REVIEW ARTICLE

A Summary of the Third Global Interferon-γ Release Assay Symposium

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Studies over the past several decades have dramatically increased our understanding of the immune response to *Mycobacterium tuberculosis* infection, and advances in proteomics and genomics have led to a new class of immune-diagnostic tests, termed interferon-γ (IFN-γ) release assays (IGRAs), which appear to obviate many of the problems encountered with the tuberculin skin test (TST). Worldwide, 2 IGRAs are currently commercially available. QuantiFERON-TB Gold In-Tube (Cellestis) is a third-generation product that uses an enzyme-linked immunosorbent assay to measure IFN-γ generated in whole blood stimulated with *M. tuberculosis*–specific antigens. T-Spot-TB (Oxford Immunotec) employs enzyme-linked immunosorbent spot technology to enumerate the number of purified lymphocytes that respond to *M. tuberculosis*–specific antigens by producing IFN-γ. These in vitro tests measure the host immune response to *M. tuberculosis*–specific antigens, which virtually eliminates false-positive cross reactions caused by bacillus Calmette-Guérin vaccination and/or exposure to environmental nontuberculous mycobacteria that plague the interpretation and accuracy of the tuberculin skin test (TST). The high specificity of IGRAs, together with sensitivity commensurate with or better than that of the TST, promises an accurate diagnosis and the ability to focus tuberculosis-control activities on those who are actually infected with *M. tuberculosis*. The Third Global Symposium was held over a 3-day period and was presented by the University of California, San Diego, Continuing Medical Education department; slides and sound recordings of each presentation are available at http://cme.ucsd.edu/igras/syllabus.html. A moderated discussion is also available at http://cme.ucsd.edu/igrasvideo. This document provides a summary of the key findings of the meeting, specifically focusing on the use of IGRAs in screening healthcare worker populations.

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For over a century, exploring the immune response to *Mycobacterium tuberculosis* has provided important insights into understanding the natural history of tuberculosis (TB) as well as information about methods of diagnosis. In 1909, von Pirquét was the first to measure delayed-type-hypersensitivity reactions to cutaneous application of tuberculin as a means of identifying patients with active TB.[1] He found that many children in his clinic had a reaction to tuberculin but did not have active TB. He concluded that these children had latent *M. tuberculosis* infection (LTBI).

In the 1960s, the US Public Health Service undertook a series of studies to understand the risk of developing active TB. They used the Mantoux technique of intradermal injection of purified protein derivative (PPD) and quantified the reaction by measuring the millimeters (mm) of induration 48 hours later. Results demonstrated that those with a tuberculin skin test (TST) reaction greater than 12 mm in diameter had a higher risk of developing active TB than did those who had a TST reaction less than 5 mm in diameter.[2] These studies provided the basis for the cutoff points used for the TST in the United States. Remembering this history, we should be mindful that the TST cutoffs are risk based and that a positive result should not be considered definitive for infection.

Studies over the past several decades have dramatically increased our understanding of the immune response to *M. tuberculosis* infection, and advances in proteomics and genomics have led to the identification of highly specific TB antigens. In the past decade, these scientific advances have led to a new class of immune-diagnostic tests, termed interferon-γ (IFN-γ) release assays (IGRAs), that appear to eliminate many of the problems encountered with the TST. These in vitro tests measure the host immune response to TB-specific antigens, which virtually eliminates false-positive cross reactions caused by nontuberculous mycobacteria (NTM) such as bacillus Calmette Guérin (BCG) and/or environmental strains that plague the interpretation and accuracy of the TST.[2] The first-generation QuantiFERON-TB (QFT; Cellestis) used PPD as the antigen and required a technician to pipet aliquots of blood. The second-generation QuantiFERON-TB Gold used the highly specific TB antigens ESAT6 and CFP10. The third-generation QuantiFERON-TB Gold In-Tube uses ESAT6 and CFP10 but also uses an additional highly specific antigen, TB7.7. The In-Tube version

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uses tubes that are precoated with the antigens so that no pipetting is needed for processing. T-Spot-TB (T-Spot; Oxford Immunotec) employs enzyme-linked immunospot technology to enumerate the number of purified lymphocytes that respond to TB-specific antigens ESAT6 and CFP10 by producing IFN-γ. The high specificity of IGRAs, in combination with their sensitivity, which is equal to or better than that of the TST, promises that they will provide an accurate diagnosis and the ability to focus TB-control activities on those who are actually infected with *M. tuberculosis*.

