 12 months of study observation. The lower limit of detection of the assay was 400 copies/mm\(^3\).

Immunophenotyping was performed at enrollment and every 3 months for 12 months. Whole blood was freshly collected in sodium heparin tubes, and 200 \(\mu\)L was aliquotted to each of the 12 \(\times\) 75 mm test tubes. For flow cytometry, the following
Antibodies were used: anti-CD4 allophycocyanin (APC), anti-CD8 APC, anti–HLA-DR phycoerythrin (PE), anti-CD38 or anti-CD62L phycoerythrin Cy5 (PECy5), and anti-CD45RO fluorescein isothiocyanate (FITC). Mouse monoclonal isotypic control conjugated with PE, PECy5, FITC, and APC was used to determine nonspecific binding and to set gating boundaries. Antibodies were obtained from BD Pharmingen.

Cells were acquired using a FACS Calibur flow cytometer (BD Bioscience) using Cellquest software (BD Bioscience) with additional analysis performed using FlowJo Software (Tree Star). A total of 25,000–50,000 cells were analyzed for each condition. Lymphocytes were selected on the basis of forward and side scatter characteristics, and CD4+ or CD8+ T cell populations were then determined. T cells were then evaluated for proportion of cells expressing HLA-DR and CD38 or CD45RO and CD62L, with gating strategies identical to those we previously reported [31]. All immunologic studies were performed at the Joint Clinical Research Center in Kampala, Uganda.

Whole-blood ELISAs were performed at enrollment and every 3 months for 12 months to measure IFN-γ in response to phytohemagglutinin (PHA), as well as M. tuberculosis– and HIV-specific antigens. Whole blood was diluted 1:5 with RPMI 1640 and cultured in 48-well tissue culture plates (1 mL/well) with medium alone, PHA (5 μg/mL; Sigma), M. tuberculosis culture filtrate (10 μg/mL; Colorado State University), M. tuberculosis antigen 85B (20 μg/mL; Colorado State University), and purified p24 protein from HIV clade A or HIV clade D, the 2 prominent HIV clades in Uganda (10 μg/mL; Case Western Reserve University, Molecular Core). After 7 days of incubation, cell-free supernatants were collected and cryopreserved for batch testing with ELISA. IFN-γ concentration (pg/mL) was determined in duplicate wells by using sandwich ELISA with the anti–IFN-γ antibody pairs ENM-700A and biotinylated ENM-701B (Pierce). Background IFN-γ production (response to medium alone) was subtracted from IFN-γ production in response to each individual stimulus, and values were log transformed prior to statistical analysis.

### Table 1. Demographic and Baseline Clinical Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Tuberculosis treatment alone (n = 36)</th>
<th>HIV-tuberculosis treatment (n = 38)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median years (range)</td>
<td>32 (19–54)</td>
<td>32 (23–47)</td>
<td>.50</td>
</tr>
<tr>
<td>Female sex, %</td>
<td>36 (n = 13)</td>
<td>39 (n = 15)</td>
<td>.77</td>
</tr>
<tr>
<td>BMI, median (range)</td>
<td>9.1 (15.6–26.0)</td>
<td>9.7 (15.2–29.2)</td>
<td>.56</td>
</tr>
<tr>
<td>CD4 cell count, median (range)</td>
<td>609.5 (374–1368)</td>
<td>532.5 (283–1415)</td>
<td>.15</td>
</tr>
<tr>
<td>Log viral load, median (range)</td>
<td>4.4 (2.0–5.9)</td>
<td>4.5 (2.0–5.9)</td>
<td>.84</td>
</tr>
<tr>
<td>Extent of disease according to chest radiograph, %</td>
<td></td>
<td></td>
<td>.83</td>
</tr>
<tr>
<td>Normal/minimal</td>
<td>17</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Moderately advanced</td>
<td>36</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Far advanced</td>
<td>44</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>AFB smear, median grade (%)</td>
<td>3+ (62)</td>
<td>3+ (74)</td>
<td>.25</td>
</tr>
</tbody>
</table>

**NOTE.** AFB, acid-fast bacilli; BMI, body mass index; HIV, human immunodeficiency virus.
copies/mL, IQR 3.6–4.6) and 12 (4.2 log_{10} copies/mL, IQR 3.4–4.9) months (Figure 1B).

