

Persistently Retained Interferon-Gamma Responsiveness in Individuals with a History of Pulmonary Tuberculosis

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The interferon gamma (IFN- γ) release assays (IGRAs) are the best method of detecting *Mycobacterium tuberculosis* infection. However, reports on IGRAs results obtained during and right after the treatment of tuberculosis (TB) have presented differing results. Some studies have shown declining responses, whereas other reports described persistent, fluctuating, or increasing responses. We postulated that the IGRA-positivity will decrease or revert long time after treatment of TB, and thus, evaluated the response of IGRA in subjects with a history of pulmonary TB. Seventy subjects (M:F = 51:19; age = 53.2 ± 11.8 years) underwent tuberculin skin tests (TSTs) and IGRA. The interval of time elapsed after the completion of anti-TB treatment was < 10 years for 16 subjects, 10-20 years for 13 subjects, 20-30 years for 16 subjects, and ≥ 30 years for 25 subjects. The TST was positive in 49 subjects (74%) and negative in 17 subjects (26%). The IGRA was positive in 52 subjects (74%) and negative in 18 subjects (26%). The IFN- γ level and the size of induration showed good correlation ($r = 0.525$, $P < 0.001$). However, the correlation between time elapsed after the completion of anti-TB treatment and the size of induration or that between time and the IFN- γ level was not significant. The TST and IGRA were positive in 72.7% and 68.0% of subjects ≥ 30 years after the treatment of pulmonary TB. In conclusion, majority of subjects with a history of pulmonary TB are IGRA-positive, even a few decades after the completion of anti-TB treatment.

Keywords: history of pulmonary tuberculosis; interferon-gamma release assays; latent tuberculosis; reversion; tuberculin skin test

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Introduction

The World Health Organization (WHO) reported that there was an estimated 8.7 million incident cases of tuberculosis (TB) globally in the year 2011, with approximately one-third of the world's population infected with *Mycobacterium tuberculosis* (*M.TB*) (World Health Organization 2012). Therefore, the diagnosis and treatment of latent TB is important not only for prevention of the transition from latent to active TB, but also for control of TB globally.

For several decades, the tuberculin skin test (TST) was the only available method for detecting latent *M.TB* infection. Recently, interferon gamma (IFN- γ) release assays (IGRAs) have been developed for detecting latent TB. IGRAs are whole blood tests based on the ability of the *M.TB* antigens to stimulate production of IFN- γ from T lymphocytes. If the subject has been infected *M.TB* before, T lymphocytes produce IFN- γ after incubation of whole

blood with TB specific antigens. The advantages of IGRAs over TST are that subjects do not need to return to the clinic for measurement of the response and that IGRAs can be repeated without the concern of sensitization and boosting (Dinnes et al. 2007). Additionally, since IGRAs are *M.TB*-specific, these assays are not affected by previous Bacille Calmette-Guerin (BCG) vaccination (Brock et al. 2004).

The immunity gained from TB treatment is not protective. Recent studies have shown that exogenous re-infection, rather than endogenous reactivation may be the primary cause of relapse (van Rie et al. 1999; Caminero et al. 2001). The individuals who have been successfully treated for TB are at higher risk of developing TB from re-infection than the general population (Verver et al. 2005). However, there was a lack of detection methods for re-infection of TB after the completion of treatment for active or latent TB. If the IGRA-positivity wanes after treatment of the infection, IGRAs can be used for detecting re-infection in subjects with a history of TB.

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Reports on IGRA test results obtained during and after the treatment of active or latent TB have presented differing results. Some studies have shown declining responses during and after the treatment (Kobashi et al. 2009a; Connell et al. 2010; Lee et al. 2010b), whereas other reports described persistent, fluctuating, or increasing responses (Ferrand et al. 2005; Pai et al. 2006; Dyrholm-Riise et al. 2010). These discrepancies were attributed to variations in incubation periods, antigens, and assay formats (Pai et al. 2006). We hypothesized that the relatively short time lapse after the treatment of TB is a key factor contributing the discrepancies, and that the IGRA results would be negative after a few decades. To examine this possibility, we evaluated the results of the QuantiFERON[®]-TB Gold In-Tube test (QFT-GIT; Cellestis Ltd., Carnegie, VIC, Australia) among subjects with a history of pulmonary TB.

