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ORIGINAL INVESTIGATION

Treatment of latent tuberculosis infection induces changes in multifunctional *Mycobacterium tuberculosis*-specific CD4^{+} T cells

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Abstract To ascertain whether multiparametric flow cytometry assessment of multifunctional *Mycobacterium tuberculosis* (*Mtb*)-specific CD4^{+} and CD8^{+} T cells can distinguish between untreated and treated patients with latent tuberculosis infection (LTBI), we enrolled 14 LTBI subjects treated with isoniazid (INH) therapy, 16 untreated LTBI patients, and 25 healthy controls. The analysis of mono-functional CD4^{+} and CD8^{+} T cells producing single cytokines showed significant differences only between uninfected and infected LTBI subjects (both treated and untreated). Conversely, the analysis of multifunctional CD4^{+} T cells revealed a significant reduction in the frequency of two CD4^{+} T cells subsets, those producing IFN-γ, IL-2, and TNF-α simultaneously (triple positive; $p = 0.005$) and those producing IL-2 alone ($p = 0.0359$), as well as a shift towards T cells producing only one cytokine in treated as compared to untreated LTBI subjects. Assigning a triple-positive CD4^{+} T cells a cut-off >0.082 %, 94 % of untreated LTBI patients were scored as positive, as compared to only 28 % of treated LTBI patients and none of the healthy controls. No significant differences between untreated and treated LTBI subjects in terms of *Mtb*-specific CD8^{+} T cell cytokine profiles ($p > 0.05$) were identified. The significant changes in the cytokine profiles of *Mtb*-specific T cells after INH therapy suggest that analysis of multifunctional T cells may be a promising means for the monitoring of LTBI treatment success.

Keywords Multifunctional T cells · Cytokines · *M. tuberculosis* · Latent tuberculosis infection · TB treatment · Intracellular cytokine flow cytometry

Introduction

It is estimated that more than two billion people worldwide have latent *Mycobacterium tuberculosis* (*Mtb*) infection [1]. Since 5–10 % of these individuals will go on to develop active tuberculosis (TB) during their lifetime [2], the accurate diagnosis and treatment of the latent infection (LTBI) are essential components of TB control programmers [3].

However, there is currently no reliable test or surrogate marker that enables monitoring of patients' response to preventive treatment for LTBI. Although previous studies have suggested interferon gamma release assays (IGRAs) as a useful means of monitoring the response to anti-TB treatment, results remain controversial in both active and latent TB. Indeed, despite some authors having shown declining T cell responses during treatment using this method of assessment [4–6], in other studies IGRAs have revealed apparently unchanging, fluctuating, or increasing responses to treatment [7–9].

With a view of improving discrimination between active TB and LTBI, alternative immunological methods have also been investigated in recent years [10–16]. In particular, the determination of multifunctional *Mtb*-specific T cells secreting a range of cytokines (IFN-γ, IL-2, and TNF-α) by multiparameter intracellular cytokine flow cytometry (ICCFC) appears to show a better diagnostic yield than

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Table 1 Characteristics of the three groups of patients

Characteristics	Untreated ($n = 16$)	Treated ($n = 14$)	Healthy Controls ($n = 25$)
Sex (male/female)	6/10	8/6	13/12
Age (years; median/range)	39/31–48	46/36–67	48/32–69
Risk factors for LTBI (*n*/%)			
Birth in a TB endemic area	5 (31.2)	5 (35.7)	0
History of household contact	0	6 (42.8)	0
Chest X-ray suggestive of LTBI	3 (18.7)	3 (21.4)	0
Previous TB	0	0	0
BCG vaccinated (*n*/%)	0	0	0
QFT-GIT (*n*%)[a]			
Positive	16 (100)	11 (78.5)	0
Negative	0	3 (21.5)	25 (100)
Indeterminate	0	0	0
TST (*n*/%)[a]			
Positive	16 (100)	12 (82.7)	0
Negative	0	2 (14.3)	25 (100)

LTBI latent tuberculosis infection, *TB* tuberculosis, *BCG* Bacille Calmette-Guerin, *QFT-GIT* QuantiF-ERON-TB Gold In-Tube, *TST* tuberculin skin test

[a] The immunological tests were performed at the time of LTBI diagnosis

IGRA to detect TB infection [17–20]. Indeed, ICCFC has been reported to improve discrimination between active TB and LTBI [10–16] and to enable more reliable monitoring of responses to TB treatment [11–13, 21, 22].

To our knowledge, no study exploring the effect of LTBI treatment on multifunctional *Mtb*-specific T cell responses has yet been carried out. Here, we investigate whether multiparameter flow cytometry assessment of multiple cytokines secreted by *Mtb*-specific T cells could enable discrimination between untreated LTBI patients and those treated by chemoprophylaxis.

Materials and methods

Study populations

We retrospectively enrolled 55 adult subjects (27 men and 28 women, age range 31–69 years) at the Department of Public Health and Infectious Diseases, "Sapienza" University, Rome, Italy. Subjects were classified into the following 3 groups: (1) 14 subjects with LTBI who had received a 9-month course of isoniazid (INH) prophylaxis; (2) 16 subjects with LTBI in which INH prophylaxis had not been started yet; (3) 25 healthy subjects.