Most developed countries now have guidelines and recommendations on the use of these IGRAs for TB control.[3] Over the past decade, there have been more than 700 publications on IGRAs. To assist our understanding of the performance of these IGRAs, the academic community has held a series of global symposia, the first of which was held in 2006, the second in 2009, and the third in January 2012. These meetings have provided a forum in which to present and discuss the developing state of knowledge regarding the use of IGRAs.

The Third Global Symposium was held over a 3-day period and was presented as a program by the University of California San Diego Continuing Medical Education (UCSD CME); slides and sound recordings of each presentation can be viewed at http://cme.ucsd.edu/igras/syllabus.html. A moderated discussion is available at http://cme.ucsd.edu/igrasvideo. UCSD CME credits can be earned through the website. This document provides a summary of the key findings from the meeting and specifically focuses on the use of IGRAs in screening healthcare worker (HCW) populations. The conference presenters are listed in the Acknowledgments.

THE USE OF IGRAS AMONG HCWS

TB among HCWs reflects the rate of infection in the community from which they come as well as the TB risk that they encounter because of close contact with patients who have active TB. In low-prevalence settings, some HCWs are at low risk for TB, because many do not encounter patients with active TB; however, some do encounter patients with TB, and recently infected HCWs are indeed at high risk to become infected. Additionally, HCWs who develop active TB also pose a risk to their patients, many of whom may have immunosuppressive conditions. For these reasons, screening HCWs is seen as a very important TB control strategy.

The risk of active TB in HCWs was the focus of a large study in Portugal presented by J. Torres Costa. Over 6,000 HCWs were screened for TB from 2005 to 2010. Of these, 62 received a diagnosis of active TB. Active TB was diagnosed in 19 HCWs in 2005, 13 in 2006, and 14 in 2007. In 2007, young HCWs were screened with QFT, and preventative treatment was recommended to those with positive test results. Presumably as a result of this intervention, the number of cases of active TB among HCWs decreased to 6 in 2008, 5 in 2009, and 5 in 2010. Clearly, the prevalence of active TB among HCWs was still high (92 cases per 100,000 HCWs) in 2010, but even in this high-prevalence situation, the usefulness of screening for TB and treatment of QFT-positive HCWs resulted in marked improvement in TB control.

Among German HCWs, it was found that, regardless of TST status, no HCW who had a negative QFT result developed active TB, despite the fact that none were offered treatment for LTBI. Guidelines require that all HCWs with a positive QFT result be offered treatment, so the natural history of these individuals is not known. In Poland, BCG vaccination is mandatory for newborns, and during the period 1955–2006, vaccination was repeated at 6, 12, and 18 years of age. In this setting, 88 (61%) of 145 HCWs tested had a TST response greater than 10 mm, whereas 46 (32%) had a positive QFT result. Analysis revealed that QFT positivity was related to age and the incidence of TB in the community, but prevalence was greater among HCWs than among the general population. QFT appears to be a better indicator of who is infected than TST. Even so, the rate of positivity according to QFT identified more HCWs than could be treated because of limited resources. Even in this setting, a negative QFT has a strong negative predictive value (NPV).

Under the TB Epidemiologic Studies Consortium, the US Centers for Disease Control and Prevention (CDC) has conducted a large multicenter study of the use of IGRAs among HCWs. In a preliminary report, 2,767 HCWs were enrolled from 4 healthcare centers in the United States. Each completed a structured interview in addition to the 3 tests approved by the Food and Drug Administration as aids in the diagnosis of TB. TST, QFT, and T-Spot were performed at baseline and again at 6, 12, and 18 months. In this relatively low-prevalence population, it was not surprising that there was a good deal of discordance among the 3 tests; even so, 81% of the HCWs had negative results for all 3 tests, and 1.4% had positive results for all 3 tests. The number of HCWs with conversion, defined as going from below the cutoff point for a positive result to above that cutoff point, was lowest with TST, higher with QFT, and highest with T-Spot (0.9%, 6.1%, and 8.3%). Notably, a high proportion of those who experienced conversion reverted to being negative when retested and remained negative, which suggests that many conversions were actually "false conversions."