Methods

Subjects and data collection

Seventy-two subjects with a history of pulmonary TB treatment were enrolled in this study. Two subjects with indeterminate QFT-GIT results were excluded. Chest radiographs from these patients were reviewed by a radiologist (Kwon, W.). The inclusion criteria were as follows: (1) subjects who remembered the exact period of previous treatment for active pulmonary TB; (2) findings from chest radiographs were compatible with old TB and showed no difference from the radiographs taken at least 1 year earlier; and (3) no obvious history of close contact of the subject with active TB patients after their own treatment. The interval of time elapsed after the completion of TB was < 10 years for 16 subjects, 10-20 years for 13 subjects, 20-30 years for 16 subjects, and ≥ 30 years for 25 subjects. Korean government initiated the national BCG vaccination project in 1952 (Joung and Ryoo 2013). Therefore, most of the enrolled patients were presumed to have received BCG. Written informed consent was received from all subjects. The protocol was approved by the Ethics Review Committee of Ulsan University Hospital (Ulsan, Republic of Korea).

TST and QFT-GIT assay

All subjects underwent QFT-GITs. TST was performed after sampling for QFT-GITs. Four subjects declined TSTs after sampling for QFT-GITs. For TST, 2 TU (tuberculin unit) PPD (purified protein derivative) RT 23 (Statens Serum Institut, Copenhagen, Denmark), which is recommended by WHO as a standard TST survey and the standard dose used in Korea, was injected intradermally into the volar aspect of the forearm, and the transverse diameter of induration was measured 48-72 hours later. If this induration was greater than 10 mm, TST was considered positive. TST was repeated after 1-3 weeks if the result of the first test was negative. However, because 7 out of 17 subjects declined a second TST, only the first TST result was analyzed.

QFT-GIT is one of the commercially available IGRAs. It is an in-tube collection system that consists of three special heparinized tubes, nil, TB antigen and mitogen. QFT-GITs were performed and interpreted according to the manufacturer's recommendations. Whole blood was collected in three special heparinized tubes: one coated with the *M.TB* specific antigens ESAT-6, CFP-10, and TB7.7; one coated with mitogen, as a positive control; one without antigen coat-

ing, as a negative control. The tubes were incubated for 24 hours at 37°C, centrifuged, and stored in the cold until testing. The plasma IFN- γ concentration was measured using ELISA. An IFN- γ response to the ESAT-6/CFP-10/TB7.7 mixture with a value ≥ 0.35 IU/mL above the nil control value (and ≥ 25% of the nil control) was considered a positive test result. If the response (corrected for the nil control) was < 0.35 IU/mL and the response to the mitogen-positive control was > 0.5 IU/mL, the result was considered negative. If the nil-corrected IFN- γ responses were < 0.35 IU/mL and the mitogen-positive control responses were < 0.5 IU/mL, the response was considered indeterminate.

Statistical analysis

The concordance between the TST and QFT-GIT results was analyzed using kappa coefficients. The relationship between time and TST or QFT-GIT was analyzed using χ^2 test. The correlation between time, TST, and QFT-GIT was analyzed by Pearson's correlation test. All tests for significance were two-sided and *P* values < 0.05 were considered significant.

Results

Clinical characteristics

Two subjects were excluded because the QFT-GITs were indeterminate. As a result, a total of 70 subjects were analyzed. The subjects attended the hospital for medical check-ups (*n* = 22), other respiratory diseases (*n* = 31), scheduled operations (*n* = 8), and other diseases (*n* = 9). The clinical characteristics of the subjects are shown in Table 1. The mean age was 53.2 ± 11.8 years. The male:female ratio was 51:19. The mean interval between treatment of TB and QFT-GIT tests was 22.3 ± 12.8 years.

