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# 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

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**Abstract** Purified protein derivative (PPD) or tuberculin skin testing is used to identify infected individuals with *Mycobacterium tuberculosis* (Mtb) and to assess cell-mediated immunity to Mtb. In the present study, we compared PBMC cultures in the presence of tuberculin or Candida antigens using cytokine bead arrays and RNA microarrays. Measurements of different cytokines and chemokines in supernatants of PMBC cultures in the presence of PPD showed increased levels of interferon (IFN)-$\gamma$ in active tuberculosis infection (ATBI) and latent TB infected (LTBI) compared to controls, and increased levels of TNF-$\alpha$ in ATBI compared with LTBI. Also, we found increase of IL-6 in cultures of PPD positive and controls but not in the cultures with Candida. We also report the molecular signature of tuberculosis infection, in ATBI patients, the following genes were found to be up-regulated and absent in LTBI individuals: two kinases (JAK3 and p38MAPK), four interleukins (IL-7, IL-2, IL-6, and IFN$\beta$1), a chemokine (HCC-4) a chemokine receptor (CxCR5), two interleukin receptors (IL-1R2 and IL-18R1), and three additional ones (TRAF5, Smad2, CIITA, and NOS2A). By contrast, IL-17 and IGFBP3 were significantly up-regulated in LTBI. And, STAT4, GATA3, Fra-1, and ICOS were down-regulated in ATBI but absent in LTBI. Conversely, TLR-10, IL-15, DORA, and IKK-$\beta$ were down-regulated in LTBI but not in ATBI. Interestingly, the majority of the up-regulated genes found in ATBI were found in cultures stimulated with tuberculin (PPD) or

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Candida antigens, suggesting that these pathogens stimulate similar immunological pathways. We believe that the molecular signature distinguishing active from latent tuberculosis infection may require using cytokine bead arrays along with RNA microarrays testing cell cultures at different times following in vitro proliferation assays using several bacterial antigens and PPD.

**Keywords** Latent tuberculosis (LTBI) · Active tuberculosis (ATBI) · IL-17 · Cytokines · Chemokines

## Background

Cellular immunity plays an important role in the response to infection with *Mycobacterium tuberculosis* (Mtb). Macrophages, T cells, and other cell types secrete pro-inflammatory cytokines, such as TNF-$\alpha$ and IFN-$\gamma$, in order to mediate control of infection with Mtb [1]. Pulmonary tuberculosis (TB) is diagnosed mainly by demonstration of the acid-fast bacilli on sputum samples. Tuberculin or purified protein derivative (PPD) skin testing is the main screening method used to test both latent tuberculosis infection (LTBI) or active tuberculosis infection (ATBI) [2].

It is generally accepted that primary infections with Mtb produce active disease in approximately 10% of those infected [3]. In the majority of infected persons, Mtb exists in the latent state, contained in the lungs, in the form of granulomas or Ghon complex. In this regard, generally unknown host immune system mechanisms are involved in preventing the disease progression and immune-compromised individuals are at a higher risk to reactivate the disease [4]. A precise definition of the latent state is lacking mainly because of the need to develop laboratory methods to distinguish active from latent TB as well as the mechanism involved in the protection from infection in highly exposed individuals.

However, several cell types have been reported to confer resistance to Mtb infection and to also lead to maintenance of the disease [4]. One major cell type involved in innate immunity and protection from TB are macrophages [5, 6]. Activated macrophage products such as the L-arginine dependent pathway [4, 7, 8] may protect by generation of reactive nitrogen intermediates (RNI) of nitric oxide (NO). Such mechanisms of cytotoxic activity are influenced by cytokines such as interferon gamma (IFN-$\gamma$) and tumor necrosis factor alpha (TNF-$\alpha$), as demonstrated in several mouse studies [9–11]. It is important to mention that the mycobacterium has developed evolutionary survival mechanisms in which the antimicrobial activity has been evaded [7, 8]. Mtb prevents fusion with the phagosomal molecules within the lysosomal compartment of macrophages [12–14].

