Clinical, Immunological, and Epidemiological Importance of Antituberculosis T Cell Responses in HIV-Infected Africans

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Background. Human immunodeficiency virus (HIV)–associated tuberculosis is a major cause of mortality in Africa. The assay of T cell interferon-γ released in response to antigens of greater specificity than purified protein derivative is a useful improvement over the Mantoux tuberculin skin test, but few studies have evaluated interferon-γ secretion in HIV-infected individuals.

Methods. Mycobacterium tuberculosis antigen–specific interferon-γ secretion was assessed by whole blood assay and enzyme-linked immunospot, which were compared with the Mantoux tuberculin skin test in HIV-infected and HIV-uninfected individuals without active tuberculosis and HIV-infected patients with pulmonary tuberculosis in Khayelitsha, South Africa.

Results. The skin test and whole blood assay responses to purified protein derivative in HIV-positive subjects were decreased, compared with responses in HIV-negative subjects (P < .001). By contrast, the responses to M. tuberculosis antigens (early secreted antigenic target 6, culture filtrate protein 10, TB10.3, and α-cristallin 2) were less affected, indicating a high prevalence of latent tuberculosis (~80%) in both HIV-negative and HIV-positive subject groups. Whole blood assay responses did not differ between the HIV-positive subjects without tuberculosis and HIV-positive subjects with tuberculosis, but the enzyme-linked immunospot method response to early secreted antigenic target 6 and culture filtrate protein 10 was higher in the group of HIV-infected subjects with tuberculosis (P ≤ .04), although this group had lower CD4+ cell counts. A ratio of the combined enzyme-linked immunospot method response divided by the CD4+ cell count of >1.0 had 88% sensitivity and 80% specificity for active pulmonary tuberculosis in HIV-infected individuals.

Conclusions. Interferon-γ release appears to be less impaired than skin testing by HIV coinfection. The novel potential to relate the enzyme-linked immunospot method and CD4+ cell count to assist diagnosis of active tuberculosis in patients with HIV infection is important and deserves further evaluation.

Tuberculosis (TB) is the major cause of death in HIV-infected individuals in Africa. Although it has only 11% of the world’s population, Africa accounts for more than one-quarter of the global burden of TB, with an estimated 2.4 million TB cases and 540,000 TB deaths annually [1]. Although it is very well recognized that the degree of CD4+ T cell depletion relates to the risk of HIV-associated TB [2, 3], surprisingly few studies have examined in detail the T cell response to Mycobacterium tuberculosis antigens in HIV-infected individuals in regions of endemicity. Some early studies of antigens not restricted to the M. tuberculosis complex showed the cellular proliferative and IFN-γ response to various antigenic preparations to be markedly decreased both in HIV-infected patients with active TB and in HIV-infected patients without TB [4, 5]. By contrast, other analyses reported proliferative responses and found that the production of TNF was 3–10 times

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higher in patients than in control subjects [6]. IFN-γ responses to the *M. tuberculosis*-specific antigens culture filtrate protein 10 (CFP-10) and early secreted antigenic target 6 (ESAT-6), measured by enzyme-linked immunospot (ELISPOT) assay, also appear to be relatively unimpaired by HIV infection [7, 8]. ESAT-6 and CFP-10 are encoded by a genomic segment (RD1) absent from all bacille Calmette-Guérin strains [9–11], and ELISPOT responses to these antigens differentiate immune sensitization by bacille Calmette-Guérin from infection by pathogenic mycobacteria [12]. In addition, sensitivity for the detection of overt TB infection (i.e., detection of culture-positive patients) is 80%–90% [12–14]. In outbreak circumstances, a positive ELISPOT response more clearly correlates with the degree of exposure to the index case than does the tuberculin skin test (TST) [15, 16].

