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Low Antigen-specific T-cell Response in Pulmonary Tuberculosis is Associated with Impaired Phenotype and Functions of Interferon-α Induced Dendritic Cells

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Abstract

The phenotype and functions of monocyte-derived dendritic cells (DCs), generated upon stimulation with GM-CSF and IFN-α, were investigated in pulmonary tuberculosis (TB). Patient INF-DC cultures were characterized by increased number of $CD14^+$ cells, lowered level of $CD25^+$ cells and increased expression of B7-H1. Besides, DCs produced enhanced levels of IL-6 and IL-10 and differed by reduced secretion of INF-γ and INF-α. Patient IFN-DCs had lower capacity to stimulate allogeneic T-cell proliferation and induce $CD3^+IFN\text{-}\gamma^+$ T cells but increased ability to activate $CD3^+IL\text{-}4^+$ T cells in mixed lymphocyte culture. The most pronounced increase of IL-10 production, as well as the decrease of allostimulatory activity and the alteration of T1/T2 stimulatory activity of DCs were registered in patients with low PPD-induced proliferative response. The data obtained evidence the tolerogenic phenotype of monocyte-derived IFN-DCs and the association of DC impairment with decreased antigen-specific T cell response in TB patients.

Keywords: Pulmonary tuberculosis; Dendritic cells; Interferon-α

Introduction

Pulmonary tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is still remaining one of the most widespread infectious diseases. Pathogenesis and disease course are in part determined by immune system impairments specifically by the defective antigen-specific T-cell response. The deficiency of antigen-specific response in patients with TB is manifested by skin test anergy to tuberculin purified protein derivative (PPD), as well as decrease of T-cell proliferation and interferon-γ (IFN-γ) production in PPD-stimulated cultures [1]. The suppression of antigen-specific immune response is registered on average in 40% of TB patients, and is associated with anergy and increased T-cells apoptosis, as well as generation of regulatory T-cells [2,3].

One of the possible causes of impaired cell immunity in pulmonary TB may be the defect of dendritic cells (DCs), known to be the most potent professional cells, capable of antigen presentation and activation of naive T-cells [4]. In human, the DC precursors are located in bone marrow. Besides, the important source of DCs is the circulating monocytes, which can differentiate into DCs upon stimulation with various cytokines. *In vitro* DCs are effectively generated by culturing of monocytes upon of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) followed by maturation with lipopolysaccharide (LPS) or proinflammatory cytokines [5,6]. Besides, the monocytes may differentiate into DCs when cultured with GM-CSF and interferon- α (IFN-α) [7,8]. This way seems to be more natural, because IFN-α belongs to cytokines of the "first wave" that are produced in response to different pathogen stimuli and play the important role in TB [9,10]. IFN-α induced DCs (IFN-DCs) show the high engulfing activity, keep stable in the absence of cytokines and exceed the IL-4-DCs in migratory activity and ability to stimulate CD8 T-cells [7,11-13].

DCs play an important role in triggering and maintaining of immune response to *M. tuberculosis* [14,15]. On the other hand, there are evidences of impaired DC functions in TB infection. Dysfunctions of the DCs in pulmonary TB may be result of the direct influence of mycobacterial antigens on DCs or monocytes (being the potential source of DCs). For example, Hanekom et al. showed that DCs infected with *M. tuberculosis* are characterized by reduced expression of activation/maturation markers (CD25 and CD83) and decreased capacity to stimulate T-cell proliferation [16]. The impaired maturation and functional activity of DCs which consists in increased production of TNF-α and IL-10 along with decreased ability to induce T-cell proliferation and functional polarization of T-cells was also demonstrated for DCs generated from monocytes infected with *M. tuberculosis* [17].

