Journal of Infection (2016) 73, 588–597

BIAM British Infection Association

www.elsevierhealth.com/journals/jinf

First characterization of the CD4 and CD8 T-cell responses to QuantiFERON-TB Plus

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Accepted 25 September 2016
Available online 4 October 2016

KEYWORDS
QuantiFERON-TB Plus;
CD8;
CD4;
Tuberculosis;
LTBI;
Active TB

Summary *Introduction:* QuantiFERON®-TB Gold Plus (QFT-Plus) is the new generation of QuantiFERON-TB Gold In-Tube test to identify latent tuberculosis infection (LTBI). QFT-Plus includes TB1 and TB2 tubes which contain selected *Mycobacterium tuberculosis* (Mtb) peptides designed to stimulate both CD4 and CD8 T-cells. Aim of this study is the flow cytometric characterization of the specific CD4 and CD8 T-cell responses to Mtb antigens contained within QFT-Plus.
Methods: We enrolled 27 active tuberculosis (TB) patients and 30 LTBI individuals. Following stimulation with TB1 and TB2, antigen-specific T-cells were characterized by flow cytometry. Data were also correlated with the grade of TB severity.
Results: TB1 mainly elicited a CD4 T-cell response while TB2 induced both CD4 and CD8 responses. Moreover, the TB2-specific CD4 response was detected for both active TB and LTBI patients, whereas the TB2-specific CD8 response was primarily associated with active TB ($p = 0.01$).

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http://dx.doi.org/10.1016/j.jinf.2016.09.008

Conclusions: To our knowledge, we report the first characterization of the CD4 and CD8 T-cell response to QFT-Plus. CD8 T-cell response is mainly due to TB2 stimulation which is largely associated to active TB. These results provide a better knowledge on the use of this assay.

Introduction

Tuberculosis (TB), being responsible for 9.6 million cases and 1.5 million deaths annually, represents a major public health problem.[1] Moreover, latent TB infection (LTBI), which is estimated to affect one-third of the world's population, may progress to active disease in about 3—15% of the LTBI individuals during their lifetimes.[2,3] Considering that LTBI subjects are the seedbed of TB disease, diagnosing and treating LTBI is one of the main goals to control the TB epidemic.[4—7] Tuberculin skin test (TST) and T-cell interferon-γ release assays (IGRAs) are the routine diagnostic tools to identify LTBI. Two IGRAs are commercially available: the QuantiFERON®-TB Gold In-Tube (QFT-GIT) (Qiagen, Hilden, Germany) and the T-SPOT.TB (Oxford Immunotec, Abingdon, UK). IGRAs have several advantages: the results are not affected by Bacille Calmette—Guérin (BCG)-vaccination[4—7] and by the majority of environmental mycobacteria; moreover, only one patient-visit is required. However, since these assays are based on an immune response detection, they have a poor sensitivity in children and in immune-compromised subjects[7—9] furthermore, they do not discriminate between active TB and LTBI[5] and poorly correlate with the risk of developing active disease.[3,10,11]

In the last few years several studies have described the role of CD8 T-cell responses in TB. Mtb-specific CD8 T cells have been associated with active TB, both in HIV-uninfected and infected patients,[12—15] and to recent infections, in adults and young children recently exposed to a smear-positive active TB case.[16,17] An increase of the CD8 T-cell responses associates with Mtb load, as found for both humans and animal models.[12,18—20] Importantly, longitudinal studies have shown a decrease of the CD8 T-cell response during anti-TB treatment.[12,13,21]

Interestingly, a study on QFT-GIT performance has shown that the addition of peptides for eliciting CD8 T-cell responses to QFT-GIT tubes increases the sensitivity of the test for LTBI detection.[22] On the base of this evidence, recently QuantiFERON®-TB Gold Plus (QFT-Plus),[23—28] has been proposed as a new generation of QFT-GIT. QFT-Plus includes two tubes, called TB1 and TB2 respectively, with *Mycobacterium tuberculosis* (Mtb) antigens to elicit a specific immune response. The TB1 tube, contains peptides derived from ESAT-6 and CFP-10 (TB-7.7, present in QFT-GIT, has been removed), and it is designed to induce a specific CD4 T cells response. TB2 contains newly designed peptides stimulating interferon (IFN)-γ production by both CD4 and CD8 T cells.[29] However, as previously described, due to the lack of a gold standard for LTBI detection, active TB cases are used as surrogate reference standard for evaluating test accuracy.[5]

At present, the specific response to QFT-Plus TB1 and TB2 tubes has not been characterized. Therefore, the main aim of this study is to evaluate by flow cytometry the specific CD4 and CD8 T-cell responses to the Mtb antigens contained within the QFT-Plus test in patients with active TB and LTBI.

