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Restoration of mycobacterial antigen-induced proliferation and interferon-γ responses in peripheral blood mononuclear cells of tuberculosis patients upon effective chemotherapy

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Abstract

Peripheral blood mononuclear cells (PBMC) were obtained from culture-proven tuberculosis (TB) patients before and after 2 and 6 months of chemotherapy with a multi-drug regimen. PBMC were tested for cellular responses in antigen-induced proliferation and interferon-γ (IFN-γ) assays in response to complex mycobacterial antigens (whole cell *Mycobacterium bovis* BCG and *M. tuberculosis*, cell walls and short-term culture filtrate [ST-CF] of *M. tuberculosis*), fractionated ST-CF antigens (fractions F1–F10) and ESAT-6. The responses in TB patients before anti-TB treatment were low (median stimulation index (SI) = 1–7, median delta IFN-γ = 0–12 U ml^{-1}, and percent responders = 13–67%) to all the antigenic preparations. Following the administration of anti-TB chemotherapy for 2 months, there were significant ($P < 0.05$) improvements in the cellular responses (median SI = 9–76, median delta IFN-γ = 3–70 U ml^{-1}, and percent responders = 33–100%) to most of the antigenic preparations tested. However, concanavalin A-induced proliferation responses of PBMC from the same patients before and after 2 months of chemotherapy were high and comparable (median SI = 101 and 114, respectively, $P > 0.05$, 100% responders). A further increase in IFN-γ responses (median delta IFN-γ = 14–250 U ml^{-1} and percent responders = 43–100%) to mycobacterial antigens was observed in patients receiving chemotherapy for 6 months. Among the ST-CF fractions, F1 and F2 containing low molecular mass proteins resulted in the highest responses, whereas ESAT-6 showed responses comparable to these fractions only in a minority of the patients. HLA-DR typing of these patients showed heterogeneity in the expression of molecules encoded by *HLA-DRB* genes. These results show that effective chemotherapy restores cellular responses of TB patients to a large number of *M. tuberculosis* antigens, which could be useful in monitoring the efficacy of anti-TB treatment.

Keywords: Tuberculosis; Chemotherapy; Peripheral blood mononuclear cell; Proliferation; Interferon-γ secretion

1. Introduction

Tuberculosis (TB) remains the leading cause of morbidity and mortality throughout the world [1]. Developing countries are particularly affected, but even in the developed countries the incidence of TB has increased in the recent past [1]. In addition to other factors, the emergence of the HIV-AIDS pandemic has a strong influence on the increased incidence of TB particularly in the developing world [2]. In addition, multidrug-resistant strains of *Mycobacterium tuberculosis* are spreading all over the world [3]. This has led the World Health Organization to declare the TB situation a 'global emergency'.

Most (90%) people infected with *M. tuberculosis* remain clinically well and are able to induce a life-long protective immune response. However, about 5% of the infected develop disease within the first year of infection (primary TB) and the other 5% develop the disease later in life (reactivation TB). The activation of Th1 cells indicated by high interferon-γ (IFN-γ) production has been considered the hallmark of protective immunity against TB [4,5]. Peripheral blood mononuclear cells (PBMC) from healthy contacts of TB patients and patients with limited disease produce large quantities, whereas PBMC from active TB

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patients with advanced disease produce low quantities of IFN-γ in response to complex mycobacterial antigens such as whole cell mycobacteria, purified protein derivative (PPD) of *M. tuberculosis*, secreted antigens present in the short-term culture filtrates (ST-CF) of *M. tuberculosis*, antigenic fractions of ST-CF, and single antigens like ESAT-6, 16-kDa and 38-kDa proteins etc. [6–11]. Effective treatment of active TB patients with anti-TB drugs has been shown to improve cellular responses (antigen-induced proliferation and IFN-γ secretion) of PBMC to various complex mycobacterial antigens and some immunodominant single antigens, e.g. ESAT-6, 16-kDa and 38-kDa proteins [12–14]. However, it is not known if the improvement in cellular responses is limited to these proteins or is a generalized improvement of cellular responses to a large number of antigenic proteins of *M. tuberculosis*. Furthermore, no information is available on the role of HLA molecules in the restoration of cellular responses in the treated TB patients, particularly HLA-DR molecules, which mostly restrict the responses of mycobacterial antigens to CD4+ Th1 cells [15–17]. This study was designed to assess the cellular responses of PBMC from TB patients before and after chemotherapy to complex mycobacterial antigens (whole cell *M. bovis* BCG; whole cells, cell walls and ST-CF of *M. tuberculosis*) as well as to 10 purified antigenic fractions of ST-CF and the low molecular mass *M. tuberculosis* antigen ESAT-6. In addition, we have also determined the HLA-DR types of the patients at 6 months of treatment to study if the restoration of the cellular response was generalized or restricted to some HLA-DR types.

