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Interferon- γ and Interleukin-17 production from PPD-stimulated PBMCs of patients with pulmonary tuberculosis

Abstract

Purpose: The purpose of this study was to evaluate Interferon (IFN)- γ and Interleukin(IL)-17 profiles in patients with different clinical presentations of pulmonary tuberculosis (TB) and to compare them with those of tuberculin-negative and tuberculin-reactive healthy controls

Methods: Peripheral blood mononuclear cells (PBMCs), isolated from patients (n=52) and controls (n=30), were stimulated *ex vivo* with purified protein derivative (PPD) and IFN- γ and IL-17 levels in the supernatant were measured.

Results: At baseline, PBMCs from patients with TB released a significantly lower amount of IL-17 (p=0.043) than PBMCs from healthy controls, whereas IFN- γ levels were similar in the two groups. After PPD stimulation, a significant rise in IL-17 levels was found only among healthy controls (p=0.02). This rise in IL-17 levels was similar between tuberculin-reactive and tuberculin-negative subjects. After PPD stimulation, patients with infiltrative TB secreted higher levels of IL-17 and IFN- γ than those affected with chronic, miliary and cavitary TB (p<0.01). IFN- γ production from patients with infiltrative TB was even higher than for healthy controls (p<0.01). PBMCs from tuberculin-reactive patients released higher levels of IFN- γ than tuberculin-negative subjects after PPD stimulation (p<0.01).

Conclusion: *Ex vivo* PPD stimulation of PBMCs from patients with pulmonary TB does not significantly stimulate IL-17 release; however, higher IL-17 and IFN- γ levels are found in patients with infiltrative disease, in comparison with those affected with miliary, cavitary and chronic TB.

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Despite the high infectivity of *Mycobacterium tuberculosis* (Mtb), only 5-10% of infected individuals develop clinical symptoms and radiological evidence of active disease [1]. Also, clinical presentation in patients with active tuberculosis (TB) varies substantially from mild symptoms to severe or fatal TB, the former being characterized by an infiltrative-exudative pulmonary lesion and the latter by large, cavitory lung lesions or miliary TB.

Cell-mediated immunity represents the main component of host defense against Mtb [2]; the T-helper 1 (Th1) immune response, which is characterized by the production of IL-12, TNF- α and IFN- γ [3-6], implies a close interplay between CD4+ T-cells and macrophages and is usually able to control bacterial proliferation and to prevent TB clinical progression. In particular, IFN- γ , produced by CD4+ T-cells, plays a key role in the host response to Mtb, given its activating effect on macrophages is responsible for the intracellular killing of mycobacteria [7,8]. Recent studies have focused on a new CD4+ T-cell subset, Th17 cells, as an important mediator of immune responses against Mtb [9]. Th17 cells produce IL-17, a potent proinflammatory cytokine that has been implicated in many autoimmune and inflammatory diseases, including rheumatoid arthritis, multiple sclerosis, asthma and systemic lupus erythematosus [10,11]. Generation of human Th17 cells depends on IL-23, IL-1 β , TGF- β and IL-6 stimulation [12-14]. In the lungs, IL-17 triggers neutrophil chemotaxis and induces the production of chemokines, growth factors and adhesion molecules [15]. The IL-23-IL17 axis has recently been shown to participate in the immune response to Mtb [16-19]. Both *in vitro* and *in vivo* experiments have shown defective TH1 and TH17 response in patients with TB in comparison with healthy tuberculin reactors, and a negative correlation between IFN- γ and IL-17 levels and disease severity [14,16,21-24]. A clear-cut shift towards TH2 immune response has been demonstrated [25]; in fact, increased levels of IL-4, the prototypical TH2 cytokine, as well as IL-5 and IL-10, have been found in patients with TB and have been related to a more severe clinical state [26-28]. Other studies have reported opposing results, describing higher levels of IL-17- producing CD4+ T cells among subjects with active TB and a direct correlation between the frequency of IL-17 secreting cells and disease severity [29-31].

In the present study, IFN- γ and IL-17 levels were determined in the supernatant of peripheral blood mononuclear cells (PBMCs) isolated from patients with different clinical and radiological features of pulmonary TB and stimulated *in vitro* with purified protein derivative (PPD). IFN- γ and IL-17 expression profiles for patients with pulmonary TB were com-

pared with those of a group of tuberculin-negative and tuberculin-reactive healthy controls.