Materials and methods

Population characteristics

This study was approved by the Ethical Committee of "L. Spallanzani" National Institute of Infectious Diseases (INMI), approval number 72/2015. Written informed consent was required to participate in the study that was conducted at INMI. We prospectively enrolled HIV-uninfected patients with pulmonary active TB and LTBI. Active TB microbiologically diagnosed was defined based on the Mtb isolation from sputum culture. Active TB clinically diagnosed was defined based on the clinical and radiological lung lesions associated with TB in the absence of Mtb isolated in the sputum that completely recovered after TB-specific treatment for 6 months. Microbiological TB was characterized by first line Mtb drug-sensitive isolates. Patients were enrolled within 7 days of starting the specific treatment.

In the absence of clinical, microbiological and radiological signs of active TB, LTBI was defined based on a positive score to QFT-GIT (Qiagen, Hilden, Germany). The LTBI group included subjects with a remote infection (reported contact with a smear-positive pulmonary TB patient at least 3 years before the enrollment) and subjects reporting a recent contact (no more than 3 months), LTBI subjects reporting the time of exposure between "more than 3 months" and "3 years" were not enrolled.[30,31] None of the subjects enrolled had previously undergone treatment with immunosuppressive drugs. Demographic and epidemiological information were collected at enrollment and are reported in Table 1.

Chest X-ray evaluation

All chest X-rays (rendered anonymous) were evaluated for the presence of nodules, fibrosis, infiltrates, cavitation, bronchial spread, miliarity, pleural effusion and adenopathy, as previously reported.[32] Cavity size in centimeters was recorded ($<$4 cm, $\geq$4 cm). The proportion of the affected lung was analyzed by a visual estimate of the extent of parenchymal infiltrates; a proportion of 30% of affected lung was used as our internal cut-off value to grade TB severity. In agreement with literature data[33] and on the basis of experience, the disease was graded (by DG, FP, RU) using a sliding scale of severity as follows: 0: normal chest X-rays; 1: mild grade (nodules and or infiltrates with proportion of lung affected $<$30%); 2: intermediate grade (infiltrates with proportion of lung affected $>$30% and/or cavitation $<$4 cm in diameter); 3: high grade

Table 1 Demographic characteristics of enrolled patients.

	Active TB	Remote LTBI	Recent LTBI	Total
N (%)	27 (47)	18 (32)	12 (21)	57 (100)
Age Median (IQR)	38 (28–44)	41 (35.7–60.7)	47 (28.5–60.5)	40 (32–51)
Sex				
Female N (%)	13 (48)	11 (61)	8 (67)	32 (56)
Origin				
West Europe (%)	10 (37)	11 (61)	9 (75)	30 (53)
East Europe (%)	10 (37)	6 (33)	2 (17)	18 (31)
Asia (%)	4 (15)	0 (0)	0 (0)	4 (7)
Africa (%)	2 (7)	1 (6)	1 (8)	4 (7)
South America (%)	1 (4)			
BCG				
Vaccinated (%)	17 (63)	7 (39)	3 (25)	27 (47)

TB: tuberculosis; LTBI: latent tuberculosis infection; BCG: bacillus Calmette–Guérin, IQR: interquartile range.

(an infiltrate of any percentage of extension with cavitation >4 cm in diameter and/or bronchial spread and/or miliarity and/or pleural effusion and/or adenopathy). All subjects underwent standard chest X-rays at the time of TB diagnosis.

QFT-GIT and QFT-Plus

QFT-GIT and QFT-Plus assay was performed for each patient. QFT-Plus kits were donated by Qiagen and used according to manufacturer's instructions.[29]

Levels of IFN-γ were quantified by ELISA and the QFT-Plus Analysis Software (available from www.quantiFERON.com) was used to analyze raw data and to calculate the results in international units per milliliter (IU/ml). The software performs a quality control assessment of the assay, generates a standard curve and provides a test result for each subject. Test results were interpreted according to manufacturer's criteria.[29]

Intracellular staining assay

Intracellular staining (ICS) was performed, concomitantly to QFT Plus, for each patient. Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll density gradient centrifugation and resuspended in complete RPMI-16-40 medium (Gibco, CA, USA) with 10% fetal bovine serum (PAA Laboratories GmbH, Pasching, Austria). To characterize by flow cytometry the Mtb-specific T-cell response, 1×10^6 PBMC resuspended in 1 ml of medium, were dispensed in TB1, TB2, Mitogen and Nil tubes of QFT-Plus kit. After a 1-h incubation, PBMC were transferred in polystyrene round-bottom tubes, 1 μl/ml of Golgi plug (BD Biosciences San Josè , USA) was added to inhibit cytokine secretion and anti-CD28 and anti-CD49d monoclonal antibodies (mAb) at 2 μg/ml each, were added to costimulate cells.