We considered affected by LTBI the subjects tested positive for either tuberculin skin test (TST) or QuantiFERON-TB Gold In-Tube (QFT-GIT). All subjects had also one of the following risk factors: chest radiograph results (apical pleural thickening, pulmonary nodules, upper lobe bronchiectasis, interstitial granulomatous calcification, and

lymph node or pericardial calcification), a history of exposure to a case of active TB, originating from an area with a high prevalence of TB infection. None of the individuals had clinical, radiological, and microbiological evidence of active TB, and none had received prior TB treatment for old TB. The healthy subjects were unexposed individuals with no previous history of TB, no known TB contact, and tested negative for TST and QFT-GIT. The treated LTBI subjects were examined 1 year after treatment completion. The demographics and clinical characteristics of the patients are shown in Table 1.

The study received approval from the local Ethics Committee of Azienda Policlinico Umberto I, Rome, Italy (Reference number 2669), and informed written consent was obtained from all individual participants included in the study.

QuantiFERON-TB Gold In-Tube (QFT-GIT)

The QFT-GIT (Cellestis Limited, Carnegie, Victoria, Australia) was carried out and interpreted by the same trained technicians according to the manufacturer's instructions. Analysis of data was carried out by the QuantiFERON®-TB Gold Analysis Software.

Intracellular cytokine flow cytometry (ICCFC)

For intracellular cytokine flow cytometry, heparinized peripheral blood was collected, and 0.5 ml of whole blood was added to three test tubes containing, respectively, saline (negative control), phytohaemagglutinin (PHA), and
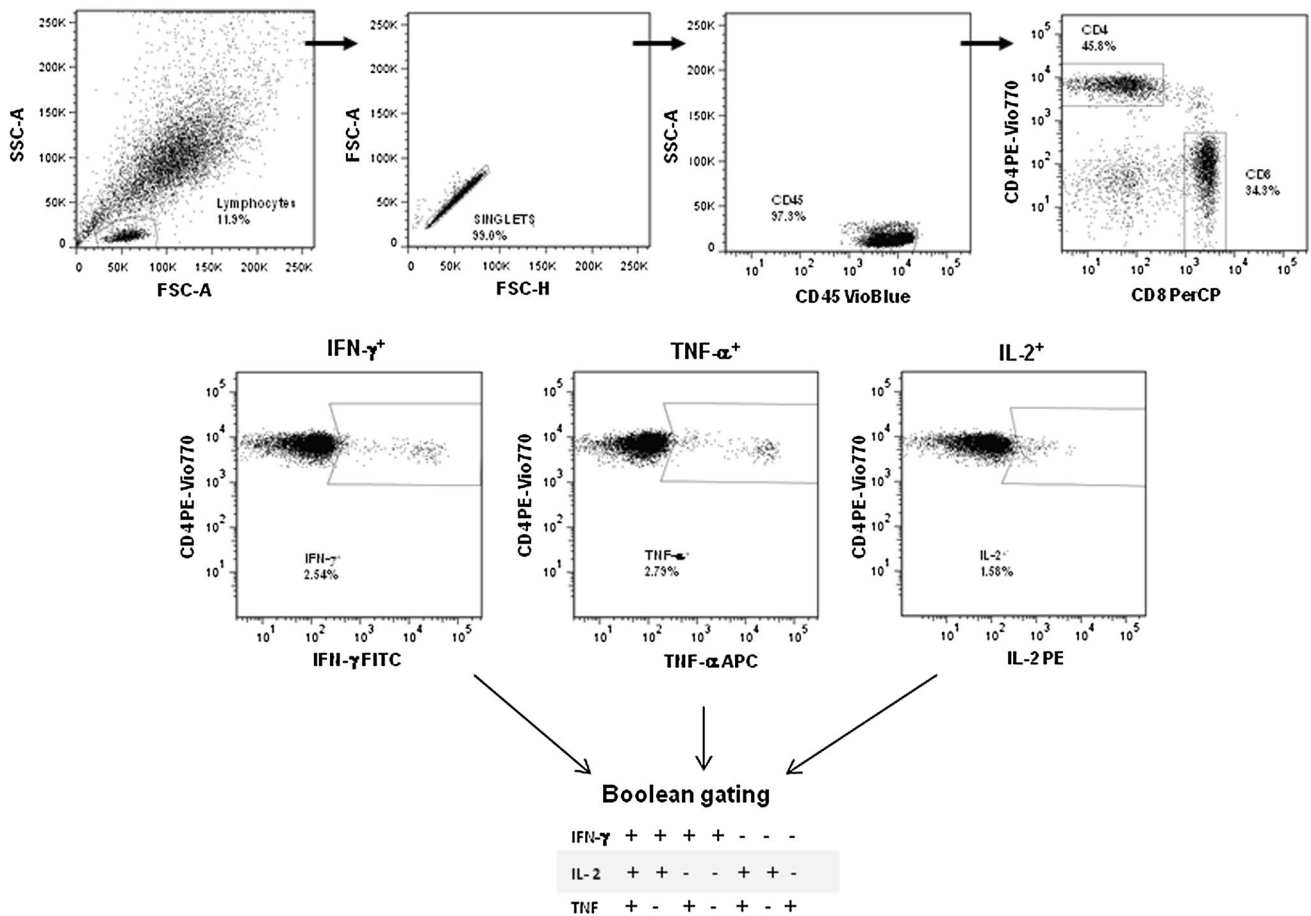
Fig. 1 Representative flow cytometry analysis showing the gating strategy for identification of multifunctional CD4$^+$ and CD8$^+$ T cells. Stimulated whole blood was analysed by flow cytometry for intracellular production of IFN-γ, IL-2, and TNF-α. CD4$^+$ and CD8$^+$ T cells were gated from single lymphocytes (FSC-H/FSC-A). Activated cells were gated individually for each T cell subsets and activation markers, and then Boolean gating combinations were computed