Materials and Methods

Study subjects

Fifty-two consecutive patients with pulmonary TB, diagnosed at the Unit of Infectious Diseases of Catania, Eastern Sicily, Italy, were enrolled for the study between January 2010 and January 2012; diagnosis was based on clinical history, chest X-ray, intradermal tuberculin PPD test and positive detection of acid-fast bacilli in sputum smear or isolation of mycobacteria in sputum cultures. All patients with a positive sputum culture for mycobacteria also had molecular characterization of Mtb as determined by polymerase chain reaction (PCR). On the basis of specific clinical and radiological features, patients were divided into four subgroups as follows: 1), patients with infiltrative TB (n=17); 2), patients with cavitory TB (n=15); 3), patients with miliary TB (n=10); and 4), patients with chronic fibrous TB (n=10).

Subgroup 1: Cases of infiltrative TB were characterized by radiological evidence of apical exudative infiltration without micro- or macronodules or cavitations.

Subgroup 2: Patients with cavitory TB had single unilateral (n=8) or multiple bilateral (n=7) lung cavities.

Subgroup 3: Patients with miliary TB were defined on the basis of the presence of widespread micronodules (<2 mm) on chest CT scan, with or without extrapulmonary involvement (2 out of 10 miliary cases had a concurrent meningeal disease).

Subgroup 4: Radiological evidence of lung fibrotic bands and/or pleural calcifications associated with a history of previous untreated or incorrectly treated TB, with repeated positive sputum smears throughout a period of at least 72 months, was consistent with the diagnosis of chronic fibrous TB.

Blood samples were taken from all TB patients at diagnosis, within two days of hospital admission and before starting antitubercular treatment.

The control group consisted of 30 healthy subjects, 15 of whom were PPD-positive and 15 were PPD-negative. Participants had not been vaccinated with BCG, were serologically negative for HIV infection and had no known condition associated with overt immune depression, such as steroid or other immunosuppressive drug intake, cancer or onco-haematological disease, primitive immune deficiency, chronic or acute renal failure, or poorly controlled diabetes.

Written informed consent was obtained from patients and control subjects for blood sampling and PPD skin test.

TABLE 1. Clinical and epidemiological characteristics of the study population

	Pulmonary TB	Healthy controls
N	52	30
Age (years $\pm$ SD)	43 $\pm$ 17	39 $\pm$ 14
Gender: male/female*	30(57.7)/22(42.3)	17(56.7)/13(43.3)
Autochthonous/immigrant population*	31(59.6)/21(40.4)	18(60)/12(40)
Tuberculin skin test: positive/negative subjects*	42(80.8)/10(19.2)	15(50)/15(50)
Quantiferon assay: positive/negative*	44(84.6)/8(15.4)	15(50)/15(50)
Acid-fast bacilli in sputum smear*	46(88.5)	----
Mycobacteria in sputum culture*	52(100)	----
Detection of Mycobacterium tuberculosis by PCR*	52(100)	----
Clinical features of TB		----
Infiltrative disease*	17(32.8)	----
Cavitary disease*	15(28.8)	----
Miliary disease*	10(19.2)	----
Chronic fibrous disease*	10(19.2)	----

*N(%)

The main clinical and epidemiological characteristics of the study population are shown in Table 1.

Cell preparation and in vitro cultures

Heparinized peripheral blood, drawn from patients and healthy controls, was diluted 1:4 with PBS, overlaid on Ficoll-Hypaque (Sigma, Milan, Italy) and centrifuged at 1600 rpm for 20 minutes. The mononuclear cell layer was then removed from the plasma/Ficoll interface, washed twice with PBS and resuspended in Roswell Park Memorial Institute (RPMI) 1640 medium, supplemented with fetal calf serum (10%), penicillin/streptomycin (1%) and L-glutamine (1%). Cells were plated in 24-well tissue culture plates (Falcon) and incubated with or without PPD (1 μ g/mL, Biocine PPD liofilo, Sclavo, Siena, Italy) for 72 hours, at 37°C in a 5% CO₂ humidified atmosphere. After 72-hour incubation, supernatants were collected, centrifuged and stored frozen (-20°C) until use.

