MAJOR ARTICLE

Evaluation of a Whole-Blood Interferon-γ Release Assay for the Detection of *Mycobacterium tuberculosis* Infection in 2 Study Populations

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(See the editorial commentary by Nadal on pages 1457–9.)

A whole-blood interferon-γ release assay (IGRA) is being evaluated for its potential to replace the tuberculin skin test (TST) for detecting *Mycobacterium tuberculosis* infection. To test the assay in a population in which tuberculosis is highly endemic and in another population that is representative of an urban United States population, 253 volunteers from Ethiopia and 175 volunteers from Baltimore were studied for responsiveness on IGRA compared with a simultaneously performed TST. The agreement between the 2 tests, beyond that due to chance, was 68% among subjects from Baltimore and only 35% among those from Ethiopia. IGRA had a sensitivity of 71%, compared with 95% sensitivity for the TST, among 21 subjects who had undergone treatment for culture-confirmed tuberculosis. The specificity was 85% for IGRA and 96% for TST among 52 subjects with no known history of exposure to tuberculosis. In its current form, with purified protein derivative used as the stimulation antigen, the IGRA was found to perform poorly in comparison to the TST in diagnosing *M. tuberculosis* infection.

Identification and treatment of individuals with latent infection is an important component of tuberculosis control. The HIV epidemic and the resurgence of tuberculosis have made early diagnosis and treatment for those coinfected with HIV and *Mycobacterium tuberculosis* even more important [1]. For years, the tuberculin skin test (TST) has been the only screening tool available for the detection of latent *M. tuberculosis* infection. Despite dependence on the TST and its widespread use, the test is subject to significant variations and limitations. It cannot reliably differentiate between *M. tuberculosis* infection, BCG vaccination, and exposure to mycobacteria other than *M. tuberculosis* [2]. An improved screening method would reduce inappropriate initiation of preventive treatment (with its potential for medication-related toxicities) for persons with nonspecific reactivity to tuberculin and unnecessary use of health care resources for uninfected persons. Recently, a new whole-blood IFN-γ release assay (IGRA) has been introduced that has the potential to replace the TST. The assay measures IFN-γ released in whole blood after overnight incubation with PPD. The few studies published to date have shown mixed correlation between

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the IGRA and the TST [3–8]. In the present study, we sought to evaluate the IGRA in a population that is representative of the general US population and in a population with a large pool of healthy persons latently infected with *M. tuberculosis*. Therefore, we studied use of the IGRA among volunteers from Baltimore who were at varying risk of exposure, to represent the general US population, and among volunteers from Addis Ababa, Ethiopia, where tuberculosis is highly endemic. The Baltimore subjects were part of a larger multicenter study evaluating the IGRA [8].

SUBJECTS, MATERIALS, AND METHODS

Study population. The study in Baltimore was conducted at Johns Hopkins University (JHU) Bloomberg School of Public Health and included 202 subjects. Of these, 27 were excluded, in accordance with the JHU and Centers for Disease Control and Prevention (CDC) protocols, because TSTs for these subjects were not read during the optimal time frame (i.e., between 48 and 72 h). Therefore, 175 subjects in Baltimore who fulfilled the criteria of the study protocol were included in the analysis. These included students and prospective employees from countries where the prevalence of tuberculosis is low who came to the Johns Hopkins Hospital for preenrollment or preemployment screening for tuberculosis ("low risk"), contacts of patients with infectious tuberculosis or persons from countries where tuberculosis is prevalent ("intermediate risk"), persons being evaluated for active tuberculosis ("tuberculosis suspects"), and persons who had completed treatment for culture-confirmed tuberculosis within the previous 2 years ("tuberculosis treatment completers"). These 175 Baltimore subjects were part of a United States–based 5-center study of IGRA that has been reported elsewhere [8]. None of the Baltimore subjects were tested for HIV reactivity. Those who self-reported as HIV seropositive were not enrolled in this multicenter study (in accordance with the JHU and CDC protocols).