T cells and antigen presenting cells (APC), such as macrophages and/or dendritic cells, and their interaction, may lead to the production of cytokines and chemokines and potentially to changes in gene expression profiles. It is suggested that infection is mainly regulated by Type 1 helper (Th1) cytokines. IFN-$\gamma$ is usually considered to be the major Th1 cytokine. In this regard, high levels of IFN-$\gamma$ have been detected in the lungs and bloodstream of patients with active TB [15–17] along with increased levels of IL-6 in the serum on these same patients [18]. However, the role of Th1 cytokines especially IL-17 and RNA microarrays in humans with active disease compared with persons with latent tuberculosis has yet to be investigated.

In this preliminary study, we performed gene chip micro-array and cytokine bead array (CBA) analyses of PBMC cultures stimulated with tuberculin or PPD and Candida antigens. Our aim was to compare the levels of cytokines in supernatants from these cultures and to

determine the gene expression profiles of both active and latent TB in order to identify genes permitting us to distinguish between these two presentations of TB infection.

## Methods

### Patients and control subject

This study was approved by the institutional review boards of Harvard University and the Dana Farber Cancer Institute, Boston, MA. The samples from three ATBI and three LTBI individuals were collected in Colombia, South America, and processed in Boston 12 h after collection. Patients with active tuberculosis had Mtb in the sputum, evidence of Ghon complex in chess X-rays, and positive delayed type hypersensitivity (DTH) by skin reaction to tuberculin. Individuals with latent TB had absence of Mtb in the sputum, evidence of Ghon complex in chess X-rays, and DTH by skin reaction to tuberculin. Three control subjects had normal X-rays and were negative for DTH to tuberculin.

### Proliferation assays

We isolated peripheral blood mononuclear cells (PBMC) from blood samples from each subject. PBMCs ($1 \times 10^5$ cells) were incubated with two different antigens: *Candida albicans* (20 μg/ml, Green laboratories, Lenoir, NC), and PPD or tuberculin (10 μg/ml, Mycos Research LLC, Loveland, CO) [19]. The cells from 5-day cultures were labeled with [$^3$H] Thymidine (1 μCi/well) for 12 h as previously performed [20–22]. Plates were then harvested and the radioactivity was monitored by Wallac liquid scintillation counter (Perkin-Elmer, Boston, MA). Supernatants (50 μl) of triplicate cultures were collected 24 h after antigen stimulation and the pools of these triplicate cultures were saved at −80°C for cytokine assays.

### RNA and Superarray

On day 5 of the proliferation assays, RNA from PBMC from one patient with active TB, one with latent TB, and one healthy control were isolated from the blood using RNA STAT 60 (Tel-Test, Inc., Friendswood, TX). The triplicate cultures were pooled and treated with DNase I (Qiagen). To determine the influence of PPD and Candida on the expression of genes including those involved in inflammation, GEArray S series human immune and inflammatory gene array kit (Superarray Bioscience, Frederic, MD), containing 364 known genes that encode for inflammatory chemokines, cytokines and their receptors, and T helper cell surface markers was used. RNA samples were reverse transcribed with AmpoLabelling LPR kit (Superarray Bioscience) according to the manufacturer's protocol to produce biotin-16 dUTP (Roche, Indianapolis, IN) labeled probes. Heat denatured probes were hybridized to array membranes at 60°C for 18 h in hybridization buffer containing 100 ng/ml heat denatured salmon sperm DNA (Sigma). Array membranes were washed and developed according to the manufacturer's protocol. Intensities of the spots were extracted from scanned negatives using ScanAlyze software (Eisen software). Gene expression profiles were analyzed using the Excel program as previously described [22]. All intensities were corrected to background (pUC spots) and normalized to GAPDH spots. Expression level of each gene from PBMC samples derived from TB patients was

normalized using the values of those demonstrating lack proliferation with either PPD or Candida; up-regulation or down-regulation higher than six-fold was considered significant.