Enzyme linked immunosorbent assay (ELISA) for human IFN- γ and IL-17

IL-17 concentration in cell supernatant was measured using a solid-phase enzyme amplified sensitivity immunoassay performed on microtiter plate, purchased from Biosource Europe S.A. (Biosource IL-17 Cytoscreen kit, Nivelles, Belgium), following the manufacturer's instructions. The lower limit of detection of the assay was 2 pg/mL. IFN- γ concentration in cell

supernatant was measured by ELISA (Predicta, Genzyme Diagnostics, Cambridge, USA). The lower limit of detection for the assay was 3 pg/mL.

Statistics

Data were expressed as means $\pm$ standard deviation (SD). Categorical variables were analyzed by using the Fisher's exact test. Intergroup comparisons were performed using the paired and unpaired Student's *t* test. Statistical analysis was performed using Prism software (GraphPad, La Jolla, USA).

Results

IFN- γ and IL-17 levels in the supernatant of PPD-stimulated PBMCs from patients with different patterns of pulmonary TB and healthy controls were analyzed.

Baseline and post-PPD levels of IL-17

Baseline levels of IL-17 were significantly lower among patients with TB in comparison with healthy controls (205 $\pm$ 57 vs 75 $\pm$ 11 pg/mL, *p*=0.05). Mean IL-17 concentration after PPD-stimulation was 153 $\pm$ 88 pg/mL in TB patients, which was again significantly less (*p*=0.043) than in healthy controls. After PPD stimulation, a significant rise from basal IL-17 values was found among healthy controls (*p*=0.02), whereas in patients with TB no significant increase was seen in comparison with baseline values (Figure 1a). PPD-induced IL-17 produc-

FIGURE 1.

a. IL-17 levels in the supernatant of peripheral blood mononuclear cells (PBMCs) from patients with TB (n=52) and healthy controls (n=30), at baseline and after 72-hour stimulation with PPD (purified protein derivative). Baseline levels were significantly higher in healthy controls in comparison with patients with TB (**p=0.05). A significant rise in IL-17 levels was found only in healthy controls (**p=0.02). After PPD stimulation, IL-17 levels were significantly higher in healthy controls in comparison with patients with TB (*p=0.043).

b. IL-17 levels in the supernatant of peripheral blood mononuclear cells (PBMCs) from healthy controls whose tuberculin skin test was positive (n=15) and negative (n=15) at enrollment. A significant rise in IL-17 levels was found in tuberculin-negative (*p=0.05), as well as tuberculin-reactive subjects (**p<0.01), when comparing baseline and post-PPD values.

c. IL-17 levels in the supernatant of peripheral blood mononuclear cells (PBMCs) from healthy controls (n=30) and patients with pulmonary TB (n=52), divided into four groups on the basis of their clinical state, at baseline and after 72-hour stimulation with PPD (purified protein derivative). Baseline (*p<0.01) and post-PPD levels (**p<0.01) were significantly higher in healthy controls in comparison with the other groups; in addition, post-PPD levels were significantly higher in patients with infiltrative TB (n=17), compared with those with cavitary (n=15), miliary (n=10) and chronic fibrous (n=10) TB (**p<0.01). After PPD stimulation, a significant rise in IL-17 levels was found in patients with infiltrative (α p<0.01), cavitary (β p=0.048) and chronic fibrous (γ p=0.038) TB, but not in patients with miliary TB.

FIGURE 2.

a. IFN- γ levels in the supernatant of peripheral blood mononuclear cells (PBMCs) from patients with TB (n=52) and healthy controls (n=30), at baseline and after 72-hour stimulation with PPD (purified protein derivative). A significant rise in IFN- γ levels was observed in TB patients (**p<0.01), as well as in controls (*p<0.01). PPD-induced IFN- γ production was significantly higher in controls, in comparison with TB patients (***p<0.01).

b. IFN- γ levels in the supernatant of peripheral blood mononuclear cells (PBMCs) from healthy controls whose tuberculin skin test was positive (n=15) and negative (n=15) at enrolment. A significant rise in IFN- γ levels was detected only in tuberculin-reactive subjects (*p<0.01). Post-PPD levels were significantly higher among tuberculin reactive subjects, in comparison with tuberculin negative patients (**p<0.01).