The study in Ethiopia was conducted at the Armauer Hansen Research Institute and included 275 volunteers. Of these, 22 were excluded from the analysis (8 failed to return for TST reading, blood samples from 3 did not generate detectable IFN-γ responses to mitogen, an inadequate amount of blood was drawn from 4, and 7 had chest radiographic abnormalities other than tuberculosis). The remaining 253 subjects, including 56 who were HIV seropositive, were included in the subsequent analysis. All Ethiopian subjects were screened for HIV by double ELISA. Subjects were recruited from healthy volunteers among the employees of the Faculty of Medicine, Addis Ababa University, and persons who came to the Tuberculosis Center for screening for tuberculosis and had negative results of chest radiographic and sputum examination for active tuberculosis. Sputum was said to be negative when all results were negative after 3 samples had been tested by the Ziehl-Neelsen technique by trained technicians at the center, followed by another Ziehl-Neelsen test and culture with use of Löwenstein-Jensen medium. Chest radiography was also done for all subjects, and radiographs were read by 2 independent radiologists (1 from Ethiopia and 1 from the United States). Informed consent was obtained from subjects in both locations, and the studies were approved by the institutional review boards of JHU, the CDC, and the National Ethical Committee of Ethiopia.

Laboratory methods. The commercially available IGRA QuantiFERON-TB (Cellestis) was used according to the manufacturer's instructions. Blood (10 mL) was collected in heparinized Vacutainer tubes (Becton Dickinson) immediately before the TST was performed. Within 6 h of collection, 1-mL aliquots were dispensed into each of 4 wells of a 24-well tissue culture plate. One of the 4 different antigens supplied with the kit (PBS [negative control], human tuberculin PPD, avian tuberculin PPD, and phytohemagglutinin [positive control]) was added to each well and incubated for 16–24 h at 37°C. Plasma supernatant (300–400 μL) was collected, and the levels of IFN-γ released were quantified by ELISA. Optical densities were read at 450 nm (Immunoskan MS [Biological Diagnostic Supplies]; Labsystems Genesis, version 3.03 [Labsystems]) and converted to international units per milliliter by means of a standard curve constructed from the standard plasma provided with the kit. The results were calculated, according to the manufacturer's instructions, by subtracting the subject's unstimulated IFN-γ level (background) and calculating the responses to human and avian PPD as percentages of the same subject's maximal response. "Maximal response" was defined as the IFN-γ level released in response to phytohemagglutinin minus the background. A positive result for *M. tuberculosis* infection is defined by the manufacturer as an IFN-γ response of $\geqslant$15% to human PPD, and a positive result for *Mycobacterium avium* complex (MAC) infection is defined as a response of $\geqslant$20% to avian PPD and a difference between responses to human and avian PPD of less than $-$10% [8]. All other combinations of outcomes are defined as negative IGRA results for *M. tuberculosis* infection, except for phytohemagglutinin (positive control) responses of <0.5 IU/mL, which were considered to be indeterminate.

For TST, 0.1 mL (5 TU) of PPD tuberculin (Tubersol; Pasteur Mérieux Connaught Laboratories) was injected intracutaneously on the volar surface of the forearm, and the transverse diameter of induration was measured 48–72 h later. Induration of $\geqslant$5 mm was defined as a positive reaction in the Ethiopian cohort; for the Baltimore cohort, induration of $\geqslant$10 mm was defined as a positive reaction for the intermediate-risk subjects, tuberculosis suspects, and tuberculosis treatment completers, and induration of $\geqslant$15 mm was considered to be a positive reaction for the low-risk subjects [9].

Table 1. Characteristics of subjects from Baltimore or Ethiopia in a study comparing the tuberculin skin test with a whole-blood IFN-γ release assay for the detection of *Mycobacterium tuberculosis*.

Characteristic	Percentage of subjects	
	From Baltimore (n = 175)	From Ethiopia (n = 253)
Sex		
Male	41.1	48.2
Female	58.9	51.8
BCG vaccination		
Yes	16.6	42.3
No	83.4	57.7
History of contact		
Household	8.6	24.9
Nonhousehold	28.5	25.7
None	62.9	49.4
HIV status		
Negative	NA	77.8
Positive	NA	22.2
History of culture-confirmed tuberculosis	6.8	4.3

NOTE. NA, not assessed.

Statistical analysis. Comparisons between the IGRA and the TST were made with κ statistics for agreement, Pearson's correlation coefficient, and sensitivity and specificity estimations. All statistical analyses were 2-sided, and $P < .05$ was considered to be statistically significant. SPSS statistical software, version 10.0, was used for all analyses.

RESULTS

The demographic characteristics of the subjects from the 2 study sites are shown in table 1. Of the 175 subjects from Baltimore, 59% were female. There were 52 subjects (30%) in the low-risk group, 108 (61%) in the intermediate-risk group, 3 (2%) in the group of tuberculosis suspects, and 12 (7%) in the group of subjects who had been treated for culture-confirmed tuberculosis. Of the 253 subjects from Ethiopia, 131 (51.8%) were female; 11 (4.3%) of the 253 subjects had been treated for culture-confirmed tuberculosis, and 2 of those were HIV seropositive.

