Clinical Significance of Interleukin-2/Gamma Interferon Ratios in *Mycobacterium tuberculosis*-Specific T-Cell Signatures

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In countries with low tuberculosis (TB) incidence rates, such as Switzerland, targeted testing for latent *Mycobacterium tuberculosis* infection (LTBI) among risk groups, such as health care workers (HCW), is an important measure for preventing active TB disease (3, 7). Occupational health programs are increasingly turning to gamma interferon (IFN-γ) release assays (IGRA), such as the QuantiFERON-TB Gold in-tube test (QFT test; Cellestis Inc., Australia), to screen employees for LTBI (4, 5, 9). Diel et al. prospectively followed QFT test-positive, recent-TB contacts who had refused isoniazid treatment. All contacts who subsequently developed active TB disease within the follow-up period were strong responders in the QFT assay, with IFN-γ plasma levels above the 10-IU/ml upper limit for the test’s enzyme-linked immunosorbent assay (ELISA) (6). These results suggest that a correlation exists between the amount of IFN-γ released upon *M. tuberculosis*-specific stimulation of CD4+ T cells and the probability of developing active TB disease.

A dynamic relationship between antigen load and distinct IFN-γ and interleukin-2 (IL-2) profiles of antigen-specific CD4+ T cells has been demonstrated in viral infections (10). Typically, antigen clearance is associated with IL-2-dominant functional T-cell signatures. In contrast, high antigen loads are associated with IFN-γ-dominant functional T-cell signatures (10). A similar relationship between the IFN-γ and IL-2 profiles of *M. tuberculosis*-specific T cells and antigenic load was reported in patients who were treated for active TB disease (1, 2, 8). Simultaneous measurement of IFN-γ and IL-2 secretion at the single-cell level revealed a codominance of CD4+ T cells that secrete only IFN-γ and those that secrete both IFN-γ and IL-2 in patients with active TB disease. A shift to dominance of CD4+ T cells secreting both IFN-γ and IL-2 and of newly detectable CD4+ T cells secreting only IL-2 has been demonstrated both during and following TB treatment. Thus, three main functional patterns were observed: a dominant IL-2 response, a multifunctional (IL-2 and IFN-γ) response, and a dominant IFN-γ response. Determination of the levels of IL-2 and IFN-γ secretion has been proposed as an adequate marker for clinical monitoring (10). The net result of functional T-cell signatures would be detectable by assessing the *M. tuberculosis*-specific IL-2/IFN-γ ratio in QFT test plasma supernatants. Thus, the simultaneous analysis of both IFN-γ and IL-2 in QFT test plasma supernatants might provide (i) a more precise basis for assessing an individual’s risk of developing active TB disease and (ii) information concerning the interpretation and clinical significance of weakly positive QFT test results (IFN-γ, \( \geq 0.35 \) to \(< 1.0\) IU/ml), which, in our program (11), make up almost 50% of all positive IGRA results. The QFT assay was performed according to the manufacturer’s instructions. HCW were stratified retrospectively into three groups on the basis of the QFT test results (IFN-γ in IU/ml): group A consisted of weak responders (\( \geq 0.35 \) to \(< 1.0\) \( n = 21 \)), group B consisted of intermediate responders (1.0 to 5.0) \( n = 32 \), and group C consisted of strong responders (\( > 5.0 \) \( n = 21 \)). In addition, QFT test plasma supernatants from patients with culture-confirmed active TB disease \( n = 10 \) were included as a control group. Concentrations of IFN-γ and IL-2 were assessed in duplicate by an ELISA (Mabtech AB, Germany) in QFT test plasma supernatants (kept at \(-20^\circ\)C) after *M. tuberculosis*-specific stimulation of the T cells. Values were log transformed, and groups were assessed for normal distribution (D’Agostino-Pearson omnibus normality test, \( P > 0.05 \)) and homogeneity of variance (Bartlett’s test for equal variances, \( P = 0.263 \)). Significant differences were determined by a one-sided analysis of variance (ANOVA) and the Tukey-Kramer posttest by using GraphPad Prism v.5.01.

In LTBI patients (groups A to C), the IL-2/IFN-γ ratios were inversely associated with the QFT test results (Fig. 1). The means of the IL-2/IFN-γ ratios were shown to differ significantly by a one-sided ANOVA \( (P < 0.001) \). The Tukey-Kramer posttest comparison of all pairs of columns showed significant differences between groups A and B \( (P < 0.01) \) and between groups A and C \( (P < 0.01) \) but not between groups B and C. Group C was most similar to the group of patients with active TB disease, which is associated with IFN-γ-dominant functional T-cell signatures (1, 2, 8). Overall, our results are compatible with a shift to dominance of both CD4+ T cells dually secreting IL-2 and IFN-γ and CD4+ T cells secreting only IL-2 as a function of decreasing QFT test results. This was

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pronounced in group A, i.e., the group of low responders in the QFT test.

The raw IFN-γ and IL-2 concentrations determined with the Mabtech ELISA were plotted against their respective QFT test results. This analysis revealed a positive correlation between both IFN-γ and IL-2 secretion and the respective QFT test results (Fig. 2) (Spearman’s rank correlation coefficient \( r \) is 0.92 for IFN-γ and 0.59 for IL-2). Only those values within the linear range of the QFT test were used for plotting. The analysis of cytokine secretion levels did not permit discrimination of active TB from LTBI.

These results suggest that QFT test results around the cutoff may indicate potential shifts of the functional T-cell signature toward an IL-2-dominant T-cell response. Distinct IFN-γ and IL-2 profiles of antigen-specific CD4+ T cells have recently been associated with different clinical disease states and antigen loads in viral infections (10) and TB (1, 2, 8). Thus, the current concept of LTBI, defined by the biomarker of antigen-specific T cells in the absence of clinical symptoms, may not faithfully represent the full diversity of immunological responses. Recent publications revealed a considerable heterogeneity of host immune responses to Mycobacterium tuberculosis infection (1, 2, 8), which is compatible with the existence of distinct stages of Mycobacterium tuberculosis infection (12).

In summary, the simultaneous analysis of IFN-γ and IL-2 in plasma supernatants permits the detection of shifts in M. tuberculosis-specific T-cell signatures. The IL-2/IFN-γ ratio of the QFT test’s low responders (IFN-γ <1.0 IU/ml) revealed a significant shift toward an IL-2-dominant functional T-cell response compared to those of the intermediate (IFN-γ <1.0 to 5.0 IU/ml) and high (IFN-γ >5.0 IU/ml) responders. This type of response has been shown to be associated with the elimination of M. tuberculosis infection (1, 2, 8) and suggests that such patients may not require prophylactic therapy. Preliminary results obtained from selected patients appear to confirm our findings at the single-cell level (data not shown). The clinical relevance of these findings needs to be further investigated by analyzing more samples and by prospectively following the patients over time.

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