Comparison of a Tuberculin Interferon-γ Assay with the Tuberculin Skin Test in High-Risk Adults: Effect of Human Immunodeficiency Virus Infection

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A novel, whole blood interferon-γ (IFN-γ) assay was evaluated to determine its suitability for detecting *Mycobacterium tuberculosis* exposure in intravenous drug users with or without human immunodeficiency virus (HIV) infection. Whole heparinized blood was incubated overnight in separate wells with tuberculin purified protein derivative (PPD), saline, and mitogen controls. Levels of IFN-γ in plasma supernatants were determined by rapid ELISA. Participants were then administered the tuberculin skin test (TST) and tested for cutaneous anergy. The whole blood IFN-γ test agreed (89%–100%) with a positive TST in both HIV-seropositive and -seronegative subjects, but reactivity to PPD was more detectable by the whole blood assay among those with negative TSTs or anergy. TST induration diameter and IFN-γ responses were correlated (Spearman's $\rho = .45$, $P = .0001$), but both responses were blunted by HIV infection. In summary, tuberculin reactivity appears to be more detectable by the whole blood IFN-γ assay than by TST, and the assay requires no return visit for test reading.

The tuberculin skin test (TST) is widely used to diagnose infection with *Mycobacterium tuberculosis* [1, 2]. The TST is predictive of subsequent tuberculosis (TB) disease in both human immunodeficiency virus (HIV)–seronegative [1, 2] and –seropositive persons [3, 4] and, as a result, has remained the cornerstone for determining which patients should receive chemoprophylaxis for TB [5, 6]. While the TST requires minimal time to inject tuberculin, it has several limitations, including poor specificity [7], limited sensitivity [4, 8–10], requirement for a return visit for skin test reading [11], difficulty in application, and lack of reliability [12–15]. For example, not all persons infected with TB respond to the TST: Some with radiographic- or sputum-positive evidence of TB disease do not produce a TST response [2]. Other individuals with specific or nonspecific skin test anergy go on to develop TB disease [4]. The obligation for patients to return in 2 to 3 days for reading of the TST also means that some individuals are lost to follow-up [11].

When the TST was first devised in the 1930s, the role of lymphocytes in mediating the response was unrecognized. Subsequent recognition of the T lymphocyte role has made it possible to develop in vitro assays to measure purified protein derivative (PPD) responsiveness. However, such methods requiring the isolation of appropriate cells are impractical for routine use because they are labor and time intensive and require sterile conditions [16]. The time-intensive factors include lymphocyte isolation, cell counting, and incubation for periods of 2–7 days, depending on the parameter of cell-mediated immunity being measured. In Australia, Wood and colleagues [17, 18] developed a whole blood assay for the diagnosis of bovine TB that overcame many of the shortcomings associated with the TST. The assay is based on the incubation of whole blood with PPD and the subsequent measurement of interferon (IFN)-γ released from PPD-reactive T cells in infected animals. The assay system was shown to be both highly sensitive and specific for the detection of *Mycobacterium bovis* infection in cattle [17, 18]. An equivalent assay (*Quanti*FERON-*TB* [QIFN]; CSL, Ltd.) in humans for the detection of *M. tuberculosis* and other mycobacterial infections has been developed and shown to be similarly specific and effective at discriminating between TB and *M. avium-intracellulare* complex infection [19, 20].

In this study, we evaluated the QIFN test in a population of injection drug users (IDUs). Our goal was to find a TB test that is easily performed and readily standardized, eliminates a reading visit, and has better sensitivity with at least as good specificity as the TST. An additional goal was to determine the assay's performance in HIV-infected, immunosuppressed individuals. IDUs have high rates of HIV and TB infection and TST anergy [4, 8, 9]. The requirement for a return visit for test reading can be particularly problematic in substance abusers, which makes the use of a one-visit blood test particularly attractive in this population.

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Informed consent was obtained from patients, and human experimentation guidelines of Johns Hopkins University were followed in the conduct of this research.