c. IFN- γ levels in the supernatant of peripheral blood mononuclear cells (PBMCs) from healthy controls (n=30) and patients with pulmonary TB (n=52), divided into four groups on the basis of their clinical state, at baseline and after 72-hour stimulation with PPD (purified protein derivative). Baseline levels were similar. Post-PPD levels were higher among patients with infiltrative TB in comparison with the other groups (*p<0.01). Patients with cavitary (n=8), miliary (n=5) and chronic (n=5) TB did not show a significant increase in IFN- γ levels, whereas significant differences were observed for patients with infiltrative (n=7) TB (**p<0.01).

tion varied significantly among patients with different TB clinical features. In fact, patients with miliary TB did not exhibit a significant increase in IL-17 production after PPD stimulation (61 ± 7 vs 71 ± 38 pg/mL); in contrast, IL-17 levels rose significantly after PPD stimulation among those subjects with infiltrative TB (87 ± 13 vs 350 ± 99 pg/mL, $p < 0.01$), cavitary TB (51 ± 10 vs 132 ± 78 pg/mL, $p = 0.048$) and chronic fibrous TB (81 ± 10 vs 178 ± 68 pg/mL, $p = 0.032$) (Figure 1c).

Among healthy controls, baseline and PPD-induced IL-17 did not significantly differ between tuberculin-reactive and tuberculin-negative subjects. After PPD challenge, IL-17 levels significantly rose from baseline both in tuberculin-negative ($p = 0.05$) and in tuberculin-reactive ($p < 0.01$) subjects (Figure 1b).

Baseline and post-PPD levels of IFN- γ

At baseline, IFN- γ levels were similar between healthy controls and patients with TB; after PPD stimulation, a significant increase in IFN- γ levels was observed for both TB patients (from 19.6 ± 9.4 pg/ml to 121.8 ± 159.1 pg/ml, $p < 0.01$) and healthy controls (from 17.8 ± 6.3 pg/ml to 205.5 ± 85.8 pg/ml, $p < 0.01$) (Figure 2a). In detail, IFN- γ concentration in PBMCs supernatants was significantly higher after PPD-stimulation in tuberculin-reactive controls in comparison with those tuberculin-negative patients whose levels did not rise significantly from baseline (Figure 2b). When considering only the TB cohort, subgroup analysis showed a significant increase in IFN- γ levels among patients with infiltrative TB ($p < 0.01$). In contrast, PPD stimulated-PBMCs from patients with chronic, miliary and cavitary TB did not produce higher levels of IFN- γ in comparison with unstimulated PBMCs (Figure 2c).

Discussion

In the present study, PBMCs from patients with TB were found to release a significantly lower amount of IL-17 than PBMCs from healthy controls; moreover, our findings support the existence of a clear-cut correlation between IL-17 levels and clinical presentation of pulmonary TB. In fact, in patients with infiltrative TB, usually considered as the clinical prototype of a strong but pathologic cell-mediated response to Mtb (mostly described among young and immunocompetent adults [4]), a consistently higher production of PPD-induced IL-17 was seen in comparison with other clinical forms of pulmonary TB, such as cavitary and miliary TB, but not in comparison to healthy controls. In addition, a significantly higher production of IFN- γ was observed in subjects with infiltrative disease,

when compared with healthy controls and subjects with other clinical features of TB, in keeping with the major role of Th1 cytokines during the host response to Mtb [8].