Following an incubation of 16–24 h, the ICS was performed. As previously described,[14,34–36] PBMC were stained with anti-CD4 peridinin chlorophylprotein (PerCp)-Cy5.5 conjugate, anti-CD8 allophycocyanin (APC)-H7 conjugate, anti-CD3 Horizon V500 conjugate and anti-IFN-γ Pacific Blue (PB) conjugate (all from BD Biosciences). The Mtb-specific T-cell response was characterized evaluating the frequencies of IFN-γ CD4 and IFN-γ CD8 T cells (Fig. 1). At least 200,000 lymphocytes were acquired with a FACS CANTO II (BD, Biosciences). Cytometry data were analyzed using FloJo software. Background cytokine production in the Nil tube was subtracted from each stimulated condition. If the background was higher than half of the antigen-specific response, the results were scored as negative. A frequency of IFN-γ-producing T cells of at least 0.03% was considered as positive response.

Statistical analysis

Data were analyzed using SPSS software (Version 19 FOR Windows, Italy SRL, Bologna, Italy). The median and interquartile ranges (IQRs) were calculated for continuous measures. The Chi-Square test was used for proportions. The Kruskal–Wallis test was used for comparison among several groups and the Mann–Whitney U test was used for pairwise comparison. The Spearman rank correlation was used to correlate continuous variables; $r_s \geq 0.7$ was considered a high correlation, $0.7 < r_s > 0.5$ was considered a moderate correlation and $r_s \leq 0.5$ was considered a low correlation. The Fisher exact test was used for categorical variables.

Results

Features of the population

A total of 57 participants were enrolled: 27 with active pulmonary TB (23 microbiologically diagnosed and 4 clinically diagnosed) and 30 with LTBI (18 remote LTBI and 12 recent LTBI). Fifty-five percent of the enrolled subjects were from Western Europe; no significant differences were found for sex, age, origin and BCG vaccination among the different groups (Table 1).

QFT-Plus evaluation among active TB and LTBI subjects

QFT-GIT and QFT-Plus assay were performed for each patient (Fig. 2 and Table 2). To evaluate the sensitivity of