### Cytokine and chemokine measurements in proliferation assay experiments by FACS analysis

Supernatants from from pools of triplicate 24-h cultures after antigen challenge were obtained from PBMC incubated in the presence or absence of PPD or Candida antigens of ATBI and LTBI individuals and uninfected controls. Levels of cytokine/chemokine production were measured using Th1/Th2 cytokine bead arrays according to the manufacturer's protocol (BD Biosciences, CA)[23]. Levels of cytokine production were determined by FACSCalibur (BD Biosciences, CA) and analyzed by CBA software (BD Biosciences, CA).

### Statistical analysis

An unpaired Student *t* test was used to determine the statistical significance for all comparisons (StataCorp). The changes in gene expression levels of the control with tuberculin or PPD and candida were analyzed and used to correct the responses of ATBI and LTBI to these antigens. For the RNA superarray, fold increases of seven-fold or higher were considered statistically significant for both the up-regulated and down-regulated genes.

## Results

### Proliferation studies using PBMC from TB patients and controls stimulate with PPD or Candida antigens

The 5-day cultures of three LTBI, three ATBI, and three controls cultured with PPD or Candida were compared with cultures of PBMC without antigens using $^{3}H$ incorporation expressed as stimulation index (SI) = $^{3}H$ uptake of cultures with antigen over cultures without antigen. The SI of both groups that were PPD-positive (LTBI and ATBI) treated with PPD was higher than the controls. However, subjects that were Candida-positive were not different (Fig. 1).

PBMC cultures incubated with tuberculin showed stimulation indexes of 4, 5, and 5.5 (mean of 4.8) in controls, and higher SI in PPD-positive individulas of 50, 53, and 96.3 (66.4) in ATBI and 108, 126.5, and 146 (126) in LTBI. By contrast, the SI using Candida was not different in the three groups: 17, 22.3, and 48 (29.1) in controls; 19, 27.9, and 58 (34.9) in ATBI and 11.7, 59.9, and 93.7 (55.1) (Fig. 1).

### Cytokine measurements

Measurements of cytokines in the supernatants of PBMC of patients with active (three patients) or latent disease (three individuals) demonstrated statistically significantly higher levels (tuberculin) of IFN-$\gamma$ in both ATBI and LTBI than controls ($P < 0.002$) based on measurement in ATBI of 1310, 5000, and 10000; in LTBI of 4691, 9822, and 9920 and in controls 16, 382, and 902 (Fig. 2). Those for Candida were lower in ATBI, 15, 26, and 31 than in LTBI (63, 248, 390 ($P = 0.05$)). Control values were 7, 146, and 202.

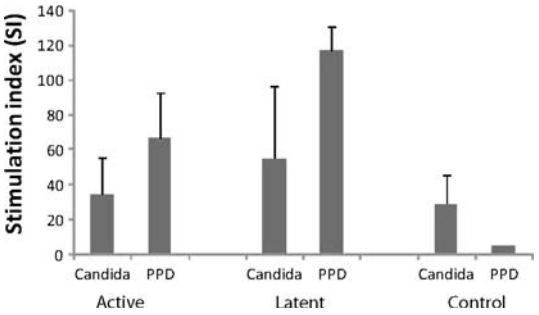


**Fig. 1** Proliferation studies using PBMC from TB patients stimulated with either PPD or Candida antigen. PBMC were obtained from the peripheral blood of three ATBI patients, three LTBI patients, and three control subjects cultured with two different antigens (PPD or Candida). Additional controls included PBMC alone. PBMC proliferation using $^{3}H$ incorporation is presented, by dividing this value and the corresponding control CPM without antigen, stimulation index (SI). There was higher SI in both groups of PPD positive (LTBI and ATBI) treated with PPD but responses to Candida were not different in the three groups