Defining conversions for IGRAs, rather than simply moving from positive to negative around the respective cutoffs, has been a major point of discussion in the literature. For QFT, van Zyl Smit and colleagues[4] proposed a change of 2.6 times over baseline, and Pai and colleagues[5] suggested a cutoff point of greater than 0.7 IU. Using these cutoff points for defining conversions in the US HCW study, the rate for QFT was reduced to 4.5% and 2.3%, respectively. For T-Spot, the proposed conversion points were more than 7 spots and more than 9 spots and resulted in conversion rates of 6.1% and 5.2%, respectively. Another interesting observation was that, with both IGRAs, the rate of conversions was reduced after repeated testing over the period of 6, 12, and 18 months. In

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summary, on initial testing, IGRAs are less likely to be positive in HCWs with previous BCG vaccination than is the TST, and positive IGRA results have been associated with occupational risk factors in several studies. Clearly, both previous and repeated TST can affect the results of IGRA tests, but this is likely a short-term phenomenon. Within-person variability for IGRAs occurs and can lead to a change in IGRA result. Conversion rates are significantly higher with IGRAs than with TST, and reversion is common with all of the tests.

D. Marder presented a retrospective analysis of 20,543 tests performed on 8,111 HCWs at a single center. Again, the vast majority (89.5%) had a negative result and required no additional testing; 9.4% had a positive result, and 1.1% had an indeterminate result. To analyze results from HCWs who were tested more than once, Marder concentrated on those with positive results and categorized them according to their TB-Antigen minus Nil result as low positive (greater than or equal to 0.35 and less than 0.7 IU/mL), intermediate positive (greater than or equal to 0.7 and less than 1.0 IU/mL), and high positive (greater than or equal to 1 IU/mL). Of those HCWs with a positive QFT result, 49% were classified as high positive, 14% as intermediate positive, and 37% as low positive. All of these HCWs had at least 1 follow up QFT. Among those classified as low positives, 82% remained low positive or became negative on repeated testing, and 6% and 12% became intermediate or high positive, respectively. Among intermediate positives, 58% were negative or low positive when they underwent retesting, whereas 14% remained intermediate positive and 28% became high positive. Among high positives, 57% remained so at retesting, whereas 36% became low positive or negative and 7% became intermediate positive. A temporal analysis showed that most of the changes occurred within 3 months. It was concluded that clinical judgment is essential to interpret IGRA results. For HCWs who do not have a clinical history of exposure to TB, all results greater than 0.35 IU/mL should be considered for retesting within 1–3 months of the initial test. All individuals with QFT results that remain greater than 1.0 IU/mL for 2 serial tests should be considered persistently positive and infected and should thus be considered candidates for treatment for LTBI. A third test is generally not necessary. Clinical treatment decisions should be made within 3 months of the initial testing, and all other individuals should be followed up and tested according to their usual surveillance cycle. This approach resulted in rapid resolution of the screening process in nearly 90% of HCWs who have positive initial test results.

In another single-center retrospective study, W. Thanassi studied greater than 4,000 QFT results. A total of 85% of the HCWs tested had negative results on initial testing with QFT. Of the 15% of HCWs who had positive results on initial testing, 42% had negative results on retesting. Results from an elegant receiver operator curve (ROC) analysis demonstrated that, if the first QFT TB-Antigen minus Nil result was less than or equal to 0.9 IU/mL, there was a 71% chance that the next test result would be negative, and if the first test result was greater than 0.91 and less than or equal to 1.5 IU/mL, there was a 40% chance that the next test result would be negative. It was concluded that, if the first test result is less than or equal to 1.5 IU/mL and there are no clinical risk factors identified, the HCW should be retested, although when to retest is not known.

In summary, the evidence presented in the 3 large HCW studies in the United States shows that 80%–90% of HCWs screened had negative initial test results. Because of the high NPV, these workers need no additional testing for the year. HCWs who have high positive reactions generally have repeatedly positive results and do not need additional testing but should be evaluated for preventative therapy. However, those who have a modest IGRA reaction (below 1.0–1.5 IU/mL) on either initial testing or follow up testing would benefit from having a repeat test, because many will be transient reactors. Those who have a second positive result should be clinically evaluated and considered for treatment of LTBI.