TB antigens (ESAT-6, CFP-10, and TB 7.7) [23]. The test tubes were supplied with the QFT-GIT. The TB antigens are pools of overlapping peptides and pooled together as a single stimulation condition. Whole blood was co-stimulated with anti-CD28 plus anti-CD49d (5 µl/ml, BD Bioscience, Pharmingen, Italy) as indicated by several authors [11, 12, 24], and Brefeldin A (10 µg/ml, Sigma-Aldrich) was immediately added to each tube, as previously described [16, 20].

In brief, after 18 h of incubation, the cell surface staining was performed with the following markers: anti-CD45-VioBlue, anti-CD4 PE-Vio770, and anti-CD8 PerCP (Miltenyi Biotec, Germany) and the red cells were lysed with 1 ml FACS Lysing Solution (BD Bioscience). Cells were then permeabilized with 0.5 ml FACS Permeabilizing Solution (BD Bioscience) and intracellularly stained with anti-IFN-γ FITC, anti-TNF-α APC, and anti-IL-2 PE (Miltenyi Biotec). Cells were fixed in 1 % paraformaldehyde and analysed within 1 h using a MACSQuant Analyzer flow cytometer (Miltenyi Biotec) after calibration and automatic compensation. We acquired at least 100,000 cells in the lymphocyte gate. FlowJo Software version 7.6.5 was used to perform Boolean gate analysis of the frequency of the different combinations of IFN-γ, IL-2, and TNF-α produced by CD4$^+$ and CD8$^+$ T cells. Background cytokine production in negative control was subtracted from each stimulated condition. Figure 1 shows the gating strategy used to identify multifunctional CD4$^+$ and CD8$^+$ T cells. Intraassay coefficient of variation and interassay coefficient variation were estimated and were <5 and <10 %, respectively.

We classed T cells producing any of the 3 cytokines (IFN-γ or IL-2 or TNF-α) as "responding T cells", those producing IFN-γ alone or in combination with IL-2 and/or TNF-α as "total IFN-γ$^+$ T cells", those producing IL-2 alone or in combination with IFN-γ and/or TNF-α as "total IL-2$^+$ T cells", and those producing TNF-α alone or in combination with IL-2 and/or IFN-γ as "total TNF-α$^+$ T

cells". Similarly, we classed triple-positive T cells (those simultaneously producing all three cytokines) as "IFN-γ^+ IL-2$^+$ TNF-α^+ T cells".

Statistical analysis

Nonparametric Kruskal–Wallis ANOVA with Dunn's post-test comparison and nonparametric Mann–Whitney test were applied to compare T cell frequencies and the percentage of cytokine-secreting cells between three and two groups of patients, respectively. Receiver operating characteristic (ROC) analysis was performed to calculate optimal cut-off values for triple-positive CD4$^+$ T cells. All statistical analyses were two-sided, performed using GraphPad Prism Software version 5 (Software MacKiev), and considered significant at p values <0.05.

Results and discussion

Mtb-specific responses in QFT-GIT

The QFT-GIT was positive in all 16 untreated LTBI subjects, in 11 of the 14 (78.5 %) subjects who had received a 9-month course of INH prophylaxis, and in none of the 25 healthy controls. No QFT-GIT assay yielded indeterminate results. Overall, the levels of IFN-γ were significantly higher in LTBI-infected patients (both untreated and treated LTBI subjects) than healthy controls ($p < 0.0001$ by Kruskal–Wallis test; Fig. 2). On the other hand, the IFN-γ response to *Mtb*-specific antigens was higher in untreated LTBI subjects (median 3.23 IU/ml, range 0.68–7.5), as compared to treated subjects (1.06 IU/ml, 0.12–6.36), but the difference was not statistically significant. Thus, the analysis of the QFT-GIT results showed that these are not sufficient to differentiate between untreated and treated LTBI subjects, but only between infected and uninfected subjects.