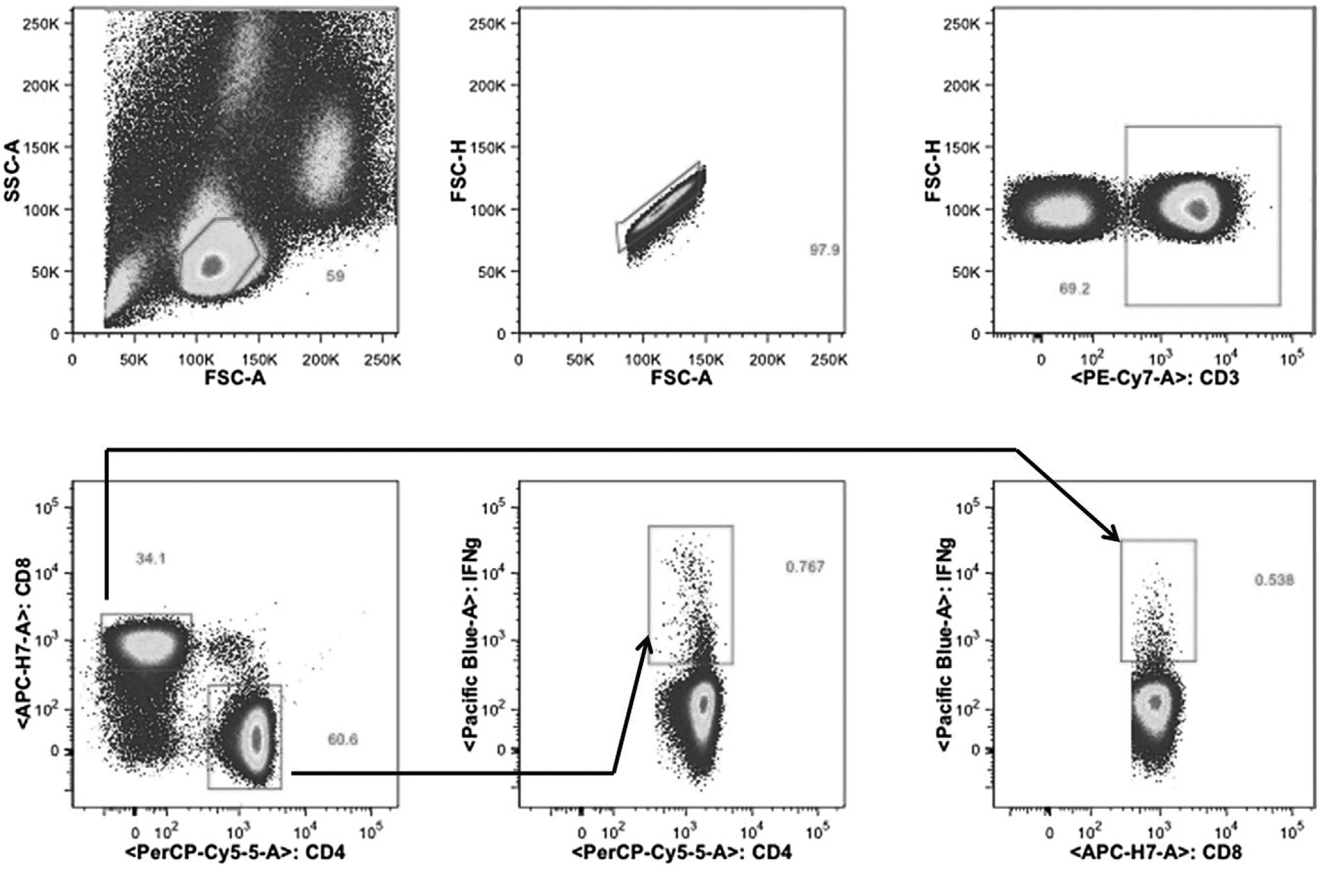

Figure 1 Representative dot plots of the TB2-induced T-cell response of an active TB patient. PBMC were stimulated overnight with TB1 and TB2 antigens and analyzed by flow cytometry for the intracellular production of IFN-γ. The frequency of Mtb-specific T cells was calculated from the proportions of CD4 IFN-γ T cells and CD8 IFN-γ T cells.

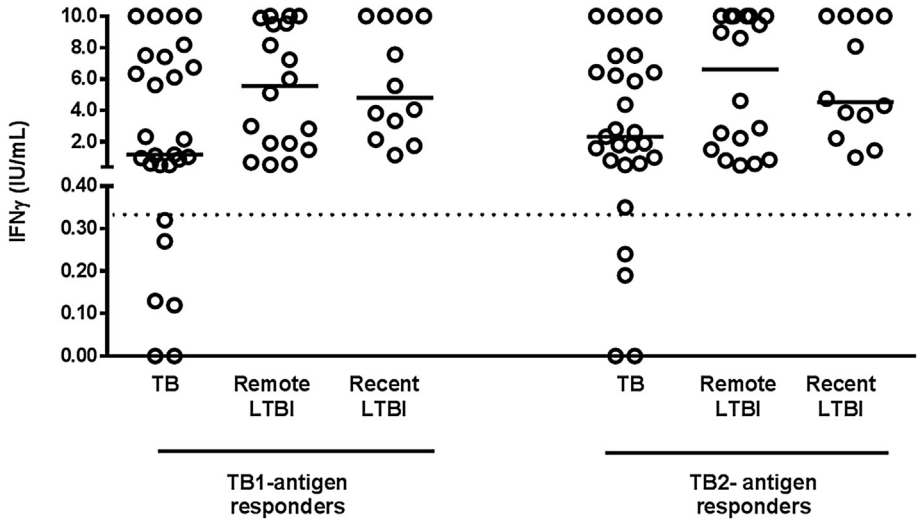

Figure 2 Evaluation of IFN-γ production using QFT-Plus kit. The IFN-γ T-cell response was evaluated in active TB patients and in LTBI subjects with recent and remote infection, one day after whole blood incubation in TB1 and TB2 tubes of the QFT-Plus kit. Horizontal lines indicate the median. The dotted line represents the cut-off value of 0.35 IU/ml. TB: tuberculosis, LTBI: latent tuberculosis infection; IU: international unit; IFN: interferon; QFT: QuantiFERON.

Table 2 Mtb specific response to TB1 and TB2 stimulation in the different groups.

		Antigen response to	N (%) of responders			
			Active TB 27 (47)	Remote LTBI 18 (32)	Recent LTBI 12 (21)	Total 57 (100)
QFT-GIT		TB antigen	24 (89)	18 (100)	12 (100)	54 (95)
QFT-Plus		TB1	21 (78)	18 (100)	12 (100)	51 (89)
		TB2	23 (85)	18 (100)	12 (100)	53 (93)
		Concomitant TB1 and TB2	21 (78)	18 (100)	12 (100)	51 (89)
		TB1 only	0 (0)	0 (0)	0 (0)	0 (0)
		TB2 only	2 (7)	0 (0)	0 (0)	2 (4)
		TB1 or TB2	23 (85)	18 (100)	12 (100)	53 (93)
ICS	CD4	TB1	22 (81)	17 (94)	12 (100)	51 (89)
		TB2	24 (89)	15 (83)	11 (92)	50 (88)
		Concomitant TB1 and TB2	22 (82)	15 (83)	11 (92)	48 (84)
		TB1 only	0 (0)	2 (11)	1 (8)	3 (5)
		TB2 only	2 (7)	0 (0)	0 (0)	2 (4)
	CD8	TB1	4 (15)	3 (18)	4 (33)	11 (19)
		TB2	12 (44)	3 (18)	3 (25)	18 (32)
		Concomitant TB1 and TB2	4 (15)	3 (18)	3 (25)	10 (18)
		TB1 only	0 (0)	0 (0)	1 (8)	1 (2)
		TB2 only	8 (30)	0 (0)	0 (0)	8 (14)

Mtb: *Mycobacterium tuberculosis*; QFT: QuantiFERON; TB: tuberculosis; LTBI: latent tuberculosis infection; ICS: intracellular staining N: number.

the test we used as a surrogate for Mtb infection, the data generated on active TB patients. Therefore the sensibility of the tests for Mtb infection in the active TB group was similar: 89% for QFT-GIT and 85% for QFT-Plus. Among the active TB patients, the proportion of TB1-responders was 78% whereas for TB2 it was 85% (Table 2). For LTBI subjects, the proportion of QFT-Plus responders to TB1 and TB2 was 100%, both for recent and remote infection (Table 2). No significant differences were found comparing the IFN-γ production to TB1 or TB2 among groups (Fig. 2). All samples scored positive to the mitogen stimulation.