Although clinical, bacteriological and radiological improvements in TB patients are achieved early (within 2 months) upon effective chemotherapy with multidrug regimens, it requires 6 months of treatment to see complete cure and prevent frequent relapses [18]. Earlier studies assessed cellular responses in TB patients only at single time points, e.g. 4 months [13] or 6 months [11], and thus no data are available to show if there are any differences in the cellular responses at the early stages vs. the end of anti-TB chemotherapy. In this work, to study the effect of the duration of anti-TB treatment, we tested PBMC for cellular responses to mycobacterial antigens at 2 and 6 months of chemotherapy. Our results showed that, although antigen-induced proliferation responses of PBMC were restored by 2 months of effective chemotherapy, the optimal restoration of IFN-γ responses required 6 months of chemotherapy.

2. Materials and methods

2.1. Study population

Heparinized blood was collected from clinically, radiologically and bacteriologically confirmed cases of pulmonary TB ($n = 15$) attending the Chest Diseases Hospital, Kuwait. Blood samples were collected from the same group of patients following 2 months ($n = 12$) and 6 months of treatment ($n = 7$) with a multidrug regimen using directly observed therapy short course [18]. Collection of blood samples was approved by the Ethics Committee of Human Experimentation in Kuwait.

2.2. Complex and purified mycobacterial antigens and mitogen

The complex antigens used in this study were killed whole cells of *M. tuberculosis* H37Ra and *M. bovis* BCG [19,20], *M. tuberculosis* cell walls (provided by P.J. Brennan, Colorado State University, Fort Collins, CO, USA, through the repository of TB research materials, NIAID, NIH contract no. AI-25147, USA) and *M. tuberculosis* ST-CF, a preparation highly enriched for secreted antigens of *M. tuberculosis* with only trace amounts of intracellular soluble antigens [21].

The antigenic fractions of ST-CF (F1–F10) were prepared by using preparative sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) according to methods described previously [21]. The protein fractions were eluted from the preparative SDS–PAGE overnight with 0.1% (w/v) SDS in phosphate-buffered saline. The fractions were then passed through Extractigel columns (Pierce Europe, Oud-Beijerland, The Netherlands) to remove SDS. The protein concentration in the fractions was determined by the Micro BCA method (Pierce). All fractions were stabilized with 2% human AB serum and stored frozen at −20°C until use. The recombinant ESAT-6 antigen was expressed in *Escherichia coli*, as previously described [22], and purified by preparatory SDS–PAGE, and residual endotoxin was depleted by passage through a lipopolysaccharide affinity column (Detoxi-Gel, Pierce). The mitogen used was concanavalin A (Con A, Sigma Chemical, St. Louis, MO, USA).

2.3. Isolation of PBMC

PBMC were isolated from the heparinized blood of TB patients according to standard procedures [19]. In brief, the blood from each individual was diluted with warm tissue culture medium (RPMI 1640) at a ratio of 1:2 and gently mixed. Two volumes of the diluted blood were loaded on top of 1 volume of a Lymphoprep gradient (Pharmacia Biotech, Uppsala, Sweden). After centrifugation, the white ring of PBMC between the plasma and the Lymphoprep was removed and washed three times with RPMI 1640. The cells were finally suspended in complete tissue culture medium (RPMI 1640+10% human AB serum+penicillin (100 U ml^{-1})+streptomycin (100 μg ml^{-1})+gentamicin (40 μg ml^{-1})+fungizone (2.5 μg ml^{-1})) and counted with a Coulter counter (Coulter Electronics, Luton, UK).