An alternative to ELISPOT analysis is the determination of the IFN-γ content of supernatants derived from stimulated whole blood. Evidence suggests that whole blood assays based on ESAT-6 and CFP-10 are also of increased specificity and sensitivity, compared with the TST [17]. However, no study has systematically evaluated the whole blood assay response to ESAT-6 and CFP-10 in the context of HIV infection. This is important, because the skin response to tuberculin PPD used in the TST is impaired by HIV [18]. Were it to be convincingly shown that in vitro tests of sensitization were less impaired than other tests by HIV, this might justify their wider implementation in resource-poor environments, despite their inherently greater complexity and expense. Indeed, a goal of the global Stop-TB partnership is to implement, by 2012, “a diagnostic toolbox to accurately identify people with latent TB infection and those at high risk of progression to disease” [19, p. 16]. In Africa alone, this plan envisages that 21 million people living with HIV infection and/or AIDS will be screened for latent TB and, of these, 2.4 million people will be offered isoniazid preventive therapy in the year 2015. This will be difficult to achieve without technological improvement.

In this study, we employed a research whole blood assay that involves dilution of the blood, followed by culture at 72 h after collection of the sample (as opposed to overnight) [20, 21]. In addition to assaying the response to ESAT-6 and CFP-10, we included PPD and 2 additional novel and immunodominant antigens. TB 10.3 is a member of the ESAT-6 family, but it is not restricted to pathogenic mycobacteria [22]; it has recently been implicated in protection against experimental bovine TB [23]. The antigen α-cristallin 2 (Acr2) is a member of the α-cristallin family of small heat shock proteins that we have previously found to be expressed—and, therefore, recognized by T cells—shortly after *M. tuberculosis* infection [24, 25]. Our first aim was to determine differences in *M. tuberculosis* antigen recognition between HIV-infected and uninfected individuals living in the same area. Second, what differences exist between HIV-infected individuals with and HIV-infected individuals without active pulmonary TB? Third, we were interested in determining whether differences in the pattern of antigen recognition between groups correlated with TB disease status and whether we could thereby gain insight into the potential diagnostic utility of these responses.

**MATERIALS AND METHODS**

**Participants.** This study was approved by the University of Cape Town (Cape Town, South Africa) Research Ethics Committee (reference numbers 443/2004 and 173/2005). Written consent for inclusion was provided by all participants. Recruitment was conducted at the Ubuntu HIV–TB clinic in the socioeconomically deprived periurban township of Khayelitsha (Cape Town, South Africa). There is an extremely high burden of both TB and HIV infection in this township, with an annual TB incidence of 1612 cases per 100,000 population and an antenatal HIV seroprevalence of 33% in 2005. HIV-seropositive persons with newly diagnosed pulmonary TB (the TB-PTB group) were enrolled within 5 doses after starting anti-TB chemotherapy; all of these patients were culture-positive or sputum smear-positive for *M. tuberculosis*. Persons with a new diagnosis of HIV infection but without signs or symptoms of active TB (the HIV-nonTB group) and healthy persons found to be HIV seronegative (the HIV-negative group) were enrolled sequentially shortly after attending the same voluntary counselling and testing clinic for HIV infection. Thus, we were able to recruit similar groups that differed only by their HIV-infection status. Both HIV-nonTB and HIV-negative subjects were screened for active TB by a symptom score questionnaire that was based on validated approaches that demonstrate that chest radiography does not increase diagnostic yield [26, 27]. The presence of any 1 of the following signs or symptoms formed exclusion criteria and led to referral: cough, chest pain, weight loss, night sweats, fever, loss of appetite, lymphadenopathy, and generalized tiredness. Furthermore, any other suspicious finding on clinical examination also gave rise to an investigation according to national guidelines. Receipt of current antibiotic, antiviral, and steroid therapy were exclusions, as were pregnancy, age >18 years, and any acute illness other than TB. HIV-nonTB subjects who qualified for antiretroviral therapy under current guidelines (CD4+ cell count <200 cells/mm3 or World Health Organization clinical stage 4) were referred to the antiretroviral clinic.

**Mantoux TST.** For each subject, a dose of 2TU tuberculin PPD RT23 was injected intradermally into the volar aspect of the left forearm. Transverse induration was recorded at 72 h after injection. The presence or absence of induration was recorded, and induration was recorded at 48 h. Positivity was defined as an induration ≥15 mm in diameter in the HIV-negative group and ≥5 mm in diameter in the HIV-nonTB group and the HIV-PTB group. HIV-nonTB subjects who experienced Mantoux reactions ≥5 mm in diameter were
offered isoniazid preventive therapy according to South African national guidelines.

