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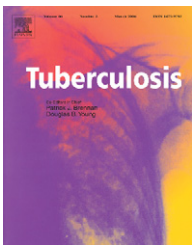

Relationship between whole-blood interferon-gamma responses and the risk of active tuberculosis

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Summary
We have analyzed the relationship between the responses to the diagnostic method for *Mycobacterium tuberculosis* (*Mtb*) infection, QuantiFERON®-TB Gold (QFT-G), and the risk of developing active tuberculosis (TB). Contacts under 42 years old who were exposed to a patient with infectious pulmonary TB were tested using QFT-G during an investigation. Among 172 contacts, 111 (64.5%) were QFT-G positive. All subjects were evaluated for active TB by chest X-ray examination and, if needed, by CT scan at the time of the QFT-G test and 39 were diagnosed with active TB based on radiological abnormalities consistent with TB. Of these, 35 (89.7%) were QFT-G positive. Statistically the geometric mean of interferon-gamma (IFN-γ) production levels of the active TB group was significantly larger than that of the latent TB infection group ($p = 0.013$). The results of the multivariate analysis clearly showed that a combined parameter of ESAT-6 and CFP-10 significantly contributes to disease risk for the infected subjects. Our results suggest that subjects with high levels of IFN-γ production in response to either ESAT-6 and/or CFP-10 in the QFT-G test have a higher possibility of developing active TB than QFT-G positive subjects with lower levels of IFN-γ.

Tuberculosis (TB) is still one of the major infectious diseases in the world. It has been estimated that one-third of the world's population is infected with *Mycobacterium tuberculosis* (*Mtb*), a causative agent of TB.[1] Although many individuals are infected, more than 90% are thought to develop a protective response to *Mtb* and successfully control *Mtb* growth.[2] This protective response of hosts against *Mtb* is mediated by cell-mediated immunity, in which several cytokines including interferon-gamma (IFN-γ) play a crucial role.[3] In fact, individuals who have genetic defects in their IFN-γ system are known to be highly

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sensitive to mycobacterial infection.[3] Thus, *Mtb* infection generally induces *Mtb*-antigen specific IFN-γ responses to protect hosts from developing TB.

By applying this immune response, two new diagnostic methods for *Mtb* infection, QuantiFERON®-TB Gold (QFT-G) and T-SPOT®. TB, which measure IFN-γ production in response to the *Mtb*-specific antigens, ESAT-6 and CFP-10, has been developed.[4] We previously evaluated QFT-G using TB patients and healthy subjects in Japan and demonstrated that QFT-G has high specificity and sensitivity for diagnosing *Mtb* infection without influence of prior BCG vaccination.[5] Moreover, we and others have shown that QFT-G can detect latent TB infection (LTBI).[6–9] IFN-γ is mainly produced by antigen-specific effector T cells with assay systems having incubation times of less than 24 h.[10] Since the frequency of the effector T cells is thought to rely on the antigen load,[10] the level of IFN-γ production is supposed to be related to the bacterial load in the hosts. Therefore, the high level of IFN-γ production in response to *Mtb*-specific antigens may imply a risk factor for developing active TB. Indeed, it has been demonstrated that the level of IFN-γ responses to ESAT-6 correlates with the degree of pathology or the bacterial load in animal models.[11–14] Furthermore, Doherty et al.[15] have shown that a high level of IFN-γ responses to ESAT-6 correlates with the subsequent development of active TB in healthy household contacts of TB patients. Based on these observations, the hypothesis that high and/or rising levels of IFN-γ in response to ESAT-6 might serve as a prognostic marker for the subsequent development of the disease has been recently proposed.[16] This hypothesis may be in line with the observation that the successful chemotherapy in TB patients generally decreases the level of IFN-γ production in response to ESAT-6 and/or CFP-10.[17–20] Very recently, Adetifa et al.[21] have shown that IFN-γ producing T cell numbers increased prior to developing TB, which also supports this hypothesis. In the present study, we analyzed the relationship between the level of IFN-γ responses to *Mtb* antigens and development of TB among QFT-G positive individuals identified in a contact investigation.