Mono-functional *Mtb*-specific CD4$^+$ and CD8$^+$ T cell responses

Multiparameter flow cytometry measurement of the frequency of responding T cells, those producing any of the three cytokines (IFN-γ, IL-2, or TNF-α), showed a higher frequency of CD4$^+$ T cells in response to *Mtb*-specific antigens in untreated LTBI subjects (median 1.301 %, range 0.23–5.76 %) than in treated subjects (0.81 %, 0–5.36 %) and healthy controls (0.05 %, 0–0.31 %), although these differences were only significant between healthy controls and infected subjects (both untreated and treated LTBI subjects; $p < 0.0001$ by Kruskal–Wallis test; Fig. 3a left panel). Similarly, the frequency of responding CD8$^+$ T cells was
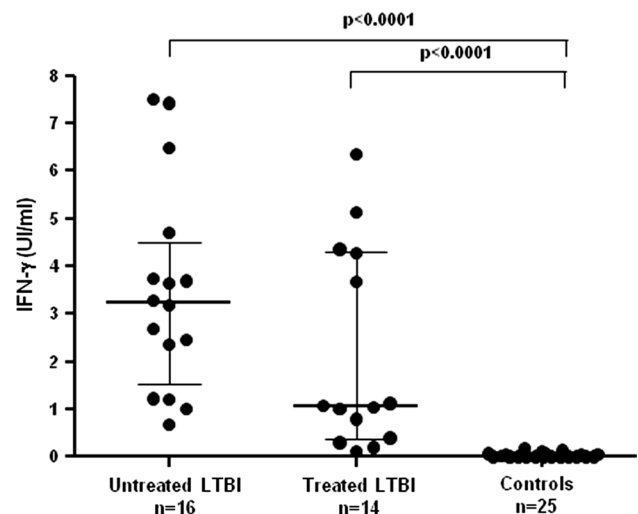

Fig. 2 Analysis of *Mtb*-specific responses in QFT-GIT. Individual IFN-γ response (UI/ml) to *Mtb*-specific antigens in 16 untreated LTBI subjects, in 14 LTBI subjects treated with isoniazid, and in 25 healthy controls. *Horizontal bars* represent the median values with interquartile range. Statistical analysis was performed using Kruskal–Wallis ANOVA with Dunn's post-test comparison, and significant p values are indicated in the figure

significantly higher in both untreated (median 0.39 %, range 0–1.79 %) and treated LTBI subjects (0.29 %, 0–4.21 %) with respect to healthy controls (0 %, 0–0.25 %, $p < 0.0001$ by Kruskal–Wallis test; Fig. 3a right panel). Comparing CD4$^+$ and CD8$^+$ T cell responses to *Mtb*-specific antigens in all groups of subjects, we observed a predominance of CD4$^+$ T cell responses, which were greater in untreated than in treated patients with LTBI (Fig. 3a).

The frequency of responding T cells in response to mitogen (PHA) was similar among untreated, treated, and healthy controls within both CD4$^+$ (median 19.03 %, 15.95 %, 15.5 %, respectively; $p > 0.05$ by Kruskal–Wallis) and CD8$^+$ T cell subsets (median 13.13, 14.77, 14.3.5 %, respectively; $p > 0.05$ by Kruskal–Wallis; Fig. 3b).

Assessing the cytokines production by T cell at the single level, we observed that the frequencies of "total IFN-γ^+", "total IL-2$^+$", and "total TNF-α^+" CD4$^+$ T cells (as defined in the "Materials and Methods" section) after *Mtb*-antigen stimulation were all higher in untreated LTBI subjects than in treated and healthy subjects. These differences were only statistically significant between healthy and infected subjects (both untreated and treated LTBI subjects; $p < 0.0001$ for IFN-γ; $p < 0.0001$ for IL-2; $p < 0.0001$ for TNF-α by Kruskal–Wallis test; Fig. 4a). There was also a slightly lower frequency of both "total IFN-γ^+ T cells" and "total IL-2$^+$ T cells" in treated, with respect to untreated patients, although this difference was not significant ($p > 0.05$ by Kruskal–Wallis test).
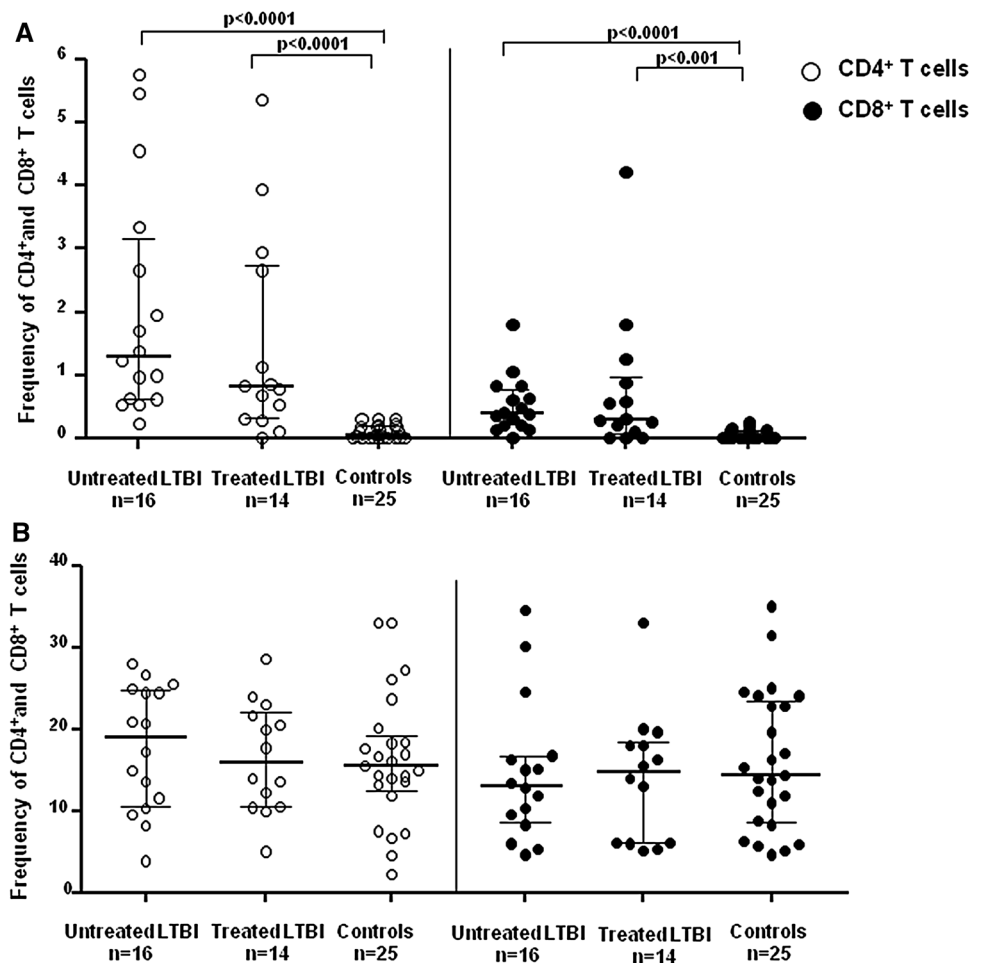
Fig. 3 Analysis of cytokine production by CD4^{+} and CD8^{+} T cells at the single-cell level. Frequencies of responding CD4^{+} T cells (*white circles*) and CD8^{+} T cells (*black circles*) producing any of the three cytokines (IFN-γ, IL-2, or TNF-α) in 16 untreated LTBI subjects, in 14 LTBI subjects treated with isoniazid, and in 25 healthy controls are shown in response **a** to *Mtb*-specific antigens and **b** to PHA. *Horizontal bars* represent the median values with interquartile range. Statistical analysis was performed using Kruskal–Wallis ANOVA with Dunn's post-test comparison