As for TB patients, the data concerning the phenotype and functions of monocyte-derived DCs are few. For example, Rajashree et al. showed that TB patient DCs generated under GM-CSF and IL-4 displayed the low expression of CD1a, MHC II, CD80 and CD83, decrease allostimulatory activity, high production of TNF-α and IL-6 and low level of IL-12 [18]. Data on IFN-DCs in TB patients are still absent. Therefore, the present study was aimed to assess the phenotype and functions of IFN-DCs in patients with pulmonary TB. We also attempted to clear up whether IFN-DCs differed in PPD-anergic and reactive patients in order to evaluate the potential role of DCs in T-cell dysfunctions during active pulmonary tuberculosis.

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Materials and Methods

Study subjects

Ninety TB patients (47 males and 43 females aged from 20 to 64 years) were recruited for the study including 60 subjects with fibrocavernous TB, 24 - infiltrative and 6 - disseminated TB. Positive for *M. tuberculosis* (Mtb+) sputum specimens were revealed in 61 patients (68%). Multidrug resistance (MDR) was registered in 31 patients (34%). The TB patients underwent to the standard antimicrobial treatment, including first-line drugs (combination of tubazid, rifampicin, streptomycin, ethambutol and pyrazinamide) and in patients with MDR – the second-line drugs (the combination of fluoroquinolones with amikacin or kanamycin, capriomycin, cycloserine). The control group included 38 sex and age matched healthy donors. The signed informed consent was obtained before the examination from all the patients.

Cell isolation and evaluation of proliferative activity

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by density gradient centrifugation using Ficoll-Verografin and cultured at 0.1×10^6/well in 96-well plates (Nunc, Denmark) in RMPI-1640 (Sigma-Aldrich, USA) medium with 0.3 mg/ml L-glutamine, 5 mM HEPES-buffer, 100 μg/ml gentamicin and 10% inactivated human AB (IV) serum at 37°C in CO_2 incubator. To stimulate cell proliferation, the PPD ("Biopreparat", Russia) was used at 50 μg/ml. The proliferation rate was estimated on 6th day based on 3H-thymidine incorporation added during the last 18 hours.

DC generation and phenotyping

The DCs were obtained by 4-days cultivation of adherent fraction of PBMCs in RPMI-1640/5% FCS with human recombinant GM-CSF (40 ng/ml, Sigma-Aldrich) and IFN-α (1,000 U/ml, Roferon-A, Roche, Switzerland) followed by maturation with lipopolysaccharide (LPS, 10 μg/ml, Sigma-Aldrich) for 24 h. Analysis of IFN-DC phenotype was performed by one- or two-color flow cytometry assays using FITC-, PE- or APC-conjugated antibodies (CD1a, CD11c, CD14, CD25, CD83, CD123, B7-H1; Pharmingen, USA).

Cytokine measurements

The cytokines were assessed in 5-day DC culture supernatants which were collected and stored at -80°C until measurement. The concentrations of TNF-α, IFN-γ IFN-α, IL-6, IL-10 and IL-18 were evaluated by commercial ELISA kits ("Vector-Best", Russia). The production of IFN-α was measured in DC cultures which were washed after 4 days of culture to remove the residual IFN-α and then maturated for the last 48 h with LPS (10 μg/ml).

Intracellular cytokine assay

The intracellular cytokine expression was evaluated in T-cells stimulated with allogeneic DCs using 3-color flow cytometry (FASC Calibur, Becton Dickinson). For this, donor PBMCs devoid of adherent cells were cultured with or without DCs (control) of healthy donors and TB patients in the ratio 10:1 for 5 days. Subsequently, MLC cultures were re-stimulated with the same DCs for additional 48 hours (secondary MLC). Brefeldin A (10 μg/ml, ICN) was added to the cultures during the last 18 h to accumulate most of the cytokine in the Golgi complex. Then the cells were collected, treated with APC-labeled anti-CD3 monoclonal antibodies and fixed with 1% paraformaldehyde. Cell permeabilization was performed using 0.2% Tween-20 solution. After this, the cells were treated with FITC-labeled anti-IFN-γ monoclonal antibodies and PE-labeled anti-IL-4 monoclonal antibodies (Becton Dickinson, USA). Samples were analyzed by flow cytometer using the CellQuest Software. Stimulation index (SI) was calculated as number of IFN^+CD3^+ cells in DC-stimulated cultures/number of IFN^+CD3^+ cells in cultures without DCs.