A model has been developed that analyzes the impact of sensitivity and specificity on overall test accuracy in a population in which the prevalence of a disease such as LTBI is 20%. If one estimates that the TST has a sensitivity of 70% and specificity of 65%, then the overall test accuracy is 68%. However, in the case of QFT, for which the sensitivity is estimated to be 84% and the specificity is 99%, the test accuracy is 96%. Specificity is a critical value that has a major impact on test accuracy.

In summary, the immune response to TB is complex and continuously being remodeled. Those who are infected may "self-cure" or contain the infection using acquired immune mechanisms that are in a continual state of flux. TB is not latent but subclinical and is also continuously changing. Over the past century, the TST has been a useful tool to study TB and the risk of developing active TB. However, with the advent of IGRAs, we have a new and improved tool that is far more specific than the TST. Additionally, because IGRAs do not affect the immune response of the subject with regard to repeated testing, we can now better study the dynamics of the immune response to *M. tuberculosis* infection, which is something that was difficult with the TST. Clearly, the dynamic nature of the human response to TB is revealed, and we need to understand this new perspective on the immune response and how to use it to assess the risk of developing active TB.

Although the approved cutoff points for IGRAs are appropriate for testing those with known exposure or risk, the application of IGRAs to serial testing may require revision of the tests' interpretation criteria as well as our thinking and practices. Clearly, the IGRAs are at least as effective as TST in identifying those infected with *M. tuberculosis*, plus they are much more specific. Increased specificity is critical in screening for TB in HCWs who are not exposed to patients with active TB. In most low-prevalence settings, the vast majority of HCWs have a negative IGRA result, and the large body of data on the performance of IGRAs demonstrates that

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they have a very high NPV. The clinical significance of the smaller number of HCWs with a positive IGRA result using the currently approved cutoffs requires additional consideration. This is particularly important when the tests are used serially to test populations with relatively low risk of being newly infected. In such settings, it seems prudent to review the clinical status and risk of exposure of any HCW with a positive IGRA result, and it may be prudent to repeat IGRAs in some or (potentially) all HCWs with a positive IGRA result.

PROGRESSION TO ACTIVE TB

Are IGRAs useful to detect those people who are likely to progress to active TB? It appears that the NPV for progression to active TB of QFT is very high and that QFT-negative people in both HCW and household contact situations are very unlikely to develop active TB. In contrast, the positive predictive value (PPV) for progression to active TB is much more complex and requires analysis beyond the IGRA tests results alone. A positive IGRA result tells you that the patient is infected; however, assessing the likelihood of that individual progressing to active TB requires different types of information and is a completely separate decision from determining infection status. There is some evidence that the amount of IFN-γ detected by the IGRA tests may provide some indication of the degree of infection. Patients with active TB generally have higher levels of IFN-γ than those with latent TB. Perhaps those with LTBI and high levels of IFN-γ may be at higher risk of developing active TB than those with lower levels.

In his presentation, P. Haldar addressed the question of whether a positive IGRA test predicts progression to TB disease. Two recent meta-analyses that addressed the topic reached very different conclusions. The heterogeneity of the populations studied likely led to those differences in results. The analysis by Rangaka et al[6] covered studies conducted in high-prevalence and low- and middle-income settings and excluded studies in low-prevalence settings, because the authors believed that there was potential for incorporation bias. The analysis included, without discrimination, studies conducted using both commercial and noncommercial IGRAs as well as IGRAs with prolonged incubation times. In contrast, the review by Diel et al[7] included only studies from low-prevalence settings (albeit involving high-risk subjects) and only those studies in which commercial IGRAs had been used. Rangaka et al[6] showed a low rate of progression and similar PPVs for IGRAs and the TST, whereas Diel et al[7] found a high PPV for the IGRAs that was significantly better than that for the TST. In the Diel et al[7] meta-analysis, there was a higher likelihood of recent infection and, thus, progression. Meta-analyses may therefore be inaccurate for determining PPV for progression because of the high rate of heterogeneity in TB prevalence, risk groups studied, meta-analysis design, and data presentation. However, IGRAs seem to be more likely to be predictive of progression to active TB in the setting of recent infection.

The issue of the PPV of IGRAs in low-burden settings was discussed further. In a prospective study involving a population of close contacts to acid-fast bacilli (AFB) smear-positive cases, those with a positive QFT result (10% of adults, 20% of children 6–16 years old, and 50% of children less than 6 years of age) went on to develop active TB, with mean time from test to TB being 4.5 months for children and 12.5 months for adults. The NPV of QFT was 100%, with none of the QFT-negative persons of any age developing active TB over the minimum of 2 years of follow-up.