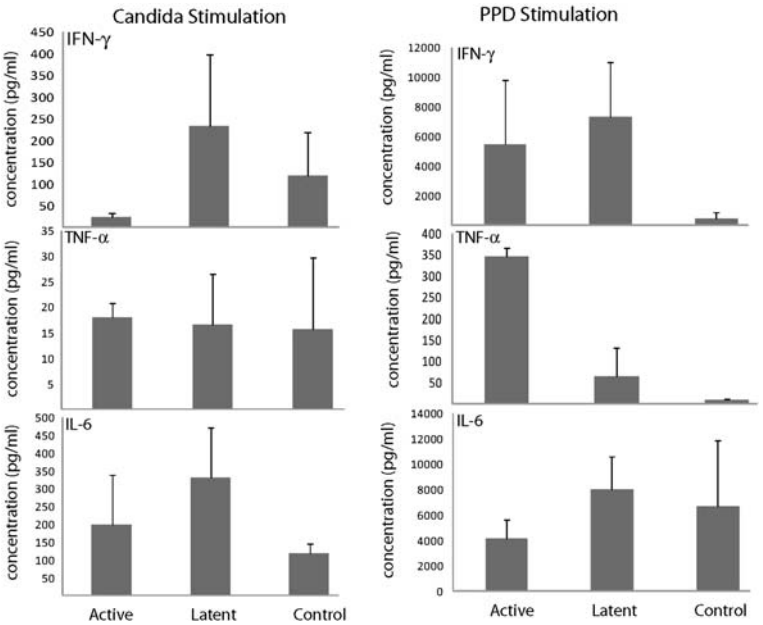


**Fig. 2** Measurements of cytokines in PMBC cultures after in vitro antigenic stimulation with tuberculin. Levels of IL-6, IFN-$\gamma$, and TNF-$\alpha$, from the triplicate supernatants of 24-h cultures of three ATBI patients, three LTBI, and three normal control subjects are presented. PPD cultures demonstrated higher levels of IFN-$\gamma$ in both ATBI and LTBI compared to controls ($P < 0.002$). By contrast, TNF-$\alpha$ was higher in the cultures of ATBI compared to LTBI ($P < 0.01$) and compared to controls ($P < 0.004$). An increased level of IL-6 in the cultures with tuberculin but not with Candida in the three groups of individuals tested that included the controls in the comparison ($P < 0.004$). Levels of IL-1$\beta$ and IL-12, IL-10, IL-4, and IL-12 were variable

Measurements of TNF-$\alpha$ for tuberculin cultures were higher in ATBI of 321, 345, and 366 than in LTBI of 25, 20, and 141 ($P < 0.01$) or negative controls 6, 10, and 336 ($P < 0.004$). But the measurements for Candida were not different for the three groups.

Measurements of IL-6 showed specific stimulation in the tuberculin cultures for the three groups since there was not significant production of this cytokine in the cultures with Candida (Fig. 2). However, the combined response of PPD versus Candida showed significant increase ($P < 0.004$). The levels of IL-12, IL-10, IL-4, and IL-2 were variable in ATBI and LTBI individuals and the uninfected control that did not respond to either PPD or Candida (data not shown).

Up-regulated genes in PBMC of ATBI cultured in the presence of PPD or Candida

Several cytokines, chemokines, and receptors were up-regulated from the patient with active TB but not in the LTBI or the control individuals in the presence of either PPD or Candida antigens. As shown in Table 1, cytokines and chemokines such as IL-7, IL-2, IL-6, IFN-$\beta$1, CXCL13, CXCR5, IL-18R1, p38 MAPK, and TRAF5 were up-regulated.

Up-regulated genes in the PBMC of the patient with active TB in the presence of PPD or Candida

PPD was used to examine whether there were differential expression of genes that would be up-regulated in a patient with active TB. PBMC incubated with PPD antigen from a patient with ATBI had several genes that were up-regulated. IL-8, CIITA, JAK3, Smad 2, TECK, CXCL13, and nitric oxide sythase (NOS) were up-regulated more than seven-fold only in the TB patient with active disease (Table 1).

Up-regulated genes in PBMC of an LTBI individual in the presence of either PPD or Candida

IL-17 was the only up-regulated gene product out of 364 genes in the PBMC of the LTBI individual. In the presence of PPD there was a nine-fold increase, whereas in the presence of Candida a 36-fold increase was demonstrated (Table 1).

Down-regulated genes in the PBMC of active and latent TB individuals

Several genes were differentially down-regulated in the ATBI individual versus LTBI individual in the presence of PPD: Fra-1 ICOS, STAT4 were down-regulated in the patient with active TB, in the LTBI individual; however, only two genes were down-regulated: IL-15 and dimethyl sulfoxide reductase A (DORA) (Table 2).