It is well known that a switch from Th1 to Th2 cell response takes place in clinically overt TB. Some recent studies suggested a role for impaired Th17 response in determining the severity of pulmonary TB. Chen *et al.* [18] reported the Th17 response to be suppressed in patients with active TB: flow cytometry analysis showed that the frequency of a nonspecific Th17 response was significantly lower in patients with active TB disease than in healthy controls and subjects with latent TB infection (LTBI) and, of importance, suppressed Th17 response correlated with the severity of disease since those patients with pulmonary TB exhibiting meningeal complications had a significantly lower frequency of Th17 cells than patients with uncomplicated pulmonary TB. Kumar *et al.* [22] found similar results in a population of children affected with active TB: children with TB had diminished Th1 and Th17 cytokine responses in comparison with controls, either spontaneously *in vitro* or following PBMCs stimulation with mycobacterial antigens. In addition, children with active TB complicated by microbial dissemination and neurological involvement showed the most profound defect in production of both IFN- γ and IL-17. Stern *et al.* [32] reported a 9-fold increase in the levels of IL-17 mRNA after PPD stimulation of PBMCs drawn from individuals with LTBI. Consistent with the results of the present study, Scriba *et al.* [16] reported a reduced Mtb-specific Th17 response in patients with active TB in comparison with healthy donors. Other authors reported opposing results: Jurado *et al.* [29] recently demonstrated that in patients affected with TB, stimulation of lymphocytes with sonicated Mtb antigens induced a significantly lower amount of IFN- γ and higher concentration of IL-17 than healthy controls. The authors showed that IL-17 was secreted by CD4⁺ IFN- γ ⁺ IL-17⁺ lymphocytes, whose proportion directly correlated with the severity of disease. Similarly, Marin *et al.* [30] reported that patients with active TB had a higher frequency of circulating IL-17-producing CD4⁺ T cells after PPD stimulation than subjects with LTBI. In contrast to the results of the present study, Sargentini *et al.* [33] did not observe any significant difference in IL-17 release from PBMCs between healthy controls and patients with active TB after 72-hour stimulation with PPD; however, in this study, a very limited number of individuals (14 patients and 6 controls) was enrolled. In a recent paper of Cowan *et al.* [34], the authors found that the frequency of IL-17-producing CD4⁺ T cells was lower in patients with active TB in comparison with healthy controls and subjects with LTBI. After a 7-day stimulation of PBMCs with my-

cobacterial antigens, Cowan and coworkers detected a significant higher increase in IL-17-expressing CD4⁺ T cells among patients with active TB, in comparison with healthy controls. In contrast with our observation of similar response to PPD stimulation among tuberculin-negative and tuberculin-reactive subjects, Babu *et al.* [35] found that the production of Th17 cytokines, such as IL-17 and IL-23, was significantly lower in tuberculin-reactive individuals after challenging with PPD and Mtb culture filtrate.

Our study has a number of limitations: firstly, it was conducted on a relatively small number of patients. Secondly, since PPD, which is not a Mtb specific antigen, was used to stimulate PBMCs, further research is required to establish whether our findings may be reproduced when stimulating PBMCs with specific Mtb antigens, such as ESAT-6 and CFP-10. Thirdly, cytokine production was measured by ELISA so further evaluation by using surface and intracellular cytokine flow cytometry should be worthwhile. In addition, it would be interesting to focus on the possible role of macrophages and dendritic cells, considering that their interaction with Mtb antigens and their subsequent cytokine secretion may modulate host response to Mtb [36].

In conclusion, IL-17 production is inhibited in patients with pulmonary TB, with particular reference to cavitary or miliary TB, both of which are characterized by a weaker cell-mediated immune response.

The protective and pathological mechanisms of immune response to Mtb are complex and still unclear. More research is needed to understand how Th17 cytokines may influence the severity of TB presentation. Further investigations are required in order to shed light on the complex network of cytokine responses that mediate resistance or susceptibility to Mtb infection and determine the variability of clinical presentation of pulmonary TB.