**CD8^+ but Not CD4^+ T Cell Subsets Are Altered by Combined HIV-Tuberculosis Therapy**

Total CD4^+ and CD8^+ T cell counts, as well as the proportions of naive (CD45RO^−/CD62L^+), memory (CD45RO^+/CD62L^−), and effector (CD62L^−) CD4^+ and CD8^+ T cells, were measured at enrollment and every 3 months during the 12-month study period. There was no significant change in absolute CD4^+ or CD8^+ T cell counts during the 12-month study period in either arm (Figure 1 C,D). At the 6-month time point, participants who received combined HIV-tuberculosis therapy had a significant increase from baseline in the proportion of naive CD8^+ T cells, as well as a significant decrease from baseline in proportion of effector CD8^+ T cells, when compared with participants who received tuberculosis treatment alone (P = .01 and P = .04, respectively, Table 2). By 12 months, however, these differences were no longer observed. Although there was a trend toward restoration of the naive CD4^+ T cell pool in both arms during the 12-month study period, these changes were not significant (P = .84 and P = .92 at 6 and 12 months, respectively, Table 2).

**Tuberculosis Treatment Alone and Combination HIV-Tuberculosis Therapy Both Significantly Reduce CD4^+ and CD8^+ T Cell Activation**

T cell activation was assessed by measuring the expression of CD38 and HLA-DR on CD4^+ and CD8^+ T cells by means of flow cytometry at enrollment and every 3 months for 12 months. Among patients treated for tuberculosis alone, there was a significant reduction in median proportion of CD38^+/CD8^+ and CD38^+/HLA-DR^+/CD8^+ T cells at all time points, compared with baseline (Figure 2A,C). The median proportion of HLA-DR^+/CD8^+ T cells was significantly decreased at 3 and 6 months, compared with baseline (data not shown). Participants who received combined HIV-tuberculosis therapy had a significant reduction in median proportion of CD38^+/CD8^+ and CD38^+/HLA-DR^+/CD8^+ T cells at 3, 6, and 12 months, compared with baseline (Figure 2B,D). Among intervention participants, the median proportion of HLA-DR^+/CD8^+ T cells was not significantly altered at any time point among control arm participants (data not shown). Comparison of the change in proportion of CD38^+/HLA-DR^+/CD8^+ T cells from baseline between the control and intervention arms revealed a more significant reduction in median proportion of CD38^+/HLA-DR^+/CD8^+ T cells among the intervention arm at 3 (P = .01).
and 6 ($P = .007$) months. However, this reduction was no longer significantly different between arms by 9 months ($P = .70$).

Patients in both arms showed significant reductions in CD38 and HLA-DR expression on CD4$^+$ T cells, although this was more pronounced in the intervention group. The median proportion of CD38$^+$/CD4$^+$ T cells was significantly reduced from baseline only at 12 months among control patients, yet in the intervention arm, significant reductions from baseline were