Allostimulatory activity of DCs

To assess the allostimulatory activity in mixed lymphocyte culture (MLC), donor and patient DCs, generated as described above, were washed and cultured with allogeneic donor PBMCs (0.1×10^6/well) at 1:10 for 5 days in 96-well plates at 37°C. The intensity of proliferation was assessed by ^{3}H-thymidine incorporation.

Statistical analysis

The statistical analysis was carried out using software package "Statistica 6.0". To reveal significant difference of values compared, non-parametric Wilcoxon-Mann-Whitney U-test was employed. The level of $p<0.05$ was considered significant. Spearman rank correlation was used to investigate relationships between characteristics.

Results

Characteristics of PPD-reactive and PPD-anergic TB patients

In healthy donors PPD-induced proliferative response of PBMCs was averaged 28,450 ± 4,100 cpm (with median 22,300 and the interquartile range from 12,500 to 33,800 cpm). TB patients (n=90) differed by a distinct tendency to decrease in PPD-reactivity of T-cells (20,500 ± 1,900 cpm; Me 15,580 cpm; IR 9,100–30,450 cpm; pU=0.06). Among them, 40 patients had T-cell proliferative response which did not exceed the low quartile of normal range (<12,500 cpm; "PPD-anergic" patients) while 50 patients showed proliferative response above 12,500 cpm ("PPD-reactive" patients) (Table 1). The mean value of PPD-response in PPD-anergic patients was virtually 4.5 times lower than values of both healthy donors and PPD-reactive TB patients. Despite the differences in PPD-response the patients of the opposite groups did not differ significantly in age, gender, the number of sputum smear positive cases, clinical forms of pulmonary TB and drug resistance.

Phenotype of cultured IFN-DCs

As compared with the healthy donors, the patient IFN-DCs were notable for the higher level of $CD14^+$ cells (Table 2). Of note, in PPD-anergic patients the number of $CD14^+$ cells in IFN-DC cultures was 2 times higher than in PPD-reactive patients, and 4 times exceeded the mean values of $CD14^+$ cells in healthy donors. Besides, regardless of the PPD-reactivity, patient DCs were characterized by lower level of $CD25^+$ cells known to be the marker of mature/activated DCs [16]. However, we did not find any differences in the number of $CD1a^+CD83^-$ (immature), $CD1a^+CD83^+$ (semi-mature) and $CD1a^-CD83^+$ (mature) cells in IFN-DC cultures of TB patients and healthy donors.

IFN-DCs are known to express CD123 molecule, which is the specific marker of plasmacytoid DCs [7,19,20]. On the other hand, the expression of this marker by myeloid DCs may reflect the immaturity of monocyte-derived IFN-DCs, since the CD123 is decreased during DC differentiation/maturation, and this process is inhibited by IFN-γ. The number of $CD123^+$ cells in DC cultures of TB subjects and healthy donors did not differ. In addition to stage-specific markers, we also

Parameters	TB patients (n=90)	PPD-responsive patients (n=50)	PPD-anergic patients (n=40)
Age, years (M ± SE)	34.8 ± 1.6	34.7 ± 2.2	34.5 ± 2.6
Male	47 (52%)	27 (54%)	20 (50%)
Female	43 (48%)	23 (46%)	20 (50%)
Sputum smear positive cases (%)	61 (68%)	34 (68%)	27 (68%)
Forms of TB:			
- fibrocavernous	60 (67%)	35 (70%)	25 (63%)
- infiltrative	24 (27%)	11 (22%)	13 (33%)
- disseminated	6 (7%)	4 (8%)	2 (5%)
Drug resistance (MDR+)	31 (34%)	17 (34%)	14 (35%)
PPD-induced proliferative response of PBMCs, cpm (M ± SE)	20,500 ± 1,900	27,680 ± 2,300	6,230 ± 1,130 *

Note: * - pU<0.05 vs PPD-responsive patients.