Analysis of CD8^{+} T cells showed no significant differences in the frequencies of "total IFN-γ^{+}", "total IL-2^{+}", and "total TNF-α^{+}" between untreated and treated LTBI subjects. However, differences between infected (LTBI treated and untreated) and healthy subjects were found to be significant ($p = 0.0008$ for IFN-γ; $p = 0.0004$ for IL-2; $p = 0.0002$ for TNF-α by Kruskal–Wallis test; Fig. 4b).

Comparing mono-functional CD4^{+} and CD8^{+} T cell responses to *Mtb*-specific antigens in all groups of subjects, we observed a predominance of CD4^{+} T cell responses, which were greater in untreated than in treated patients with LTBI and healthy subjects. Thus, the mono-functional analysis, within both CD4^{+} and CD8^{+} T cell subsets, allowed the discrimination only between *Mtb*-infected and uninfected patients, without a clear distinction between untreated and treated LTBI individuals. This response is quite similar to QFT-GIT results that were found to be positive in both groups, without a clear discrimination between LTBI-treated and LTBI-untreated subjects.

Multifunctional *Mtb*-specific CD4^{+} and CD8^{+} T cell responses

In order to detect a possible difference between treated and untreated LTBI subjects, in the second cytokine analysis, we performed a broad characterization of the functional profiles of *Mtb*-specific CD4^{+} and CD8^{+} T cells. A modest reduction in the frequency of all combinations of cytokine-producing CD4^{+} T cells was seen after INH therapy. However, we detect a significant reduction in the frequency of both "IFN-γ^{+} IL-2^{+} TNF-α^{+}" (triple positive) and "IL-2^{+}" in treated, as compared to untreated LTBI patients ($p = 0.005$ and 0.0359, respectively, by Mann–Whitney test; Fig. 5a). The frequency of other single- or double-cytokine-secreting CD4^{+} T cells did not differ significantly between these two groups.

The proportions of CD4^{+} T cell subsets producing 1, 2, or 3 cytokines in untreated and treated LTBI patients are