To determine whether the 15% IFN-γ response level recommended by the company as a breakpoint for positivity for the IGRA is optimal, other breakpoints were examined. Positive TST reactions have been defined on the basis of the frequency distribution curves of the different sizes of reactions [2]. As shown in figure 1*A*, the TST distributions in our study were bimodal, with a clear distinction between subjects with positive or partial reactions and those with negative reactions. In contrast, no bimodality in the distribution of IFN-γ responses was seen in either study population (figure 1*B*), and it was not possible to use the frequency distribution curves to designate a breakpoint for the IGRA. In place of frequency distribution analysis, we evaluated the IGRA responses in persons with known immunologic exposure to tuberculosis, compared with those in persons with a low risk of exposure, to seek a logical breakpoint. We designated the 21 subjects who had been treated for culture-confirmed tuberculosis (table 2; 12 subjects from Baltimore and 9 HIV-seronegative subjects from Ethiopia) as "true-positives" for *M. tuberculosis* infection and the 52 low-risk volunteers from Baltimore who had no known history of exposure to tuberculosis as "true-negatives" and determined

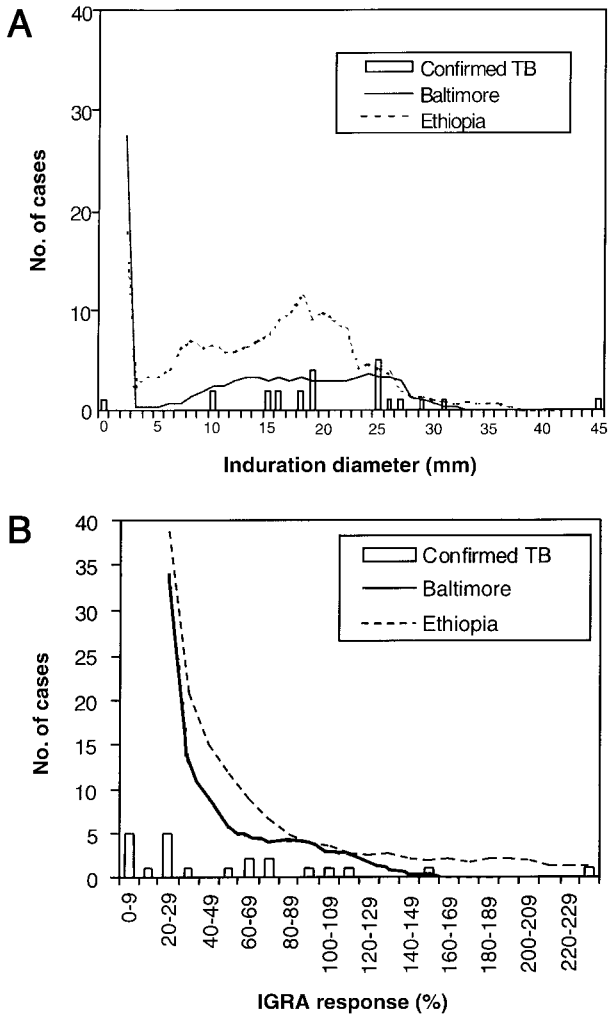

Figure 1. Distribution of tuberculin skin test results *(A)* and IFN-γ responses (according to a whole-blood IFN-γ release assay [IGRA]; *B*) to human PPD antigen for 253 subjects from Ethiopia and 175 subjects from Baltimore, 21 of whom had been treated for culture-confirmed tuberculosis (confirmed TB). IGRA responses are given as the percentage of the subject's maximal response. Frequency values are displayed as a 5-point moving average to minimize bias due to terminal digit preference: $(n_{-2} + n_{-1} + n + n_1 + n_2)/5$.

Table 2. Distribution of diameter of induration in response to the tuberculin skin test and IFN-γ response to human PPD among subjects from Baltimore or Ethiopia who had completed treatment for culture-confirmed tuberculosis.