Table 1: Characteristics of TB patients.

Marker (% cells)	Healthy donors (n=13)	TB patients		
		The total group (n=27)	PPD-responsive (n=17)	PPD-anergic (n=10)
$CD14^+$	8.7 ± 1.4	19.6 ± 2.5 *	17.2 ± 2.7 *	30.0 ± 5.4 *#
$CD25^+$	25.1 ± 3.5	9.6 ± 1.2 *	8.8 ± 1.3 *	12.1 ± 2.9 *
$CD1a^+$	11.8 ± 2.5	11.8 ± 1.4	10.7 ± 1.4	14.6 ± 3.7
$CD83^+$	23.3 ± 2.7	32.4 ± 2.9	34.2 ± 3.7	27.8 ± 3.4
$CD11c^+$	28.3 ± 2.6	30.2 ± 2.7	29.5 ± 3.2	34.2 ± 5.3
$CD123^+$	27.5 ± 2.3	25.2 ± 1.8	24.0 ± 2.0	25.0 ± 2.7
$CD1a^+CD83^-$	6.9 ± 2.0	5.3 ± 0.8	4.5 ± 0.7	7.5 ± 2.0
$CD1a^+CD83^+$	5.0 ± 1.0	6.5 ± 1.0	6.2 ± 1.1	7.1 ± 2.3
$CD1a^-CD83^+$	18.9 ± 2.6	25.9 ± 2.8	28.0 ± 3.6	20.7 ± 2.9

Note: Analysis of IFN-DC phenotype was performed by one- or two-color flow cytometry assays using FITC-, PE- or APC-conjugated antibodies (CD1a, CD11c, CD14, CD25, CD83, CD123; Pharmingen, USA). * - pU <0.05 vs healthy donors; # - pU <0.05 vs PPD-responsive patients.

Table 2: Immunophenotypic characteristics of patient IFN-DCs.

investigated the expression of co-inhibitory B7-H1 (PD-L1) molecule which mediates negative signals upon interactions with PD-1 on T-cells. As shown in Figure 1, the patient DC cultures contained significantly higher numbers of the $B7\text{-}H1^+$ cells as compared with the donor DCs (61.0 ± 2.9 vs 42.3 ± 4.4%, respectively; pU <0.05). Increased level of $B7\text{-}H1^+$ DCs was seen in both PPD-reactive and PPD-anergic patients, though PPD-anergic patients were characterized by more pronounced increase in $B7\text{-}H1^+$ DCs.

Cytokine production by IFN-DCs

The analysis of DC-derived cytokines showed that patient IFN-DCs exhibited significantly lower IFN-γ and IFN-α production and increased level of IL-6 and IL-10, as compared with the donor IFN-DCs (Figure 2), but we did not found significant differences in production of IL-18 and TNF-α between patient and donor DCs. The decreased IFN- γ production was shown in cultured DC of both PPD-reactive and PPD-anergic patients while secretion of IFN- α was significantly reduced in PPD-anergic patients. The enhancement of IL-6 production was mainly observed in PPD-reactive patients. At the same time the level of IL-10 was shown to be increased in patients with both decreased and preserved T-cell proliferative response to PPD, though PPD-anergic patients differed by the highest IL-10 level (pU<0.05). Notably, IL-10 directly correlated with B7-H1 expression both in total group of studied patients (r=0.51; p=0.0004; n=44) and in healthy donors (r=0.82; p=0.0005; n=13). Such strong correlation evidently indicates the participation of IL-10 in autocrine regulation of B7-H1 molecule expression on DCs.