The index case was a male teacher in a cram school. He developed fever and cough in the early January 2005. When his symptoms persisted, he visited three medical facilities in late March. However, he was not diagnosed with TB at this point. On 6th April, upon revisiting one of the facilities, he was first suspected to have pulmonary TB based on the chest X-ray examination, and he was hospitalized on 8th April. He was coughing severely at this point. He was diagnosed with pulmonary TB with a sputum smear heavily positive for acid-fast bacilli. By the time of contact investigation by a public health center, four students had already developed TB. Twenty-eight teachers, 47 guardians who had consulted with the index case, and 118 students who were registered in this school from December 2004 to early April 2005 were subjects in a contact investigation that was offered in late June. Each contact was examined with a chest X-ray, and if TB was suspected, computed tomography (CT) was employed for confirmation, in addition to the bacteriological examinations. The QFT-G test and the tuberculin skin test (TST) were applied for those under 42 years old, and carried out as described previously.[16] Those with LTBI were judged so if they had no abnormality on chest X-ray or CT scan examination and they were positive in the QFT-G test. The IFN-γ responses to ESAT-6/CFP-10 of those who had already developed TB at the QFT-G test were compared with those who did not develop TB.

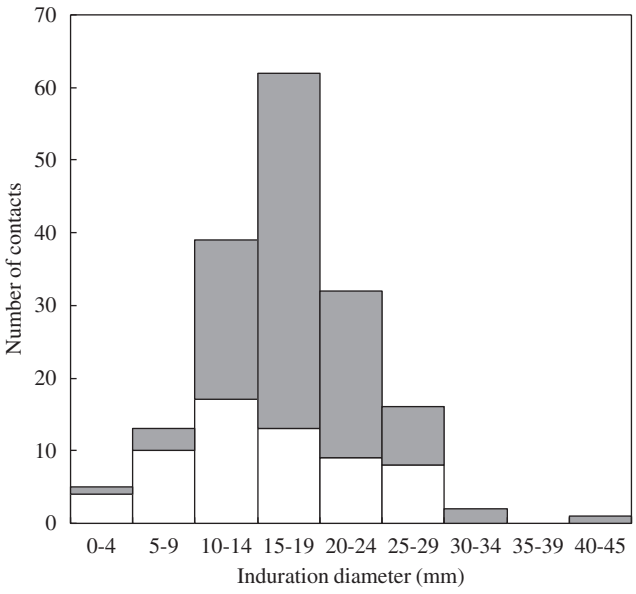

Figure 1 Distribution of induration diameters. Bars represent the number of subjects with different TST size, giving either a QFT-G positive (gray) or a QFT-G negative (white).

QFT-G was used with 172 contacts. The mean age of the 172 contacts was 18.98 years (range: 6–42). Figure 1 shows the distribution of the contacts according to the induration size of TST. The arithmetic mean of the indurations was 17.0 mm, and 89.4% of the tested subjects showed reaction greater than or equal to 10 mm. Although all of the subjects reported a history of vaccination with BCG at least once in their childhood, the distribution profile suggested there was extensive transmission of *Mtb*. Of the 172 contacts, 111 (64.5%) were QFT-G positive, also indicating that very extensive transmission of *Mtb* had occurred in this cram school. The majority of QFT-G positives (96.4%) showed the induration size of greater than 10 mm (Figure 1). As a result of the evaluation for active TB, 39 (22.7%) were diagnosed with active TB based on the radiological abnormalities consistent with TB. None of them was proven as positive on bacteriology. Four of these 39 patients (10.3%) were QFT-G negative.

Figure 2 shows comparison of the distributions of higher IFN-γ production level in response either to ESAT-6 or CFP-10 in two groups, i.e., one group including those who were QFT-G positive without developing TB (LTBI), and the other group including those who were QFT-G positive with developing TB (active TB). Comparison of geometric means of IFN-γ response values between active TB group and LTBI group was tested with *t*-test, assuming the different variances. Statistically the geometric means of the active TB group was significantly larger than that of the LTBI group ($p = 0.013$).

To further analyze the factors related to the risk of clinical development of TB among positive QFT-G responders, we first used univariate analyses (Table 1). The risk of clinical development of TB was evaluated according to their characteristics. The risk was calculated as a rate of those