Characterization of the CD4 and CD8 T-cell responses: evaluation of the proportion of responders to QFT-Plus by flow cytometry

Our first goal was to compare the CD4 and CD8 T-cell responses elicited by the same antigen stimulation (Fig. 3). To assess the specificity of the ICS assay, 10 healthy donors not exposed to Mtb and scored negative to QFT-Plus, were enrolled. We found that the specificity of the ICS assay to detect a TB infection (latent or active TB status) was 100% (data not shown).

Among those with active disease, TB1 induced a CD4-specific T-cell response in 81% of subjects and a CD8-specific response in only 15% of them. On the other hand, TB2 induced a CD4-specific T-cell response in 89% of subjects and a CD8-specific response in 44% of those (Fig. 3, Table 2).

Among the remote LTBI subjects, TB1 induced a CD4-specific T-cell response in 94% and a CD8-specific T cell response in only 18% of them (Table 2). The TB2 induced a CD4-specific T-cell response in 83% of the sample evaluated and a CD8-specific T cell response in 18% of them (Table 2).

Lastly, for the recent LTBI subjects, TB1 induced a CD4-specific T-cell response in 100% and a CD8 T-cell response in 33%. After TB2 stimulation we found a CD4-specific T-cell response in 92% and a CD8 T-cell response in 25% for both, CD4 and CD8 T-cell subsets (Table 2). Interestingly a selected CD8 response to TB2 stimulation and not to TB1 was found only in patients with active TB (8 of them) (Table 2).

These data indicate that TB1 stimulation induces mainly a CD4 T-cell response in all studied groups, while TB2 stimulation elicits a CD4 response in all groups and a selective CD8 T-cell response in subjects with active Mtb replication, either with active disease or with a recent Mtb exposure.

Evaluation of CD4 and CD8 T-cell frequencies to TB1 and TB2 by flow cytometry

To better characterize the CD8-specific T cells, we evaluated the frequency of the responses to TB1 and TB2. Among active TB patients, the CD8 response to TB2 was significantly higher than that one generated by TB1 ($p = 0.01$; Fig. 3B). Moreover, the active TB group has a frequency of CD8 response to TB2 stimulation significantly higher compared to that one elicited in the remote LTBI subjects ($p = 0.04$). These data demonstrate that the Mtb-specific CD8 T-cell response associates with TB2 stimulation in active TB patients.