Fig. 4 Analysis of cytokine production by CD4$^+$ and CD8$^+$ T cells at the single-cell level. Frequency of "total IFN-γ$^+$", "total IL-2$^+$", "total TNF-α$^+$" *Mtb*-specific CD4$^+$ (**a**) and CD8$^+$ T cells (**b**) in untreated LTBI patients (*n* = 16, *white circles*), in treated LTBI patients (*n* = 14, *black circles*), and in healthy controls (*n* = 25, *grey circles*) are shown. *Horizontal bars* represent the median values with interquartile range. Statistical analysis was performed using Kruskal–Wallis ANOVA with Dunn's post-test comparison, and significant *p* values are indicated in the figure
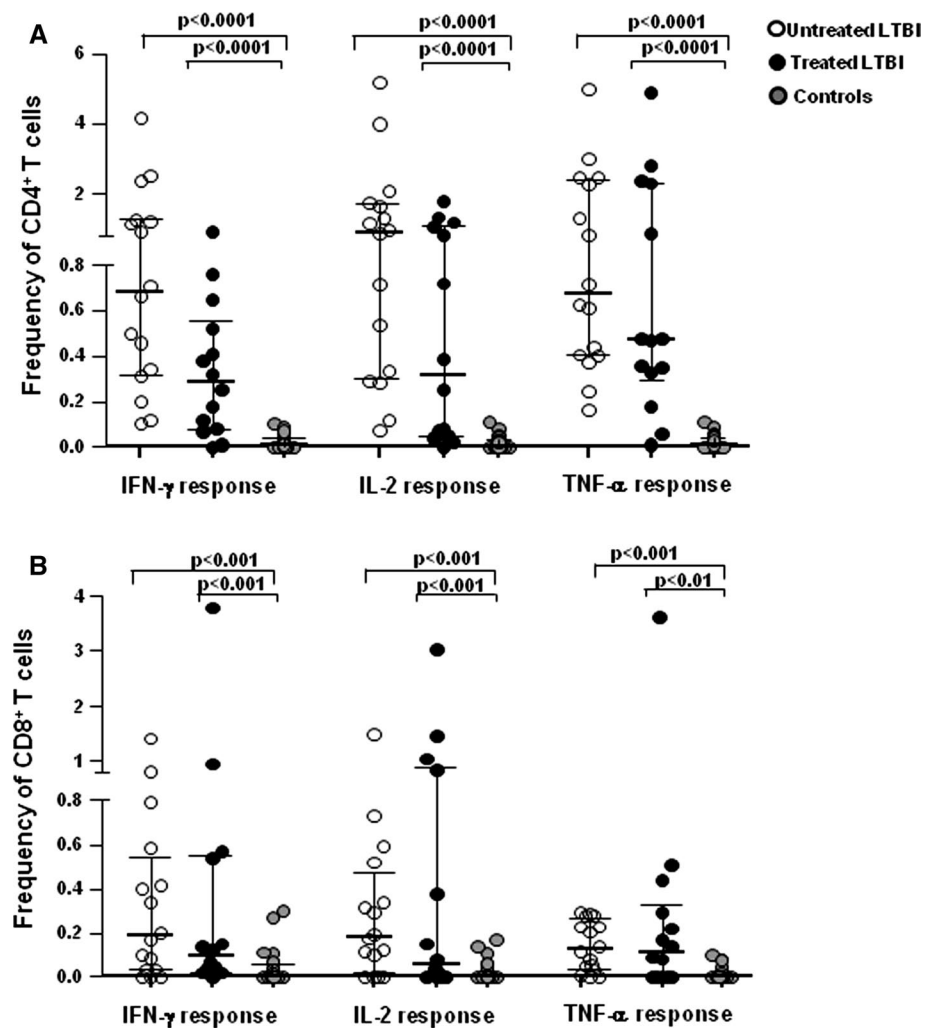
shown in Fig. 5b. Treated individuals showed a smaller proportion of CD4$^+$ T cells producing the 3 cytokines simultaneously—which constituted only 10 % of the total cytokine response—with respect to untreated patients 22 %. Furthermore, the treated patients also showed a greater proportion of single-cytokine-producing T cells (70 %), as compared to untreated individuals (64 %), and those secreting TNF-α alone constituted 49 % of the total cytokine response.

Taken together, these data indicate that INH therapy induces changes in the cytokine profiles of *Mtb*-specific CD4$^+$ T cells in LTBI patients. In particular, it induces a small shift towards T cells that produce only one cytokine, but a striking reduction in the frequency of CD4$^+$ T cells producing IFN-γ, IL-2, and TNF-α simultaneously and IL-2 alone. Indeed, ROC analysis of our findings revealed that a cut-off >0.082 % for triple-positive CD4$^+$ T cells was able to differentiate between untreated and treated LTBI subjects with a best combination of sensitivity (93.75 %, 95 % CI 69.7–99.8 %) and specificity (71.4 %,

95 % CI 41.9–91.6 %; AUC 0.8482, 95 % CI 0.7078–0.9887 %; *p* = 0.001; Fig. 5c). Using this cut-off to score ICCFC responses as either positive or negative, we observed a positive response (>0.082 %) in 15/16 (94 %) untreated LTBI patients, in 4/14 (28 %) of treated LTBI patients, and in none of the healthy controls (Fig. 5d).

Changes in the cytokine profiles of *Mtb*-specific T cells have previously been observed in subjects with active TB disease during and after treatment [11–13, 21, 22]. In particular, both Millington [21] and Caccamo [11] have shown a decline in the frequency of specific CD4$^+$ T cells accompanying the considerable reduction in the *Mtb* load induced by chemotherapy in active TB. Although there is a far smaller bacterial load in latent than in active infection, it seems likely that the decline in multifunctional *Mtb*-specific CD4$^+$ T cells observed in our treated subjects may reflect decreasing numbers of viable bacilli during chemoprophylaxis.