Lymphocyte proliferation induced by IFN-DCs

Evaluation of the allostimulatory activity revealed that the ability of patient IFN-DCs to stimulate T cell proliferation in MLC was significantly lower compared with donor DCs (Figure 3). The impairment of allostimulatory activity was most prominent in PPD-anergic patients. The MLC proliferative response stimulated by DCs from PPD-reactive patients was on average 6,173±1,126 cpm (from 1,446 to 28,446 cpm), whereas the level of the MLC proliferative response induced by DCs of PPD-anergic patients was 3 times lower (1,983 ± 366 cpm).

Activation of T cells producing IFN-γ and IL-4 by cultured IFN-DCs

To assess the capacity of IFN-DCs to activate T-cell type 1 and type 2 responses we studied the number of $CD3^+$ T-cells with intracellular IFN-γ and IL-4 in a secondary MLC, induced by patient and donor DCs (Table 3). Evidently, the donor IFN-DCs possessed mainly T1 stimulatory activity, since induced more than 4-fold increase of $CD3^+IFN^-\gamma^+$T-cells, and did not influence the number of $CD3^+IL\text{-}4^+$T-cells. As compared to donor DCs, DCs from TB patients, especially those of PPD-anergic subjects, were characterized by lower capacity to stimulate $CD3^+IFN\text{-}\gamma^+$T-cells, still having the increased ability to activate the $CD3^+IL\text{-}4^+$T-cells.

Discussion

Recent studies provided a lot of evidence that antigens of *M. tuberculosis* inhibit differentiation of human DCs and suppress their functions [17,21,22]. These data along with findings on impaired DC functions in *M. tuberculosis* infection in experimental models suggest that DC dysfunctions may result to the decrease of antigen-specific T-cell response in patients with active TB. Nevertheless, the issues concerning the circulating and/or monocyte-derived DCs in TB patients remain virtually unstudied.

In the present study we have characterized for the first time the phenotype and functions of monocyte-derived DCs generated in the presence of GM-CSF and IFN-α from peripheral blood of TB patients, and have compared DCs in PPD-anergic and PPD-reactive patients. Our findings revealed that patient DCs had an increased CD14 and decreased CD25 expression thus indicating an impairment of differentiation and activation/maturation of patient DCs. The data obtained are consistent with those of Rajashree et al., which studied monocyte-derived DCs generated in presence of GM-CSF and IL-4 and found the inhibition of DC differentiation/maturation in pulmonary TB patients [18]. Besides, we for the first time have demonstrated that patient DCs had the increased expression of B7-H1 (PD-L1), known to induce T cell apoptosis and anergy upon interactions with PD-1 on T-cells [23]. The impairment of IFN-DC differentiation/activation in TB patients was associated with marked DCs dysfunction, in particular with altered cytokine production, decreased allostimulatory activity and the shift toward T2-stimulatory activity. Importantly, these phenotypic and functional alterations were most pronounced in PPD-anergic patients.