2.4. HLA typing of PBMC

PBMC were HLA-typed genomically by using sequence-specific primers in polymerase chain reaction (PCR), as described previously [22,23]. In brief, the high molecular mass genomic DNAs were isolated by treatment of PBMC with proteinase K and salting out in miniscale. HLA-DR 'low resolution' kits containing the primers to type for *DRB1*, *DRB3*, *DRB4* and *DRB5* alleles were purchased from Dynal AS (Oslo, Norway) and used in PCR according to the manufacturer's instructions. DNA amplifications were carried out with a Gene Amp 2400 PCR system (Perkin-Elmer, Cetus), and the amplified products were analyzed by gel electrophoresis using standard procedures. The genotypes were identified from the size of the amplified products and serologically defined HLA-DR specificities were determined from the genotypes by following the guidelines provided by Dynal AS.

2.5. Antigen and Con A-induced proliferation of PBMC

Antigen and Con A-induced proliferation of PBMC was performed using standard procedures [24,25]. In brief, PBMC (2×10^5 cells per well) suspended in 50 μl of complete tissue culture medium were seeded into the wells of 96-well tissue culture plates (Nunc, Roskilde, Denmark). Antigen or Con A in 50 μl of complete medium was added to the wells in triplicate at an optimal concentration of 5 μg ml^{-1}. Whole bacilli were used at a concentration of 10 μg (wet weight) per ml. The final volume of the culture in each well was adjusted to 200 μl. The plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The Con A-stimulated cultures were pulsed on day 3 and the antigen-stimulated cultures were pulsed on day 6 with 1 μCi of [^{3}H]thymidine (Amersham Life Sciences, Little Chalfont, UK) for 4 h and harvested on filter mats with a Skatron harvester (Skatron Instruments, Oslo, Norway), and the radioactivity incorporated was measured by liquid scintillation counting.

2.6. Interpretation of proliferation results

The radioactivity incorporated was obtained as counts per minute (cpm). The average cpm was calculated from triplicate cultures stimulated with each antigen or Con A as well as from triplicate wells of negative control cultures lacking antigen or mitogen. Cellular proliferation results are presented below by using the stimulation index (SI), which is defined as follows: SI = cpm in antigen- or mitogen-stimulated cultures/cpm in cultures without antigen or mitogen. An SI of >5 was considered a positive proliferative response [25].

2.7. IFN-γ assay

Supernatants (100 μl) were collected from antigen-stimulated cultures of PBMC (96-well plates) before being pulsed with [^{3}H]thymidine. The supernatants were kept frozen at −70°C until assayed for IFN-γ activity. The amounts of IFN-γ in the supernatants were quantified by using immunoassay kits (Coulter/Immunotech, Marseille, France) as specified by the manufacturer. The detection limit of the IFN-γ assay kit was 0.08 U ml^{-1}. Secretion of IFN-γ in response to a given antigen was considered positive when delta IFN-γ (the IFN-γ concentration in cultures stimulated with antigen minus the IFN-γ concentration cultures without antigen) was >5 U ml^{-1} [25].

2.8. Statistical analysis

The results of proliferation and IFN-γ assays with PBMC from the TB patients before and after chemother-

Table 1
Mycobacterial antigen-induced proliferation responses of PBMC obtained from TB patients before and after 2 and 6 months of anti-TB chemotherapy

Mycobacterial antigen	Proliferation (median SI) of PBMC obtained from TB patients		
	Before treatment	Treated for 2 months	Treated for 6 months
M. bovis BCG	7	32	30
M. tuberculosis H37Ra	4	76*	20*
Cell walls	7	72*	36*
ST-CF	4	52*	30*
ESAT-6	2	3	4
F1	4	23	17*
F2	4	32*	22
F3	3	17*	15*
F4	2	19*	18*
F5	2	15*	13*
F6	2	14*	9*
F7	3	13*	9*
F8	1	11*	7*
F9	1	9*	7*
F10	2	15*	12*

*$P<0.05$, statistical significance by Mann–Whitney *U*-test when the responses of the treated patients are compared with the responses of patients before treatment.

apy were statistically analyzed for significant ($P < 0.05$) differences using the non-parametric Mann–Whitney *U*-test.