**IFN-γ release assays.** Laboratory workers were blind to the clinical status of participants. ELISPOT and whole blood assay analysis were performed as previously described [21, 28, 29]. Antigenic stimuli were endotoxin free and were assayed in duplicate wells. The concentrations used were 5 μg/mL of ESAT-6, CFP-10, and TB10.3 and 20 μg/mL of Acr2. PPD was used at 200 IU/mL (4.6 μg/mL). Control wells included phytohaemagglutin (5 μg/mL) and no antigenic stimulus. A 3-day whole blood assay was performed [20], in which we found a minimal further increase in IFN-γ production when extending cultures to 5 days. ELISPOT plates were read on an Immunospot Series 3B Analyzer (Cellular Technology), and plates were retained for visual inspection and confirmation in case of anomaly.

**Analysis.** Because the aim of the study was to determine the effect of HIV infection on the recognition of TB antigens and to further compare the response in HIV-infected persons with and HIV-infected persons without active TB, the HIV-nonTB subject group was used as a reference for comparisons, with and HIV-infected persons without active TB, the HIV-PTB group showed a markedly skewed distribution of TST responses, which tended to be either completely negative or strongly positive. Although the median value was greater in the HIV-PTB group than in the HIV-nonTB group, this difference was not statistically significant.

**IFN-γ secretion in whole blood assays.** The whole blood assay was performed for 115 of 123 participants. Five subjects (12.2%) in the HIV-PTB group and 3 subjects (7.3%) in the HIV-nonTB group had an insufficient sample. There was a nonsignificant trend towards lower IFN-γ secretion in response

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV-PTB group (n = 41)</th>
<th>HIV-nonTB group (n = 41)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HIV-negative group (n = 41)</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median years (range)</td>
<td>32.1 (19–54)</td>
<td>29.9 (18–44)</td>
<td>NS</td>
<td>31.5 (14–52)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>19:22</td>
<td>14:27</td>
<td>NS</td>
<td>16:25</td>
<td>NS</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; cell count, median cells/mm&lt;sup&gt;3&lt;/sup&gt; (IQR)</td>
<td>167 (89–291)</td>
<td>464 (283–592)</td>
<td>&lt;.001</td>
<td>NA</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI, median value (IQR)</td>
<td>20.7 (19.2–22.8)</td>
<td>27.3 (24.4–31.2)</td>
<td>&lt;.001</td>
<td>29.7 (21.8–35.6)</td>
<td>NS</td>
</tr>
<tr>
<td>BCG vaccination,c % of subjects</td>
<td>55</td>
<td>50</td>
<td>NS</td>
<td>65</td>
<td>NS</td>
</tr>
<tr>
<td>Mantoux tuberculin skin test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induration, median cm (IQR)</td>
<td>18 (0–20)</td>
<td>8 (0–18)</td>
<td>NS</td>
<td>19 (0–22)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Positive result,d % of subjects</td>
<td>65</td>
<td>51</td>
<td>NS</td>
<td>69</td>
<td>NS</td>
</tr>
<tr>
<td>Recent contact with tuberculosis, % of subjects</td>
<td>11</td>
<td>5</td>
<td>NS</td>
<td>17</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NOTE.** BCG, bacille Calmette-Guérin; BMI, body mass index (calculated as weight in kilograms divided by the square of the height in meters); HIV-negative group, HIV-uninfected subjects without tuberculosis; HIV-nonTB, HIV-infected subjects without tuberculosis; HIV-PTB group, HIV-infected subjects with pulmonary tuberculosis; IQR, interquartile range; NA, not applicable; NS, nonsignificant.

<sup>a</sup> HIV-PTB group vs. HIV-nonTB group.

<sup>b</sup> HIV-nonTB group vs. HIV-negative group.