Rajashree et al. also found the increased IL-6 production by

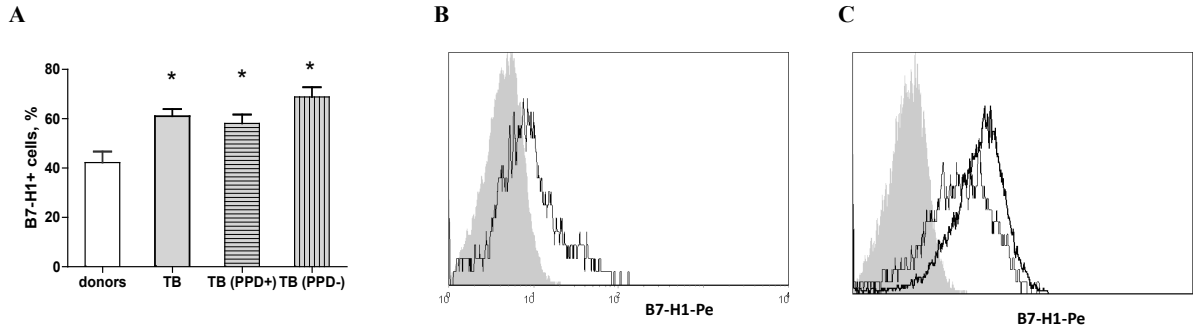

Figure 1: B7-H1 expression in patient and donor DC cultures. A. Data are presented as percentage of B7-H1$^+$ DCs (M ± S.E.) in DC cultures of healthy donors (donors; n=19) and TB patients (TB; n=44), including PPD-reactive (PPD+; n=32) and PPD-anergic patients (PPD-; n=12); *-p_U<0.05 vs healthy donors. **B.** Open histogram represents stained cells (donor DCs) and the filled histogram represents isotype specific control. **C.** B7-H1 expression in PPD-reactive (grey line) and PPD-anergic (black line) patient DC cultures.

