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Dynamics of immune parameters during the treatment of active tuberculosis showing negative interferon gamma response at the time of diagnosis

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SUMMARY

Objectives: In the performance of interferon gamma release assays (IGRA) for the diagnosis of tuberculosis (TB) infection, false-negative results are a major obstacle. In active TB patients, treatment-dependent changes of the negative test results remain unknown.

Methods: The treatment course of 19 smear-positive/culture-confirmed TB patients who had IGRA-negative results by QuantiFERON-TB in-tube (QFT-IT) method at the time of diagnosis (month 0) in a previous study, were monitored in the present study. Blood was further collected at months 2 and 7, and the concentrations of 27 immune molecules were measured in the plasma supernatants remaining after performing the IGRA, using a suspension array system.

Results: After initiating treatment, eight of the 19 QFT-IT-negative patients showed positive conversion, whereas the remaining 11 (58%) did not; the interferon gamma (IFN-γ) response was restored to levels higher than 1 IU/ml in only three of the eight patients with positive conversion. Plasma concentrations of interleukin 1 receptor antagonist, interleukin 2, and interferon gamma-induced protein 10 remained low after *Mycobacterium tuberculosis*-specific antigen stimulation at months 2 and 7 in the continuously QFT-IT-negative group, whereas the parameters were elevated only in the transiently QFT-IT-negative group.

Conclusions: It was demonstrated that a majority of active TB patients showing negative IGRA results did not regain sufficient levels of immune responsiveness despite successful treatment.

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1. Introduction

The interferon gamma release assay (IGRA) is currently used as one of the representative tests to diagnose tuberculosis (TB) infection.[1] In this test, the cellular response to *Mycobacterium tuberculosis* is assessed by measuring the interferon gamma (IFN-γ) released from peripheral blood lymphocytes after stimulation with *M. tuberculosis*-specific antigens.[1]

The QuantiFERON-TB Gold In-Tube test (QFT-IT) is a commercially available IGRA based on the ELISA method; it has a sensitivity of 78–83% and specificity of 98–100%.[1] This imperfect sensitivity causes difficulties in ruling out TB infection, particularly when the

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prevalence of TB infection is high, and the low negative predictive value of the test may reduce the chance of a possible therapeutic intervention.

To assess the sensitivity of the QFT-IT, patients with bacteriologically proven active TB disease have often been recruited as surrogates for individuals with latent TB infection. The IGRA results also provide a clue to suspect active TB disease clinically.

Although weakened immunity in severe TB may affect the test results,[2] treatment-dependent changes of the negative IGRA results with a very low IFN-γ response have not been investigated fully.

This study group has recently reported the results of a cross-sectional study on the sensitivity of the QFT-IT method in Hanoi, Vietnam, and demonstrated that aging, emaciation, HIV co-infection, and a particular HLA genotype, DRB1*07:01, lowered the sensitivity of the test in active pulmonary TB patients.[3] In the present study, 19 of the 24 patients who showed false-negative results at the time of diagnosis were monitored. Further analysis of the treatment response and dynamics of immune parameters was performed, with the measurement of the concentrations of various cytokines and chemokines in the plasma supernatants remaining after use in the IGRA assay.

2. Methods

2.1. Study subjects and IGRA

From July 2007 to March 2009, whole blood was collected from 504 adult patients in Hanoi, Vietnam, who had smear-positive/culture-confirmed pulmonary TB and a history negative for TB treatment. The blood was collected in heparinized tubes before anti-TB treatment was initiated (month 0).[3] The patients were tested with a commercially available ELISA-based IGRA (QFT-IT; Cellestis, Victoria, Australia), as reported previously.[3] Plasma supernatants were separated at 4000 rpm for 15 min (Model 2010; Kubota Co., Tokyo, Japan) and stored at −80 °C until measurement. The cut-off value to interpret the QFT-IT results was set at 0.35 IU/ml, as per the manufacturer's instructions.