<sup>c</sup> Scar noted or clear documentation.

<sup>d</sup> Defined as ≥5 mm induration for HIV-infected patients and ≥15 mm induration for HIV-uninfected patients.
to the *M. tuberculosis*–specific antigens in the HIV-nonTB group, compared with the HIV-negative group, with the exception of TB10.3, for which the responses in the 2 groups were similar (table 2). IFN-γ secretion in response to PPD, however, was markedly decreased in the HIV-nonTB group, compared with the HIV-negative group (an 11.4-fold decrease; 913 μg/mL vs. 10,433 μg/mL; *P* < .001). When comparing the HIV-nonTB group to the HIV-PTB group, patients with active TB had a stronger response to Acr2, TB10.3, and PPD and a weaker response to ESAT-6 and CFP-10. However, responses were markedly heterogeneous within the groups, and none of these differences were statistically significant (table 2).

**IFN-γ ELISPOT analysis.** A total of 31 subjects in the HIV-PTB group and 16 subjects in the HIV-nonTB group provided a sufficient amount of blood to isolate adequate numbers of PBMCs to also perform an ELISPOT assay using ESAT-6 and CFP-10. The median responses to ESAT-6 (353 spot-forming cells [interquartile range (IQR), 99–977 spot-forming cells] vs. 120 spot-forming cells [IQR, 20–369 spot-forming cells]; *P* = .038), CFP-10 (376 spot-forming cells [IQR, 45–1092 spot-forming cells] vs. 20 spot-forming cells [IQR, 3–110 spot-forming cells]; *P* = .005), and PPD (279 spot-forming cells [IQR, 130–693 spot-forming cells] vs. 25 spot-forming cells [IQR, 3–92 spot-forming cells]; *P* = .001) were significantly higher in the HIV-PTB group, compared with the HIV-nonTB group (figure 1A).

**Stratification of responses by CD4+ cell count.** The conditions of both IFN-γ release assays favor the detection of *M. tuberculosis*–specific CD4+ cells and will, therefore, be influenced by the absolute CD4+ cell count. Therefore, we stratified the whole blood assay and ELISPOT results by CD4+ cell count. In both the HIV-nonTB group and the HIV-PTB group, the responses to PPD, ESAT-6, and CFP-10 decreased with decreasing CD4+ cell count. PPD-stimulated whole blood IFN-γ values significantly correlated with CD4+ cell count in the HIV-PTB group (*r* = 0.4436; *P* = .012); this correlation was not statistically significant for ESAT-6 and CFP-10. In contrast, a lower-level response to TB10.3 and Acr2 was preserved irrespective of CD4+ cell count; the response to Acr2 was, in fact, slightly higher in those patients in the HIV-PTB group who had the greatest CD4+ cell depletion.

The ELISPOT response to PPD also correlated with CD4+ cell count in the HIV-nonTB group (*r* = 0.5489; *P* = .034); otherwise, correlations were nonsignificant, although the responses to ESAT-6 and CFP-10 again decreased with decreasing CD4+ cell count. However, in the HIV-PTB group, the ELISPOT responses had no discernible relationship to CD4+ cell count and were as likely to be elevated in subjects with severe immune depletion as they were to be elevated in subjects with higher CD4+ cell counts (figure 1B). When the ELISPOT frequency (spot-forming cells per 10⁶ PBMCs) for each antigen was arbitrarily divided by the total CD4+ cell count, an interesting relationship became apparent (figure 1C). When considering all antigens, the median value of this ratio in the HIV-PTB group was 18.6 times higher than it was in the HIV-nonTB group (2.05 spot-forming cells per 10⁶ PBMCs per mm³ [IQR, 0.61–6.39 spot-forming cells per 10⁶ PBMCs per mm³] vs. 0.11 spot-forming cells per 10⁶ PBMCs per mm³ [IQR, 0.018–0.38 spot-forming cells per 10⁶ PBMCs per mm³]; *P* < .001, by Mann-Whitney U test).