### Table 2. Changes in CD4$^+$ and CD8$^+$ T Cell Subsets during 12 Months of Immunologic Follow-up

<table>
<thead>
<tr>
<th>Subset</th>
<th>Tuberculosis treatment alone</th>
<th>HIV-tuberculosis treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive, % RO$^-$/62L$^+$ (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>47 (42.1–53.5)</td>
<td>44.1 (36.0–58.0)</td>
</tr>
<tr>
<td>6 months</td>
<td>47.5 (38.8–53.1)</td>
<td>46.5 (36.3–59.0)</td>
</tr>
<tr>
<td>12 months</td>
<td>49.1 (39.6–57.4)</td>
<td>48.3 (36.8–59.4)</td>
</tr>
<tr>
<td>Memory, % RO$^+$/62L$^+$ (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>38.6 (32.9–41.8)</td>
<td>39.3 (30.2–43.9)</td>
</tr>
<tr>
<td>6 months</td>
<td>41.5 (34.9–49.1)</td>
<td>38.3 (30.2–45.2)</td>
</tr>
<tr>
<td>12 months</td>
<td>35.3 (32.7–43.9)</td>
<td>36.3 (27.2–44.7)</td>
</tr>
<tr>
<td>Effector, % 62L$^-$ (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12.8 (9.5–17.3)</td>
<td>14.8 (9.1–19.0)</td>
</tr>
<tr>
<td>6 months</td>
<td>11.3 (8.9–14.2)</td>
<td>13.7 (7.0–20.6)</td>
</tr>
<tr>
<td>12 months</td>
<td>15.7 (7.3–17.8)</td>
<td>11 (8.7–16.9)</td>
</tr>
</tbody>
</table>

**NOTE.** IQR, interquartile range.

$^b P < .05$, nonparametric cross-sectional comparison between treatment arms.

Figure 2. Changes in CD8$^+$ T cell activation markers in response to tuberculosis treatment alone and combined human immunodeficiency virus (HIV–tuberculosis treatment. Percentage expression of CD38 and CD38/HLA-DR on CD8$^+$ T cells at baseline and 3, 6, 9, and 12 months was measured for participants receiving tuberculosis treatment alone (A,C) and combined HIV-tuberculosis treatment (B,D). The boxes indicate the interquartile ranges, the horizontal lines transecting the boxes indicate the medians, and the whiskers indicate the highest and lowest values. Nonparametric rank tests were performed to compare median changes in percentage of CD38$^+$ and CD38$^+$/HLA-DR$^+$ CD8$^+$ T cells within each treatment group from baseline to months 3, 6, 9, and 12. Significant $P$ values are indicated. TB, tuberculosis.
seen at 3, 6, 9, and 12 months (Figure 3A,B). A significant reduction in the median proportion of CD38+/HLA-DR+/CD4+ T cells from baseline was seen in the control arm at 3, 6, and 12 months (Figure 3C). The median proportion of CD38+/HLA-DR+/CD4+ cells was significantly decreased, compared with baseline, at all study time points among intervention participants (Figure 3D). The median proportion of HLA-DR+/CD4+ T cells was significantly decreased from baseline only at 12 months in the control group, yet in the intervention arm, significant decreases from baseline were seen at all study time points (data not shown).

Combination HIV-Tuberculosis Treatment Improves T Cell Response to Mitogen but Has No Effect on HIV or M. tuberculosis–Specific Responses

T cell effector function was assessed by means of IFN-γ production in response to 7-day incubation of whole blood with mitogen PHA, as well as M. tuberculosis–specific (M. tuberculosis culture filtrate, Ag85B) and HIV-specific (p-24 protein from HIV clade A and clade D) proteins. Whole-blood IFN-γ responses were measured with use of ELISA. Participants in the intervention arm had significant increases in IFN-γ production, compared with baseline (2.8 log₁₀ pg/mL), in response to PHA at 6 (3.3 log₁₀ pg/mL; P = .002), 9 (3.3 log₁₀ pg/mL; P = .01), and 12 (3.5 log₁₀ pg/mL; P = .002) months (Figure 4B). Control patients did not show significant changes in IFN-γ production in response to PHA (Figure 4A).

In both study arms, there were no significant changes in IFN-γ production in response to M. tuberculosis culture filtrate, M. tuberculosis Ag85B, HIV p-24 protein/clade A, or HIV p-24 protein/clade D during the study period (P > .05) (Figure 4C,D). In addition, changes in IFN-γ production in response to these antigens were not significantly different between the 2 arms at any time point (P > .05).

