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Characterization of the CD4 and CD8 T-cell response in the QuantiFERON-TB Gold Plus kit

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ABSTRACT

Objective/background: QuantiFERON-TB Gold In-Tube (QFT-GIT, Qiagen, Hilden, Germany) is an interferon- γ (IFN- γ) release assay designed to detect latent tuberculosis infection (LTBI). Although QFT-GIT has several advantages (mainly that it is not affected by the Bacille Calmette–Guérin vaccination), it has a poor sensitivity in immune-compromised individuals as it involves an immune response-based detection. Recently, QuantiFERON-TB Gold Plus (QFT-Plus) assay has been proposed as a new generation of QFT-GIT. QFT-Plus includes two tubes, TB1 and TB2 with *Mycobacterium tuberculosis* antigens to elicit a specific immune response. TB1 contains peptides derived from the antigens 6 kDa early secretory antigenic target (ESAT-6) and 10 kDa culture filtrate protein (CFP-10) (TB-7.7, present in QFT-GIT, has been removed), and it is designed to induce a specific CD4 T-cell response. TB2 contains newly designed peptides stimulating IFN- γ production by both CD4 and CD8 T cells. The additional peptides for eliciting CD8 T-cell responses have been included to increase the sensitivity of the test for LTBI detection.

The aim of the study was to evaluate specific CD4 and CD8 T-cell responses to the *M. tuberculosis* antigens contained within the QFT-Plus test by flow cytometry in individuals with active TB and LTBI.

Methods: We enrolled 23 individuals with active TB and 30 individuals with LTBI. QFT-Plus assay and intracellular staining were performed. One million of peripheral blood mononuclear cells in 1 ml of complete medium (RPMI 1640) were dispensed in QFT-Plus tubes. Following 16–24 h stimulation, antigen-specific T cells were characterized by flow cytometry evaluating CD4, CD8, CD3 markers, and IFN- γ production. For statistical analysis, non-parametric tests were performed.

Results: We found that CD4 T-cell responses were induced by both TB1 and TB2. Differently, the CD8 T-cell response was mainly induced by TB2 and was significantly higher than that induced by TB1 ($p = 0.01$). The frequency of *Mtb* specific T-cells observed in individuals with active TB was significantly higher than in those with LTBI ($p = 0.04$). Finally, TB2-specific CD8 T-cell responses in individuals with active TB were associated with high radiological

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severity of lung lesions and microbiological diagnosis (based on *M. tuberculosis* isolation in sputum culture).

Conclusion: This is the first characterization of CD4 and CD8 T-cell responses to QFT-Plus TB1 and TB2 tubes in individuals with active TB and LTBI enrolled in a low TB-endemic country such as Italy. We demonstrated that the increased sensitivity is a consequence of the ability of TB2 to induce a CD8 T-cell response which is mainly associated with active TB. This assay has the potential to be very useful in conditions of immune depression due to CD4 T-cell impairments.

Conflicts of interest

The authors have no conflicts of interest to declare.