## Discussion

Mtb infection is controlled by the effective activation of macrophages and CD4+ T lymphocytes secreting Th1 cytokines such as IFN-$\gamma$, TNF-$\alpha$, and IL-6 [24]. It is known that IFN-$\gamma$ was shown to induce autophagic control of tuberculosis infection [25], thus influencing the waining of specific response with time after infection [26], and high levels of IFN-$\gamma$ have been found in the bloodstream [15, 27] and lungs [16, 17] of TB patients with active disease. Also, TNF-$\alpha$ is involved in the initial and long-term progression of TB and in macrophage activation along with recruitment of cells to the infected area [5]. Neutralization of this cytokine leads to lack of control of initial or chronic TB infection, and loss of granuloma structure [5]. Our results of increased secretion of this cytokine in the

**Table 1** Up-regulated messenger RNA analysis in the PBMC of TB patients incubated with etiher PPD or Candida

| Gene name | Symbol | Active TB | | Latent TB | | Control | |
|---|---|---|---|---|---|---|---|
| | | PPD | Candida | PPD | Candida | PPD | Candida |
| Kinases | | | | | | | |
| JAK3 | JAK3 | 8 | 2 | 1 | 0 | 1 | 1 |
| p38 MAPK | MAPK14 | 64 | 21 | 1 | 0 | 1 | 1 |
| Toll-like receptors | | | | | | | |
| TLR8 | TLR8 | 2 | 7 | 1 | 2 | 2 | 1 |
| Interleukins | | | | | | | |
| IL-7 | IL7 | 94 | 39 | 1 | 0 | 1 | 1 |
| IL-2 | IL2 | 27 | 13 | 1 | 1 | 1 | 1 |
| IL-6 | IL6 | 22 | 20 | 1 | 0 | 1 | 1 |
| IL-8 | IL8 | 11 | 5 | 1 | 1 | 1 | 0 |
| IFN-$\beta$1 | IFN$\beta$1 | 9 | 7 | 0 | 1 | 1 | 1 |
| IL-17 | IL17 | 0 | 0 | 9 | 36 | 1 | 1 |
| Interleukin receptors | | | | | | | |
| IL-1R2 | IL1R2 | 15 | 8 | 0 | 1 | 1 | 1 |
| IL18R1 | IL18R1 | 7 | 13 | 1 | 0 | 1 | 1 |
| IL-15Ra | IL-15Ra | 30 | 24 | 25 | 12 | 1 | 1 |
| Chemokine | | | | | | | |
| HCC-4 | CCL16 | 31 | 35 | 0 | 2 | 1 | 1 |
| SCYB13 | CXCL13 | 7 | 4 | 1 | 1 | 2 | 2 |
| Chemokine receptor | | | | | | | |
| CXCR5 | BLR1 | 7 | 9 | 0 | 1 | 0 | 1 |
| Other genes and proteins | | | | | | | |
| TRAF5 | TRAF5 | 12 | 8 | 1 | 1 | 1 | 1 |
| CIITA | MHC2TA | 9 | 5 | 1 | 1 | 1 | 2 |
| Smad2 | MADH2 | 8 | 2 | 1 | 0 | 1 | 1 |
| NOS | NOS2A | 7 | 2 | 1 | 1 | 1 | 1 |

*Note*: Expression of mRNA of each transcript in the PBMC cultured for 5 days with either PPD or Candida was compared with unstimulated controls. Numbers indicate fold increase in the TB (LTBI and ATBI) patients as compared with three PPD negative controls. Individuals with negative proliferation in the presence of Candida were used for corrections. Higher than six-fold increases were considered significant

cultures on ATBI but not in LTBI are consistent with such findings. Also, increased levels of IL-6 have been shown to be present in plasma of patients with active tuberculosis [28]. Furthermore, exposure of THP-1 human monocytes to Mtb H37Rv strain induced the phosphorylation of p38 mitogen-activated protein kinase (MAPK) along with an upregulation of IL-6 [28]. This is consistent with the increased secretion of cytokines such as IFN-$\gamma$, in the cultured supernatants of PPD-positive individuals (Fig. 2). However, IFN-$\gamma$ and TNF-$\alpha$ expression were not significantly up-regulated in the microarrays possibly because they were tested only in 5-day cultures. Three unexpected findings that need to be confirmed were (i) cultures with PPD but not with Candida in the 3 PPD-negative controls showed comparable levels of IL-6 secretion to those produced by the 6 PPD-positive individuals (LTBI and ATBI), suggesting that PPD elicited primary in vitro stimulation;