**Comparison of in vitro responses with the TST.** Because there is no gold standard for the diagnosis of TB, estimation of sensitivity to detect TB has to rely on the response in overtly infected individuals (i.e., individuals with culture-positive or smear-positive disease). Using results from 21 individuals in the HIV-PTB group for whom the results of all 3 assays were available, we calculated the sensitivity of each method for detection of active TB (table 3). Of the patients in the HIV-PTB

<table>
<thead>
<tr>
<th>Antigen</th>
<th>HIV-PTB group (n = 36)</th>
<th>HIV-nonTB group (n = 38)</th>
<th>HIV-negative group (n = 41)</th>
<th><em>P</em>&lt;sup&gt;a&lt;/sup&gt;</th>
<th><em>P</em>&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESAT-6</td>
<td>377 (0–1276)</td>
<td>895 (0–2685)</td>
<td>1510 (0–3642)</td>
<td>.40</td>
<td>.34</td>
</tr>
<tr>
<td>CFP-10</td>
<td>489 (0–1599)</td>
<td>936 (0–2784)</td>
<td>1862 (264–5478)</td>
<td>.54</td>
<td>.13</td>
</tr>
<tr>
<td>Acr2</td>
<td>469 (0–1296)</td>
<td>260 (0–645)</td>
<td>460 (0–1500)</td>
<td>.38</td>
<td>.41</td>
</tr>
<tr>
<td>TB10.3</td>
<td>600 (0–1715)</td>
<td>361 (32–1015)</td>
<td>320 (0–3789)</td>
<td>.71</td>
<td>.55</td>
</tr>
<tr>
<td>PPD</td>
<td>1602 (340–5794)</td>
<td>913 (50–3614)</td>
<td>10433 (1136–22616)</td>
<td>.20</td>
<td>&lt;.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> HIV-PTB group vs. HIV-nonTB group.

<sup>b</sup> HIV-negative group vs. HIV-nonTB group.

<sup>c</sup> Statistically significant.
Figure 1. IFN-γ enzyme-linked immunospot (ELISPOT) response to antigens of *Mycobacterium tuberculosis*. A, Data are shown as spot-forming cells (SFC) per 10⁶ PBMCs. Shaded symbols indicate HIV-infected individuals with pulmonary tuberculosis (HIV-PTB group), and unshaded symbols indicate HIV-infected individuals without tuberculosis (HIV-nonTB). Horizontal lines indicate median values. The HIV-PTB group had a significantly greater response to all 3 antigens, compared with the HIV-nonTB group (†). B, In the HIV-PTB group, ELISPOT responses had no discernible relationship to CD4⁺ cell count and were as likely to be high in individuals with severe immune depletion as in individuals with more moderate depletion of CD4⁺ cells. C, ELISPOT frequencies for each antigen were divided by the corresponding total CD4⁺ cell count and were plotted against CD4⁺ cell count. When considering all antigens, the median value of this ratio in the HIV-PTB group (shaded symbols) was 18.6 times greater than that in the HIV-nonTB group (unshaded symbols) (†, P < .001, by Mann-Whitney U test).

**Table 3.** HIV-infected subjects with pulmonary tuberculosis exhibiting a positive response to tests for tuberculosis.

<table>
<thead>
<tr>
<th>Test, cut-off value</th>
<th>Sensitivity, %</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mantoux tuberculin skin test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induration diameter &gt;15 mm</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Induration diameter &gt;5 mm</td>
<td>67</td>
<td>.75</td>
</tr>
<tr>
<td>Whole blood assay response&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10 µg/mL</td>
<td>90</td>
<td>.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>&gt;100 µg/mL</td>
<td>81</td>
<td>.18</td>
</tr>
<tr>
<td>Enzyme-linked immunospot assay&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;25 spot-forming cells per 10⁶ PBMCs</td>
<td>90</td>
<td>.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Compared with the Mantoux tuberculin skin test with an induration diameter cut-off value of >15 mm.

<sup>b</sup> Response to either early secreted antigenic target 6 or culture filtrate protein 10.