DISCUSSION

In this prospective cohort study of immunologic changes associated with tuberculosis therapy alone in comparison with...
combined HIV-tuberculosis treatment among HIV-infected Ugandan adults with pulmonary tuberculosis and preserved CD4$^+$ T cell counts, we have found that tuberculosis therapy alone produces significant reduction in T cell activation and maintains stable HIV viral load and CD4$^+$ T cell counts. A punctuated course of ART given concurrently with tuberculosis treatment suppressed HIV replication, as expected, and allowed for a more significant reduction in CD8$^+$ T cell activation than did tuberculosis therapy alone. However, these virologic and immunologic benefits were not sustained once ART was withdrawn. Significant improvements in T cell cytokine production in response to M. tuberculosis– and HIV-specific antigens were not seen in either group. Thus, it seems that tuberculosis treatment alone has a substantial effect on immune activation and that the addition of ART during tuberculosis treatment does not result in additional sustained improvements in T cell activation or antigen-specific T cell function for HIV-1 infected persons with preserved immune function.

Reports on the effects of tuberculosis treatment on immune activation in HIV-tuberculosis coinfection are conflicting. Two studies from sub-Saharan Africa reported reduction in serum markers of immune activation among ART-naive, HIV-1–infected adults undergoing treatment for pulmonary tuberculosis, and we previously reported that tuberculosis treatment alone results in decreased T cell activation in HIV-tuberculosis coinfection in persons with less advanced HIV disease [31, 34, 35]. However, Morris et al found that markers of T cell activation did not decrease among HIV-tuberculosis coinfected South African patients with advanced immunosuppression treated for tuberculosis [36]. Although the exact mechanism(s) of increased T cell activation in HIV is not understood, there is a well-recognized correlation between viral replication and CD38 expression on CD8$^+$ T cells [20, 37], and reduction of viral load with ART clearly reduces immune activation in HIV infection alone [29, 30]. Studies comparing T cell activation among individuals with tuberculosis or HIV alone with individuals with HIV-tuberculosis coinfection reveal that dual infection is associated with higher levels of CD38 and HLA-DR expression on T cells [26, 27]. Therefore, simultaneous treatment of both infections would be expected to maximize reduction in T cell activation.

The comparative analysis performed in this study reveals that combined HIV-tuberculosis treatment allows for a more significant reduction in CD38/HLA-DR coexpression on CD8$^+$ T cells than does tuberculosis therapy alone. However, this benefit over tuberculosis therapy alone is lost once ART is withdrawn and...
viral load rebounds to levels equivalent to those in the control arm. Although viral load was maintained at baseline levels in the control arm, a significant reduction in T cell activation was still observed. Moreover, at month 12, participants in both study arms maintained significant reductions in T cell activation. Thus, the relationship between viral replication and immune activation in HIV-tuberculosis coinfection seems not to be linear. In HIV-tuberculosis coinfection, active mycobacterial disease is a major driver of immune activation, and treatment of tuberculosis alone results in substantial reduction in immune activation.

Several groups have investigated the mechanism(s) of increased T cell activation in HIV-tuberculosis coinfection. *M. tuberculosis* can induce HIV replication in lymphocytes from HIV-infected individuals with *M. tuberculosis* infection, suggesting that an antigen-specific response against *M. tuberculosis* induces T cell activation and results in enhanced HIV replication [23]. The excess of proinflammatory cytokines, such as tumor necrosis factor-α, that are characteristic of tuberculosis drives viral replication [38]. Chemokines upregulated at sites of tuberculosis disease, such as monocyte chemotactic protein–1, increase HIV replication and are associated with advanced HIV disease [24, 39, 40]. Therefore, treatment of tuberculosis alone may provide sufficient reduction in systemic inflammation to reduce drivers of T cell activation and thus HIV replication.