3. Results

3.1. Cellular responses of TB patients' PBMC to complex mycobacterial antigens and Con A

To determine the cellular responses of PBMC isolated from the peripheral blood of active TB patients (before starting chemotherapy with the multidrug regimen) to complex mycobacterial antigens, the cells were stimulated with whole cell *M. bovis* BCG and *M. tuberculosis* H37Ra, the cell walls and ST-CF of *M. tuberculosis*. The results showed weak cellular responses to the complex mycobacterial antigens in this patient group as indicated by weak proliferation (median SI = 4–7) (Table 1) and low levels of IFN-γ secretion (median delta IFN-γ = 1–12 U ml^{-1}) (Table 2). The proliferation and IFN-γ responses of PBMC to the complex mycobacterial antigens were significantly elevated following the administration of chemotherapy for 2 months (median SI = 32–76 and median delta IFN-γ = 25–70 U ml^{-1}). In contrast to the depressed proliferative responses to the mycobacterial antigens, the proliferation of PBMC from the same group of TB patients to the mitogen Con A, before and 2 months after chemotherapy, was high (median SI = 101 and 114, respectively) and comparable ($P > 0.05$). The IFN-γ responses to the complex mycobacterial antigens further improved after 6 months of chemotherapy (median delta IFN-γ = 210–250 U ml^{-1}) (Table 2).

3.2. Cellular responses of TB patients' PBMC to ST-CF fractions and ESAT-6

To identify the antigenic fractions responsible for cellular responses induced by the ST-CF, PBMC isolated from the blood of TB patients before and after chemotherapy were stimulated for proliferation and IFN-γ secretion in response to the ST-CF fractions F1–F10. Consistent with the low cellular responses to the complex mycobacterial antigens, all the ST-CF fractions induced low proliferation and IFN-γ responses, i.e. median SI = 1–4 (Table 1) and median delta IFN-γ = 0–3 U ml^{-1} (Table 2) in patients before treatment. Following the treatment of TB patients with anti-TB drugs, significant ($P < 0.05$) improvements in proliferation responses to almost all ST-CF fractions were observed after 2 months of chemotherapy (Table 1), but significant elevation of IFN-γ responses required 6 months of treatment (Table 2). Although, following chemotherapy, improved cellular responses were observed to all the antigenic fractions of ST-CF, the highest responses were seen to fractions F1 and F2 (Tables 1 and 2), which contain proteins of low molecular mass.

To identify a single antigen in the low molecular mass fractions stimulating the cellular responses, we evaluated the responses of the patients to the low molecular mass protein ESAT-6. Both proliferation and IFN-γ responses to ESAT-6 in patients before treatment were low (median SI = 2 and median delta IFN-γ = 1 U ml^{-1}) (Tables 1 and 2). The proliferation responses did not show a significant increase upon chemotherapy (Tables 1 and 2), but IFN-γ responses to ESAT-6 were significantly ($P < 0.05$) elevated at 6 months of anti-TB therapy (median delta IFN-γ = 14 IU ml^{-1}) (Table 2). Furthermore, a comparative analysis

Table 2
Mycobacterial antigen-induced IFN-γ responses of PBMC obtained from TB patients before and after 2 and 6 months of anti-TB chemotherapy

Mycobacterial antigen	Median delta IFN-γ responses of PBMC obtained from TB patients		
	Before treatment	Treated for 2 months	Treated for 6 months
M. bovis BCG	4	25	220*
M. tuberculosis H37Ra	12	70	220*
Cell walls	6	25	250*
ST-CF	1	60	210*
ESAT-6	1	6	14
F1	3	25	220*
F2	2	35	190
F3	2	16	190*
F4	1	6	100*
F5	1	17	90*
F6	1	15*	80*
F7	1	3	25*
F8	0	10	60*
F9	1	5	40*
F10	2	10	60*

*$P < 0.05$, statistical significance by Mann–Whitney *U*-test when the responses of the treated patients are compared with the responses of patients before treatment.