Diameter of induration, mm	No. of subjects, by IFN-γ response[a]						Total no. of subjects
	0%–19%	20%–39%	40%–59%	60%–79%	80%–99%	⩾100%	
0–4	0	1	0	0	0	0	1
5–9	0	0	0	0	0	0	0
10–14	0	2	0	0	0	0	2
15–19	3	1	0	0	1	0	5
20–24	1	0	1	1	0	1	4
25–29	2	2	0	1	0	2	7
30–34	0	0	0	1	0	0	1
35–39	0	0	0	0	0	0	0
40–44	0	0	0	0	0	0	0
45–49	0	0	0	1	0	0	1
Total	6	6	1	4	1	3	21

[a] IFN-γ response is given as a percentage of the subject's maximal response.

the sensitivities and specificities of the IGRA for different IFN-γ response levels. Breakpoints between IFN-γ responses of 8% and 15% were found to be optimal. When the 15% breakpoint was used, the IGRA showed a sensitivity of 71.4% (15 true positives among 21 tuberculosis treatment completers), compared with 95.2% (20 of 21) for the TST (table 3), and the specificity of the IGRA was 84.6% (44 true negatives among 52 low-risk subjects), compared with 96.2% for the TST (50 of 52) (table 3). Use of an 8% breakpoint gave 86% sensitivity and 75% specificity for the IGRA.

In the study done in Baltimore, subjects for whom the results of the initial TST and IGRA were discordant were asked to participate in repeat testing. Of the 29 subjects with discordant results, a second set of tests was done for 11 subjects 1–12 weeks after the initial tests were performed. The rest of the subjects were lost to follow-up. Of those 11 subjects, 7 were from the intermediate-risk category (4 of whom had been vaccinated with BCG), 3 had been treated for culture-confirmed tuberculosis, and 1 was from the low-risk category. For the 3 subjects who had been treated for culture-confirmed tuberculosis, only the IGRA was repeated. We conducted an assessment of the reproducibility of IGRA results on the basis of data from these 11 subjects. As is shown in table 4, 2 (25%) of 8 subjects showed an interval interpretation change in the TSTs; in both cases, the change was from negative to positive (classic booster phenomenon). A total of 4 (50%) of 8 subjects showed some degree of boosting in the second TST, and there were no instances of positive TST reactions reversing to negative on repeat testing. In contrast, for IGRA (using ⩾15% as the breakpoint for positivity), 6 (55%) of 11 subjects demonstrated an interval interpretation change: results for 5 (45%) converted from negative to positive, and results for 1 (9%) converted from positive to negative. Seven (64%) of the 11 subjects had higher IFN-γ responses on the second test than on the first, whereas 4 (36%) of 11 had lower values.

Figure 2 shows scatter diagrams of the correlation between the TST induration diameter and the magnitude of IFN-γ response on the IGRA for the subjects from Baltimore and Ethiopia. There was a moderate correlation (Pearson's correlation coefficient, $r = .58$; $P = .01$) in the Baltimore study population. Although the correlation was significant in the Ethiopian study population (Pearson's correlation coefficient, $r = .43$; $P = .01$), it was lower than that for the subjects from Baltimore. In the Ethiopian group, 7.4% of the subjects who had 0 mm of induration on the TST had IFN-γ responses of ⩾100%, and

Table 3. Results of the tuberculin skin test (TST) and a whole-blood IFN-γ release assay (IGRA) for 21 subjects who had completed treatment for culture-confirmed tuberculosis (TB) and for 52 low-risk subjects from Baltimore who had no known history of exposure to TB.

Test, response	No. (%) of subjects	
	Treated for culture-confirmed TB	At low risk for TB
TST[a]		
⩾15 mm	20 (95.2)	2 (3.8)
0–14 mm	1 (4.8)	50 (96.2)
IGRA[b]		
⩾15%	15 (71.4)	8 (15.4)
0%–14%	6 (28.6)	44 (84.6)

[a] Induration was measured. Sensitivity, 95.2%; specificity, 96.2%.
[b] IFN-γ response is given as a percentage of the subject's maximal response. Sensitivity, 71.4%; specificity, 84.6%.

Table 4. Results of initial and repeat testing with the tuberculin skin test (TST) and a whole-blood IFN-γ release assay (IGRA) to human PPD for 11 subjects from Baltimore for whom the results of initial testing were discordant.

Risk category, subject	TST result, mm of induration		Interpretation, first/second TST	IFN-γ response on IGRA, %		Interpretation, first/second IGRA	BCG vaccination	History of exposure to TB
	First	Second		First	Second			
Low risk: A	0	16	−/+	20	12	+/−	No	None
Intermediate risk								
B	0	0	−/−	55	95	+/+	Yes	Born in India[a]
C	0	0	−/−	61	54	+/+	Unknown	Born in China[a]
D	0	0	−/−	43	93	+/+	No	Contact with an individual with active TB
E	0	12	−/+	44	24	+/+	Yes	Born in Peru[a]
F	12	25	+/+	10	17	−/+	Yes	Born in Pakistan[a]
G	14	20	+/+	12	32	−/+	Yes	Born in China[a]
H	22	15	+/+	6	21	−/+	Unknown	Born in South Korea[a]
TB treatment completers								
I	20	ND	+/ND	9	31	−/+	No	Treated for TB
J	25	ND	+/ND	7	4	−/−	No	Treated for TB
K	25	ND	+/ND	9	64	−/+	No	Treated for TB

NOTE. Subjects who had been treated for culture-confirmed TB were not tested a second time, in accordance with the study protocol. ND, not done; TB, tuberculosis.