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Materials and Methods

Study population. The ALIVE (AIDS Linked to Intravenous Experiences) study is a longitudinal cohort study of the natural history of and risk factors for acquiring HIV infection among inner-city drug users. The study initially screened 2921 IDUs in 1988 and early 1989. Of these, 630 were HIV-seropositive and 2291 were HIV-seronegative. A further 251 have seroconverted during the subsequent 8 years of follow-up. Currently, 618 HIV-seropositive and 955 HIV-seronegative IDUs remain in the study and are seen semiannually. Details of the study design and methods are described elsewhere [21]. Tuberculin skin testing and anergy testing were first offered in 1990 [9] and are repeated semiannually for active participants in the TB-ALIVE substudy. Participants in the TB-ALIVE substudy do not differ demographically or in their HIV or TB risk profiles from the remainder of the ALIVE cohort population. From participants in the TB-ALIVE substudy, we randomly recruited 67 participants with known prior skin test results. We aimed to recruit individuals in the following 6 groups: A, HIV seronegative and TST positive; B, HIV seropositive and TST positive; C, HIV seropositive and skin test anergic; D, HIV seronegative and TST negative; E, HIV seronegative and skin test anergic; F, HIV seropositive and TST negative.

TST positivity among HIV-seropositive IDUs was defined as a ⩾5-mm diameter induration response 48–72 h after intradermal injection of 5 tuberculin units (TU). Among HIV-seronegative subjects, TST positivity was defined as a ⩾10-mm diameter induration response to tuberculin [9]. Anergy was defined as a <3-mm diameter induration response read 48–72 h after intradermal injection of 0.1 mL each of 4 cfu of mumps antigen, 1:100 dermatophyton ''O,'' candida antigen, and 0.08 Lf tetanus toxoid [9].

Blood collection and skin test procedures. Participants of the TB-ALIVE study were asked at their regular semiannual visit to participate in the interferon-γ (IFN-γ) study: 67 consented and had 8 mL of blood collected into a heparinized tube. The blood samples were transported at ambient temperature within 5 h to the TB immunology laboratory at Johns Hopkins School of Hygiene and Public Health. Next, skin test antigens (tuberculin PPD, mumps, candida, and tetanus toxoid) were injected intradermally on the volar aspect of the participants' forearms, with each injection site separated by >6 cm. Participants returned 48–72 h later (66 returned, 1 late return was excluded from analysis) for test readings.

Laboratory methods. The QIFN test was done according to the manufacturer's instructions. In brief, the blood was first gently resuspended by inverting the tubes several times, and, for each individual tested, 1 mL was pipetted into each of 5 wells of a 24-well tissue culture plate (Falcon; Becton-Dickinson, Lincoln Park, NJ). Next, 3 drops of the appropriate control (phytohemagglutinin [PHA] or saline) or tuberculin (of *M. tuberculosis, M. avium,* or *M. bovis*) antigens were dispensed into the appropriate wells. Only the results for *M. tuberculosis* antigen are presented here. The plate was gently shaken on a plate shaker for 1 min and placed in a humidified (37°C) incubator. After 16 h, the plate was removed from the incubator and spun lightly (~152 *g*) in a centrifuge to facilitate removal of 300 μL of plasma supernatant. The plasma was stored at 4°C to allow batch testing.

IFN-γ in the plasma supernatant of each blood aliquot was quantified by simultaneous ELISA. Plasma samples were reacted simultaneously with anti-human IFN-γ antibodies: Both bound to the solid support and conjugated to an enzyme label (i.e., anti-human IFN-γ labeled with horseradish peroxidase). Unbound material was removed after a 1-h incubation time. Enzyme substrate was added, and the reaction was terminated after 30 min. The amount of color development was estimated spectrophotometrically in an ELISA reader (Vmax; Molecular Devices, Menlo Park, CA) at 450 nm.