Table 3
Antigen-induced proliferation and IFN-γ responses of PBMC from HLA-DR-typed patients to ST-CF fraction F1 and ESAT-6 after 6 months of chemotherapy

Patient		Proliferation (SI) in response to		delta IFN-γ in response to	
No.	HLA-DR	F1	ESAT-6	F1	ESAT-6
1	3,7,52,53	**33.1**	1.7	**210**	**7.5**
2	6,7,52	**119.1**	40.4	ND[a]	ND
3	1,4,53	**12.3**	3.9	**220**	**220**
4	2,7,51	**17.3**	2.6	**250**	**14**
5	2,51	**272.7**	**139.7**	**250**	**240**
6	5,52	**17.6**	**5.0**	ND	ND
7	2,6,51,52	**8.2**	1.1	**80**	**5.0**

The proliferation and IFN-γ responses of PBMC with SI > 5 and delta IFN-γ > 5 U ml^{-1} were considered positive, and are indicated in bold face.
[a]ND = not determined.

of the cellular responses in each patient treated for 6 months showed that all the patients responded to the low molecular mass fraction F1, by both proliferation and IFN-γ secretion (Table 3). However, PBMC of only three out of seven patients demonstrated proliferation to ESAT-6, and all the patients responded by IFN-γ secretion (Table 3). In addition, as compared to ST-CF fraction F1, the proliferation responses to ESAT-6 were weaker in all seven patients, whereas IFN-γ responses were weaker in three of the five patients (Table 3). These results suggested that in addition to ESAT-6, fraction F1 contained other low molecular mass proteins capable of inducing Th1 cell reactivity in the treated patients.

3.3. HLA-DR types expressed by TB patients' PBMC

To determine the effect of HLA-DR molecules on the restoration of cellular responses after effective chemotherapy, we determined the expression of HLA-DR molecules in all seven patients who received anti-TB treatment for 6 months and showed recovery of cellular responses to mycobacterial antigens. The results showed heterogeneity among the patients with respect to the expression of molecules encoded by *HLA-DRB1*, *-DRB3*, *-DRB4* and *-DRB5* genes (Table 3). These results suggest that the restoration of cellular responses to mycobacterial antigens after anti-TB chemotherapy was not restricted to specific HLA-DR types.

3.4. Percentages of responders to mycobacterial antigens and Con A

In addition to the absolute values of SI and delta IFN-γ presented above, the results of cellular responses of PBMC to various mycobacterial antigens and Con A were also analyzed with respect to the percentage of positive responders among the TB patients before and after anti-TB chemotherapy. This analysis showed that among the

Table 4
Percentage responders among TB patients before and after 2 and 6 months of chemotherapy to mycobacterial antigens in proliferation and IFN-γ assays

Mycobacterial antigen	Percentage of TB patients responding to mycobacterial antigens					
	Before treatment		Treated for 2 months		Treated for 6 months	
	Proliferation	IFN-γ	Proliferation	IFN-γ	Proliferation	IFN-γ
M. bovis BCG	53	40	92	81	100	100
M. tuberculosis H37Ra	47	53	100	81	100	100
Cell walls	67	60	100	67	100	100
ST-CF	40	27	92	73	100	100
ESAT-6	13	13	33	55	43	100
F1	40	40	83	73	100	100
F2	47	40	83	64	100	100
F3	33	27	75	64	190	100
F4	33	33	75	64	100	100
F5	27	27	83	64	100	100
F6	20	20	92	73	100	100
F7	27	40	75	46	86	100
F8	33	13	83	55	86	100
F9	27	20	75	55	71	100
F10	33	27	83	64	86	100

A patient was considered a responder to a given antigen in proliferation and IFN-γ assays when PBMC showed SI > 5 and delta IFN-γ > 5 U ml^{-1}.