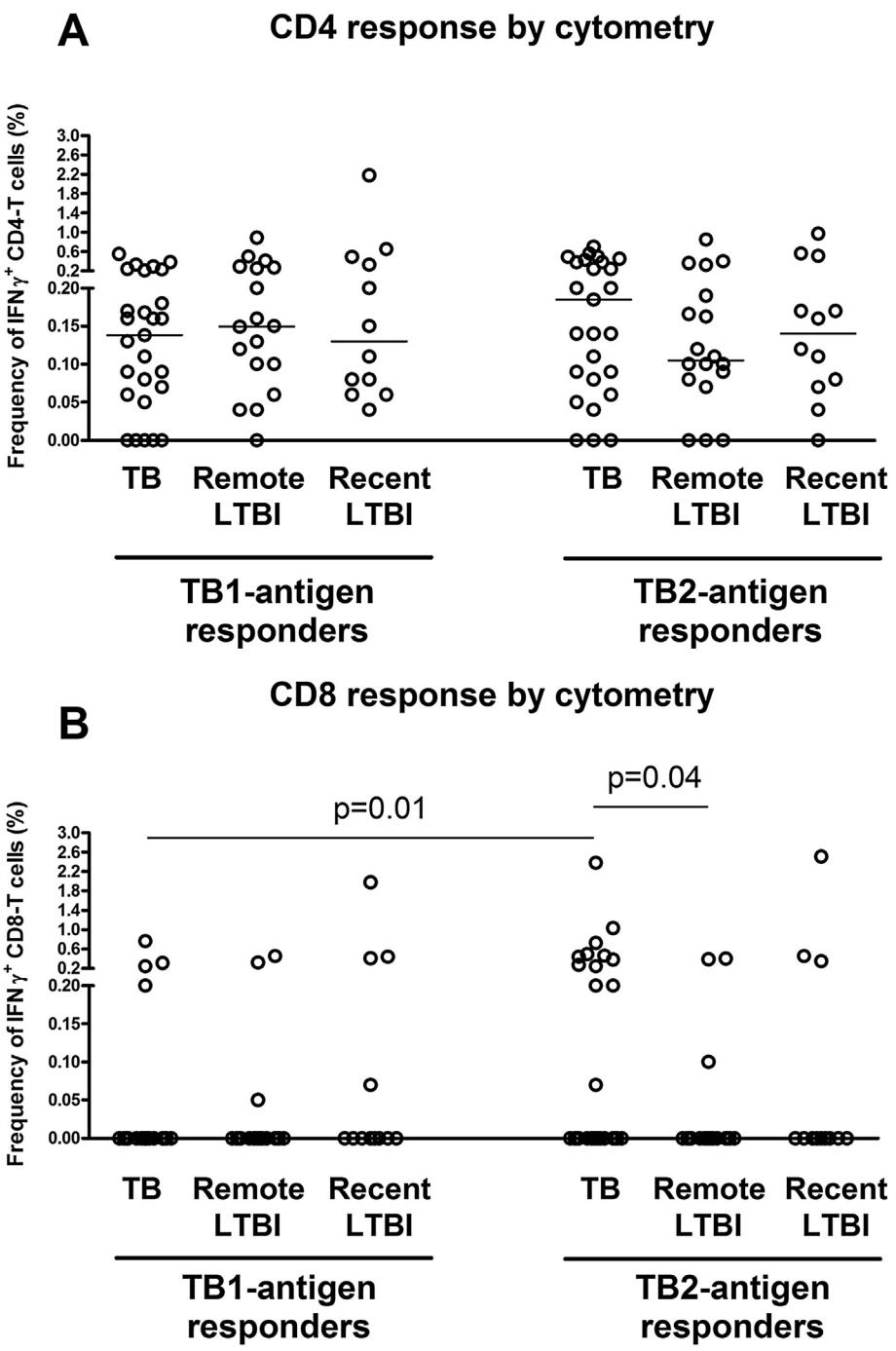

Figure 3 TB2 stimulation induces a CD8 T-cell response in active TB patients. Flow cytometric evaluation of CD4 (A) and CD8 (B) T-cell to TB1 and TB2 antigens in active TB patients and LTBI subjects with recent and remote infection. The response was scored positive if the frequency of IFN-γ producing T cells was at least 0.03%. The horizontal lines represent the medians. Statistical analysis was performed using the Mann—Whitney U test and the p value was considered significant if $\leq$0.05. TB: tuberculosis, LTBI: latent tuberculosis infection.

Then we compared the frequency of antigen-specific CD4 and CD8 T cells in response to the same stimulation. In LTBI subjects, a significantly higher frequency of the CD4 response to TB1 ($p < 0.0001$) and TB2 ($p = 0.0005$) was found in comparison to that one generated by CD8 T cells (Table 3). These data indicate that TB2 stimulation seldom induces a CD8 response in remote LTBI subjects.

Active TB patients showed a significantly higher frequency ($p < 0.0001$) of TB1-induced CD4 T cells compared to the levels found for the CD8 T cells. Since TB2 stimulation induced both a CD4 and a CD8 T-cell response we did not find significant differences comparing the frequency of the two antigen specific T-cell subsets. Interestingly, in active TB patients we found a positive

Table 3 Comparison of the frequencies of the CD4 and CD8 T-cell response to TB1 and TB2 in the different groups.

	Frequency of the CD4 vs CD8 T-cell response					
	TB1			TB2		
	Active TB p^*	Remote LTBI p^*	Recent LTBI p^*	Active TB p^*	Remote LTBI p^*	Recent LTBI p^*
Active TB	**<0.0001**	–	–	0.1	–	–
Remote LTBI	–	**<0.0001**	–	–	**0.0005**	–
Recent LTBI	–	–	**0.02**	–	–	**0.02**

Mtb: *Mycobacterium tuberculosis*; TB: tuberculosis; LTBI: latent tuberculosis infection; p^* = significance according to the Mann–Whitney U test.
Significant differences are indicated in bold.

and significant correlation between the frequencies of CD4 T cells and that one of the CD8 T cells in response to TB2 stimulation ($r_s = 0.76$, $p < 0.0001$). These results highlight the capacity of the cells from active TB patients to respond with both CD4 and CD8 T-cell subsets to TB2 stimulation.

Impact of the severity of TB disease and mycobacterial load on QFT-Plus results

Finally, we evaluated whether the severity of TB disease may influence the response to QFT-Plus. Among the parameters used to estimate the clinical severity we used the evaluation of the lung lesions based on the radiology findings. To better analyze the results, the data from patients with intermediate/high severity (grades 2 and 3) were combined and compared to those from patients with low severity (grade 1). As shown in Table 4 the patients with intermediate/high severity TB had a similar proportion of responders (84%) to QFT-Plus assay compared to patients with low TB severity (88%). Analyzing the specific immune results, CD8 T-cell response to TB2 was found in 53% (10 out 19) of patients with intermediate/high severity TB and only in 25% (2 out 8) of the low severity TB group (Table 4, Fig. 4). Moreover stratifying the active TB patients

Table 4 Response to TB1 and TB2 stimulation according to the severity of active TB and the positivity of the mycobateria culture.