It is unclear whether tuberculosis treatment has a significant impact on other markers of HIV disease progression, such as T cell counts and changes in T cell subsets. ART partially restores naïve T cell populations in individuals with HIV infection, and in some studies tuberculosis treatment increased CD4+ T cell counts among persons with HIV-tuberculosis coinfection [41, 42]. In our study, neither treatment arm experienced significant improvements in absolute CD4+ T cell counts or restoration of the naïve T cell population. Importantly, however, median CD4+ T cell counts did not decline during the 12-month study period in either treatment arm, which may be a reflection of the relatively preserved CD4+ T cell counts (>350 cells/mm³) of both groups at enrollment.

Both tuberculosis and HIV infection can inhibit T cell effector functions, such as production of IFN-γ and interleukin-2, and coinfection is associated with more profound suppression of type-1 cytokine responses [12, 14, 28, 43]. Therefore, we investigated the effect of tuberculosis treatment alone in comparison with combined HIV-tuberculosis treatment on T cell production of IFN-γ in response to the mitogen PHA, and *M. tuberculosis*– and HIV-specific antigens. Participants who received ART had significant increases in IFN-γ production in response to mitogen at 6 months that persisted throughout the study, suggesting a global improvement in T cell effector function. However, there was no significant change in cytokine production in response to *M. tuberculosis*– and HIV-specific antigens in either group. These results are consistent with those of longitudinal studies of tuberculosis patients who have depressed purified protein derivative–induced IFN-γ production for 18 months or more after completion of treatment [12].

Our study has several limitations. The relatively preserved immunologic function of subjects in this study limits our ability to generalize our findings to HIV-tuberculosis coinfected persons with more advanced immunosuppression. Inclusion of comparison groups with tuberculosis or HIV infection alone on therapy would provide valuable information on the contribution of each individual infection to immune dysfunction. In addition, because subjects in our intervention arm received only 6 months of ART, we cannot determine whether continued ART would have provided more long-term immunologic benefit, ie, increased CD4+ T cell counts or improved T cell effector function. The triple nucleoside reverse transcriptase inhibitor treatment regimen used in this study minimized the possibility of drug-drug interactions, but may not have provided optimal virologic suppression in comparison with regimens with a protease inhibitor or nonnucleoside reverse transcriptase inhibitor. In addition, because immunologic follow-up was limited to 12 months, we may have missed delayed improvements in immune phenotype or function. Subjects in both treatment arms received cotrimoxazole and rifampin, so the extent to which these medications with known anti-inflammatory properties contributed to decreasing immune activation cannot be excluded. Despite these limitations, to our knowledge, this is the first randomized study to compare immunologic changes associated with tuberculosis treatment alone with those associated with tuberculosis treatment plus ART among HIV-infected individuals with pulmonary tuberculosis and preserved immunologic function.

ART has revolutionized the care of individuals with HIV by substantially reducing mortality and progression to AIDS and thus the many opportunistic infections associated with very low CD4+ T cell counts [44–46]. However, tuberculosis remains the major opportunistic infection worldwide in part because it occurs at all levels of HIV-induced immunodeficiency, as emphasized by our study population, in which 25% of participants had CD4+ T cell counts >350 cells/mm³. Our study comparing the immunologic effects of tuberculosis treatment alone with the effects of tuberculosis treatment combined with punctuated ART suggests that treatment of tuberculosis alone has a major effect on immune activation that lasts at least 1 year from the time of initiation of tuberculosis treatment. Despite recent recommendations that all HIV-infected individuals with tuberculosis receive ART, access to these drugs in places where tuberculosis is endemic remains highly variable and unpredictable. Therefore, if immune activation is a major driver of HIV disease progression, our findings suggest that, for patients with HIV and preserved (>350 cells/mm³) CD4+ T cells who receive a diagnosis of and successful treatment for pulmonary tuberculosis, delaying the initiation of ART for up to 12 months may not accelerate a decline in immunologic function.
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