The negative ICCFC response observed in 10 out of 14 (71 %) treated patients after INH treatment could be
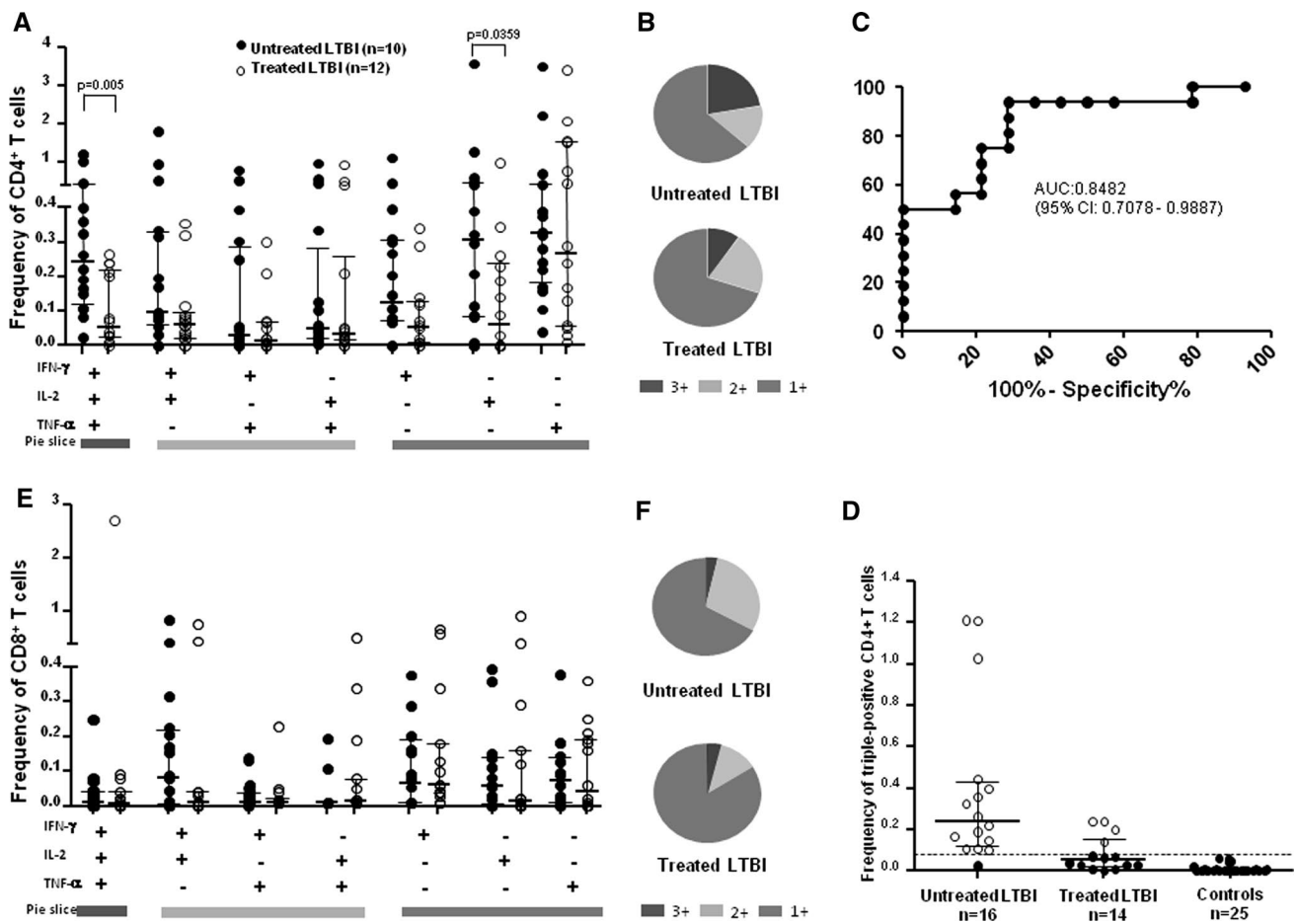
Fig. 5 Multifunctional cytokine analysis of *M. tuberculosis* (*Mtb*)-specific CD4$^+$ and CD8$^+$ T cells. **a**, **e** Frequency of *Mtb*-specific CD4$^+$ and CD8$^+$ T cells producing all combinations of IFN-γ, IL-2, and TNF-α in untreated LTBI patients (*n* = 16, *black circles*) and in LTBI subjects treated with isoniazid (*n* = 14, *white circles*). *Horizontal bars* represent the median values with interquartile range. Statistical analysis was performed using Mann–Whitney test, and significant *p* value is indicated in the figure. **b**, **f** *Pie charts* represent the relative proportions of cytokine-producing T cell subsets in each group after *Mtb*-specific stimulation, summarizing the fractions of single (1+), double (2+), and triple (3+) producers of IFN-γ, IL-2, and TNF-α.