		Antigen response to	Active TB N (%) of responders			
			Radiological classification		Microbiological classification	
			Low severity TB 8 (30)	Intermediate high severity TB 19 (70)	Microbiological confirmed TB 23 (85)	Clinical TB 4 (15)
QFT-GIT		TB antigen	8 (100)	16 (84)	20 (87)	4 (100)
QFT-Plus		TB1	7 (88)	14 (74)	18 (78)	3 (75)
		TB2	7 (88)	16 (84)	20 (87)	3 (75)
		Concomitant TB1 and TB2	7 (88)	14 (74)	18 (78)	3 (75)
		TB1 only	0 (0)	0 (0)	0 (0)	0 (0)
		TB2 only	0 (0)	2 (11)	2 (9)	0 (0)
		TB1 or TB2	7 (88)	16 (84)	20 (87)	3 (75)
ICS	CD4	TB1	7 (88)	15 (79)	19 (83)	3 (75)
		TB2	7 (88)	17 (89)	21 (91)	3 (75)
		Concomitant TB1 and TB2	7 (88)	15 (79)	19 (83)	3 (75)
		TB1 only	0 (0)	0 (0)	0 (0)	0 (0)
		TB2 only	0 (0)	2 (11)	2 (9)	0 (0)
	CD8	TB1	2 (25)	2 (11)	3 (13)	1 (25)
		TB2	2 (25)	10 (53)	11 (48)	1 (25)
		Concomitant TB1 and TB2	2 (25)	2 (11)	3 (13)	1 (25)
		TB1 only	0 (0)	0 (0)	0 (0)	0 (0)
		TB2 only	0 (0)	8 (42)	8 (35)	0 (0)

Mtb: *Mycobacterium tuberculosis*; QFT: QuantiFERON; TB: tuberculosis; LTBI: latent tuberculosis infection; ICS: intracellular staining, N: number.

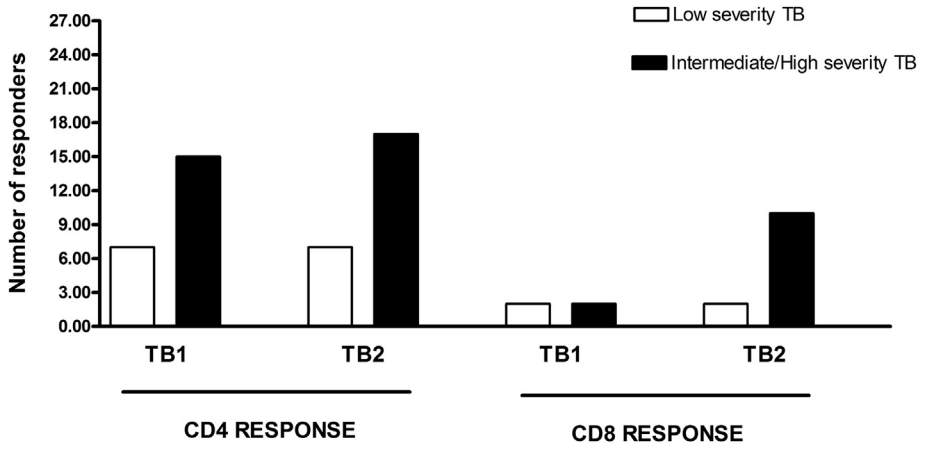

Figure 4 Impact of TB disease severity on CD4 and CD8 T-cell responses. Flow cytometric evaluation of CD4 and CD8 responses to TB1 and TB2 antigens in active TB patients. Patients were stratified according the grade of TB severity: low and intermediate/high severity. The bars represent the number of patients responding to TB1 and TB2 stimulation. TB: tuberculosis.

according to the microbiological diagnosis, we found that the TB2-induced CD8 response was associated with a microbiological diagnosis of TB more than to a clinical TB diagnosis (48% vs 25%) (Table 4). To note that among the active TB, the CD8 responders either classified as severe TB (8/10) or by a microbiological diagnosis (8/11), a selective TB2 response was observed (Table 4). These results suggest that CD8 T cell response associate with the radiological severity of TB disease and with the mycobacterial load.

Discussion

This is the first characterization of the CD4 and CD8 T-cell responses to TB1 and TB2 tubes of QFT-Plus assay in a cohort of subjects with active TB disease and LTBI enrolled in a low TB endemic country such as Italy. We demonstrated that both, TB1 and TB2 induce a CD4 T-cell response. On the other hand, CD8 T-cell response is mainly due to TB2 stimulation which is largely associated to active TB.