Comparison of TSTs and QFT-GITs

Both TST and QFT-GIT results were available for 66 subjects (Table 2). TSTs were positive in 49 subjects (74%) and negative in 17 subjects (26%). Of the 17 subjects with negative results, ten were retested after 1-3 weeks and

Table 1. Characteristics of the study population.

Clinical characteristics	Values (<i>n</i> = 70)
Age (mean ± S.D.)	53.2 ± 11.8
Gender (M : F)	51 : 19
Reasons for visit	
Operation	8
Other respiratory disease	31
Medical check up	22
Other diseases*	9
Years after the completion of anti-tuberculosis treatment	
< 10	16
10-20	13
20-30	16
≥ 30	25

*Ureter stone, gastroesophageal reflux disease, thyroid nodule, unstable angina, adrenal mass, atrial fibrillation, chronic hepatitis B, osteoarthritis, constrictive pericarditis.

Table 2. Comparison of TST and QFT-GIT results for subjects with a history of pulmonary tuberculosis.

QFT-GIT	TST		Total
	Negative	Positive	
Negative	10 (15%)	7 (11%)	17 (26%)
Positive	7 (11%)	42 (63%)	49 (74%)
Total	17 (26%)	49 (74%)	66 (100%)

TST, tuberculin skin test; QFT-GIT, QuantiFERON®-TB Gold In-Tube.

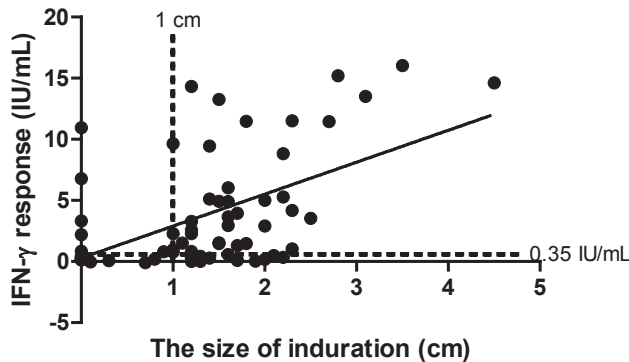


Fig. 1. Correlation between IFN- γ response and tuberculin skin test ($n = 66$). The IFN- γ level and the induration size of tuberculin skin test showed positive correlation ($r = 0.525$, $P < 0.001$).

seven subjects declined a second TST. Of the ten subjects retested, three converted to positive and seven remained negative. The mean induration after the first TST among the subjects who converted positive in the second test was 4.0 mm and the mean TST induration of persistently negative subjects was 0.14 mm. The QFT-GIT was positive in 52 subjects (74%) and negative in 18 subjects (26%). The concordance between the first TST and QFT-GIT was fair-to-good ($\kappa = 0.445$). The IFN- γ level and the size of induration had a good correlation ($r = 0.525$, $P < 0.001$; Fig. 1).

The relationship between follow-up time and TST or QFT-GIT

After the treatment of pulmonary TB, the TST and QFT-GIT were still positive in 72.7% and 68.0% of subjects ≥ 30 years (Table 3). The relationship between time elapsed after the completion of treatment and TST or QFT-GIT is shown in Fig. 2 and Fig. 3. Neither the TST results nor the IFN- γ levels in QFT-GITs of subjects with a history of pulmonary TB changed even after 30 years of the completion of treatment. The correlation between time elapsed after the completion of treatment and size of induration ($r = -0.057$, $P = 0.652$; Fig. 2) or that between the time and IFN- γ level ($r = 0.045$, $P = 0.713$; Fig. 3) was not significant.

QFT-GIT at different cut-off values

QFT-GIT positivity at different cut-off values is shown

in Table 4. Although QFT-GIT positivity changed according to cut-off values, there was no significant difference in QFT-GIT positivity between groups of subjects with various time lapses after the completion of anti-TB treatment.