Test validation was assessed by examination of the mean absorbance of the human IFN-γ standard plasmas. Using SOFTmax (Molecular Devices) software, a linear standard curve of known IFN-γ standards was plotted, and the IFN-γ concentration for each test sample was calculated. The tuberculin PPD IFN-γ–specific response was calculated as the percent increase in IFN-γ produced by the tuberculin-stimulated cells, subtracting background response of cells incubated with saline only, divided by IFN-γ produced by PHA mitogen–stimulated cells (also subtracting background). That is, the formula for calculating the percent of tuberculin response (% Hu; i.e., % response to *M. tuberculosis* antigens in the test kit compared with response to mitogen) of each patient's blood cells is

$$\frac{[\text{IU/mL IFN}-\gamma\ (\text{tuberculin}) - \text{IU/mL IFN}-\gamma\ (\text{saline})] \times 100}{\text{IU/mL IFN}-\gamma\ (\text{PHA}) - \text{IU/mL IFN}-\gamma\ (\text{saline})}.$$

On the basis of prior studies in low- and high-risk populations, a ⩾15% tuberculin response is considered to be a positive result when the response to mitogen is ⩾0.5 IU/mL [19, 20]. This cutoff also gave the best agreement with the TST in our study population.

Data analysis. Two sets of analyses were done to compare the agreement between skin test results and the QIFN test results. First, the agreement between the skin test result at the concurrent study visit was compared directly with the QIFN results. Since reactivity to tuberculin PPD appeared to be more detectable by the QIFN assay than by the skin test, we conducted a second set of analyses in which currently TST-negative subjects (i.e., those who were TST negative at the blood-draw visit) who had a positive QIFN test result were reclassified as ''agreeing'' if they had a prior positive TST result in the ALIVE study.

The effect of HIV infection, degree of immunosuppression, TST induration diameter, and sociodemographic variables on QIFN positivity were assessed using χ^2 and Mantel-Haenszel trend tests. The correlation between QIFN response level and the TST induration diameter was assessed by Spearman's rank correlation test. *t* tests and Wilcoxon rank sum test were used when appropriate.

Results

The demographics for and HIV status of the study population are presented in table 1. Almost all participants were African American, most were men, and most were long-standing IDUs who were ⩾35 years old. About half were HIV-seropositive, and 30% had a positive TST. In contrast, 65% had a positive QIFN test. Although all had injected drugs in the past, 64% reported injecting drugs in the recent past (6 months).

Agreement between QIFN and TST by study group. The number of participants with positive and negative QIFN results

Table 1. Characteristics of 66 injection drug users (IDUs) participating in a study comparing a whole blood tuberculin IFN-γ assay (QIFN) with the TST for detection of *Mycobacterium tuberculosis* exposure.

Variable	No. of subjects	% of total
HIV seropositive	34	52
Age ⩾35 years	55	83
African American	64	97
Male	53	80
IDU ⩾10 years	43	65
Current IDU	42	64
QIFN positive	43	65
Currently TST positive	20	30

in each of the 6 study groups (A–F) is shown in table 2. The results are presented for the current TST results and for the percent agreement between the QIFN test and the TST adjusted for a prior positive TST. For current TST results, the agreement in group A (HIV negative and TST positive) was 100% and for group B (HIV positive and TST positive) was 89% (i.e., only 1 person was QIFN negative). In the skin test–anergic or TST-negative groups, the QIFN assay was positive in 52% overall. In particular, 7 of 15 skin test–anergic subjects were positive in the QIFN assay, suggesting prior exposure or infection to *M. tuberculosis.* Among those with a negative TST, the QIFN assay was positive in 17 of 31 subjects, but positivity of the assay was higher among HIV-seronegative subjects. Of the 12 QIFN-positive, HIV-seronegative, TST-negative subjects, 5 had been TST positive in the last 12 months. Overall, the percent agreement between the two tests was higher among HIV-seropositive subjects (62%) than among HIV-seronegative subjects (33%). However, after adjusting for a recent prior positive TST among those with a current negative TST and a current positive QIFN result, the percent agreement improved among groups C, D, and F. Group C (HIV positive and anergic) agreement improved from 67% to 73%, group D (HIV negative and TST negative) from 33% to 46%, and group F (HIV positive and TST negative) from 62% to 73%.

Effect of HIV infection and immunosuppression. The agreement between the QIFN assay and TST results was higher among HIV-seropositive than HIV-seronegative subjects, but similar trends were observed in both groups (table 3). Among TST-positive subjects, the agreement with the QIFN assay was very high among both HIV-seropositive and -seronegative persons, ranging from 89%–100%. However, among TST-negative subjects, the QIFN assay was positive in 60%–71% (percent agreement, 29%–40%) of the HIV-seronegative subjects and 27%–36% (percent agreement, 64%–73%) of the HIV-seropositive subjects.