**Table 2** Down-regulated messenger RNA analysis in the PBMC of TB-infected individuals incubated with etiher PPD or Candida

| Gene name | Symbol | Active TB | | Latent TB | | Control | |
|---|---|---|---|---|---|---|---|
| | | PPD | Candida | PPD | Candida | PPD | Candida |
| Transcription factors | | | | | | | |
| STAT4 | STAT4 | 7 | 1 | 1 | 2 | 3 | 1 |
| Toll-like receptor | | | | | | | |
| TLR10 | TLR10 | 4 | 4 | 47 | 5 | 0 | 1 |
| Interleukin | | | | | | | |
| IL-15 | IL15 | 0 | 0 | 19 | 1 | 0 | 0 |
| Other genes and proteins | | | | | | | |
| Fra-1 | FOSL1 | 16 | 1 | 2 | 1 | 1 | 2 |
| ICOS | ICOS | 8 | 1 | 1 | 1 | 1 | 1 |
| DORA | IGSF6 | 0 | 0 | 8 | 1 | 0 | 0 |

*Note*: Messenger RNA expression of each transcript in the PBMC with either PPD or Candida antigen were compared with controls. Numbers indicate fold decreases in the TB patients as compared with unstimulated controls. For Candida, individuals with negative proliferation in its presence were used for corrections. Six-fold decreases or higher were considered significant

(ii) higher secretion of TNF-$\alpha$ in cultures with PPD in ATBI but not in LTBI or controls; and (iii) higher secretion of IFN-$\gamma$ in cultures with PPD in LTBI and ATBI.

Importantly, Mtb may alter the cytokine balance in the inflammatory environment by modulating expression of both inflammatory and anti-inflammatory molecules/cells. Promotion of an inflammatory milieu would increase the production of IFN-$\gamma$, resulting in an increased expression of HLA on APC [29].

Microarray analyses of messenger RNA has been used to investigate the gene expression of PBMC from cattle infected with *Mycobacterium bovis* [30]. The pattern of expression in PPD bovine stimulated PBMC provided the first description of *M. bovis* specific signature of infection. These studies were performed in cultures after 3–24 h showing several up-regulated genes: IFN-$\gamma$, TNF-$\alpha$, IL-12, and class II MHC molecules. However, these studies did not investigate the signature profile of cattle with latent infection. Additionally, their results were difficult to compare with our results, since we did not investigate gene expression after 3–24 h of incubation. Also, associations between cytokine gene expression and the severity of the infection measured between animals with pulmonary pathology in *M. bovis* infected cattle. Cells from animals with high pathology secreted higher amounts of IFN-$\gamma$, TNF-$\alpha$, iNOS, and IL-4 than animals in the low pathology group at early times post-infection. Also, the expression of IL-10 decreased with time and IFN-$\gamma$ and iNOS gene expression were significantly greater in tissues of infected than uninfected animals [31]. But these reports did not investigate cattle with latent infection.