<sup>c</sup> Statistically significant.
In HIV-infected individuals, the in vivo (TST) and in vitro (whole blood assay) response to PPD was markedly decreased ($P < .001$). By contrast, the response to *M. tuberculosis* antigens (ESAT-6, CFP-10, TB10.3, and Acr2) was not significantly decreased. Whole blood assay responses did not differ between the HIV-nonTB and HIV-PTB groups, but the IFN-$\gamma$ ELISPOT response to ESAT-6, CFP-10, and PPD was higher in the HIV-PTB group ($P = .04$ in all cases), despite the fact that the HIV-PTB group had significantly lower CD4$^+$ cell counts. A consequence of these contrasting trends was that a ratio of combined ESAT-6 and CFP-10 ELISPOT count divided by the CD4$^+$ cell count of $>1$ had 88% sensitivity and 80% specificity for active pulmonary TB in HIV-infected individuals.

Although our whole blood assay performed as well as the ELISPOT for the overall detection of TB infection (table 3), was considerably easier to perform, and (as a consequence of dilution) required much less blood, it had poor specificity for the detection of active TB, even when results were stratified by CD4$^+$ cell count. The result that underlies this difference between the overnight ELISPOT technique and the 3-day culture is the high ELISPOT response to CFP-10 and ESAT-6 in the HIV-PTB group (figure 1). We believe that this relates to bacillary burden, which was likely to be very high in our culture-positive or sputum-smear positive patients. A close relationship between the overnight IFN-$\gamma$ response to these antigens, which are secreted by actively dividing bacilli, and the number of viable bacilli recovered from organs has been observed experimentally [33, 34]. Furthermore, the IFN-$\gamma$ ELISPOT response to these antigens decreases during therapy for both active and latent TB in humans, an effect that presumably reflects a decreasing bacillary load [28, 35]. The ELISPOT assay primarily detects activated effector T cells [29], whereas the longer 3-day culture may permit the differentiation of—and, therefore, IFN-$\gamma$ production by—memory T cells, the frequency of which is less influenced by antigen load.

Overall, our results support findings that ELISPOT analysis is less impaired by HIV infection than is the TST [7, 8]. Our analysis of these responses also suggests a simple way that the ELISPOT test could be incorporated into practice in the context of HIV infection. If the test has positive results and the sum of ESAT-6 and CFP-10 responses per 10$^6$ PBMCs divided by the CD4$^+$ cell count of $>1$, this strongly suggests active disease that should be investigated and treated. If the ratio is $<1$, active disease is not excluded but, if disease is undetected, the patient should at least be treated for latent TB infection. We intended this study to be a pilot, and we acknowledge that the confidence limits of our analysis are wide and that the tests we used were “in house” assays. Our results support a larger prospective study of commercial tests that should be extended to the difficult diagnostic area of extrapulmonary TB, which is a common
problem in HIV-infected individuals. It would be necessary to perform a simultaneous CD4+ cell count, but this requirement is not problematic in the developed world. In the developing world, the cost-effectiveness and feasibility of the test should also form a major component of evaluation.

Our results also indicate a very high prevalence of latent TB infection among people attending voluntary HIV counselling and testing facilities in Khayelitsha. The >80% prevalence of latent TB infection that we have detected in adults aged 30 years is consistent with estimates of the annual rate of infection made in nearby areas of Cape Town, where the incidence of TB is lower [36]. TB is very difficult to control in Khayelitsha, where the overall incidence of TB is 1612 cases per 100,000 population and where the rate of TB is much greater in the population of HIV-infected individuals [37]. The World Health Organization has called for extraordinary and unprecedented actions in such circumstances to reverse the rising tide of infection and disease. On the basis of this study, we are now considering mass isoniazid preventive therapy for all HIV-infected individuals without Mantoux TST. Because there is evidence from similar communities that individuals who transmit TB may, in fact, be oligosymptomatic chronic excretors [38, 39], consideration of targeted radiographic screening and greater use of isoniazid preventive therapy, irrespective of HIV-infection status, may also be pertinent. Our findings also underscore the need for novel preventive therapies, as well as a pre- or postinfection vaccine that might prevent reactivation and thereby prevent transmission [40].

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