Factors associated with having a positive QIFN test are presented in table 4. Subjects seropositive for HIV were less likely to be QIFN positive (50% vs. 81%, $P = .008$). Similarly, as immunosuppression increased, QIFN positivity decreased (table 4). Only 30% of HIV-seropositive persons with <200 CD4 cells/μL were QIFN positive. The mean percentage of IFN-γ responses to tuberculin by QIFN assay was also significantly lower among HIV-seropositive participants (47% vs. 152%, $P = .004$). Figure 1 presents the median percentage of IFN-γ responses to tuberculin according to HIV serostatus and CD4 lymphocyte count. Increasing immunosuppression was associated with decreasing magnitude of the IFN-γ response and the proportion of subjects who were QIFN positive. In our series, only 1 TST-positive subject was QIFN negative. That individual was HIV seropositive and had the lowest CD4 T cell level, 320/μL, in group B. On the other hand, the QIFN test detected IFN-γ secretion to PPD tuberculin in skin test–anergic subjects with CD4 T cell levels below 100/μL, suggesting that immunosuppression as measured by CD4 level is not the main reason for this anomalous result.

Correlation between TST induration diameter and percent IFN-γ response to tuberculin stimulation. Overall, the correlation between current TST induration diameter and the magnitude of the IFN-γ response to tuberculin was significant and of moderate strength (Spearman's $\rho = 0.45$; $P = .0001$). This

Table 2. Agreement between whole blood tuberculin IFN-γ assay (QIFN) and TST results by study group at current study visit and adjusting for prior TST results.

	Current TST result			Adjusted for prior positive TST result		
	QIFN			QIFN		
Study group	Negative	Positive	% agreement	Negative	Positive	% agreement
A (HIV negative and TST positive)	0	11	100	0	17	100
B (HIV positive and TST positive)	1	8	89	1	11	92
C (HIV positive and anergic)	8	4	67	8	3	73
D (HIV negative and TST negative)	6	12	33	6	7	46
E (HIV negative and anergic)	0	3	0	0	2	0
F (HIV positive and TST negative)	8	5	62	8	3	73

Table 3. Agreement between TST and whole blood tuberculin IFN-γ assay (QIFN) by HIV serostatus.

	HIV seronegative			HIV seropositive		
	QIFN			QIFN		
	Negative	Positive	% agreement	Negative	Positive	% agreement
Currently TST negative	6	15	29	16	9	64
Currently TST positive	0	11	100	1	8	89
Adjusted TST negative	6	9	40	16	6	73
Adjusted TST positive	0	17	100	1	11	92

correlation was only slightly higher among HIV-seropositive than HIV-seronegative participants (Spearman's $\rho = 0.50$ vs. 0.43, respectively). After we took into account prior positive TST results, the overall correlation improved to 0.61 ($P = .0001$). Improvements in correlation were of similar magnitude among both HIV-seropositive and -seronegative participants.

Figure 2 shows the median percentage of IFN-γ responses according to current visit induration diameters of 0, 1–9, 10–19, and >20 mm and stratified by HIV serostatus. Although IFN-γ responses were more attenuated among the HIV-seropositive subjects, a clear dose-response relationship was demonstrated for both HIV-seropositive and -seronegative study participants.

Table 4. Relationship of HIV serostatus, immunosuppression, and other risk factors to positive results on whole blood tuberculin IFN-γ assay (QIFN).

	No. of subjects	% QIFN positive	P*
HIV positive with CD4 <200/μL	10	30	
HIV positive with CD4 ≥200/μL	23	57	
HIV negative	32	81	.008
IDU			
≥10 years	43	72	
<10 years	22	50	.07
Current IDU			
Yes	42	74	
No	24	50	.05
Cocaine IDU			
Yes	38	74	
No	28	54	.09
Heroin IDU			
Yes	42	74	
No	24	50	.05
Male	53	68	
Female	13	54	.34
Previous chemoprophylaxis			
Yes	27	78	
No	39	56	.07

NOTE. IDU = injection drug use.
* Determined by χ^2 analysis.