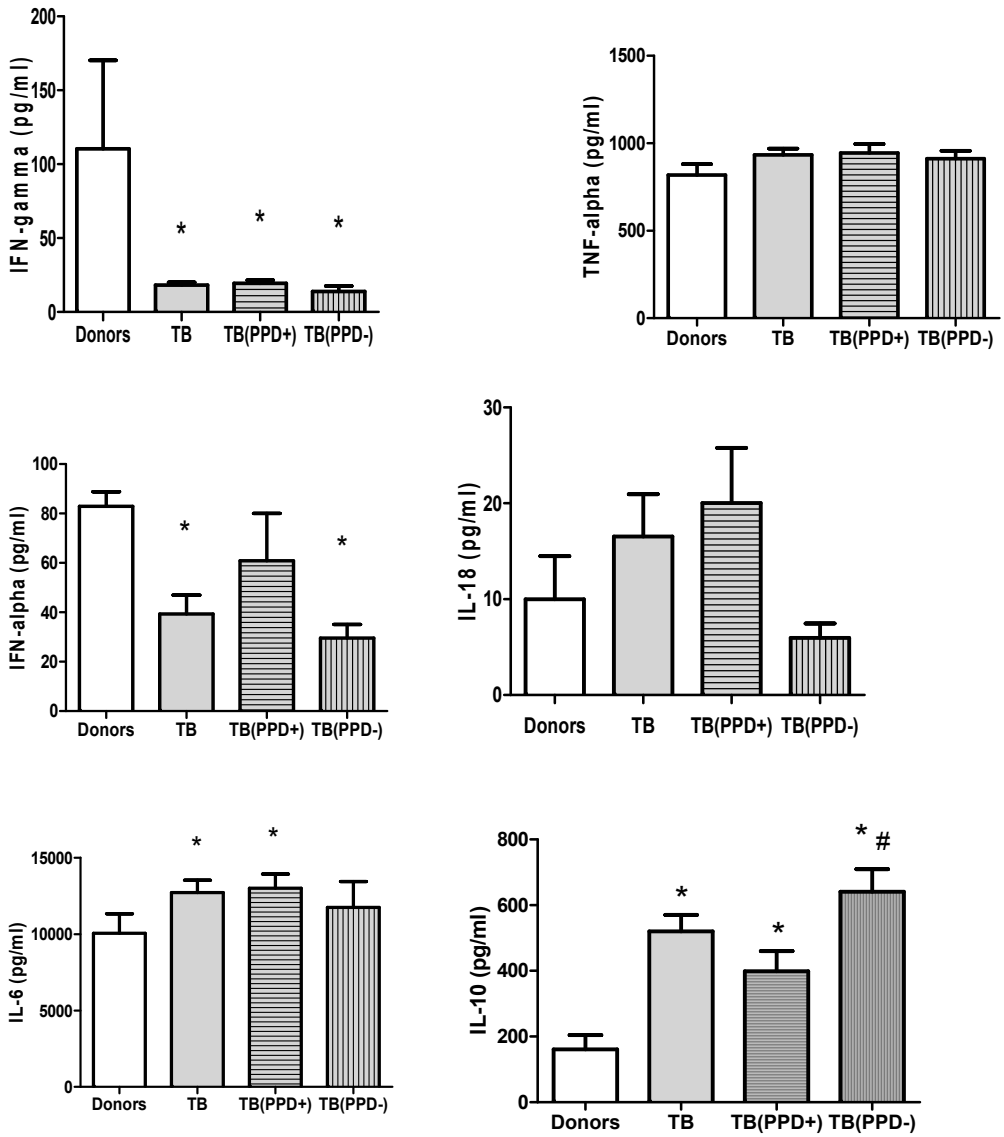

Figure 2: Production of cytokines by IFN-DCs. The results are expressed as M ± S.E. The levels of IFN-γ, TNF-α, IFN-α, IL-18, IL-6 and IL-10 in 5-day DC culture supernatants were determined by ELISA in healthy donors (n=20) and TB patients (n=40), including PPD-reactive (PPD+; n=24) and PPD-anergic patients (PPD; n=16). *-p_U<0.05 vs healthy donors; #-p_U<0.05 vs PPD-reactive patients.

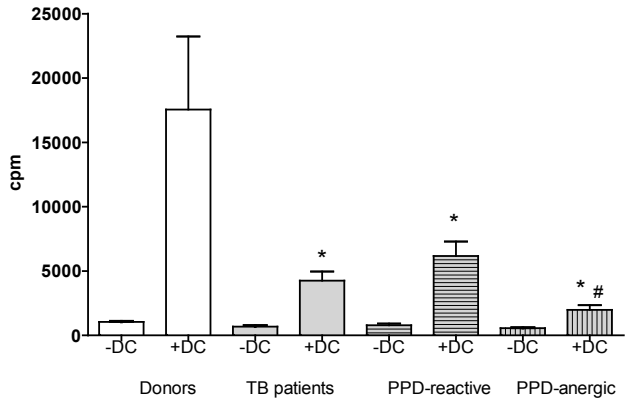

Figure 3: Induction of PBMCs proliferation by cultured DCs. To test the allostimulatory activity, DCs of healthy donors (donors; n=24) and TB patients (TB; n=55), including PPD-reactive (PPD+; n=31) and PPD-anergic (PPD-; n=24) patients were cultured with allogeneic PBMCs at a stimulator/responder ratio of 1:10 for 5 days. Control cultures included PBMCs alone (-DC). The data are expressed as M±S.E. *-p_U<0.05 vs healthy donors; #-p_U<0.05 vs PPD-reactive patients.

IFN-DCs	$CD3^+IFN\text{-}\gamma^+$ T-cells (%)			$CD3^+IL\text{-}4^+$ T-cells (%)		
	0	+DC	SI	0	+DC	SI
Healthy donors (n=20)	1.9 ± 0.3	6.1 ± 0.5	4.8 ± 0.9	1.8 ± 0.1	2.2 ± 0.3	1.3 ± 0.2
TB patients (n=36)	1.9 ± 0.1	3.1 ± 0.4*	1.8 ± 0.2*	2.0 ± 0.2	4.7 ± 0.6*	2.2 ± 0.2*
PPD-responsive patients (n=17)	1.7 ± 0.1	3.7 ± 0.6	2.4 ± 0.3*	1.8 ± 0.2	2.8 ± 0.4	1.6 ± 0.1
PPD-anergic patients (n=19)	2.1 ± 0.1	2.7 ± 0.5*#	1.3 ± 0.2*#	2.4 ± 0.3	6.7 ± 1.1*#	2.8 ± 0.3*#

Note: The percentage of $CD3^+$T-cells with intracellular expression of IFN-γ ($CD3^+IFN\text{-}\gamma^+$T-cells) and IL-4 ($CD3^+IL\text{-}4^+$T-cells) was assessed in the cultures of donor PBMCs, depleted from monocytes (0), and activated with allogeneic DCs (+DC) during 5 days and than re-stimulated with the same DCs for additional 48 h. SI: Stimulation Index of DCs; *-p_U<0.05 vs healthy donors; #-p_U<0.05 vs PPD-reactive patients