References

1. Flynn JL (2004) Immunology of tuberculosis and implications in vaccine development. *Tuberculosis (Edinb)* 84:93-101.
2. Tufariello JM, Chan J, Flynn JL (2003) Latent tuberculosis: mechanisms of host and bacillus that contribute to persistent infection. *Lancet Infect Dis* 3:578-590.
3. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR (1993) An essential role for interferon γ in resistance to Mycobacterium tuberculosis infection. *J Exp Med* 178:2249-2254.
4. Orme IM, Roberts AD, Griffin JP, Abrams JS (1993) Cytokine secretion by CD4 T lymphocytes acquired in response to Mycobacterium tuberculosis infection. *J Immunol* 151:518-525.
5. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeiffer K, Lowenstein CJ, Schreiber R, Mak TW, Bloom BR (1995) Tumor necrosis factor- α is required in the protective immune response against Mycobacterium tuberculosis in mice. *Immunity* 2:561-572.
6. Mohan VP, Scanga CA, Yu K, Scott HM, Tanaka KE, Tsang E, Tsai MM, Flynn JL, Chan J (2001) Effects of tumor necrosis factor alpha on host immune response in chronic persistent tuberculosis: possible role for limiting pathology. *Infect Immun* 69:1847-1855.
7. Rich EA, Torres M, Sada E, Finegan CK, Hamilton BD, Toossi Z (1997) Mycobacterium tuberculosis (MTB)-stimulated production of nitric oxide by human alveolar macrophages and relationship of nitric oxide production to growth inhibition of MTB. *Tuber Lung Dis* 78:247-255.
8. MacMicking JD, Taylor GA, McKinney JD (2003) Immune control of tuberculosis by IFN- γ -inducible LRG-47. *Science* 302:654-659.
9. Curtis MM, Way SS (2009) Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. *Immunology* 126:177-185.
10. Fouser LA, Wright JF, Dunussi-Joannopoulos K, Collins M (2008) Th17 cytokines and their emerging roles in inflammation and autoimmunity. *Immunol Rev* 226:87-102.
11. Doe C, Bafadhel M, Siddiqui S, Desai D, Mistry V, Rugman P, McCormick M, Woods J, May R, Sleeman MA, Anderson IK, Brightling CE (2010) Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. *Chest* 138:1140-1147.
12. Wilson NJ, Boniface K, Chan JR, *et al.* (2007) Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* 8:950-957.
13. Volpe E, Servant N, Zollinger R, Bogiatzi SI, Hupé P, Barillot E, Soumelis V (2008) A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. *Nat Immunol* 9:650-657.
14. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F (2007) Interleukins 1 beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17 producing human T helper cells. *Nat Immunol* 8:942-949.
15. Laan M, Cui ZH, Hoshino H, Lötval J, Sjöstrand M, Gruenert DC, Skoogh BE, Lindén A (1999) Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J Immunol* 162:2347-2352.
16. Scriba TJ, Kalsdorf B, Abrahams DA, Isaacs F, Hofmeister J, Black G, Hassan HY, Wilkinson RJ, Walzl G, Gelderbloem SJ, Mahomed H, Hussey GD, Hanekom WA (2008) Distinct, specific IL-17- and IL-22-producing CD4⁺ T cell subsets contribute to the human anti-mycobacterial immune response. *J Immunol* 180:1962-1970.
17. Dheda K, Chang JS, Lala S, Huggett JF, Zumla A, Rook GA (2008) Gene expression of IL17 and IL23 in the lungs of patients with active tuberculosis. *Thorax* 63:566-568.