Relation of other variables to QIFN positivity. We examined by χ^2 analysis a range of other risk factors for TB infection that might also influence QIFN positivity (table 4). Although only marginally statistically significant (P range, .05–.09), current injection drug use, longer duration of injection drug use, and use of heroin and cocaine were all associated with a higher likelihood of QIFN positivity. IFN-γ levels tended to be lower in those who had recently stopped injecting than in those who had continued ($P = .073$). This association was only observed in HIV-seronegative subjects ($n = 32$, $P = .09$) and not in the HIV-seropositive subjects ($n = 34$, $P = .48$). QIFN positivity was also more frequently observed in the TST–negative, nonanergic, HIV-seronegative or -seropositive subjects who were currently using drugs, but the groups were too small to observe a statistically significant association ($P > .3$, Fisher's exact test; $P > .19$, Mantel-Haenszel; but $P = .12$ when the groups are combined). Similarly, those who had received prior isoniazid chemotherapy (within or outside the ALIVE study) were more often QIFN positive ($P = .07$). Age, race, and gender were unrelated to QIFN positivity.

Among TST-negative participants, we found that the mean number of previous TSTs in the QIFN-positive and QIFN-

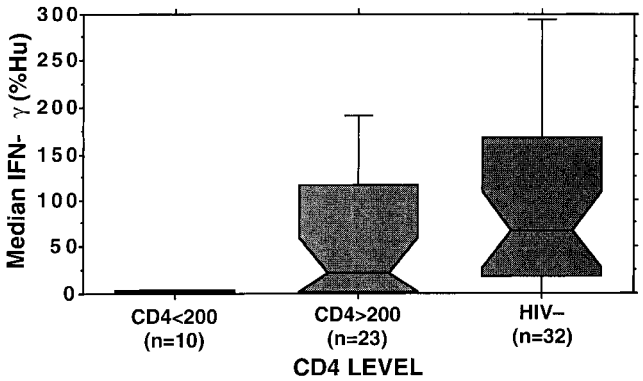

Figure 1. Median (interquartile range) % IFN-γ responses to tuberculin purified protein derivative stimulation (% Hu; i.e., % response to *M. tuberculosis* antigens, as described in Materials and Methods), according to HIV serostatus and CD4 T lymphocyte count. CD4 cell counts of <200 and ≥200 = HIV seropositive; HIV– = HIV seronegative.

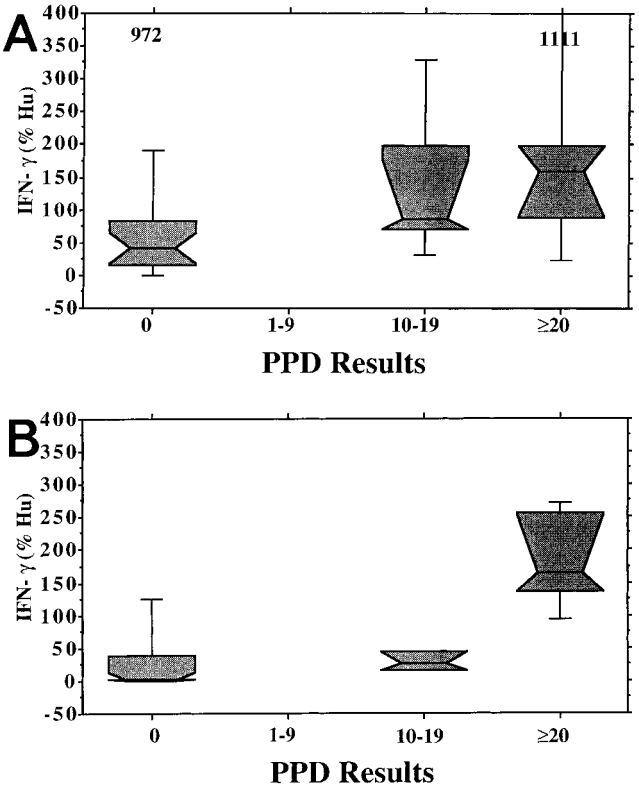

Figure 2. Median (interquartile range) % IFN-γ responses to tuberculin stimulation (% Hu; i.e., % response to *M. tuberculosis* antigens, as described in Materials and Methods) in HIV-seronegative (**A**) and -seropositive (**B**) subjects, according to induration diameter (mm) of tuberculin purified protein derivative (PPD) skin test at current study visit. Two outlier values of 972 and 1111 IU/mL were observed and are indicated in **A**.

negative subjects was nearly identical (3.1 and 3.29, respectively; $P = 1$), ruling out any ''booster effect'' of frequent skin testing.