THE DYNAMIC NATURE OF THE IMMUNE RESPONSE TO *M. TUBERCULOSIS* INFECTION

Pioneering work presented by C. Barry in both animal models and human TB looking at granulomas in the lung visualized with computed tomography and positron emission tomography showed convincing evidence that TB granulomas are highly dynamic in both their location and their size within the lung. Sequential scans showed granulomas resolving and new granulomas appearing within the lungs of infected animals. The overall message was that TB is a dynamic state with large changes in granulomas and lesions over time. One could conclude that, similar to pathological indicators, the immune response to TB is also dynamic and that it should not be surprising that IGRA responses of an individual will change over short periods of time.

The concept that "there is nothing latent about latent TB" was further developed in D. Lewinsohn's presentation. The extent of *M. tuberculosis* infection and the associated immune response are dynamic, interactive, and constantly adjusting to a variety of factors. For many people exposed to *M. tuberculosis*, the innate immune system may eliminate the organism, and in others, elimination of *M. tuberculosis* may be achieved by the adaptive immune response. In those who do not clear the infection, the immune response may be highly variable in both mode and intensity with time. Variation may be attributable to factors such as nutrition or may be caused by how well and how long subclinical infection is maintained and whether progression to clinical disease occurs. The amount of IFN-γ produced in an IGRA will vary depending on the individual's mycobacterial load, the degree of engagement of different lymphocyte phenotypes at different times, and other stresses to the immune system. These factors all relate to the risk of developing active TB, and it was stressed by several speakers that it is necessary to also consider epidemiologic risk factors, comorbidities, and many other clinical factors, not just an IGRA result, in evaluating the risk of progression.

Much has been said about conversions and reversions of IGRAs, but these observations are at least in part a reflection of the dynamic nature of the immune response. In fact, similar results have been observed with TST. Specifically, Friedman et al[8] found that 45% of HCWs with a positive TST

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result had reverted to a negative TST result on retesting. For the TST, the medical community has dealt with this by establishing criteria for conversion, and it has been suggested this should be done for IGRAs. Data also suggest that potentially there may be information to be gained from the degree of an individual's IGRA response, with responses greater in individuals with recent infection than in those with older infection. These and other observations have led D. Lewinsohn and E. Nardell to raise the likelihood of transient exposure and self-cure.

Tumor necrosis factor α (TNF-α) blockers play an important role in the biology of TB disease and LTBI. TNF-α is a key cytokine involved in cell-mediated immunity, and blocking its action in vivo is used with increasing frequency as a treatment for autoimmune disorders, such as rheumatoid arthritis. However, TNF-α blockade leads to disruption of the immune system, which in turn leads to impaired containment of granulomatous diseases, particularly TB. R. Wallis presented a review of the global experience with TNF-α-blocking agents, in which both infliximab and etanercept increase reactivation; however, the mean time to reactivation was 20 weeks in those treated with infliximab, compared with 54 weeks in those receiving etanercept. In Spain, use of the 2-step TST to identify those infected with *M. tuberculosis* was successful in preventing TB disease but judged to be cumbersome and not specific. Conversely, in France, using unboosted TST was insensitive in preventing TB. R. Wallis concluded that TNF-α blockers, because of their propensity to promote reactivation of LTBI, have provided a unique window into the protective immune response to *M. tuberculosis* infection. In the discussion that followed, many concluded that those individuals about to receive TNF-α blockade treatments need better screening for LTBI than that provided by the TST.

Active pulmonary TB is often associated with depression of Th1 responses because of transient overproduction of interleukin 10 and transforming growth factor β. When active TB is treated, a rapid decrease in IFN-γ production follows. It appears that latency requires active immune surveillance to maintain intact granulomas, which is a view strongly supported by the data emerging from use of TNF-α blockade therapy. In each setting, IGRAs work very well to exclude those who are not infected, and it is speculated that IFN-γ production may be greater in progressive primary active TB than in reactivation TB. In conclusion, latency requires continuous immune surveillance, including surveillance of IFN-γ production, and although one could consider LTBI to be clinically latent, it is clearly not immunologically latent.

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