Our results although preliminary are the first to compare ATBI with LTBI individuals, by bead arrays after tuberculin activation of PBMC the measurements of cytokine and chemokines showing similar cytokine levels in ATBI and LTBI. In this study, we present exploratory results using both bead and RNA arrays. We showed that using RNA microarrays with 364 genes after PBMC antigen challenge for 5 days demonstrated differences between active from latent TB infection. Among the 14 genes that were up-regulated, IL-2 and IL-6, IFN$\beta$-2, IL-8 and IL-7 were important in communication between T cells and macrophages. For example, infection of macrophages with Mycobacteria leads

to effective IL-7 secretion maintaining cellular immune responses of T cell subsets [32], and enhanced survival of Mtb-infected BALB/c mice [33]. Also, it was shown that IL-7 induces anti-mycobacterium avium activity in macrophages[34] and IL-2 was increased in patients with active TB [18]. Other up-regulated genes included p38 MAPK which leads to the production of both TNF-$\alpha$ and IL-6 in human monocytes through TLR interaction [35]. CCL16 was markedly increased in the PBMC of the patient with active TB but not in the LTBI individual. CXCR5 has been shown to be increased in B cells of pulmonary lymphoid tissues [8]. CXCL13 is negatively regulated by the presence of B cells, as its production is elevated in lungs of B cell-deficient (B cell(−/−)) mice [8], suggesting a role of B cells in TB. Smad 2 was up-regulated in the patient with active TB and is a member of the R-Smads, which is activated by phosphorylation by type I receptors. Furthermore, transduction of TGF-$\beta$ signaling depends on activation of Smad2 and Smad3 by heteromeric complexes of ligand-specific receptors [36].

Importantly, NOS, expressed in macrophages of human TB lesions, may control the different stages of TB including possible innate protection from infection [37–39]. This is consistent with the up-regulation of NOS2A only in the PBMC of the active TB patient (Table 2) and the production of NO by infected macrophages correlated with the function and life span of gamma/delta T cells at the site of TB infection [40].

Only one gene, IL-17, was up-regulated in the LTBI individual. IL-17 is an inflammatory cytokine produced by T lymphocytes in response to IL-23. In non-infected and Mtb-infected mice, IL-17 production may be secreted by gamma/delta T cells and other non-CD4+ CD8+ cells, rather than CD4+ T cells [41]. IL-23 was detected in the lungs during early onset of infection and the IL-17-producing cells may be involved in the innate protective response to infection[41]. Also, IL-17 was up-regulated in response to Candida, which was reported to be required for anticandida host defense[42]; memory T cells specific for Candida are mainly in the Th-17 subset [43]. It is possible that IL-17 induces neutrophil-mediated inflammation and potentially protect from TB by inducing T cells to produce less IFN-$\gamma$ in respone to *M. bovis* bacille Calmette Guerin (BCG) [43, 44]. In additon, memory T cells express high levels of IL-17 [42, 45] which was up-regulated in latent TB consistent with the possible role of this cytokine in controling the reactivation of the disease in LTBI individuals.

Also, it is worth mentioning the higher expression of this cytokine in T regulatory susceptible to HIV infection in patients with chronic infection and high plasma levels of bacterial liposaccharide. Although we did not study LPS in our culture experiments, the studies with Candida suggest that mycobacterial infections are influenced by co-infections and that IL-17 is a good candidate to explain the production of latency to disease as well as mechanism to lose latency after HIV infections [46].

As described in our results, the down-regulated genes in active TB were ICOS, Fra-1 IL-15, DORA, and TLR10. These can be involved in the production of granulomas with inactivation of the infection but can also indicate protection from infection in individuals highly exposed to infected TB patients but without evidence of infection. Our preliminary findings may constitute the basis for future large number cohorts of patients with active and latent TB comparing PBMC in the presence or absence of tuberculin to ascertain whether these genes are involved in protection from infection or used as a marker.

In summary, 17 genes were found up-regulated in active TB and one in latent TB. Also, four genes were found down-regulated in active TB and four in latent TB that distinguish latent from active disease. Our results demonstrated that bead arrays used to measure cytokines in PMBC cultures can distinguish ATBI from LTBI since cultures with tuberculin had higher secretion of TNF in ATBI than in LTBI and cultures with Candida higher

secretion of IFN in LTBI than ATBI. The up-regulation of IL-17 and the down-regulation of IL-15 and TLR10 are candidate genes that could be involved in the definition of latent TB. Of importance, we found 11 out of 16 genes that were up-regulated in active TB showing similar patterns in the presence of either tuberculin or Candida, suggesting the importance of the similar gene networks involved in cellular immune responses involved in the pathogenesis of infectious diseases.

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