Discussion

In a country with intermediate TB burden, this study is the first study that evaluated the usefulness of QFT-GIT in subjects with a history of pulmonary TB. Even a few decades after anti-TB treatment, the QFT-GIT positivity and TST results did not differ.

Theoretically, the response of IGRAs decreases after the treatment of active infection. The effector-memory T (T_{EM}) cells, which are easily activated during acute infection, decline once the infection is resolved, whereas long-term memory T cells [referred to as central-memory T (T_{CM}) cells] remain elevated even after complete treatment of acute infection (Todryk et al. 2009). Because T_{CM} cells require a longer period of incubation than T_{EM} cells, the overnight incubation step selectively amplifies replication of T_{EM} . IGRA results are more likely to be positive for individuals who have recently been infected (Diel et al. 2011) and the positivity decreases after the treatment of active infection. However, TST is more likely to identify individuals with long-standing cellular immune responses to these antigens (Pollock et al. 2008).

However, in actual practice, the rate of reversion of IGRA during or after treatment of latent or active pulmonary TB is variable and there exist controversies regarding the reversion of IGRAs (Chiappini et al. 2012b). Some authors have reported that reversion of IGRAs does not occur after the completion of anti-TB treatment (Pai et al. 2006; Chiappini et al. 2012a; Denkinger et al. 2013), whereas others reported decreased IGRA responses after treatment (Ewer et al. 2006; Lee et al. 2010a, b). Pai et al. reported that the decline of IFN- γ response was not statistically significant during the treatment of latent TB infection (LTBI). The QuantiFERON®-TB Gold (QFT-G) assay result continued to be positive in 9 out of 10 participants (Pai et al. 2006). In contrast, Lee et al. reported that IFN- γ level decreased in 97.3% of 214 Korean subjects and that the reversion rate of QFT-GIT was relatively high (41.9%) during treatment of LTBI (Lee et al. 2010a). Aiken et al. reported that successful treatment of active TB was accompanied by a significant reduction in the *M. TB*-specific anti-

Table 3. TST and QFT-GIT results for subjects with a history of pulmonary TB, stratified by years after the completion of anti-TB treatment.

Years after the completion of anti-TB treatment	TST positive	QFT-GIT positive
< 10	13/16 (81.3%)	13/16 (81.3%)
10-20	11/13 (84.6%)	11/13 (84.6%)
20-30	9/15 (60.0%)	11/16 (68.8%)
≥ 30	16/22 (72.7%)	17/25 (68.0%)
Total	49/66 (74.2%)	52/70 (74.3%)

TB; tuberculosis; TST, tuberculin skin test; QFT-GIT, QuantiFERON®-TB Gold In-Tube.

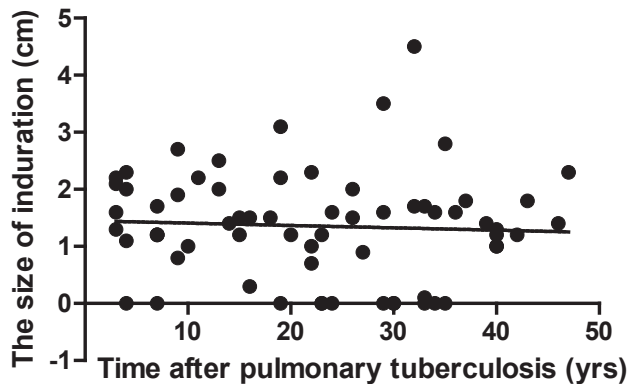


Fig. 2. Correlation between the size of induration and history of pulmonary tuberculosis ($n = 66$). The induration size of tuberculin skin test did not correlate with time elapsed after pulmonary tuberculosis ($r = -0.057$, $P = 0.652$).