patients before treatment, there were only 13–67% and 13–60% responders in proliferation and IFN-γ assays, respectively (Table 4). After 2 months of chemotherapy, the percentage of responders to mycobacterial antigens increased to 33–100% and 46–81% in proliferation and IFN-γ assays, respectively (Table 4). However, in response to Con A stimulation of PBMC, positive proliferation responses were observed in 100% of patients before as well as after 2 months of chemotherapy (data not shown). Following 6 months of anti-TB treatment, the percentage of responders to mycobacterial antigens further increased to 43–100% in proliferation assays (Table 4), whereas all the patients (100%) responded to the various preparations of mycobacterial antigens in IFN-γ assays (Table 4).

4. Discussion

This study demonstrates that PBMC from active TB patients with advanced to far-advanced disease exhibit weak cellular responses to complex mycobacterial antigens including whole cell *M. bovis* BCG, whole cells, cell walls and ST-CF of *M. tuberculosis* as well as to 10 different antigenic fractions (F1–F10) of ST-CF containing low (<10 kDa) to high molecular mass (>97.4 kDa) culture filtrate proteins [6], and a single low (6 kDa) molecular mass antigen ESAT-6 [26]. Previous works have also shown the depressed proliferation of PBMC from patients with pulmonary and pleural TB to crude extracts of mycobacterial antigens such as PPD [13,27], whole cell *M. tuberculosis* [27], whole cell *M. bovis* BCG [9] and to defined antigens such as peptides from the 16-, 19- and 38-kDa molecules of *M. tuberculosis* [12]. In addition, our study confirms the previous reports showing cellular low responsiveness of PBMC from active TB patients to ST-CF fractions [6,7]. Furthermore, our results show that the low responsiveness of active TB patients' PBMC to mycobacterial antigens was not due to a generalized suppression of cellular responses because the PBMC from the same group of patients showed strong proliferation in response to the mitogen Con A. Normal polyclonal cellular responses of active TB patients' PBMC to another mitogen (phytohemagglutinin) have also been reported previously [13,27].

To assess cellular responses of PBMC, we have used assays for antigen-induced proliferation and quantitation of IFN-γ secreted in the culture supernatants. Both of these assays are considered to reflect Th1 cell-mediated immunity, which has been shown to provide protection against TB in animal models [4,5]. In addition, IFN-γ levels have also been shown to correlate with the clinical manifestations of the disease in humans, e.g. low quantities of IFN-γ are produced by PBMC from pulmonary TB patients with advanced disease [8–11], whereas IFN-γ levels are high in healthy contacts of TB patients and patients with early disease [8,9,11,27]. The low level of cellular responses to mycobacterial antigens in the active TB patients studied in this work and the increase in cellular responses after effective chemotherapy further support the importance of Th1 cytokines in mediating protective immunity against TB.

The cellular responses of PBMC from our patients to mycobacterial antigens were significantly improved after chemotherapy with the multidrug regimen. It is important to note that proliferation responses were restored to the same level after 2 months of treatment as after 6 months, whereas 6 months of chemotherapy was required for a significant rise in IFN-γ levels. Although our results confirm the previous findings showing that effective chemotherapy against active TB restores the protective (Th1) type of immunological reactivity to mycobacterial antigens [12–14], this is the first study to demonstrate that proliferation responses are restored earlier than IFN-γ responses. This is also the first study to examine the cellular responses of TB patients to a battery of highly complex (whole cells, cell walls and culture filtrates) and fractionated ST-CF preparations before and after 2 and 6 months of anti-TB chemotherapy. Such improved Th1 cell reactivity can be helpful in fast recovery from the active disease and minimize the possibility of reactivation disease by interfering with the spread of infection from the hidden sites.