In an effort to find additional tools for performing better diagnosis using biomarkers,[37] the QFT-Plus is a new generation IGRA designed to offer high sensitivity and specificity for LTBI diagnosis.[29]

In the absence of a gold standard for the diagnosis of LTBI, active TB patients are used as a surrogate to validate the test. In line with this, a recent study demonstrated that QFT-Plus, in comparison to QFT-GIT, improves the sensitivity for active TB detection maintaining a high specificity.[23] Other two studies performed in low TB endemic country demonstrated that the performance of QFT-Plus is as accurate as that of QFT-GIT.[27,28] Here we confirm that QFT-GIT and QFT-Plus have similar sensitivity for active TB diagnosis. Moreover in the LTBI population we confirmed by QFT-Plus the data generated by QFT-GIT.

Several studies have described that CD8 T cells play a unique function in the recognition and containment of intracellular infection with Mtb, recognizing and eliminating heavily infected cells[38] including cells which do not belong to the immune system, such as the infected lung epithelial cells.[39] Studies in the mouse model demonstrated that depletion of CD8 T cells in the chronic stage of Mtb infection results in increased bacterial burden.[40] In vitro studies have shown that CD8 T cells may kill Mtb-infected human cells through granule-mediated functions such as granulysin.[41] All these data together indicate that CD8 T cells are actively involved in the immune response to Mtb and they are necessary for the control of TB infection.

In the present study only few LTBI subjects showed a CD8 T-cell response. This is in accordance to the literature and to the concept that latent TB represents a spectrum of different stages in which the immune system and the mycobacteria find a host-pathogen equilibrium.[42] In this environment, CD8 T cells are important players to control the Mtb bacterial load by emerging in the presence of replicating Mtb. This limits bacterial survival,[41] but produces tissue damage. Interestingly, the decline of CD8 T-cell response in parallel with a decrease of Mtb replication has been described in active TB patients during TB treatment.[12,21,43,44] In line with these results, we found lower CD8-specific responses in clinical TB compared to microbiologically diagnosed TB. This highlights that the monitoring of the CD8 T-cell response to TB2 by flow cytometry can be a tool to specifically evaluate treatment efficacy. Moreover we also show that TB2-induced CD8 T-cell responses associate to the grade of TB severity. The result is interesting, although the association did not reach statistical significance, probably due to the low number of subjects evaluated. It would be interesting to follow these TB patients until the end of treatment and evaluate if the CD8-T cell response change overtime. To note that the TB patients showing a CD8 response to TB2 stimulation and not to TB1, have an intermediate high severity TB and are microbiological confirmed TB, indicating that the Mtb load and the lung damage influence the ability to respond to TB2 stimulation. On the other side, independently from the stimulation tubes considered, we found that the presence of a CD8 T-cell response is associated to the CD4-specific response. This relates to the important role that CD4 T cells play in orchestrating Mtb-specific defense.

According to the manufacturer, TB1 peptides have been designed to stimulate CD4 T cells while TB2 should elicit

both CD4 and CD8 responses. Unexpectedly, in the present study we showed that in a few subjects the CD8 response was detected also following TB1 stimulation. These subjects, equally spread between all evaluated groups, showed a concomitant TB2-dependent CD8 T cell-response. This is probably due to the internalization and processing of the TB1 peptides by antigen presenting cells and their consequent presentation by the MHC I molecules to CD8 T cells.

Recently, studies using QFT-Plus kit showed that the difference in IFN-γ production between TB2 and TB1 stimulation may provide a surrogate marker of the CD8 T-cell response magnitude. This difference has been associated with smear positivity in active TB patients or with a recent exposure in TB contacts.[23] In the present study, using the same approach, we did not find any association between these two parameters, probably due to the low number of recent LTBI subjects enrolled.

In conclusion this is the first report on the characterization of the CD4 and CD8 T-cell response to TB1 and TB2 tubes of the QFT-Plus assay. We demonstrated that CD8 T-cell responses are preferentially induced by TB2 which are mainly associated to active TB. This assay has the potential to be very useful in conditions of immune depression resulting from CD4 T-cell impairments.

Conflict of interest

None of the authors has a conflict of interest.

Acknowledgments

The authors are grateful to all the patients, nurses (in particular Sara Pantanella, Daniela Milordo, Emanuela Ercoli, Giuliana Rialti, Immacolata Mauceri), microbiologist (Eugenio Bordi) and physicians who helped to perform this study. The study was supported by grants from the Italian Ministry of Health: "Ricerca Corrente" and a grant from the European Union: HEALTH-F3-2009-241642 and EC FP7 NEW-TBVAC (contract no. HEALTH.F3.2009 241745). The funders had no role in the decision to publish the study, in analyzing the data or drafting the manuscript). The Qiagen company it did not give any input into the interpretation of the data and the study was financed exclusively from institutional funds.

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