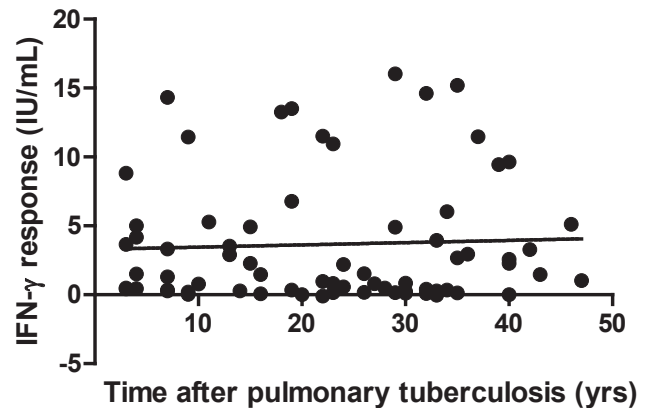


Fig. 3. Correlation between the IFN- γ level and history of pulmonary tuberculosis ($n = 70$). The IFN- γ response did not correlate with time elapsed after pulmonary tuberculosis ($r = 0.045$, $P = 0.713$).

Table 4. QFT-GIT positivity at different cut-off values, stratified by years after the completion of anti-TB treatment.

Cut-off value	Years after the completion of anti-TB treatment					<i>P</i> value
	< 10	10 - 20	20 - 30	≥ 30	Total	
0.20 IU/mL	14 (87.5%)	12 (92.3%)	11 (68.8%)	20 (80.0%)	57 (81.4%)	0.370
0.35 IU/mL	13 (81.3%)	11 (84.6%)	11 (68.8%)	17 (68.0%)	52 (74.3%)	0.592
0.50 IU/mL	9 (56.3%)	10 (76.9%)	10 (62.5%)	16 (64.0%)	45 (64.3%)	0.711
1.00 IU/mL	9 (56.3%)	9 (69.2%)	6 (37.5%)	15 (60.0%)	39 (55.7%)	0.347
3.00 IU/mL	7 (43.8%)	6 (46.2%)	4 (25.0%)	9 (36.0%)	26 (37.1%)	0.620
Total	16	13	16	25	70	

TB, tuberculosis; QFT-GIT, QuantiFERON®-TB Gold In-Tube.

gen ELISPOT count (Aiken et al. 2006). However, Denkinger et al. reported that only 17 of 133 subjects showed reversion at the end of therapy (Denkinger et al. 2013). Further, Ribeiro et al. reported that only 10% of the individuals showed a change from a baseline reactive test result to a negative one after 24 weeks of treatment (Ribeiro et al. 2009).

Although the response of IGRAs tended to decrease during the treatment of active or latent infection, the response did not change back immediately after the completion of treatment. One possible reason is that circulating

effector T cells may persist for longer period after the clearance of the infection.

Studies have shown that higher baseline TST induration size, elevated baseline IFN- γ level (Ewer et al. 2006; Lee et al. 2010a; Bartalesi et al. 2013), advancing age (Bartalesi et al. 2013), prior positive TST result (Ringshausen et al. 2010), low CRP levels, and absence of fever at diagnosis (Lee et al. 2010b) were factors contributing to persistent positive IGRA responses. Taken together, the reversion rate is determined by the strength of antigenic stimuli or the magnitude of the initial immune response. There are higher

antigenic stimuli in active pulmonary TB than in latent TB, and the reversion of IGRAs after the treatment of active TB may require longer time.

Recently, Bartalesi et al. reported that IFN- γ levels significantly decreased three to six months after treatment from immediate completion of treatment (Bartalesi et al. 2013). Lee et al. also reported that reversions occurred in seven (26.9%) subjects three months after the initiation of treatment for latent infection (Lee et al. 2012). The authors also reported that reversion occurred in an additional two subjects six months after treatment initiation. These findings suggested that IGRA results could show a reversion, a long time after completion of treatment.