[a] Endemic area.

31% of subjects with TST results of ⩾15 mm had IFN-γ responses of <15%.

For the Baltimore subjects, the overall agreement between TST and IGRA was 79.4% ($\kappa = 0.68$); this value did not vary significantly when we included in the analysis the 27 subjects who had been excluded from the study because their PPD results were not read during the optimal time frame. In the group of subjects from Ethiopia, agreement was lower (68%; $\kappa = 0.35$). Because the Ethiopian portion of the study was open to HIV-seropositive persons, we were able to assess the relationship between the 2 tests with respect to HIV status in that arm of the study. Among HIV-seronegative subjects from Ethiopia, the IGRA and the TST had 70% agreement ($\kappa = 0.36$). When only the HIV-seropositive subjects were considered in the Ethiopian cohort of 248 subjects (excluding the 5 who were positive for MAC), agreement between the 2 tests was lower (61%; $\kappa = 0.23$) (table 5). The mean IFN-γ response to human PPD on the IGRA among HIV-seropositive persons also was significantly lower than that among HIV-seronegative persons (7.7 vs. 34.1 IU/mL, respectively; $P < .0001$). Of the HIV-seropositive persons, 52% had positive results by TST, compared with 28% by IGRA.

Fifteen subjects from Baltimore and 5 from Ethiopia responded predominantly to avian PPD. Two of the latter were HIV seropositive. Four of these 20 had exceedingly low IGRA responses to human PPD, suggesting MAC infection rather than *M. tuberculosis* infection, whereas the IGRA response to human PPD was high in the other 16 persons.

There was no significant difference in the magnitude of responses to the IGRA or the diameter of the TST result among the different groups classified according to history of exposure to active tuberculosis cases (same household, nonhousehold, no exposure) and between the BCG-vaccinated and nonvaccinated groups in the Ethiopian cohort (data not shown).

DISCUSSION

We evaluated the usefulness of a commercially available IGRA for detecting latent *M. tuberculosis* infection in 2 populations, one with a high tuberculosis prevalence (>200 in 100,000; Ethiopia) and another with a low prevalence (15 in 100,000; Baltimore), by comparing the IGRA with the TST. The IGRA had a sensitivity of 71% (15 true positives among 21 tuberculosis treatment completers), in contrast to 95% (20 of 21) for the TST. In a similar study by Streeton et al. [3], in a group of subjects who were TST positive and had completed treatment for tuberculosis, the sensitivity of the IGRA was 80% (134 of 168). Other studies of the sensitivity of IGRA mainly have included patients with active tuberculosis and have showed sensitivities ranging from 58% to 75% [4, 6].

Among subjects from Baltimore recruited at preenrollment (students) and preemployment (job applicants) screening, our