In the previous studies, improvement of cellular responses was shown to a crude mixture of complex mycobacterial antigens represented by PPD or single purified antigens such as ESAT-6, 16-, 19- and 38-kDa antigens or mixtures of peptides to represent single antigens [12–14], whereas our results show an elevated response to all the antigenic fractions of ST-CF, with a trend towards higher activity to the low molecular mass fractions F1 and F2. Consistent with these results, it has previously been shown that in memory immune mice a significant proportion of the cellular responses are directed towards the low molecular mass ST-CF fractions F1 and F2 [26]. These fractions were later found to contain ESAT-6 as the major target for IFN-γ-secreting Th1 cells [6,26]. ESAT-6 has been described as an antigen that is specific to *M. tuberculosis* [26], and immunization with ESAT-6 induces protective immunity against TB in the mouse model [28]. To determine the contribution of ESAT-6-reactive cells in restoration of cellular responses to the low molecular mass antigenic fractions of ST-CF, we have also tested our patients' PBMC for cellular responses to ESAT-6. The overall results showed that, as compared to the low molecular mass ST-CF fractions, ESAT-6 induced weaker responses in both proliferation and IFN-γ assays (Tables 1 and 2). Further analysis of the data with respect to IFN-γ secretion in five patients after 6 months of chemotherapy showed that ESAT-6 induced comparable responses to ST-CF fraction F1 in only two of the patients (Table 3). This suggests that, in addition to ESAT-6, the low molecular mass ST-CF fractions contain other Th1 cell antigens. The possible candidates could be

other proteins belonging to the ESAT-6 family, including CFP-10, a low molecular mass Th1 cell antigen of *M. tuberculosis* present in the ST-CF [23,29].

It has previously been shown that cellular responses of mycobacterial antigens to Th1 cells are primarily restricted by the highly polymorphic HLA-DR molecules of the major histocompatibility complex (MHC) [15–17,20,22,23]. To study the effect of HLA-DR molecules on the restoration of cellular responses of PBMC to mycobacterial antigens, we studied the expression of HLA-DR molecules encoded by *HLA-DRB1*, *-DRB3*, *-DRB4* and *-DRB5* genes in the patients treated for 6 months with anti-TB drugs. The results showed that these patients were highly heterogeneous with respect to the expression of HLA-DR molecules. This suggests that the post-treatment improvements in the cellular responses of PBMC occurred over a range of MHC backgrounds of the donors.

The restoration of cellular responses in PBMC of TB patients receiving effective chemotherapy could be attributed to the increase in the proportion of peripheral CD4+ T cell that produce IFN-γ [10]. Consistent with this proposition, it has been shown that CD4+ T cells responsive to a vast array of *M. tuberculosis* epitopes are sequestered or compartmentalized at the site of the disease, and appear in the peripheral blood after effective chemotherapy, which reverses the state of anergy seen in these patients [12,13]. Another contributing factor in the restoration of cellular responses could be the shift in cytokine production by PBMC, particularly interleukin-10 (IL-10) [8,11,12] and transforming growth factor-β (TGF-β) [8]. Both of these cytokines down-regulate the activation of Th1 cells and their cytokines, with the specific inhibition of IFN-γ responses. The levels of IL-10 and TGF-β are high in active TB patients and decrease upon treatment with anti-TB drugs [8,11]. The specific inhibitory effects of IL-10 and TGF-β on IFN-γ secretion by Th1 cells could be the reason for the delayed recovery of IFN-γ responses in our patients as it took 6 months to restore IFN-γ responses as compared to 2 months for proliferation responses of PBMC.

TB remains the top infectious disease killer; it kills about 3 million people every year, which exceeds the number of deaths caused by any other infectious agent [1–3]. This is probably because inadequate or inappropriate immune responses are induced against the TB bacilli. In addition, effective chemotherapy requires a combination of different drugs given in large doses for at least 6 months leading to patients' non-compliance to treatment [18]. This has been further complicated by the emergence of multi-drug-resistant strains of *M. tuberculosis*. We have shown here that restoration of cellular responses to mycobacterial antigens correlates with effective chemotherapy, indicated by bacterial clearance and clinical improvement. Thus, monitoring of mycobacterial antigen-specific cellular responses in patients suspected of having drug-resistant TB may provide an early indication (within 1 week) of drug resistance, as compared to 4–6 weeks required to confirm drug resistance by culture techniques.

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