18. Chen X, Zhang M, Liao M, Graner MW, Wu C, Yang Q, Liu H, Zhou B (2010) Reduced Th17 response in patients with tuberculosis correlates with IL-6R expression on CD4⁺ T Cells. *Am J Respir Crit Care Med* 181(7):734-742.
19. Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, Cilley GE, Shen F, Eaton SM, Gaffen SL, Swain SL, Locksley RM, Haynes L, Randall TD, Cooper AM. (2007) IL-23 and IL-17 in the establishment of protective pulmonary CD4⁺ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. *Nat Immunol* 8:369-377.
20. Vankayalapati R, Wizel B, Weis SE, Klucar P, Shams H, Samten B, Barnes PF (2003) Serum cytokine concentrations do not parallel Mycobacterium tuberculosis-induced cytokine production in patients with tuberculosis. *Clin Infect Dis* 36:24-28.
21. Hirsch CS, Toossi Z, Othieno C, *et al.* (1999) Depressed T-cell interferon-gamma responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. *J Infect Dis* 180:2069-2073.
22. Kumar NP, Anuradha R, Suresh R, Ganesh R, Shankar J, Kumaraswami V, Nutman TB, Babu S (2011) Suppressed type 1, type 2, and type 17 cytokine responses in active tuberculosis in children. *Clin Vaccine Immunol* 18(11):1856-1864.
23. Sodhi A, Gong J, Silva C, Qian D, Barnes PF (1997) Clinical correlates of interferon gamma production in patients with tuberculosis. *Clin Infect Dis* 25:617-620.
24. Dlugovitzky D, Bay ML, Rateni L, Fiorenza G, Vietti L, Farroni MA, Bottasso OA (2000) Influence of disease severity on nitrite and cytokine production by peripheral blood mononuclear cells (PBMCs) from patients with pulmonary tuberculosis (TB). *ClinExpImmunol* 122:343-349.
25. Surcel HM, Troye-Blomberg M, Paulie S, Andersson G, Moreno C, Pasvol G, Ivanyi J (1994) Th1/Th2 profiles in tuberculosis, based on the proliferation and cytokine response of blood lymphocytes to mycobacterial antigens. *Immunology* 81:171-176.
26. Demissie A, Abebe M, Aseffa A, Rook G, Fletcher H, Zumla A, Weldingh K, Brock I, Andersen P, Doherty TM; VACSEL Study Group (2004) Healthy individuals that control a latent infection with Mycobacterium tuberculosis express high levels of Th1 cytokines and the IL-4 antagonist IL-4delta2. *J Immunol* 172:6938-6943.
27. Lienhardt C, Azzurri A, Amedei A, Fielding K, Sillah J, Sow OY, Bah B, Benagiano M, Diallo A, Manetti R, Manneh K, Gustafson P, Bennett S, D'Elia MM, McAdam K, Del Prete G. (2002) Active tuberculosis in Africa is associated with reduced Th1 and increased Th2 activity in vivo. *Eur J Immunol* 32:1605-1613.
28. Dlugovitzky D, Bay ML, Rateni L, Urizar L, Rondelli CF, Largacha C, Farroni MA, Molteni O, Bottasso OA (1999) In vitro synthesis of interferon-gamma, interleukin-4, transforming growth factor-beta and interleukin-1 beta by peripheral blood mononuclear cells from tuberculosis patients: relationship with the severity of pulmonary involvement. *Scand J Immunol* 49:210-217.
29. Jurado JO, Pasquinelli V, Alvarez IB, Peña D, Rovetta AI, Tateosian NL, Romeo HE, Musella RM, Palmero D, Chuluyán HE, García VE (2012) IL-17 and IFN- γ expression in lymphocytes from patients with active tuberculosis correlates with the severity of the disease. *J Leukoc Biol* 91(6):991-1002.
30. Marín ND, París SC, Rojas M, García LF (2012) Reduced Frequency of Memory T Cells and Increased Th17 Responses in Patients with Active Tuberculosis. *Clin Vaccine Immunol* 19(10):1667-1676.
31. Pasquinelli V, Townsend JC, Jurado JO, Alvarez IB, Quiroga MF, Barnes PF, Samten B, García VE (2009) IFN-gamma Production during Active Tuberculosis Is Regulated by Mechanisms That Involve IL-17, SLAM, and CREB. *J Infect Dis* 199:661-665.
32. Stern J, Keskin DB, Romero V, Zuniga J, Encinales L, Li C, Awad C, Yunis EJ (2009) Molecular signatures distinguishing active from latent tuberculosis in peripheral blood mononuclear cells, after in vitro antigenic stimulation with purified protein derivative of tuberculin (PPD) or Candida: a preliminary report. *Immunol Res* 45:1-12.
33. Sargentini V, Mariotti S, Carrara S, Gagliardi MC, Teloni R, Goletti D, Nisini R (2009) Cytometric detection of antigen-specific IFN- γ /IL-2 secreting cells in the diagnosis of tuberculosis. *BMC Infect Dis*
34. Cowan J, Pandey S, Filion LJ, Angel JB, Kumar A, Cameron DW (2012) Comparison of interferon-gamma, interleukin (IL)-17 and IL-22-expressing CD4⁺ T cells, IL-22-expressing granulocytes and proinflammatory cytokines during latent and active tuberculosis infection. *Clin Exp Immunol* 167:317-329.
35. Babu S, Bhat SQ, Kumar NP, Kumaraswami V, Nutman TB (2010) Regulatory T Cells Modulate Th17 Responses in Tuberculin skin test positive (TST+) individuals. *J Infect Dis* 201(1):20-31.
36. Ehlers S (2010) DC-SIGN and mannosylated surface structures of Mycobacterium tuberculosis: a deceptive liaison. *Eur J Cell Biol* 89(1): 95-101.