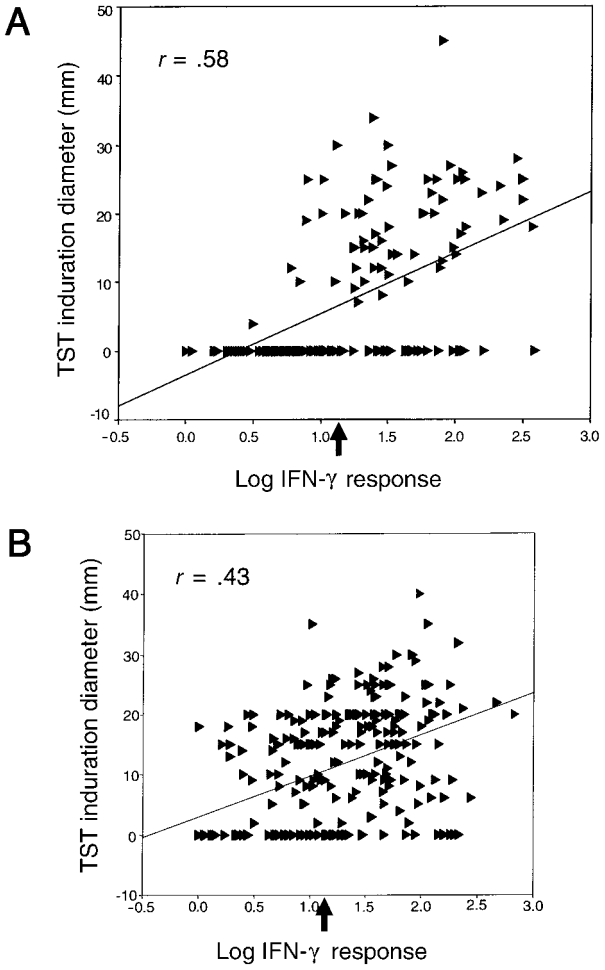

Figure 2. Correlation between diameter of induration in response to the tuberculin skin test (TST) and magnitude of IFN-γ responses to human PPD in 175 subjects from Baltimore *(A)* and 253 subjects from Ethiopia *(B)*. The regression line for each cohort is shown; the arrows indicate the 15% breakpoint for whole-blood IFN-γ release assay positivity. Pearson's correlation coefficient is shown in the upper left corner.

study found that the specificity of IGRA was 84.6% (44 true negatives among 52 low-risk subjects). This was much lower than that reported by Streeton et al. [3] (98%) and Johnson et al. [6] (97.6%). It may be that some of the apparently uninfected subjects in the low-risk category had been infected previously, because 1 low-risk subject had a boosted reaction to TST on repeat testing (table 4).

In our study, the reproducibility of IGRA results was low. Among the 11 subjects for whom the tests were repeated, the changes in the results of the TST were in the direction of boosting, whereas the changes in the results of the IGRA were random. Among the 11 subjects with negative results of the first IGRA, 5 had positive results of the second test; boosting of the IGRA by the previous PPD injection is possible in these cases. However, 1 subject with an initially positive IGRA result had a negative result on the second test. These findings are similar to those of a recent study of health care workers in Melbourne, Australia, in which positive results of a first IGRA were followed by negative results of the second assay in 36% of cases and negative results were followed by positive results in 16% of cases [10]. One possible explanation for variability in the IFN-γ responses could be that the blood samples for the first and second tests were drawn at different times of the day. It has been demonstrated that whole-blood IFN-γ production is affected by the level of cortisol in the blood. Therefore, IFN-γ production exhibits circadian rhythmicity and is maximal in the late evening and early morning, when the inhibitory effect of cortisol is less [11]. Of the 7 subjects who were tested twice and were at intermediate risk for latent *M. tuberculosis* infection (on the basis of history of exposure), only 1 showed an interval discrepancy in the TST results (table 4, subject E), whereas the result of the IGRA changed for 3 (table 4, subjects F–H). Surprisingly, the IGRA initially yielded negative results for all 3 tuberculosis treatment completers who had discordant results and were retested (table 4, subjects I–K). One reason for this finding could be that IFN-γ responses may remain depressed in some persons for a period of time after successful tuberculosis treatment [12]. Among the subjects who were tested twice, no effect due to BCG vaccination was seen, because (1) few subjects (only 4) were vaccinated and (2) those who were vaccinated likely had also been exposed to tuberculosis, and positive TST or IGRA results could, therefore, be attributed to either BCG vaccination or *M. tuberculosis* infection.

Previous studies of the IGRA that involved different exposure-risk groups in Australia and a population of intravenous drug users in the United States have shown good to fair agreement between the IGRA and the TST [3, 5]. In the present study, although the concordance between the 2 tests was good

Table 5. Diameter of induration in response to the tuberculin skin test (TST) and whole-blood IFN-γ release assay responses to human PPD, by HIV serostatus, among subjects from Ethiopia.

HIV serostatus, TST reaction	No. (%) of subjects, by IFN-γ response[a]		
	0%–14%	⩾15%	Total
Seronegative[b]			
0–4 mm	43 (22.2)	20 (10.3)	63 (32.5)
⩾5 mm	39 (20.1)	92 (47.4)	131 (67.5)
Total	82 (42.3)	112 (57.7)	194 (100)
Seropositive[c]			
0–4 mm	22 (40.7)	4 (7.4)	26 (48.1)
⩾5 mm	17 (31.5)	11 (20.4)	28 (51.9)
Total	39 (72.2)	15 (27.8)	54 (100)

NOTE. Five subjects tested positive for *Mycobacterium avium* complex and were excluded from the analysis.