In the present study, further blood samples for QFT-IT and other tests were collected and served for analysis at two more time points: after the initial phase of treatment (month 2) and close to the end of treatment (month 7). Positive conversion of the IGRA was defined by a negative result at month 0 and positive result(s) at month 2, month 7, or both time points.

2.2. Clinical data collection

The extents of cavitary lesions and infiltrates were also semi-quantitated by the grading method.[4] *M. tuberculosis* isolates were analyzed by single nucleotide polymorphism (SNP) and spoligotyping methods.[5]

2.3. Treatment course

Following the national standard regimen at that time, all patients received an 8-month course of the anti-TB treatment regimen 2S(E)HRZ/6HE, which was commonly administered during the study period in Vietnam.[5]

2.4. Immune analyte profiling by Bio-Plex assay and adiponectin ELISA assay

Immune molecules released into the plasma after TB antigen stimulation were estimated from their concentrations after a 16- to 24-h incubation with TB-specific antigens (TBAg) minus those with no antigens (Nil) obtained from the QFT-IT method. Their concentrations were determined using a human 27-plex assay (14 cytokines: interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-γ, tumor necrosis factor alpha (TNF-α); seven chemokines: eotaxin, IL-8, IFN-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory proteins MIP-1α and MIP-1β, RANTES; and six growth factors: IL-7, fibroblast growth factor (FGF) basic, granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony stimulating factor (GM-CSF), platelet-derived growth factor (PDGF)-BB, vascular endothelial growth factor VEGF) (Bio-Plex Suspension Array System; Bio-Rad, Hercules, CA, USA), following the manufacturer's instructions. All samples, standards, and controls were run in duplicate and manipulated in accordance with the manufacturer's protocol. Samples were diluted in a 1:4 volume ratio with the sample diluent and incubated for 30 min at room temperature; they were then agitated at 300 rpm to be captured with antibody-coupled magnetic beads. Following three washes in a Bio-Plex Pro Wash Station, the samples were incubated with biotinylated detection antibodies and agitated at 300 rpm in the dark for 30 min at room temperature. Each captured analyte was detected by the addition of streptavidin–phycoerythrin and quantified using a Bio-Plex array reader. The fluorescence intensities in the samples and known standards were acquired and converted to the plasma concentrations of each analyte using the Bio-Plex 200 System software (version 6.0; Bio-Rad Laboratories). When the induction of immune molecules after TB antigen stimulation was below their detection limits, these molecules were excluded from subsequent analysis.

Total human adiponectin (low, middle, and high molecular weight) levels in plasma were also measured using the Quantikine Human Total Adiponectin/Acrp30 Immunoassay Kit (R&D Systems, Inc., Minneapolis, MN, USA). The mean minimum detectable dose was 0.246 ng/ml.

2.5. Statistical analysis

Values including cytokine concentrations among groups were analyzed by Kruskal–Wallis tests with multiple comparisons for all pairs by Steel–Dwass method. The inequality of proportions among the groups was analyzed by Fisher's exact test. The statistical analysis was performed using Stata version 12 (Stata Corp, College Station, TX, USA) and JMP 9 (SAS Institute Inc., Cary, NC, USA). A p-value of < 0.05 was considered to be statistically significant. The Bonferroni correction was also used for multiple comparisons, when appropriate.

3. Results

3.1. Characteristics of the patients who completed the three-time blood collection stratified by IGRA-negative or positive result at month 0

After the cross-sectional study reported previously,[3] 19 of the 24 IGRA-negative patients with culture-confirmed active pulmonary TB at the time of diagnosis completed the three-time blood collection at months 0, 2, and 7; these patients were thus analyzed in the present study. The 351 patients who initially showed QFT-IT-positive results and completed the three-time blood collection were set as a reference. Samples showing indeterminate results before treatment were omitted from this analysis.

As expected from the results of the previous report,[3] increasing age, low body mass index (BMI) at the time of diagnosis, and the HLA-DRB1*07:01 allele were observed more frequently in the 19 QFT-IT-negative patients than in the 351 QFT-IT-positive

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