Discussion

The data presented here show several characteristics of this whole blood assay for measuring IFN-γ response to tuberculin PPD. The first is that the QIFN assay detects more reactors to PPD than does the traditional TST. For example, while nearly all TST-positive subjects were also QIFN positive, several TST-negative subjects (some of whom are documented to have been previously TST positive) were also QIFN positive, as were a subset of skin test–anergic individuals. While this finding will need to be replicated in a larger group of patients, increased sensitivity is a valuable characteristic for testing immunosuppressed patients, case contacts, and others who are at high risk for developing TB disease.

The second characteristic demonstrated is that the assay response appears to be quantifiable in a similar fashion to the induration diameter of TST. This was demonstrated by the dose-response correlation between skin test induration diameter and level of IFN-γ secreted. A key question that follows from this finding is whether increasing magnitude of the IFN-γ response predicts increased future risk of TB disease, as does increasing induration diameter following the TST. However, a cutoff of 15% appears to be suitable for both HIV-seropositive and -seronegative subjects as far as determining infection is concerned. The third characteristic of the assay demonstrated by these data is that HIV infection and immunosuppression (lower $CD4^+$ T cell counts) were associated with lower rates of QIFN positivity.

The observations indicating greater sensitivity of the QIFN assay over the TST in HIV-seronegative subjects support data reported by investigators in Australia [19, 20]. In particular, they determined that up to 50% of the TST-negative subjects who were known contacts of active TB cases were positive in the whole blood QIFN assay (i.e., IFN-γ response $\geq$15%). In those studies of >400 individuals with a mean age of 40 years, a specificity of 97% was observed. On the other hand, in the population studied in Baltimore, the data were suggestive (but not significant, possibly due to the sample size) that there may be other explanations. If it is determined that current IDUs are more likely to be QIFN positive, it could be that there is background or cross-reactive antigenic stimulation in the injected material. Alternatively, there may be an effect, direct or indirect, of certain drugs themselves on antigenically driven IFN-γ secretion. This could be investigated in vitro but may require metabolic events in vivo. There is also the possibility that the injected drugs are immunosuppressive in some individuals. Investigations of syphilis serology in the ALIVE population have found that both false-positive and false-negative reactions are found in this group (unpublished data).

There is a widely recognized need for better, more convenient methods of diagnosing TB infection than with the TST. A recent report [11] indicated that in the absence of inducements, the return rate for TST readings in an HIV clinic was only 35%. Even with inducements (food vouchers and health education), only 61% returned for readings. Financial inducements used in this study resulted in >95% return rate. The TST has other practical constraints, including difficulty in standardizing antigen placement and test reading as well as technical limitations of sensitivity, specificity, boosting, and the need for intradermal injection.

In an ongoing study, we have evaluated the impact of multiple skin testing or boosting on the QIFN test. Subjects (n = 89) who had not had a skin test in the last 5 years had a mean response of 82.5 IU/mL of IFN-γ in their plasma supernatants, whereas 66 subjects who had received regular TSTs had a mean value of 98.2 IU/mL ($P = .34$, data not shown). Therefore, we believe that boosting has no effect on the QIFN assay.

The QIFN test is designed to overcome many of the above constraints. Blood is obtained at the time of a patient's visit, the whole blood is incubated directly with antigen and controls,

and the plasma supernatant is harvested the following morning. If information regarding the patient's infection status is needed urgently, the ELISA test can be run the morning after the blood draw. Otherwise, in research settings, the sample may be stored at 4°C and then be run together with other samples at a time that is convenient for the laboratory. Therefore, the greatest obstacle in the performance of the TST is avoided and places no great burden on clinical personnel. If the QIFN test were to be used in clinical practice, a return visit would be necessary only if the sample is positive and chemoprophylaxis were to be offered.