[a] IFN-γ response is given as a percentage of the subject's maximal response.
[b] Agreement, 70%; $\kappa \pm SD = 0.36 \pm 0.14$.
[c] Agreement, 61%; $\kappa \pm SD = 0.23 \pm 0.12$.

for the Baltimore cohort (79% agreement; $\kappa = 0.68$), there was poor agreement in the Ethiopia cohort (68% agreement; $\kappa = 0.35$). The discordance was even more pronounced in the Ethiopian HIV-seropositive group (61% agreement; $\kappa = 0.23$). These findings are similar to those reported by Kimura et al. [7], who found 59% agreement ($\kappa = 0.26$) among 300 HIV-seronegative intravenous drug users from Baltimore. Although high rates of agreement between the TST and an IGRA were reported in a recent study by Black et al. [13] that included 633 HIV-seronegative, non–BCG-vaccinated persons from Malawi and that made use of grouped data, the agreement and κ values were low when data were reanalyzed at the individual level [14].

Mazurek et al. [8] reported comparable percent agreement values between the TST and the IGRA in a multicenter study involving 1226 HIV-seronegative subjects from the United States (175 of whom were included in this report) to those found in the present study. In the 1226-patient study, the overall agreement was 83% ($\kappa = 0.60$). Agreement was much stronger among subjects who had negative results of the TST. For example, among subjects at high risk of *M. tuberculosis* infection ($n = 947$), who are a prime target population for such testing, the IGRA showed 65% agreement for those who had positive results of TST and 90% agreement for those who had negative results of TST. Although our Baltimore subjects overlap with the patients described in the 1226-patient study, including them permitted us to describe these 175 subjects in greater detail than was possible in the larger study (e.g., figures 1 and 2) and to draw stronger conclusions about the IGRA than would have been possible if only the Ethiopian subjects had been included. For example, in contrast to the study by Mazurek et al. [8], the present report includes a calculation of the sensitivity of the IGRA versus that of the TST, on the basis of a detailed analysis of persons who had been treated for culture-confirmed tuberculosis. It also includes a description of follow-up testing for patients whose IGRA and TST results were discordant on initial evaluation.

A possible explanation for the discordance seen between the TST and IGRA results in several studies is the increasing evidence that suggests that different subsets of T cells may be involved in the delayed-type hypersensitivity reaction to TST and mycobacteria-specific IFN-γ responses in the peripheral blood. Hoft et al. [15] have shown that oral administration of BCG induced significant increases in mycobacteria-specific IFN-γ responses in peripheral blood mononuclear cells but inhibited mycobacteria-specific delayed-type hypersensitivity responses. Another reason could be the high prevalence of parasitic infections in the Ethiopian population. Chronic helminthic infections are believed to cause chronic activation of the immune response and shift the balance toward the Th2-type pattern [16, 17]. It has also been demonstrated that this "background" Th2 response modulates the PPD-specific Th1 response so that it becomes more like a Th2 response, as a result of the presence of IL-4 in vivo [18]. Thus, this effect of parasitic infection could have caused the overall lower IFN-γ responses in the subjects from Ethiopia. However, lowering the breakpoint for positivity for the IGRA to 8% made little difference in agreement between the 2 tests.

The effect of immunosuppression on the IGRA results was evident in the mean IFN-γ response among the HIV-seropositive subjects, which was significantly lower than that among those who were HIV seronegative. This was also true in a study by Converse et al. [5] that involved 67 intravenous drug users, in which it was found that IGRA positivity decreased as immunosuppression increased.

In our study, 20 subjects had IGRA responses to avian PPD that were predominant. In 4 (20%) of these, true MAC infection appeared to be likely, because the IGRA responses to human PPD were low. However, in the remaining 16 subjects (80%), who also had high responses to human PPD, it is possible that the high responses to avian PPD resulted from nonspecific cross-reactivity to *M. tuberculosis* antigens and/or to mycobacteria other than *M. tuberculosis.*

Despite some of the IGRA's desirable advantages, our study has found it to be less specific and less sensitive in its present form, in which PPD is used as the stimulation antigen, than is the TST. Because the IGRA makes it possible to test multiple antigens simultaneously, replacing PPD with antigens that are more specific for *M. tuberculosis,* such as early secretory antigenic target–6 (ESAT-6), and optimizing the test conditions for these antigens could refine the assay. An enzyme-linked IFN-γ immunospot assay in which ESAT-6 is used as the stimulating antigen has recently been shown in England to be an accurate method of identifying *M. tuberculosis* infection [19]. Use of combinations of *M. tuberculosis*–specific antigens is likely to increase the sensitivity without sacrificing specificity. This was demonstrated in a recent study in which a much higher sensitivity than has been reported previously was found when ESAT-6 was combined with culture filtrate protein 10 to detect *M. tuberculosis* infection [20]. Identification of factors other than the circadian rhythmicity that may affect the production of IFN-γ in whole blood might facilitate efforts to improve reproducibility.