However, in our study, we found that the results of IGRAs did not show a reversion, even a few decades after treatment. Several studies evaluated IGRAs a long time after the completion of treatment. Wu-Hsieh et al. reported that the responses to ESAT-6 were strong in at least one TB remission patient 17 years after the treatment (Wu-Hsieh et al. 2001). These authors also reported that there was no correlation between the time elapsed after the recovery and the response to ESAT-6. Although these results are consistent with our present findings, the number of subjects participated was small and ESAT-6 was the only antigen evaluated. Kobashi et al. evaluated the QFT-2G test administered a few decades after the completion of anti-TB treatment (Kobashi et al. 2009b). These authors reported that one-third of the subjects who recovered from pulmonary TB continued to show a positive QFT-2G test, and mentioned that using QFT-2G, instead of QFT-GIT which used more TB-specific antigen and provided higher sensitivity (Harada et al. 2008), was one of the major limitations of the study. Our results showed that there was no decline in the immune response, even when tested by QFT-GIT. The proportion of continued QFT-GIT-positive case in our study was 74%, which was significantly higher than that found by Kobashi et al. (2009b). This discrepancy likely originated from the test methods used and/or the differences in TB prevalence. The QFT-GIT positivity (74%) and mean IFN- γ level (3.66 ± 4.59 IU/mL) observed in this study were higher than recent Korean data (66.1% and 1.50 ± 2.12 IU/mL) (Lee et al. 2010b) and lower than Indian data (79% and 4.3 IU/mL) (Pai et al. 2007). These differences in IGRA responses likely reflected the TB burden of the countries from which the subjects were recruited. Increasing re-exposure to *M. TB* and re-infection may activate memory T cells. In the past, Korea was one of the high TB burden countries (in 1990, 163/10⁵). The incidence of TB gradually lowered and Korea is currently one of the intermediate burden countries (in 2010, 97/10⁵). Many subjects who participated in this study had completed their treatment several decades ago when Korea was a high TB burden country. The subjects might have had a higher chance of re-exposure and re-infection than those in recent Korean and Japanese data (Kobashi et al. 2009b; Lee et al. 2010b).

According to the manufacturer's specifications, the

cut-off value of QFT-GIT is 0.35 IU/mL. However, previous studies reported non-specific variations, defined as uncertainty zone that occur during IGRA serial testing (IFN- γ value between 0.20 and 0.50 IU/mL) (Pai et al. 2009; Bartalesi et al. 2013). Further, the cut-off value is for LTBI, and not for re-infection. There is a lack of consensus on whether or not this cut-off value can be used for analyzing re-infection. Therefore, different cut-off value can differentiate re-infections. We found that, irrespective of the cut-off values, there was no correlation between the time elapsed after the treatment of TB and the results of QFT-GIT or TST. Therefore, it is not likely that the low rate of reversion was due to the cut-off value.

Our study had several limitations. First, there was no data on initial TST or IGRA. Because QFT-GIT became available in Korea only recently, a long-term prospective study was not possible. Second, Korea is an intermediate TB burden country. Therefore, a possibility for re-infection after completion of treatment of TB exists. However, we excluded the subjects who had the history of close contact with active pulmonary TB patients after treatment. Third, the methods that were used for the diagnosis of TB in the subjects in our study were not known, because, many patients were diagnosed over 30 years ago in different hospitals and the medical records were not available. However, we enrolled only those subjects who remembered the diagnosis and treatment well and when the results of chest radiographs were consistent with old TB. Finally, the number of subjects was relatively small. However, because there was no decline in the QFT-GIT response in our study, we anticipate that a larger study will only reveal similar results.

In conclusion, majority of subjects with a history of pulmonary TB were QFT-GIT-positive, even a few decades after completing the anti-TB treatment. Therefore, QFT-GIT does not seem to be suitable for diagnosis of recent *M. TB* infection in subjects with a history of TB. Further studies are needed to fully test the validity of the findings.

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Conflict of Interest

The authors declare no conflict of interest.

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