Table 3: The capacity of cultured IFN-DCs to stimulate $CD3^+IFN\text{-}\gamma^+$ and $CD3^+IL\text{-}4^+$ T-cells in MLC.

monocyte-derived DCs obtained in cultures with GM-CSF and IL-4, but they did not reveal the enhancement of IL-10 [18]. On the other hand, *M. tuberculosis* was shown to induce IL-10 production in donor IFN-DC cultures [17]. With regard to IFN-α production, Lichtner M. et al. have demonstrated the decrease of circulating DCs and IFN-α production in patients with active tuberculosis, and restoration of those parameters after effective anti-tuberculous treatment. Given these data, the decreased production of IFN-α in anergic patients may be considered as an unfavorable marker associated with the active course of the TB infection [20].

The high level of IL-10 in patient DC cultures founded in our study may underline the impaired DC differentiation and increased expression of B7-H1. Indeed, the immature myeloid DCs are known to be characterized by high IL-10 production [24,25]. Besides, it is shown that IL-10 enhances the B7-H1 expression on myeloid DCs, generated in the presence of IL-4 [26]. Concerning this fact, our findings on the direct correlation between production of IL-10 and expression of B7-H1 confirm the hypothesis about possible involvement of IL-10 in the regulation of B7-H1 expression in IFN-DCs as well.

The elevated level of IL-10 may also results in low allostimulatory activity of patient DCs through the decrease of co-stimulatory molecules [27]. In fact, the low allostimulatory activity associated with decreased expression of MHC II and CD80 was also demonstrated for patient DCs generated in the presence of GM-CSF and IL-4 [18]. In addition, the current study suggests that low T cell proliferation in MLC induced by patient DCs may be also determined by high expression of B7-H1 capable of inducing T cell apoptosis/anergy through triggering of B7-H1/PD-1 pathway [26,28].

Our findings on the low ability of patient DCs to induce IFN-γ-secreting $CD3^+$T-cells and increased capacity to stimulate IL-4-secreting $CD3^+$T-cells, confirm the impaired regulatory functions of DCs in patients with pulmonary TB. Th1 response plays a key role in defense against *M. tuberculosis* [1,29]. From this point of view, the prevalent T2-stimulating activity of IFN-DCs we have found in patients with active TB and especially in PPD-anergic patients, suggests that the DC alterations may be the cause of Th1 deficiency in pulmonary tuberculosis. Generally, the decreased allostimulatory activity, the dominance of T2 stimulating activity and the increased production of IL-10 along with our previous data on pro-apoptogenic activity of patient DCs [23], give evidence of "tolerogenic" phenotype of monocyte-derived DCs in TB infection. The engagement of PD-1/PD-L1 (B7-H1) signaling pathway in DC cytotoxic activity was also demonstrated during tumor growth and in virus infections [28,30]. Possibly, the functioning of such DCs in vivo may also determine the generation of the regulatory $CD4^+$T-cells (T-reg) [31], the number of which is significantly increased and inversely correlates with antigen-specific T cell response in PPD-anergic patients [3].

The investigation of DCs in pulmonary TB is of interest not only in terms of basic research in TB infection, but also for the potential application of DCs in clinical practice. IFN-DCs seem to be an attractive candidate for cell-based therapy. Actually, IFN-DCs, as compared with the IL-4-induced DCs, are known for their high migration activity, stability in the absence of growth factors and low expression of DC-SIGN molecules, capable of binding *M. tuberculosis* [7,32]. The major promise of DC vaccines for induction of antigen-specific immune response is actively discussed not only in cancer, but also in chronic infections [33-35]. However, the clinical development of therapeutic vaccines for TB patients requires the preliminary testing and *in vitro* correction of impaired DC functions.

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