A key to colours used in the pie charts is shown at the *bottom of the panel* **a** and **e**. **c** ROC curve (plotting sensitivity vs. 1-specificity) to discriminate untreated LTBI patients from treated. The area under curve (AUC) was 0.8482, 95 % CI 0.7078–0.9887 %; *p* = 0.001. **d** Analysis of triple-positive IFN-γ$^+$ IL-2$^+$ TNF-α$^+$ CD4$^+$ T cells, using a cut-off to score responses as either positive or negative. The subjects were considered as positive (*circles white*) if the frequency of CD4$^+$ T cells was >0.082 % and negative (*circles black*) if the frequency was <0.082 %. *Horizontal bars* represent the median values with interquartile range, and *horizontal dashed line* indicates the cut-off of 0.082 %

consistent with cessation of antigen stimulation in vivo, which in turn would imply the absence of viable bacilli. In fact, these treated patients showed a negative ICCFC response, like the healthy controls, suggesting that in these therapy was successful (Fig. 5d). The positive ICCFC responses observed in 4 treated LTBI patients may imply the persistence of viable bacilli, suggested also by the higher frequencies of *Mtb*-specific CD8$^+$ T cells observed in these patients. The positive ICCFC responses seen in these subjects may indicate that the preventive treatment had reduced rather than eliminating the bacterial load. Indeed, the frequencies of triple-positive CD4$^+$ T cells were lower in these than in untreated

subjects, in whom the presence of circulating effector T cells in the absence of treatment suggests persistent antigen exposure in vivo, consistent with ongoing latent infection.

Most of LTBI subjects (100 % of untreated and 78.5 % of treated) showed a positive QFT-GIT results, with a slightly but not significant decrease in the average IFN-γ responses after INH therapy. These QFT-GIT findings highlight the inability of IGRA assay to accurately differentiate between untreated and treated LTBI subjects.

Hence, analysis of multifunctional CD4$^+$ T cells, in particular those producing IFN-γ, IL-2, and TNF-α simultaneously, may allow the discrimination between our untreated

and treated LTBI subjects, hence a promising tool for monitoring the efficacy of therapy.

INH preventive therapy is increasingly recommended in an effort to control TB, although this strategy has some caveat. Expenses associated with buying and administering INH should be considered; in addition, some patients with unrecognized active TB or LTBI receive inadequate therapy or are over-treated with increasing risk of treatment failure or development of drug resistance. In a recent trial in South Africa [25], mass screening and treatment for LTBI had no significant effect on TB control, despite the successful use of INH in preventing TB during treatment. It is conceivable that a short course of therapy for LTBI outside of settings with heavy TB transmission and the definition of a tool to monitoring therapy follow-up will be required to improve TB prevention.

As regards the cytokine profiles of Mtb-specific CD8$^+$ T cells, we found no significant differences between untreated and treated LTBI subjects ($p > 0.05$ for each T cell subset, Fig. 5e). Still, it is interesting to note, as illustrated in the pie charts (Fig. 5f), that after INH therapy the subjects showed a smaller proportion of double-cytokine-producing CD8$^+$ T cells (12 %), as compared to untreated patients (29 %). Moreover, the Mtb-specific CD8$^+$ T cells in treated patients were predominantly single-cytokine secreting (84 %), and those secreting TNF-α alone constituted 40 % of the total cytokine response, following the same trend as in CD4$^+$ T cells.

However, Rozot [26] in a study with a large number of individuals recently indicated that Mtb-specific CD8$^+$ T cell responses against ESAT-6 or CFP-10 can be detected predominantly in patients with active TB as compared to LTBI subjects, and this is consistent with the current paradigm associating CD8$^+$ T cell responses to high antigen burden [23, 24, 27, 28]. In addition, Rozot [26] found only a slight difference in the cytokine profile of Mtb-specific CD8$^+$ T cells between TB and LTBI subjects. This could explain the lack of significant differences between treated and untreated LTBI subjects in terms of Mtb-specific CD8$^+$ T cell responses in both our mono-functional (Fig. 3a–c) and multifunctional analyses (Fig. 5e). In contrast, a slight reduction in Mtb-specific CD4$^+$ T cell responses was consistently observed in patients who had received therapy (Figs. 3a, 4a, 5a).

Our study does have some limitations. First and foremost, we analysed a relatively small number of patients within each clinical group, even if the number of subjects enrolled is comparable to those described in similar reports [10, 16, 20, 29]. Secondly, this is a cross-sectional study, and determining the clinical utility of cytokine profiles of Mtb-specific T cells for treatment monitoring will require a longitudinal study.

Conclusions

There are as yet no radiological, microbiological, or clinical parameters that enable us to assess the response to treatment in LTBI, and current immunological assays, such as IGRA, which solely quantify IFN-γ secretion by T cells, may not be a sufficiently reliable biomarker of antigen load and clinical LTBI *status* for monitoring purposes. ICCFC analysis reveals a general decrease in all CD4$^+$ T cell populations after INH therapy. Considering only the mono-functional CD4$^+$ and CD8$^+$ T cell responses, we are able to distinguish only Mtb-infected subjects from uninfected subjects. On the other hand, the evaluation of multifunctional CD4$^+$ T cells response, based on all possible combinations of intracellular expression of IFN-γ, IL-2, and TNF-a, did reveal a significant reduction in the triple-positive subset, as well as a shift towards T cells producing only one cytokine, in response to INH therapy. This change in cytokine profile enabled good distinction between our untreated and treated LTBI patients, suggesting that both ICCFC and multifunctional T cells should be further investigated for their usefulness in monitoring response to treatment in LTBI. Given the need to not over-treat LTBI patients with non-efficient therapies that would involve the increase in drug-resistant Mtb strains, there is a real need to define a monitoring tool for therapy follow-up.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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