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References

1. Wagner KR, Bishai WR. Issues in the treatment of *Mycobacterium tuberculosis* in patients with human immunodeficiency virus infection. AIDS **2001**; 15(Suppl 5):S203–12.
2. Huebner RE, Schein MF, Bass JB Jr. The tuberculin skin test. Clin Infect Dis **1993**; 17:968–75.
3. Streeton JA, Desem N, Jones SL. Sensitivity and specificity of a gamma-interferon blood test for tuberculosis infection. Int J Tuberc Lung Dis **1998**; 2:443–50.
4. Pottumarthy S, Morris AJ, Harrison AC, Wells VC. Evaluation of the tuberculin gamma interferon assay: potential to replace the Mantoux skin test. J Clin Microbiol **1999**; 37:3229–32.
5. Converse PJ, Jones SL, Astemborski J, Vlahov D, Graham NM. Comparison of a tuberculin interferon-γ assay with the tuberculin skin test in high-risk adults: effect of human immunodeficiency virus infection. J Infect Dis **1997**; 176:144–50.
6. Johnson PDR, Stuart RL, Grauson ML, et al. Tuberculin–purified protein derivative–, MPT-64–, and ESAT-6–stimulated gamma interferon responses in medical students before and after *Mycobacterium bovis* BCG vaccination and in patients with tuberculosis. Clin Diagn Lab Immunol **1999**; 6:934–7.
7. Kimura M, Converse PJ, Astemborski J, et al. Comparison between a whole blood interferon-γ release assay and tuberculin skin testing for the detection of tuberculosis infection among patients at risk for tuberculosis exposure. J Infect Dis **1999**; 179:1297–300.
8. Mazurek GH, LoBue PA, Daley CL, et al. Comparison of a whole blood interferon-gamma assay with tuberculin skin testing for detecting latent *Mycobacterium tuberculosis* infection. JAMA **2001**; 286:1740–7.
9. Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med **2000**; 161:S221–47.
10. Stuart RL, Olden D, Johnson PDR, et al. Effect of anti-tuberculosis treatment on the tuberculin interferon-γ response in tuberculin skin test (TST) positive health care workers and patients with tuberculosis. Int J Tuberc Lung Dis **2000**; 4:555–61.
11. Petrovsky N, McNair P, Harrison LC. Circadian rhythmicity of interferon-gamma production in antigen-stimulated whole blood. Chronobiologia **1994**; 21:293–300.
12. Hirsch CS, Toossi Z, Othieno C, et al. Depressed T cell interferon-γ responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. J Infect Dis **1999**; 180:2069–73.
13. Black GF, Fine PEM, Warndorff DK, et al. Relationship between IFN-γ and skin test responsiveness to *Mycobacterium tuberculosis* PPD in healthy, non–BCG vaccinated young adults in Northern Malawi. Int J Tuberc Lung Dis **2001**; 5:664–72.
14. Randhawa GS, Bishai WR, Comstock GW. Analysis of data from individuals demonstrates high discordance between IFN-γ assays and PPD-tuberculin skin tests used in screening for tuberculosis infection. Int J Tuberc Lung Dis **2002**; 6:89.
15. Hoft DF, Brown RM, Belshe RB. Mucosal bacille Calmette-Guérin vaccination of humans inhibits delayed-type hypersensitivity to purified protein derivative but induces mycobacteria-specific interferon-γ responses. Clin Infect Dis **2000**; 30(Suppl 3):S217–22.
16. Bentwich Z, Kalikovich A, Weisman Z. Immune activation is a dominant factor in the pathogenesis of African AIDS. Immunol Today **1995**; 16:187–91.
17. Messele T, Abdulkadir M, Fontanet AL, et al. Reduced naive and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. Clin Exp Immunol **1999**; 115: 443–50.
18. Pearlman E, Kazura W, Hazlett FE Jr, Boom WH. Modulation of murine cytokine responses to mycobacterial antigens by helminth-induced T-helper 2 cell responses. J Immunol **1993**; 151:4857–64.
19. Lalvani A, Pathan AA, Durkan H, et al. Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. Lancet **2001**; 357:2017–21.
20. Van Pinxteren, Ravn P, Agger EM, Pollock J, Anderson P. Diagnosis of tuberculosis based on the two specific antigens ESAT-6 and CFP10. Clin Diagn Lab Immunol **2000**; 7:155–60.