In addition to the practical drawbacks (standardization and field use) of the TST, there are also issues of sensitivity, specificity, and stability of the delayed-type hypersensitivity response. Even in the absence of HIV infection or immunodeficiency due to other causes, 20%–30% of newly diagnosed untreated TB patients will not respond to tuberculin in the skin test. It has long been recognized that reactions may occur as a result of bacille Calmette-Guérin vaccination or infection with another mycobacterium [1, 2, 7, 22]. An analysis of the stability of the TST in HIV-infected and uninfected IDUs participating in the ALIVE study found ''conversions'' and ''reversions'' were commonplace regardless of HIV status, although HIV-seropositive subjects were more likely to revert [12]. Earlier studies in residents of chronic-care facilities also documented fluctuations in skin test reactivity over time in these nursing home residents [13, 14]. Reversion to negativity was also noted in the Prophit survey undertaken before World War II (and before chemotherapy) [23]. Reversion was more likely in those with reactions smaller than 15 mm. Further studies are needed in this population to determine if there is similar lability in the QIFN test. Data on this issue from Australian subjects are being analyzed by CSL Ltd.

There are preliminary data using the QIFN test that address some of these issues. First is the question of specificity. An evaluation of this system in Australian TB patients found that none of 50 subjects had stronger reactions (i.e., a dominant reaction) to *M. avium* than to *M. tuberculosis* [19]. Conversely, 10 of 10 children with *M. avium*–associated disease had a dominant response to the homologous antigen. In addition, there is inherent flexibility in the test. New antigens can be tested without injecting them into humans and without going through the appropriate regulatory and safety determinations. There is also a built-in control for anergy. The test includes a mitogen (PHA) as a positive control. Anergy to PHA was observed in only 4 persons in the present study; 3 of them were also skin test anergic. Seven HIV-seropositive and 3 HIV-seronegative and skin test–anergic subjects responded to PHA. Retesting can be done immediately with the QIFN test without concern for artifactual boosting of responses, which can confound skin test interpretation [13]. The QIFN test may also be more sensitive than the TST. We found that in the current study population, in which TB infection and disease are highly endemic, many skin test–negative individuals with a strong possibility of exposure to TB were able to produce IFN-γ in response to tuberculin in vitro. Streeton et al. [20] have evaluated this phenomenon in Australia in persons simultaneously tested with blood and skin tests: They found that most of the ''false-positives'' by the blood test had in fact been exposed (i.e., they were case contacts) and were probably ''false-negatives'' in the skin test. Future studies are planned to investigate exposure histories in subjects in Baltimore with discordant QIFN assay and TST results.

There are many candidate antigens that could replace or be added to the QIFN test. The ideal antigen should be immunogenic for circulating lymphocytes, be specific for infection with *M. tuberculosis,* be stable in inducing reactivity, and possibly wane with successful chemotherapy. Tests with the MPT64 antigen showed promise as a skin test antigen and as a reagent in the QIFN test, particularly when expressed from *Mycobacterium smegmatis* as opposed to *Escherichia coli* [24]. Since most, but not all, bacille Calmette-Guérin vaccine strains do not express this antigen, it can be useful in many populations due to its specificity for TB infection; however, it was not as immunogenic and, therefore, not as sensitive in the QIFN assay system as conventional tuberculin [24]. One antigen that has potential as an *M. tuberculosis*–specific antigen is ESAT-6 [25, 26]. This antigen is not expressed by any vaccine strains; however, whether it is also sufficiently immunogenic in the QIFN assay system remains to be determined.

We have evaluated a novel whole blood tuberculin IFN-γ assay for determining cell-mediated immunity and detecting *M. tuberculosis* infection in a cohort of HIV-seropositive and -seronegative IDUs. The test shows good agreement with a positive TST result and appears to detect more reactors to tuberculin than does the traditional TST. In a pilot test in a cross-sectional study, we are currently evaluating how well the assay discriminates infection with *M. avium-intracellulare* complex and *M. tuberculosis* compared with skin test results for antigens from the same species. Future studies are planned to address the issue of stability of responses and to formally test the issues of sensitivity and specificity.

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